PROJETO

FARMACOLOGIA DO SISTEMA NERVOSO CENTRAL (ENCÉFALO E MEDULA ESPINAL) EM MODELOS EXPERIMENTAIS DE INFLAMAÇÃO

Relatório Final

Relatório Técnico apresentado à Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG, em cumprimento ao Processo CBB 867/97, vigente no período de 25 de julho de 2000 a 24 de julho de 2002.
PROJETO

FARMACOLOGIA DO SISTEMA NERVOSO CENTRAL (ENCÉFALO E MEDULA ESPINHAL) EM MODELOS EXPERIMENTAIS DE INFLAMAÇÃO

Relatório Final

Belo Horizonte, MG.
FUNDEP
Agosto, 2002
RELATÓRIO FINAL

FAPEMIG: PROC. CBB 867/97

PROJETO: “FARMACOLOGIA DO SISTEMA NERVOSO CENTRAL (ENCÉFALO E MEDULA ESPINHAL) EM MODELOS EXPERIMENTAIS DE INFLAMAÇÃO”

PESQUISADORA: PROFA. DRA. JANETTI N. FRANCISCHI

Departamento de Farmacologia - ICB
Laboratório de Inflamação e Dor
Universidade Federal de Minas Gerais

2000-2002
BELO HORIZONTE, 23 DE AGOSTO DE 2002

DD. DIRETOR CIENTÍFICO DA FAPEMIG
PROF. DR. NAFTALE KATZ
RUA RAUL POMPÉIA, 107 – SÃO PEDRO – BH – MG

Prezado Sr.

É com satisfação que encaminhamos nosso relatório científico final referente ao projeto: “FARMACOLOGIA DO SISTEMA NERVOSO CENTRAL (ENCÉFALO E MEDULA ESPINHAL) EM MODELOS EXPERIMENTAIS DE INFLAMAÇÃO”, catalogado na FAPEMIG com o número CBB 867/97.

Conforme apresentado no relatório anexo, com a subvenção da FAPEMIG foi possível desenvolver nossos projetos de pesquisa, o que permitiu a elaboração dos seguintes trabalhos:

- 3 trabalhos completos em revistas indexadas, sendo 1 no ano de 2000 na revista “European Journal of Pharmacology” e 2 no ano de 2001 sendo 1 na revista “Inflammation” e outro na revista “Brazilian Journal of Medical and Biological Research”;

- 1 trabalho completo publicado na revista do Conselho Regional de Odontologia de Minas Gerais (CRO-MG) no ano de 2000;

- 3 trabalhos já aceitos para publicação em 2002, sendo 1 na revista “British Journal of Pharmacology”, 1 na revista “Brazilian Journal of Medical and Biological Research” e 1 na revista do “Conselho Regional de Odontologia do Estado de Minas Gerais (CRO-MG)”;

- 26 resumos apresentados sob a forma de “posters”, sendo 15 em Congressos Internacionais;
- 1 tese de Doutorado e 1 de mestrado em 2001 como orientações concluídas. O início de desenvolvimento de outras 4 teses de Doutorado e 1 de mestrado foram possibilitadas.

Convidada, pois, de que o apoio financeiro a projetos de pesquisa pelas agências financeiras, em especial o papel da FAPEMIG em Minas Gerais, é essencial para que a Ciência floresça e que dé os seus frutos no Estado, venho agradecer o apoio recebido ao nosso projeto.

Saliento ainda, que tal apoio permitiu que alcançássemos um patamar mais alto, expresso não só pela produção científica gerada, mas mais importante, contribuiu decisivamente para a formação de novos pesquisadores, quia futuros líderes da nossa Sociedade.

Atenciosamente,

Profa. Dra. Janetti Nogueira de Francisci
Coordenadora do Projeto
Departamento de Farmacologia – ICB – UFMG

P.S. Os originais dos trabalhos publicados, dos aceitos para publicação e dos resumos anexos, encontram-se em nosso poder para quaisquer conferências, uma vez que nós é impraticável enviá-los sob a forma de arquivos em disquetes.
TRABALHOS COMPLETOS PUBLICADOS (2000-2002)

- Hyperalgesia and edema responses induced by rat peripheral blood mononuclear cells incubated with carrageenin (Inflammation – 2001);

- Acute phenobarbital administration induces hyperalgesia: pharmacological evidence for the involvement of supraspinal GABA-A receptors (Brazilian Journal of Medical and Biological Research- 2001);

- Anti-inflammatory and analgesic effects of the phosphodiesterase 4 inhibitor rolipram in a rat model of arthritis (European Journal of Pharmacology- 2000);

- Avaliação da prescrição de medicamentos realizada por cirurgiões-dentistas de Belo Horizonte (Revista do Conselho Regional de Odontologia de Minas Gerais- 2000).
Hyperalgesia and Edema Responses Induced by Rat Peripheral Blood Mononuclear Cells Incubated with Carrageenin

Marcos Antonio De Resende,1 Webster Glayser Pimenta Dos Rei,1 Leani De Souza Máximo Pereira,1 Wanderley Ferreira,2 Maria Helena de Lima Perez Garcia,2 Marcelo Matos Santoro,2 and Janetti Nogueira de Francischl3

Abstract—The aim of this study was to verify the role played by mononuclear cells in an acute (nonimmune) inflammatory reaction. Mononuclear cells purified from rat peripheral blood were incubated for 1, 2, or 24 h with 100 or 250 μg/ml carrageenin (Cg). The resultant donor supernatant was injected into recipient rats to test its ability to induce hyperalgesia (reduction in threshold for paw pressure) and edema (increase in paw volume). Mononuclear cell supernatants (MnS) induced a significant time- and dose-dependent hyperalgesia and edema in rat paws, which reached a maximal effect at 3 h, lasted for 6 h, and returned to basal levels at 24 h of injection. Prostaglandins and cytokines (interleukin 1, 2, 6, 8, and tumor necrosis factor alpha) accounted for the hyperalgesia induced by MnS, as it was reduced (40 to 90%) by synthesis inhibitors such as indomethacin, dexamethasone, rolipram, and cyclosporin added to the cultures at a microgram dose-range. Edema was dependent on serotonin release in rat paws. These results indicate that mononuclear cells may be important contributors to acute inflammatory reactions, especially under those conditions where pain is an important component.

KEY WORDS: hyperalgesia; edema; rat mononuclear cells.

INTRODUCTION

Mononuclear cells are considered to play a central role in inflammatory reactions derived from immune responses. Examples of such inflammatory reactions are found in chronic diseases from human and experimental animals, such as allergies (1, 2), rheumatoid arthritis (3), systemic lupus erythematosus (4), experimental allergic encephalomyelitis—an experimental model for multiple sclerosis (5), and asthma (6, 7), where activation of monocytes/macrophages, T and B lymphocytes are key events.

Monocytes and macrophages (phagocyte mononuclear cells) (8), while essential for immune responses, are mainly associated with inflammatory reactions. These cells have been studied by both immunologists and pharmacologists for the last 40 years. Many important proinflammatory functions related to the monocyte/macrophage cells are due to cytokine release (9). For instance, interleukin 1 (IL 1), one of the oldest cytokines ascribed to macrophages (10), was shown to induce neutrophil recruitment necessary for contention by the tissues of Gram-negative bacteria spreading (11). Other cytokines released by macrophages, which
exhibit chemotactant functions are called chemokines or "small cytokine family" (12), and these control migration of specific cell lineages. One such example is neutrophil migration induced by the chemokine IL 8 (13). IL 1, tumor necrosis factor alpha (TNF-α), IL 6, and IL 8, all of which are released by macrophages, induce hyperalgesia when administered locally into rat paws (14).

T and B lymphocytes process immune responses through the release of biologically active substances such as cytokines, chemokines, and antibodies, which alone or in combination shape the signals and symptoms expressed by the host (15). In contrast to macrophages, however, data on the contribution of lymphocyte products to acute inflammatory reactions are sparse in the literature. The aim of our work was to detect and pharmacologically characterize biological active substance(s) released from cultured rat mononuclear cells (in vitro) in the presence of carrageenin, a standard proinflammatory stimulus (16). Biological activity of released substances from donor mononuclear cells was detected through the use of edema formation and hyperalgesia assays in recipient rat paws.

MATERIAL AND METHODS

Animals

Female Holtzman rats weighing 160–200 g, from Bioterium Center (CEBIO, Federal University of Minas Gerais), were used throughout the present study. The animals were left to adapt to the experimental environment for 2–3 days before use in a room with temperature control (23–25°C), food and water ad libitum and a dark–light cycle of 12/12 h (beginning at 7:00 a.m.). Ethical guidelines of the International Association for the Study of Pain in conscious animals were followed (17).

Purification of Peripheral Blood Mononuclear Cells

Blood from animals was collected by cardiac puncture (18) following complete anesthesia by an intramuscular injection of a mixture of ketamine–xylazine (90 and 15 mg/kg, respectively) under aseptic conditions. Mononuclear cells (Histopaque, Sigma) were purified when a volume of 3.0 ml blood was laid on the surface of a gradient solution of 1.038 density (3.0 ml) previously added to sterile Vacutainer tubes which were centrifuged for 30 min at 800 g. Mononuclear cells separated from other blood components in an upper layer, and were gently collected by pipette under flow chamber and washed twice with 5.0 ml RPMI 1640 medium added of heparin (5 U/ml), pH = 7.2–7.4. For supernatant culture preparation (see following), purified cells were diluted to obtain a final concentration of 1 × 10^6 cells/ml in RPMI medium. Aliquots of blood and purified cells were separated for total and specific counts under optical microscopy and were expressed as cells per cubic millimeter and percentage, respectively.

Preparation of Mononuclear Cell Supernatants (MnS)

Triplicates of purified cells (4 × 10^5 per well in 0.4 ml) were added to complete 1.0 ml with medium in 24-well plates (Limbro, Flow Labs) with or without λ-carrageenin (final concentration: 100 or 250 μg/well) and incubated for 1, 2, or 24 h under (36.5°C and CO₂ atmosphere). Cell-free carrageenin-free wells were assayed in parallel. In some experiments, either rolipram (9 or 18 μg), dexamethasone (50 μg), indomethacin (2 μg), or cyclosporin A (10 or 100 μg) was added to individual wells. Controls for the latter experiments used the same volume of vehicle under sterile conditions as follows: physiological saline for rolipram and dexamethasone; Tris 0.1 M pH = 8.0 for indomethacin and saline-diluted cremophor EL for cyclosporin. At the end of the incubation period, control wells were added of respective drugs and test wells were added of the same amount of vehicle (0.1 ml). The culture fluid was collected under sterile conditions, centrifuged to separate cells for 5 min at 1300 g, and the remaining triplicate supernatants were combined and kept in a freezer (−15°C) under use. Each pooled supernatant was used at least in two different days.

Measurement of Hyperalgesia

Hyperalgesia measurements were obtained using the Randall and Selitto method (19). Inflammation leads to hyperalgesia, an increase in pain sensation, to a normally noxious stimulus. The test consisted of measurement of the threshold for reaction (to escape or attempt) to a crecent weight (in grams, g) applied to pads of posterior paws by an experimenter using an Ugo Basile apparatus. The threshold for pain sensation was measured before (time zero) and following 1, 2, 3, 4, 6, and 24 h of intraplantar injection of defrosted 0.1 ml containing LyS or 250 μg carrageenin in one of the posterior rat pads. Contralateral paws were injected with
same volume of saline (vehicle). Some animals were also pretreated intraplantarly with 0.1 ml containing indomethacin, rolipram, dexamethasone, or vehicles 30 min before MnS or carrageenin. Results from control (vehicle) and treated animals are presented as the mean difference between the test paw threshold value and the contralateral paw value (mean ± standard error of the mean or SEM).

Edema Measurements

Edema measurements were obtained following hyperalgesia measurements. The volume (in milliliters) of the posterior paws from control and treated animals was obtained with a hydropiasthymometer (Ugo Basile 1750) at time zero and following 1, 2, 3, 4, 6, and 24 h of stimulus injection. Results are presented as mean difference between the test paw and the contralateral paw value ± standard error of the mean (mean ± SEM).

Drugs

All drugs and reagents were purchased from Sigma Co. (MI) with the exception of dexamethasone (Decadron, MSD); cyclosporin A (Sandimun, Novartis) and rolipram. Cyclosporin was a kind gift from Novartis in Minas Gerais (Brazil) and Dr. M. M. Teixeira, who kindly supplied us with rolipram.

Statistics

MnS-induced hyperalgesic and paw edema effects under various conditions tested were compared with their control groups. Differences between groups in each time were analyzed by Analysis of Variance (ANOVA t test), considering a significant difference when P < 0.05 given by a Sigma Stat program (1.0) in a Windows environment.

RESULTS

To study the potential of mononuclear cells to contribute to an acute inflammatory reaction, cells were purified from rat peripheral blood by gradient centrifugation and cultured in vitro with carrageenin, a standard proinflammatory stimulus. As shown in Table 1, lymphocytes comprised 92% of the mononuclear cell population in our purified preparation; the remaining cells were essentially monocytes (6%) and polymorphonuclear cells (eosinophils and neutrophils) (2%). The recovery rate for mononuclear cells purified from peripheral blood in 20 animals used was about 19% (Table 1). To determine whether mononuclear cells would release proinflammatory substances into the culture medium following incubation with carrageenin, mononuclear cells were cultured for different times and the resultant supernatant (MnS) was injected in recipient rat paws. Figure 1 (panels A and B) shows that intraplantar injection of 0.1 ml of the supernatant derived from 4 × 10⁷ mononuclear cells incubated with 250 μg/ml carrageenin for 2 h induced significant hyperalgesia and edema development in rat paws as compared to paws injected with culture medium devoid of cells or carrageenin (P < 0.05, ANOVA t test). The hyperalgesic and paw edema responses derived from MnS reached a maximal effect 3 h following injection, lasted for 6 h, and returned to a basal level following 24 h of injection (Fig. 1A, B). From 18 pools of 2 h mononuclear cell supernatant injected in rat paws, all presented a significant hyperalgesic activity (~44.6 ± 4.32 g), whereas only 15 have presented a significant edematogenic activ-

---

Table 1. Recovery Rate of Mononuclear Cells Following Purification of Rat Peripheral Blood by Density Gradient

<table>
<thead>
<tr>
<th>Total No. of Cells (x 10⁴)</th>
<th>Cell No. Following Purification (x 10⁴)</th>
<th>Specific Counts (%)</th>
<th>Recovery Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ly⁻ M⁻ N⁺ E⁺ B⁺</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>92 ± 1.02</td>
<td>92</td>
</tr>
</tbody>
</table>

a Blood was collected by cardiac puncture from previously anesthetized animals.

b Mean number ± standard error of the mean from 20 experiments.

c Ly = Lymphocytes; M = Monocytes; N = Neutrophils; E = Eosinophils; B = Basophils.

d Recovery rate (in %) derived by dividing data in second column by data in first column and multiplying by 100.
Fig. 1. Hyperalgesia (A) and edema (B) development in rat paws following injection of supernatant derived from incubation of mononuclear cells with carrageenin. Mononuclear cells were cultured for 2 h in RPMI medium, in the presence of 250 µg carrageenin. Controls consisted of cells with no carrageenin added, or the medium alone. Following centrifugation of the resultant fluid obtained at the end of the incubation period, supernatants were frozen until tested. When 0.1 ml was injected into one of the posterior rat paws (intraplantar). Contralateral paws were injected with the same volume of sterile physiological saline. Hyperalgesia (panel A) is described as the minimal weight in grams applied to paws necessary to provoke an escape movement by the animals at the various time points using a Randall-Selitto apparatus. Edema (panel B) was obtained by volume displacement using a plethysmometer apparatus. Results (A, in g, B, in ml) depicted in the figure refer to the mean difference between test and control values ± SEM in groups of three to five animals.

Fig. 1. Continued.

was inhibited by 97% the hyperalgesic response following 3 h of MnS injection, whereas only at a higher dose (200 µg) indomethacin could significantly affect hyperalgesic response due to direct carrageenin (250 µg) injection. On the other hand, 100 µg indomethacin did not significantly alter paw edema response due to MnS or 250 µg Cg. Only at a dose of 200 µg was indomethacin inhibited by 87 and 48% of the hyperalgesia and edema derived from 250 µg carrageenin (Table 2). In addition, previous local dexamethasone injection at 1 µg/paw significantly inhibited hyperalgesic activity due to local injection of MnS or 250 µg carrageenin by 49 and 38%, respectively (Table 2). Dexamethasone did not affect edema formation by MnS and decreased carrageenin-induced edema by 17% (Table 2). The phosphodiesterase inhibitor rolipram at 0.275 or 9 µg/paw did not affect hyperalgesic activity due to MnS, but completely inhibited hyperalgesia induced by carrageenin at both doses (Table 2). However, rolipram induced a more complex effect on paw edema due to MnS injection, either significantly increasing (20%) or reducing (45%) this response. In contrast, rolipram pretreatment of rat paws at 9 µg increased the edema by 18% following 3 h of carrageenin injection. A previous systemic dose of pizotifen (2 mg/kg), a 5-HT2 receptor antagonist, abolished paw edema by 75% due to mononuclear cell supernatant injection, although this dose of pizotifen did not affect hyperalgesic activity of the supernatant (Table 2).

To further assess the pharmacological profile of
Fig. 2. Hyperalgesia (A) and paw edema (B) dose-response curves in rat paws following injection of mononuclear cell supernatants incubated with different concentrations of carrageenan. Mononuclear cell supernatants were obtained and injected in rat paws as described in Fig. 1, and carrageenan was added at either 100 or 250 µg/ml for 2 h. Results are presented as mean ± SEM.

Fig. 3. Hyperalgesia (A) and edema (B) in rat paws following injection of lymphocyte supernatants derived from incubation of cells with carrageenan. A standard dose of 250 µg/ml was added to the cultures and left for 1, 2, or 24 h in contact with cells. (See legend to Fig. 1.) A time course of hyperalgesia and edema derived from a direct injection of 250 µg carrageenan (in 0.1 ml) in rat paws is also shown.

The substances present in MnS, standard anti-inflammatory and immunosuppressor drugs were added to the cultures. As shown in Table 3, addition of indomethacin (2 µg/ml) for 2 h to the cultures inhibited the hyperalgesic activity by 93% of the supernatant, whereas it inhibited the paw edema activity only by 66% due to the MnS injection. Dexamethasone, at 50 µg/ml, inhibited by the hyperalgesic and edematogenic activities by 62 and 50% presented by MnS (Table 3). Rolipram added
to mononuclear cell cultures at a dose of 9 or 18 μg/ml significantly inhibited the hyperalgesic activity by 77% only at the highest dose used, but did not affect the paw edema at either dose (Table 3). Finally, addition of 10 or 100 μg/ml of the immunosuppressant drug cyclosporin A to mononuclear cell cultures for 2 h completely inhibited its hyperalgesic activity in recipient animals but it did not affect the concomitant edematogenic activity of the supernatant (Table 3).

**DISCUSSION**

Mononuclear cells have been studied extensively as a cell population involved in immune function. In this context, cellular processes involved in antigen presentation, antibody production, and production of cytokines/chemokines have been thoroughly examined from the immunological point of view, as can be concluded following readings of authoritative textbooks (8, 20). Together with monocytes in the blood and macrophages in the tissues, lymphocytes comprise a major defense against invading organisms. However, there have been a more limited number of studies addressing, from a pharmacological point of view, the mononuclear cell contribution to nonimmune inflammatory reactions (21).

To evaluate the contribution of mononuclear cells to an acute inflammatory reaction, we studied in the present work the potential of the supernatant from a mononuclear cell preparation (98% pure) to induce hyperalgesia and edema in recipient rat paws. Inflammatory edema and pain are pathophysiological conditions which affect the majority of individuals at some time in their lives (22).

Data presented here showed that carrageenan added to mononuclear cell cultures induced an acute production and release of biologically active substances, i.e., hyperalgesic- and edema-producing substances. In addition, the hyperalgesic- and edema-producing effects observed following injection of 24-h supernatants derived from mononuclear cell incubation with carrageenan clearly reproduced direct injection of the latter into rat paws (Fig. 3), suggesting mononuclear cells

---

**Table 2. Effect of Anti-inflammatory Drugs on Mononuclear Cell Supernatant (MnS)- and Carrageenan (Cg)-induced Hyperalgesia and Edema in Rat Paws**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (μg/paw)</th>
<th>Hyperalgesia (MnS; Mean ± SEM)</th>
<th>Edema (Cg 250; Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MnS</td>
<td>Cg (250)</td>
</tr>
<tr>
<td>Cl</td>
<td>—</td>
<td>—30.5 ± 5.5</td>
<td>—35.3 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—0.94 ± 1.29</td>
<td>—20.6 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>NS</td>
<td>4.7 ± 4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(49%)</td>
<td>(48%)</td>
</tr>
<tr>
<td>C</td>
<td>—</td>
<td>—40.7 ± 4.1</td>
<td>—102 ± 8.7</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1</td>
<td>—20.7 ± 3.2</td>
<td>—63 ± 4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(44%)</td>
<td>(33%)</td>
</tr>
<tr>
<td>C</td>
<td>—</td>
<td>—40.8 ± 5.7</td>
<td>—53.3 ± 5.8</td>
</tr>
<tr>
<td>Rolipram</td>
<td>0.275</td>
<td>52.5 ± 3.0</td>
<td>8.5 ± 21.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4100%)</td>
<td>(720%)</td>
</tr>
<tr>
<td>C</td>
<td>—</td>
<td>—52.0 ± 4.3</td>
<td>—38.8 ± 12.9</td>
</tr>
<tr>
<td>Rolipram</td>
<td>9</td>
<td>34.0 ± 7.1</td>
<td>5.4 ± 7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4450%)</td>
<td>(1450%)</td>
</tr>
<tr>
<td>C</td>
<td>—</td>
<td>—25.5 ± 4.5</td>
<td>ND</td>
</tr>
<tr>
<td>Piroz 2</td>
<td>2</td>
<td>—31.1 ± 9.5</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N.S.)</td>
<td></td>
</tr>
</tbody>
</table>

*Hyperalgesia and edema were measured at 3 h following either MnS or Cg injection intraplantarly in a volume of 0.1 ml at time zero. NS indicates a nonsignificant statistical difference in relation to controls (C). ND = not done. Cl indicates a control paw injected with 0.1 ml Tris 0.1 M pH 8.0 at time zero. C indicates a control paw injected with 0.1 ml saline at time zero. All drugs, except pirozol, at 2 mg/kg, sc, were administered intraperitoneally 30 min before stimuli. Percentage inhibition is shown in brackets (n = 3–5 animals/group). Arrow upward and downward indicate increase and decrease of the response, respectively.*
Table 3. Effect of Anti-inflammatory and Immunosuppressive Drug Addition to Donor Mononuclear Cell Cultures on Hypersalgesic and Edematosic Activities Measured in Rat Paws

<table>
<thead>
<tr>
<th>Drug</th>
<th>[ ] = (μg/ml)</th>
<th>Hyperalgesia (in g, Mean ± SEM)</th>
<th>Edema (in ml, Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clobenacamia</td>
<td>—</td>
<td>—56.6 ± 18.9</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>2</td>
<td>—4.0 ± 11.3 (493%)</td>
<td>0.2 ± 0.01 (466%)</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>50</td>
<td>—34.7 ± 5.7</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>9</td>
<td>—13.3 ± 21.9 (462%)</td>
<td>0.05 ± 0.01 (456%)</td>
</tr>
<tr>
<td>Naproxen</td>
<td>9</td>
<td>—13.8 ± 2.9 (NS)</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>18</td>
<td>—27.4 ± 2.9 (NS)</td>
<td>0.4 ± 0.02 (NS)</td>
</tr>
<tr>
<td>Bupraden</td>
<td>18</td>
<td>—44.0 ± 5.83</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Rofinpril</td>
<td>18</td>
<td>—10.0 ± 2.99 (477%)</td>
<td>0.13 ± 0.03 (NS)</td>
</tr>
<tr>
<td>Cyclosporin 10</td>
<td>—</td>
<td>—67.0 ± 3.1</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>10</td>
<td>—6.0 ± 1.8 (7100%)</td>
<td>0.10 ± 0.01 (NS)</td>
</tr>
<tr>
<td>Cyclosporin 100</td>
<td>—</td>
<td>—66.0 ± 3.1</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>100</td>
<td>1.2 ± 3.8 (4100%)</td>
<td>0.13 ± 0.01 (NS)</td>
</tr>
</tbody>
</table>

*Mononuclear cells (4 × 10^5 cells) purified from peripheral blood were cultured in RPMI medium in the presence of 250 μg/ml α-carrageenan for 2 h. Drugs were added in the doses depicted to wells before cells and carrageenan to have a final volume of 1 ml/well.

...
unlikely that this amount could be transferred; (2) anti-
flammatory drug addition to mononuclear cell cultures or treatment of the paws prior to MnS injection gave different results from those observed following direct injection of carrageenin administration into rat paws treated with the same drugs, particularly those results from rolipram-treated animals.

The time chosen to study the release of biologically active substances by carrageenin-stimulated mononuclear cells was 2 h, and it is unlikely to detect a significant amount of protein-like substances due to synthesis of new proteins during this short period. However, a reduction in approximately 50% of the hyperalgesic activity of supernatant was observed after boiling (unpublished data), giving support to the possibility of heat-labile polypeptide/protein present in the supernatant. We do not know the chemical nature of the substances present in mononuclear cell supernatants. It is possible that the substances released, besides being prostaglandins, are cytokines/chemokines already described, such as TNFα, IL 2, IL 6, and/or IL 8. Gene expression for the latter can be inhibited by anti-inflammatory steroids, such as dexamethasone which was used in the present study. All of these substances have been demonstrated to induce hyperalgesia in rat paws (15, 34). TNFα must be considered, as rolipram which is a known inhibitor of TNFα production (30) exhibited a strong inhibitory effect on the generation of hyperalgesic activity present in the supernatant (Table 3). Interleukin-2 may also be present in our supernatants since it is produced by lymphocytes (35) and can induce hyperalgesia in rats (34). In addition, transcription of the IL 2 gene begins within 1 h following TCR-mediated stimulation of normal (human) lymphocytes (36). We can therefore speculate that carrageenin could have induced production and release of IL 2 by the cultured rat lymphocytes. Pharmacological evidence for this hypothesis was the finding that cyclosporin, a classical inhibitor of IL 2 synthesis by lymphocytes (41), following addition to mononuclear cell cultures, was also able to eliminate hyperalgesia without affecting the concomitant edematogenic activity of the supernatant. Finally, the presence of other hyperalgesic products resultant from incubation of mononuclear cells with carrageenin, such as IL 1, IL 6 and IL 8, is further suggested as anti-inflammatory steroids are able to inhibit their expression (28), and their hyperalgesic effect seems to derive from prostaglandin release in rat paws (14). As previously mentioned, addition of indomethacin to the cultures inhibited the hyper-
algesia induced by the supernatant (Table 3).

In summary, we conclude that a purified mononuclear cell preparation incubated with carrageenin acutely (following 2 h incubation) released substances able to induce hyperalgesia (the main symptom) and edema in rat paws. Prostaglandins accounted for the major hyperalgesic activity present in mononuclear supernatants, although cytokines such as TNFα, IL 1, IL 2, IL 6, and IL 8 are further suggested. Whether specifically COX-1 and/or COX-2 was/were involved in prostaglandin release by mononuclear cells in vitro is currently under investigation. Serotonin was the main mediator released by mononuclear cell supernatant in rat paws and the most important mediator involved in edema formation. The substances present in mononuclear supernatant which caused hyperalgesia were different from those which caused edema, but their exact chemical nature could not be further identified. The present data confirmed and expanded previous results from the literature, and we suggest that mononuclear cells may be prominent effectors of proinflammatory responses due to carrageenin injection in rat paws. Our results also point out a possible important role for lymphocytes, apart from monocytes, in the genesis of the pain observed during the course of an acute inflammatory reaction.

Acknowledgements—We are pleased to thank CNPq (grant 521482/96-8), FAPESP (grant CBB867/97) and CAPES for financial support. We would also like to acknowledge S. M. Bakhle and T. J. Williams for their helpful comments on this study.

REFERENCES


Acute phenobarbital administration induces hyperalgesia: pharmacological evidence for the involvement of supraspinal GABA-A receptors

Abstract

The aim of the present study was to determine if phenobarbital affects the nociception threshold. Systemic (1-20 mg/kg) phenobarbital administration dose dependently induced hyperalgesia in the tail-flick, hot-plate and formalin tests in rats and in the abdominal constriction test in mice. Formalin and abdominal constriction tests were the most sensitive procedures for the detection of hyperalgesia in response to phenobarbital compared with the tail-flick and hot-plate tests. The hyperalgesia induced by systemic phenobarbital was blocked by previous administration of 1 mg/kg picrotoxin or either 1,2-mg/kg x or 10 ng icv bicuculline. Intracerebroventricular phenobarbital administration (5 μg) induced hyperalgesia in the tail-flick test. In contrast, intrathecal phenobarbital administration (5 μg) induced antinociception and blocked systemic-induced hyperalgesia in this test. We suggest that phenobarbital may mediate hyperalgesia through GABA-A receptors at supraspinal levels and antinociception through the same kind of receptors at spinal levels.

Introduction

Barbiturates are drugs with the potential to reduce anxiety and promote sleep, to induce general anesthesia, and in special cases to inhibit tonic-clonic seizures (1). In some of these conditions, they have been replaced with other drugs such as benzodiazepines and serotonin re-uptake inhibitors. However, one such drug - phenobarbital - remains the drug of choice for long-term treatment of generalized (tonic-clonic) seizures in view of its effectiveness, low cost and low toxicity (2).

With respect to the mechanism of action, barbiturates are known to enhance the inhibitory effects of the neurotransmitter gamma-aminobutyric acid (GABA) at the GABA-A receptor level (3). To achieve this, barbiturates bind at the GABA-A receptor increasing the opening time of chloride channels, thus permitting chloride ion entry into the cells (4). In addition, activation of the GABA-A receptor has been associated with pain modulation in the central nervous system (CNS), essentially through the descending
inhibitory system (5). However, it is still a matter of discussion in the literature whether barbiturates increase (6, 7) or decrease (8, 9) the pain threshold.

Since the barbiturate phenobarbital continues to be the drug of choice to treat tonic-clonic seizures, our aim was to investigate if phenobarbital would interfere with the nociceptive threshold. For this purpose, phenobarbital was acutely tested in four algimetric assays using bicuculline and picrotoxin as pharmacological tools through various routes of administration.

Material and Methods

Animals

Experiments were carried out on male Holtzman rats (180-250 g) and Swiss mice (20-35 g), supplied by the Animal House of the Federal University of Minas Gerais. Animals were housed under controlled temperature (23 ± 2°C), on a 12-h dark/light cycle, with food and water ad libitum. The ethical guidelines of the International Association for the Study of Pain for investigations of experimental pain in conscious animals were followed (10).

Measurement of pain threshold

Phenobarbital alone or in combination with the inhibitors picrotoxin or bicuculline was tested in the following methods: tail flick (11), hot plate (12), and formalin (13) in rats, and abdominal constriction (14) in mice. To determine the nociception indices in the tail-flick and hot-plate tests the following formula was used: \( T_1 - B_1 \) (cut-off time - base), where \( T_1 \) and \( B_1 \) are test latency and baseline latency, respectively. The animals selected for testing were previously submitted to 3 sessions at 10-min intervals with baseline latencies of 3.5-4.5 s (tail flick) and 6-18 s at a temperature of 50°C (hot plate). The cut-off time for the tail-flick and hot-plate tests was 7 and 30 s, respectively. Formalin (1.25%, 50 μl) was injected into one of the hindpaws at time zero. The degree or severity of nociception is described in terms of the animal’s behavior and of how it used the injected paw, as follows: degree 0: the paw touches the box, the box wall and floor; degree I: the paw touches the wall and floor lightly and the animal limps; degree II: the paw is not used and does not make contact with any surface; degree III: the animal licks, shakes or bites the paw. The nociception rate (NR) was obtained from the formula: \( NR = (I_t + II_t + III_t) / 300 \), where \( t \) corresponds to the time (seconds) spent in each degree (I, II or III) during a period of 5 min (or 300 s) for each animal. A full abdominal constriction response to acetic acid (0.6%, w/v) was considered to be present when a wave of contraction followed by extension of the trunk and one hind limb occurred. The number of stretches was recorded during periods of 5 min over 30-min intervals for each animal in the group. The results are reported as mean number of constrictions (± SEM) for each group.

Motor coordination

Phenobarbital was also tested for motor impairment in mice conditioned to a Rotarod apparatus (Ugo Basile). The animals accepted for the test were those who fell from the Rotarod during the first 30 min of observation. The permanence time at 32 rpm was recorded for each animal before (baseline) and 15 min after intraperitoneal (ip) drug or vehicle (control) administration. The measures were made in triplicate at 5-min intervals to permit the animals to rest. The mean percentage of reduction (± SEM) in Rotarod permanence time is presented in the Results section.

Intracerebroventricular (icv) catheters

The rat was placed in a stereotaxic frame following pentobarbital anesthesia. A hole
was trephined at coordinates overlying the left lateral ventricle, i.e., 1.4 mm posterior to the bregma and 1.5 mm left to the midline, according to the atlas of Paxinos and Watson (15). The guide cannula (an 11-mm long BD-7 stainless steel cannula) was inserted 3-3.3 mm into the lateral ventricle and fixed with a polymerized acrylic cement adapted to the skull.

**Intrathecal (it) catheters**

Rats undergoing implantation of an it catheter were placed in a stereotaxic frame with the head flexed forward. Under pentobarbital anesthesia, a 7-cm long PE-10 tube was inserted into the subarachnoid space through a slit made in the atlanto-occipital membrane and advanced to the level of the lumbar spinal cord. The external part of the catheter was tunneled into the skull to exit on the parietal bone. The catheter was fixed with a small piece of acrylic placed between the atlanto-occipital membrane and the skull (16).

**Drug treatment**

Phenobarbital was diluted in vehicle consisting of propylene glycol:saline (50:50, v/v) and administered ip 20 min before the beginning of the test or either it or icv 20 min after the beginning of the test. Picrotoxin and bicuculline were dissolved in physiological saline and administered systemically by the ip or sc route 30 min before the test. Only bicuculline was administered centrally by the icv or it route 20 min after the beginning of the test. When the drug was administered systemically (ip) the volume used was 0.1 ml/100 g for rats and 0.1 ml/10 g for mice. For the icv and it injections the volume used was 10 μl 7-10 days after cannula implantation. Icv and it injections of drugs were carefully performed over a period of 90 s to avoid intracranial hypertension or drug extravasation.

**Drugs and vehicles**

The following drugs were purchased from the stated sources: picrotoxin and bicuculline (Sigma Chemical Co., St. Louis, MO, USA), phenobarbital (Rhodia, Santo Amaro, SP, Brazil), propylene glycol (Reagan, Rio de Janeiro, RJ, Brazil), acetic acid (Merck, Rio de Janeiro, RJ, Brazil) and formaldehyde (38%; Labsynth, Diadema, SP, Brazil). Formaldehyde was mixed with saline to obtain a final 1.25% formalin concentration.

**Statistical analysis**

The results are reported as mean ± SEM. Data for the treatment groups were compared by Kruskal-Wallis analysis of variance (ANOVA) on ranks followed by the Dunnett test for multiple comparisons of nonparametric data. The level of significance was set at P<0.05 and the analyses were performed using the Sigma Stat software (version 1.199).

**Results**

**Experiment 1**

Different doses of phenobarbital (2.5, 5, 10 and 20 mg/kg) or vehicle were injected ip into rats to evaluate the effects of the drug on the latency for the tail-flick reflex and the hot-plate test, as well as the nociception rate in the formalin test. In addition, the abdominal stretches in response to acetic acid were counted in mice pretreated with different doses of phenobarbital.

Systemic administration of different doses of phenobarbital (5-20 mg/kg) induced a dose-dependent reduction of the latency response in rats compared with control animals (vehicle) in the tail-flick and the hot-plate test, indicating development of hyperalgesia (Figure 1A and B; P<0.05, Kruskal-Wallis test). In addition, phenobarbital (1.25-5 mg/kg) dose dependently increased the
pain score of the formalin test in rats (Figure 1C) as well as the stretch number in mice, also indicating the development of hyperalgesia (Figure 1D). A time between 40 and 60 min was necessary to observe the maximal hyperalgesic effect in the tail-flick and hot-plate tests. The maximal hyperalgesic effect occurred at 10 and 25 min after phenobarbital administration in the constriction test and formalin test, respectively. Specifically, phenobarbital increased the nociception rate both in phases 1 and 2 of the formalin-induced response (Figure 1C).

However, the minimal dose of phenobarbital needed to induce hyperalgesia varied according to the test used: 1.25 mg/kg and 2.5 mg/kg were enough to induce a significantly increased effect in the formalin and constriction tests, whereas a 2- to 8-fold increase in the dose was necessary to induce hyperalgesia in the hot-plate and the tail-flick test, respectively. In addition, reduction in the time of permanence in the Rotarod apparatus was observed in mice with increasing doses of phenobarbital (1-35 mg/kg), indicating a dose-dependent loss of motor coordination following hyperalgesia (Table 1).

**Experiment II**

A previous (15 min) dose of 1 mg/kg picROTOXIN blocked the hyperalgesia induced by phenobarbital in all algometric assays performed ($P<0.05$, Kruskal-Wallis test). This dose of picROTOXIN induced a significant
antinociceptive effect when administered alone, which could be observed in the four tests studied (Figure 2A-D). Lower doses of picrotoxin (0.12 to 0.5 mg/kg) also blocked phenobarbital-induced hyperalgesia, but did not induce an antinociceptive effect per se (data not shown). Surprisingly, however, phenobarbital increased the antinociceptive effect of picrotoxin in two algesimetric tests performed in combination, i.e., the tail-flick (Figure 2A) and hot-plate (Figure 2B) tests.

An antinociceptive dose of systemic bicuculline (1-2 mg/kg, i.p) blocked the hyperalgesia induced by phenobarbital both in the tail-flick and constriction tests (Figure 3A and B, respectively). In addition, phenobarbital in combination with bicuculline also potentiated the antinociceptive effect induced by picrotoxin of phenobarbital-induced hyperalgesia in rats (A, B, C) and mice (D). Picrotoxin (PCHR, 1 mg/kg) was administered 10 min before ip phenobarbital (PHEN: 2.5-20 mg/kg) administration. Hyperalgesia (decrease of nociception threshold) and its consequent blockade (antinociception) were detected in the tail-flick (A) and hot-plate tests (B), reported as area under the curve (AUC) for tail-flick index (TFI) and hot-plate index (HPI) and in the formalin (C) and abdominal constriction (D) tests, reported as nociception rate and number of stretches, respectively. *P<0.05 compared to control (animals injected with vehicle), and **P<0.05 compared to picrotoxin-induced antinociception (Kruskal-Wallis test followed by Dunnett's multiple comparisons test).

Table 1: Reduction in phasic pain thresholds (P) in the formalin apparatus for mice treated with various doses of phenobarbital.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>% Reduction in phasic pain threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.0 ± 2.59</td>
</tr>
<tr>
<td>1</td>
<td>96.7 ± 1.06</td>
</tr>
<tr>
<td>5</td>
<td>92.4 ± 1.54</td>
</tr>
<tr>
<td>10</td>
<td>88.9 ± 2.66</td>
</tr>
<tr>
<td>30</td>
<td>75.5 ± 2.15</td>
</tr>
<tr>
<td>50</td>
<td>52.3 ± 1.13</td>
</tr>
<tr>
<td>100</td>
<td>38.9 ± 1.05</td>
</tr>
</tbody>
</table>

Figure 2: Blockade by picrotoxin of phenobarbital-induced hyperalgesia in rats (A, B, C) and mice (D). Picrotoxin (PCHR, 1 mg/kg) was administered 10 min before ip phenobarbital (PHEN: 2.5-20 mg/kg) administration. Hyperalgesia (decrease of nociception threshold) and its consequent blockade (antinociception) were detected in the tail-flick (A) and hot-plate tests (B), reported as area under the curve (AUC) for tail-flick index (TFI) and hot-plate index (HPI) and in the formalin (C) and abdominal constriction (D) tests, reported as nociception rate and number of stretches, respectively. *P<0.05 compared to control (animals injected with vehicle), and **P<0.05 compared to picrotoxin-induced antinociception (Kruskal-Wallis test followed by Dunnett's multiple comparisons test).
Figure 3 - Reversal of phenobarbital (PHEN)-induced hypalgesia by systemic administration of bicuculline (BIC) in the tail-flick test in rats and the abdominal constriction test induced by acetic acid in mice. Bicuculline (1 or 2 mg/kg) was administered sc 10 min before ip administration of phenobarbital. Control animals were injected with the respective vehicles at the times indicated. Results are reported as mean ± SEM (N = 8). *P<0.05 compared to control and †P<0.05 compared to bicuculline-induced antinoceptive (Kruskal-Wallis test followed by Dunnett's multiple comparisons test).

Figure 4 - Opposite (hyperalgesic vs antinoceptive) effects of phenobarbital (PHEN) administration by the lcv and iv routes in the tail-flick test. Inhibition by iv administration of phenobarbital of the hyperalgesia induced by systemic phenobarbital administration is also shown (ip + iv). A phenobarbital dose of 5 mg/kg, ip, N = 5) was used for CNS (iv and ip) administration. Systemic phenobarbital (20 mg/kg, ip, N = 5) was administered above 10 min before iv administration. *P<0.05 compared to systemic administration (Kruskal-Wallis test).

Experiment III

Phenobarbital was used at a dose of 5 μg by the lcv or iv route to study the participation of the CNS in its hyperalgesic effect, using the tail-flick method. Intracerebroventricular administration of phenobarbital (5 μg) to rats induced development of hyperalgesia as detected by the tail-flick test (Figure 4). In contrast, the same dose of phenobarbital administered iv induced an antinoceptive effect, as shown in the same figure. In addition, iv administration of phenobarbital reduced the hyperalgesia induced by its systemic administration (Figure 4).

Intracerebroventricular administration of bicuculline (10 μg) blocked both the hyperalgesic effect induced by systemic phenobarbital treatment (Figure 5) and the antinoceptive effect induced by iv phenobarbital administration (data not shown).

Discussion

We have previously shown that various anxiolytic drugs interfering with the GABA-A receptor, including barbiturates, could induce a state of hyperalgesia (7), although the literature about this subject may be considered controversial (8,9). Extending data from previous work (7), in the present study we showed that acute phenobarbital administration induced a dose-dependent hyperalgesia. This hyperalgesia was detected in four algometric assays, i.e., the tail-flick, hot-plate and formalin tests in rats, and in the abdominal constriction test in mice, thus indicating a clear-cut effect. Such effect was still more evident if one considers that at all phenobarbital doses tested a reduction of motor coordination occurred in the animals, a fact that may have theoretically impaired the observations leading to erroneous conclusions, such as a pseudo-"antinoceptive" effect, especially in mice. In fact, it should
be pointed out that if higher doses of phenobarbital (>5 mg/kg) were used in the formalin or constriction test, hyperalgesia would be probably reduced by the sedation presented by the animals.

Some barbiturates are still used today therapeutically as general anesthetics (thiopental) and anticonvulsants (phenobarbital). These drugs essentially act at the CNS level, facilitating GABAergic neurotransmission by binding at specific sites in the GABA-A receptor (4). Besides controlling seizures, GABA-A receptor activation may also be involved in pain modulation (17-19). The principal nuclei of pain neuromodulation in the CNS are the periaqueductal gray matter, nucleus raphe magnus and the spinal dorsal horn which constitute the descending inhibitory system. It has been shown that stimulation of GABAergic (inhibitory) neurones associated with the periaqueductal gray matter and nucleus raphe magnus may increase the painful afferent inputs coming from the periphery (19,20), whereas activation of GABAergic neurones at the spinal dorsal horn level may have the opposite effect, i.e., a decrease of the painful peripheral inputs (21).

The potency of phenobarbital in inducing hyperalgesia varied between tests. It has been described long ago that the tail-flick response is thought to be a spinal reflex, while the hot-plate involves at least the brainstem level since coordination of the head and limbs is necessary for the response to be observed (22). The same authors also stated that the more complex behavioral pattern of the formalin test might involve other brain regions in addition to those involved in a rapid flick of the tail. Electrophysiological studies have shown that analgesia produced by stimulation of the periaqueductal gray matter requires a significantly lower current intensity in the formalin test than in the tail-flick test (23). From a pharmacological point of view, these data may suggest that a hyperalgesic dose of phenobarbital is smaller in an algometric assay of higher complexity. In addition, our results also support the notion that the hyperalgesic effect induced by phenobarbital is not restricted to one species, since the response was consistently detected in rats and mice.

To test if GABA-A receptors could be involved in the hyperalgesic response induced by phenobarbital, we initially chose picrotoxin - a convulsive substance which blocks the chloride channel associated specifically with the GABA-A receptor (4). Indeed, subconvulsant doses of picrotoxin inhibited the phenobarbital-induced hyperalgesic response in all tests used. This antinociceptive effect of picrotoxin has been previously observed by Tatsuo et al. (7) and may derive from an action through the GABA-A receptors present in the descending inhibitory system.

The most striking result, however, was the potentiation of the picrotoxin-induced antinociceptive response when the drug was combined with phenobarbital treatment observed in 2 out of 4 tests used, i.e., the tail-flick and hot-plate test. In fact, when 1 mg/kg picrotoxin was used in the formalin test this effect was not so clear-cut (Figure 3C), but when a lower dose was used (0.12 mg/kg) this effect could be clearly demonstrated.

![Figure 5: Antinociceptive effect of bicuculline (BIC) following its acute icv administration to rats.](image-url)
(Yokoro CM and Tatsuo MAKF, unpublished observations). Some studies have shown that the site of barbiturate binding is close to the site of the neurotransmitter binding in the GABA-A receptor (4,5). On the other hand, the picrotoxin site is inside the channel where it seems to inhibit the entry of the chloride ions into the cell (24). Since phenobarbital potentiated the antinociceptive action of picrotoxin, it is our hypothesis that the binding of phenobarbital to its active site would allosterically change the GABA-A receptor, increasing the chance of picrotoxin binding at its site inside the channel.

Finally, the hyperalgesic effect induced by phenobarbital seems to derive from an action on upper rather than spinal levels, since hyperalgesia was observed when phenobarbital was injected by the icv but not by the it route. The fact that spinally administered phenobarbital induced an opposite (antinociceptive) effect as demonstrated in the present study, even blocking the hyperalgesia induced by (systemic) phenobarbital itself, supports this concept. Differences in subunit composition of the GABA-A receptor in supraspinal and spinal neurones (25) could account for the differences observed in the action of phenobarbital at the molecular level. This hypothesis was also supported by the demonstration that the specific GABA-A receptor antagonist bicuculline induced antinociception by the icv route in the tail-flick test (present study). Further studies from our laboratory have shown that icv bicuculline administration potentiated the hyperalgesia induced by systemic administration of phenobarbital itself (Yokoro CM and Tatsuo MAKF, unpublished observations).

In conclusion, the anticonvulsant drug phenobarbital when acutely administered induced hyperalgesia within a dose range of 1.25 to 20 mg/kg in four experimental pain assays. This effect was completely blocked by subconvulsant doses of picrotoxin and bicuculline, the antagonists acting on the chloride channels associated with the GABA-A receptor and the specific receptor for the inhibitory neurotransmitter GABA, respectively, clearly implicating GABA-A receptors in this hyperalgesic effect of phenobarbital. The site for this hyperalgesic response seems to be linked with upper rather than spinal levels in the CNS, since 1) phenobarbital induces hyperalgesia when administered by the icv route, reproducing the hyperalgesic effect induced by its systemic administration; 2) it administration of the drug induces an opposite effect, i.e., an antinociceptive effect; 3) the competitive GABA-A antagonist bicuculline alone induced the opposite effect of the agonist phenobarbital, i.e., antinociception, when administered systemically (ip) or icv. and 4) bicuculline in combination with phenobarbital blocked the hyperalgesic effect induced by the latter. However, phenobarbital administered by the it route induced antinociception. Since GABA-A receptors are associated with modulation of the descending inhibitory system at upper levels of the CNS, we suggest that the phenobarbital-induced hyperalgesic response may derive from an inhibitory effect on this system, favoring the painful inputs coming from the periphery. We also suggest that phenobarbital may induce an antinociceptive effect through an effect at the spinal level also involving GABA-A receptors.
References


Anti-inflammatory and analgesic effects of the phosphodiesterase 4 inhibitor rolipram in a rat model of arthritis

Janetti N. Francischia, Celina M. Yokoro, S. Poole, Wagner L. Tafuri, Fernando Q. Cunha, Mauro M. Teixeira

Departments of Pharmacologia, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
Department of Pathology, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
Department of Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil
Department of Bioquímica e Imunologia, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6577-Pampulha, 31270-901, Belo Horizonte, Brazil

Abstract

There has been much interest in strategies which modulate tumour necrosis factor-α (TNF-α) levels and/or function in rheumatoid arthritis. The elevation of intracellular levels of cyclic AMP in leukocytes by phosphodiesterase 4 inhibitors is accompanied by significant inhibition of the production of TNF-α. Nevertheless, these drugs may enhance the hyperalgesia induced by a range of inflammatory mediators, including TNF-α. In the present study, we examined the effects of the phosphodiesterase 4 inhibitor rolipram on the local inflammatory infiltrate and hyperalgesia in a rat model of adjuvant-induced arthritis. Rolipram (3 mg/kg) was administered by oral gavage from day 10 to 14 after disease induction. Pretreatment with rolipram abrogated oedema formation and significantly inhibited hyperalgesia. Histopathological analysis revealed a marked inhibition of cellular influx as well as bone and cartilage destruction. Serum and local TNF-α levels were suppressed in treated animals whereas there were little changes in interleukin-1β levels. Although cyclic AMP elevating agents may affect receptor threshold to increase the hyperalgesic responses acutely, they also possess significant anti-inflammatory activity, which may help local mediator release and/or action. The anti-inflammatory effects of rolipram predominate during this chronic arthritis model in the rat. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Arthritis; Phosphodiesterase 4; Hyperalgesia; Oedema; TNF-α (tumor necrosis factor-α); Rolipram

1. Introduction

Rheumatoid arthritis is a common chronic inflammatory disorder involving the synovial membranes of multiple joints in humans (Sewell and Trentham, 1993). Although there are reasonably good drugs used in the symptomatic relief of arthritis (e.g. non-steroidal anti-inflammatory drugs), there are few safe drugs, which modify fundamental pathologic processes responsible for the chronic inflammation (Cash and Klippel, 1994). Recently, there has been much interest in strategies, which inhibit tumour necrosis factor-α (TNF-α) levels and/or function as TNF-α appears to play a major pathophysiological role in rheuma-toid arthritis (Feldman et al., 1998). Such interest stems from the clinically beneficial effects of anti-TNF-α treatment of rheumatic patients (Feldman et al., 1998; Moreland et al., 1997).

The elevation of intracellular levels of cyclic AMP in leukocytes is accompanied by significant inhibition of the production of TNF-α (Teixeira et al., 1997, Procopio et al., 1999). In this regard, strategies which elevate cyclic AMP may be beneficial in the treatment of rheumatoid arthritis. The levels of cyclic AMP inside cells are controlled by the degree of cyclic AMP production via adeny late cyclase-coupled receptors (e.g. β-adrenoceptors) and the metabolism of cyclic AMP by phosphodiesterases (Teixeira et al., 1997). The main phosphodiesterase activity present in leukocytes is the phosphodiesterases type 4 (Teixeira et al., 1997; Torphy, 1998). Blockade of these enzymes is associated with the inhibition of several leuko-
eye functions, including inhibition of TNF-α production and the release of other inflammatory mediators and reactive oxygen species (Teixeira et al., 1997; Torphy, 1998; Au et al., 1998).

A few animal studies have evaluated the effect of phosphodiesterase 4 inhibitors in rodent models of rheumatoid arthritis (Sekut et al., 1995; Nyman et al., 1997; Ross et al., 1997). These studies demonstrate that treatment with the prototype phosphodiesterase 4 inhibitor rolipram is effective both prior to (Sekut et al., 1995) and when given after (Nyman et al., 1997; Ross et al., 1997) the arthritis-inducing stimulus. However, none of these studies evaluated any possible effect of rolipram on a major characteristic of rheumatoid arthritis, inflammatory hyperalgesia. In this respect, we have recently demonstrated that pretreatment of animals with locally injected rolipram significantly enhanced the hyperalgesia induced by several mediators, including that induced by TNF-α (Conha et al., 1999). Thus, if phosphodiesterase 4 inhibitors are to be used in the treatment of rheumatoid arthritis, it is essential that their effects on hyperalgesia in animal models of arthritis are evaluated.

In the present study, we have used a model of adjuvant-induced arthritis in rats to evaluate the effect of the phosphodiesterase 4 inhibitor rolipram on the local inflammatory infiltrate and hyperalgesia. The local (in the paw) and circulating levels of TNF-α in control and treated rats were measured to assess the effectiveness of the dose of rolipram used. For comparison, we also assessed the local and systemic levels of interleukin-1α, a cytokine known to play an important role in arthritis (Breedveld, 1999), but usually less affected by phosphodiesterase 4 inhibitor pretreatment (reviewed by Torphy, 1998). Of note, rolipram was given orally after the induction of arthritis.

2. Material and methods

2.1. Animals

Female Holtzman rats (140–170 g) were used throughout this study. Animals were kept in cages (maximum of six animals per cage) at a temperature of 26 ± 3°C, and on a 12-h light–dark cycle. Water and food were given ad libitum. All experimental procedures described below have been approved by the local animal ethics committee.

2.2. Induction of arthritis by adjuvant

Rats were injected subcutaneously with a single dose of 0.2 ml mineral oil–water emulsion (10:1, v/v) containing 400 μg of dried *Mycobacterium butyricum* into the dorsal root of the tail under ether anaesthesia. The time of adjuvant injection is referred to as day 0.

2.3. Treatment of animals with rolipram

Rolipram (3 mg/kg) or vehicle were administered via oral gavage and animals (*n* = 15) were treated for 5 days. In the first day, two oral administrations were given and this was followed by single daily administrations. Treatment was initiated on day 10, when the first signs of joint inflammation and pain are usually noted (Tatsuo et al., 1994; Francischi et al., 1997). On day 14, a group of animals (*n* = 10) was sacrificed for histopathological and serological analysis. The remaining animals (*n* = 5) were followed for a further period of 7 days. Control animals received vehicle (glycerol 0.5%). The dose of rolipram used here has been previously shown to inhibit effectively the development of chronic inflammatory diseases in rats (Sekut et al., 1995).

2.4. Measurement of hindpaw hyperalgesia and oedema

The method for measuring hyperalgesia has been previously described elsewhere (Capceta et al., 1980; Tatsuo et al., 1994; Francischi et al., 1997). Briefly, the tendency of normal (naïve), control and arthritic rats to vocalise following flexion of the tarsotibial joints of both hindpaws was tested daily for 22 days starting from day 0. The results are reported as the mean number (± S.E.M.) of vocalisations obtained following five flexions of hindlimb tarsotibial joints. Hindpaw volume (as an indicator of oedema) was
measured daily using an Ugo Basile hydroplethysmometer (model 7150) after the test for hyperalgesia. The volume (ml) of one hindpaw was essentially the same as that of the contralateral paw (data not shown) and is reported as the mean ± S.E.M. All measurements were obtained at the same time of the day.

2.5. Histopathological processing and analysis

Fragments of tarsus-metatarsal joints were collected 14 and 21 days after the induction of arthritis and fixed in 10% buffered formalin. The fragments were then treated with a 10% acidic nitric solution for decalcification, dehydrated, cleared, embedded in paraffin, cut (3–4 μm thick) and stained with Haematoxylin and Eosin. Tissue sections were analysed by one of the authors (WT) who was blind to the experimental groups. The following parameters were assessed and graded from absent to intense (− to + + +): oedema, synovial inflammation, joint-articular erosion, accumulation of neutrophils, granulomatous tissue, tendon and skeletal muscle inflammation. The joints of at least three animals were observed in each experimental group.

2.6. Measurement of systemic levels of TNF-α and interleukin-1β

Serum was prepared by allowing blood to clot and retract at 37°C for 15 min and at 4°C for 30 min, respectively. The serum collected was then centrifuged twice at 10,000 × g for 10 min and stored at −20°C until TNF-α and interleukin-1β measurements. For the measurement of tissue cytokine levels, the subcutaneous tissue of the right hindpaw and that surrounding the tarsotibial joints was removed and placed on phosphate buffered saline containing 0.05% Tween 20, 0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzamidinium chloride, 10 mM EDTA and 20 KI aprotonin A. The tissue was homogenized, centrifuged at 3000 × g for 10 min and stored at −70°C until further analysis. TNF-α and interleukin-1β levels were evaluated using a standard sandwich ELISA technique as previously described (Rees et al., 1999).

2.7. Materials

Phenylmethylsulphonyl fluoride, benzamidinium chloride, EDTA, Tween 20 and aprotonin A were from Sigma (St. Louis, USA). Rolipram was a kind gift of Dr John Fozard, Novartis, Switzerland. Recombinant rat TNF-α and interleukin-1β, the coating and biotinylated sheep anti-rat TNF-α and anti-interleukin-1β antibodies were prepared at the National Institute for Biological Standards.

Fig. 2. Effect of the treatment with rolipram on hindpaw hyperalgesia in adjuvant-induced arthritis. Adjuvant arthritis was induced and animals treated with rolipram (squares, 3 mg/kg, via oral gavage) or vehicle (circles, 1 ml/kg, via oral gavage) from days 10 to 14 after disease induction (indicated by the line). Hyperalgesia was assessed by the ability of animals to vocalize following flexion of the tarsotibial joints of both hindpaws. Results are the mean ± S.E.M. of vocalizations obtained for five flexions per paw in 10 animals (days 0 to 14) or 5 animals (days 15 to 21) in each group. *P < 0.05.

Fig. 3. Effects of the treatment with rolipram on the systemic levels of (A) TNF-α and (B) interleukin-1β in adjuvant-induced arthritis. Adjuvant arthritis was induced and animals treated with rolipram (circles, 3 mg/kg, via oral gavage) or vehicle (squares, 1 ml/kg, via oral gavage) from days 10 to 14 after disease induction (indicated by the arrows). Twenty-four hours after the last administration of rolipram, animals were killed, blood taken, serum prepared and TNF-α levels assayed by ELISA. Results are shown as the mean ± S.E.M. for five animals in each group. *P < 0.01.
and Control, United Kingdom. *M. butyricum* powder was purchased from Difco (lot 34873C, Detroit, MO).

2.8. Statistical analysis

Data are presented as the mean ± S.E.M. of the shown number of experiments. Results were analysed using analysis of variance and the Student–Newman–Keuls post-hoc test. *P* values smaller than 0.05 were considered significant.

3. Results

3.1. Effects of the treatment with rolipram on hindpaw oedema and hyperalgesia

Animals injected with adjuvant usually start demonstrating measurable oedema and hyperalgesia around the 10th day after disease induction (Francischi et al., 1997). In the present experiment, significant hindpaw oedema (Fig. 1) and hyperalgesia (Fig. 2) were observed around day 11. Treatment with rolipram starting on day 10 until day 14 abrogated paw oedema (Fig. 1). Interestingly, hindpaw oedema in arthritic animals were still significantly inhibited 7 days (day 21) after the drug had been stopped, although an upward shift was already noticeable (Fig. 1). The inhibitory effects of rolipram treatment on hindpaw hyperalgesia is shown in Fig. 2. Significant inhibition of hyperalgesia was noted from day 14 and persisted until day 21 after treatment (Fig. 2).

3.2. Effects of the treatment with rolipram on systemic and local TNF-α and interleukin-1β levels

The levels of TNF-α in naïve animals were below the detection limit of our assay (< 30 pg/ml). In the animals made arthritic by adjuvant, there was a detectable amount of TNF-α levels in serum and in the paws 14 and 21 days after disease induction (Figs. 3 and 4). Treatment of these animals with rolipram from days 10 to 14 significantly inhibited the increase in serum TNF-α levels at day 14, but had no significant effect on TNF-α levels at day 21.
Table 1

Effects of the treatment with rolipram on joint inflammation in adjuvant-induced arthritis

<table>
<thead>
<tr>
<th></th>
<th>Untreated animals</th>
<th>Treated animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema</td>
<td>++ +</td>
<td>+</td>
</tr>
<tr>
<td>Synovial inflammation</td>
<td>++ +</td>
<td>+</td>
</tr>
<tr>
<td>Intertarticular erosion</td>
<td>++ +</td>
<td>-</td>
</tr>
<tr>
<td>Cartilage and bone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumulation of neutrophils</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Osteonecrosis</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tendon and skeletal muscle inflammation</td>
<td>++ +</td>
<td>-</td>
</tr>
</tbody>
</table>

Adjuvant arthritis was induced and animals treated with rolipram (3 mg/kg) or vehicle from days 10 to 14 after disease induction. Results shown are from animals killed 7 days (day 21) after the last dose of rolipram or vehicle was given and are the average of 4 animals in each group. The following legend applies: − absent, + mild, ++ moderate and +++ intense.

(Fig. 3A). Local TNF-α levels were also significantly elevated in arthritic animals at days 14 and 21 after disease induction (Fig. 4A). TNF-α levels at days 14 and 21 were significantly inhibited by treatment with rolipram by 33% and 17%, respectively (Fig. 4A).

In contrast to its marked effects on systemic TNF-α levels, treatment with rolipram had little effect on interleukin-1β levels in serum (Fig. 3B). Nevertheless, interleukin-1β levels in the paws of treated animals 14 days after disease induction were approximately 30% lower than that in vehicle-treated animals (Fig. 4B).

3.3. Effects of the treatment with rolipram on the local inflammatory response

The paws of the mice treated with rolipram showed a pronounced reduction in inflammation. A decrease in the number and size of synovial cells was observed. A reduction in the number of neutrophils was also noted. In contrast, there was a lower degree of sub-synovial inflammation and little cartilage or bone erosion was observed (Fig. 5B). A similar histopathological picture was observed on days 14 or 21 after disease induction (data not shown). Table 1 summarises the findings obtained in control and rolipram-treated groups on day 21 after disease induction.

4. Discussion

Adjuvant-induced arthritis in rats is a well-established experimental model for the study of the pathophysiology of various types of human arthritis, in special for the study of rheumatoid arthritis (Owen, 1980; Pearson, 1986; Pearson and Wood, 1963). In addition, it is a good chronic inflammatory model for development of potential analgesic and/or anti-inflammatory drugs useful for arthritis treatment (Colpaert et al., 1982).

In the present study, we showed that daily oral administration of the prototype phosphodiesterase 4 inhibitor rolipram effectively inhibited the increase in volume of the hindpaws of arthritic rats. The inhibition of hindpaw volume was associated with inhibition of both cell infiltration and local oedema formation as assessed by histology. Moreover, there was significantly lesser tissue destruction in treated animals as compared to vehicle control. These results are in remarkably good agreement with previous studies demonstrating an inhibitory effect of rolipram in other models of arthritis in mice (Ross et al., 1997) and rats (Sekut et al., 1995; Nyman et al., 1997). In addition, our results clearly demonstrate that the anti-inflammatory effects induced by rolipram were sustained for at least 7 days after the treatment had ceased. Similarly, Nyman et al. (1997) showed that rolipram stopped disease progression for several days in a collagen-induced arthritis model in rats. Overall, these results firmly demonstrate that inhibition of phosphodiesterase 4 may be of clinical benefit in the treatment of arthritis in humans.

We have recently demonstrated that when rolipram or other cyclic AMP elevating drugs were administered locally in an acute model of hyperalgesia, there was a marked increase in hyperalgesic responses following administration of a range of different stimuli, including TNF-α (Cunha et al., 1999). Such effects of rolipram and other cyclic AMP elevating agents appeared to be related to their ability to modify nociceptor threshold in sensory nerve endings (Cunha et al., 1999). Thus, it was important to evaluate any potential increase in the hyperalgesia in our model of adjuvant arthritis. To this end, rolipram was administered on day 10, a time when hyperalgesia is already present but not maximal. In contrast to its potentiating local effect on acute inflammatory pain, rolipram had no enhancing effect in this chronic model of hyperalgesia and inflammation. In fact, this is the first study to demonstrate a significant inhibitory effect of rolipram on the hyperalgesic responses in arthritic animals. The discrepancy between the two opposing effects of rolipram are the object of active research in our laboratories. One possibility to explain such discrepancy is that in the chronic model of arthritis, rolipram may be actively inhibiting leukocyte activation and mediator release (see for example. Au et al., 1998; reviewed in Torphy, 1998). By inhibiting the release of pro-inflammatory mediators, rolipram could potentially modulate the onset and maintenance of the hyperalgesic response. Another interesting possibility that we are now examining in more detail is the possibility that rolipram and other phosphodiesterase 4 inhibitor may have differential effects when applied locally or systemically. These differences may be related to the presence of more or lesser anti-inflammatory activity, which may depend on...
the route of administration of the drug. In fact, systemic pretreatment with rolipram significantly inhibited the hyperalgesic responses and leukocyte infiltration induced by the intraplantar injection of carrageenan whereas local injection of the drug increased hyperalgesia and failed to affect leukocyte infiltration (Cunha et al., 1999 and data not shown).

There is much evidence demonstrating a direct effect of rolipram and other phosphodiesterase 4 inhibitors on the macrophage to inhibit TNF-α production (reviewed by Torphy, 1998). In addition, rolipram has been shown to block the secretion of TNF-α from macrophages by inhibiting T cell activation and expression of surface molecules (Kasyapa et al., 1999). The inhibitory effects of rolipram on TNF-α production has been shown to play a major role in the ability of the drug to inhibit collagen-induced arthritis in mice (Ross et al., 1997). Although the mechanisms underlying the anti-inflammatory effects of rolipram in our model were not determined, systemic and local TNF-α levels were substantially reduced in rolipram-treated animals arguing for a possible role of inhibition of this cytokine in the anti-inflammatory effects of rolipram. Interestingly, although systemic TNF-α levels were back to control levels after the drug was discontinued, levels of TNF-α in the paw were still significantly inhibited. Nevertheless, the level of inhibition at day 21 was lower than at day 14. This local increase in TNF-α levels may account, at least in part, for the tendency of oedema to increase towards the end of the observation period.

In addition to inhibiting TNF-α, phosphodiesterase inhibitors have been shown to modulate the production of other cytokines, including interleukin-1β (Torphy, 1998). In our experiments, treatment with rolipram significantly inhibited the increase in local, but not systemic, levels of interleukin-1β. In addition, the effects of rolipram on interleukin-1β production were marginal and not sustained. This is in agreement with previous studies demonstrating that interleukin-1β is inhibited in some but not all in vitro experiments of macrophage activation (reviewed by Torphy, 1998). The reasons for these discrepancies are not known but could be related to a greater effect of cAMP elevating agents on TNF-α mRNA stability (Verghez et al., 1995). Overall, these data argue for a possible role of inhibition of interleukin-1β for the anti-inflammatory and analgesic effects of rolipram. Thus, although rolipram may affect nociceptor threshold to increase hyperalgesic responses, it also possesses significant anti-inflammatory activity, which may hinder local mediator release and/or action. It appears that the anti-inflammatory effects of rolipram predominate during this chronic arthritis model in the rat.

In summary, we present data showing that oedema and hyperalgesia presented by arthritic rats were actively abrogated by oral administration of rolipram, a phosphodiesterase 4 inhibitor. We suggest that the reduction of hyperalgesia was secondary to the reduction of the local (at the joints) inflammatory response. Thus, phosphodiesterase 4 inhibitors may be useful in the treatment of rheumatoid arthritis as these drugs appear to modify the fundamental pathological process in the joint of affected animals. Any acute effect of these drugs on the hyperalgesic response are counterbalanced by their strong anti-inflammatory effects.

Acknowledgements

We are grateful to Webster Pimenta for his expert technical help. Work in our laboratories is supported by FAPEMIG, CNPq and FAPESP.

References


Portadores de fissura lábio-palatal

A progressão da tuberculose

Ciclosporina e tecidos gengivais

Prescrição de medicamentos
Avaliação da prescrição de medicamentos realizada por cirurgiões-dentistas de Belo Horizonte

Evaluation of drug prescription amongst dentists in Belo Horizonte – Brazil

CINTHIA MARA DA FONSECA PACHECO¹
REGINA MARIA DE MARCO TURCHETTI MAIA²
JANETTI NOGUEIRA DE FRANCISCHI³

RESUMO

O objetivo deste trabalho foi avaliar a prescrição de drogas feita por cirurgiões-dentistas atuantes na cidade de Belo Horizonte. A amostra para o estudo consistiu de 77 dentistas trabalhando em diferentes regiões da cidade. Eles responderam a um questionário contendo questões como: quais as drogas mais prescritas, as doses e as situações em que eles as utilizam. Os resultados demonstraram que analgésicos, antiinflamatórios não esteroides e antibióticos estão entre as drogas mais utilizadas na prática odontológica, mas existe uma grande divergência entre os profissionais quanto às doses e a indicação correta para utilização de tais medicamentos.

UNITERMOS

Prescrição, questionário, cirurgiões-dentistas, analgésicos, antiinflamatórios.

INTRODUÇÃO

O tratamento das infecções odontogênicas sempre envolve uma variedade de agentes terapêuticos (OWENS & SCHUMAN, 1994). De acordo com RING (1985), os tratamentos realizados no século XVII consistiam em “compressas” aplicadas externamente (na face), enquanto misturas de ervas serviam para bochechos e gargarejos, assim como, para serem ingeridos como remédios. Terapia antisséptica utilizando metais pesados como mercúrio, ouro e compostos de prata aplicados topicalmente na pele ou mucosas também era usada nesse período. Pensava-se que o acúmulo desses metais internamente retardariam os processos infeciosos; no entanto, essas substâncias se mostraram muito tóxicas para o hospedeiro (BURKE, 1939).

A era da terapia antimicrobiana começou por volta de 1800 com os trabalhos de Louis Pasteur e Joseph Lister. Suas descobertas, relacionadas ao controle do crescimento de bactérias através de procedimentos antissépticos, foram revolucionárias. A Odontolo-

¹Cirurgiã-dentista, mestre em farmacologia pela Universidade Federal de Minas Gerais.
²Professora Assistente do Departamento de Farmacologia da Universidade Federal de Minas Gerais (UFMG), mestre em biofísica pela UFMG.
³Professora Adjunta do Departamento de Farmacologia da Universidade Federal de Minas Gerais (UFMG), doutora em farmacologia pela Universidade de São Paulo, pós-doutora em farmacologia pela Universidade de Sherbrooke (Canadá) e de Oxford (Inglaterra). Pesquisadora do CNPq.
gia não podia ficar indiferente ao impacto causado pelo advento dos antibióticos. Assim, pouco após o término da Segunda Guerra Mundial, ocasião em que se generalizou o uso destes poderosos agentes antimicrobianos, começaram a aparecer na literatura odontológica de todo o mundo comunicações cada vez mais numerosas sobre o emprego dos mesmos (BEVILACQUA, 1966).

Além do controle de infecções, o controle da dor durante o tratamento odontológico é parte essencial para o sucesso na prática odontológica. Para o alívio da dor trans-operatória, os anestésicos locais são as drogas mais utilizadas. O desenvolvimento dessas drogas começou com a introdução das folhas de coca provenientes da América do Sul. Em torno de 1880, começaram a ser descritas as propriedades anestésicas locais da cocaína. Em 1904 foi sintetizada a procaina, que tornou-se o anestésico local mais utilizado até a introdução da lidocaína em 1940, um anestésico tipo amida, amplamente utilizado até os dias de hoje (MALAMED, 1992).

Se o uso de medicamentos é uma prática comum entre os dentistas, torna-se pertinente questionar se o procedimento é feito corretamente, levando-se em conta questões como indicação da droga, dose e tempo de utilização adequados. O objetivo dessa pesquisa foi avaliar a prática de cirurgiões-dentistas da cidade de Belo Horizonte quanto ao uso e prescrição de medicamentos no seu exercício clínico. Também foi objetivo do estudo estimulá os estudantes da disciplina Farmacologia Odontológica (ICB-UFMG) na avaliação de procedimentos terapêuticos de dentistas, já graduados, desde a aplicação dos questionários até a obtenção dos resultados.

**MATERIAIS E MÉTODOS**

A população estudada constiúía-se de uma amostra de 77 cirurgiões-dentistas não identificados e atuantes em postos de saúde, policlínicas, consultórios particulares e nas diversas clínicas ambulatoriais da Faculdade de Odontologia da UFMG. Os locais de estudo se localizavam nos diversos distritos satélites da Prefeitura do município de Belo Horizonte: Noroeste, Oeste, Barreiro, Norte, Nordeste, Leste e Pampulha. Em média, 10 CDs responderam ao questionário em cada região.

Para a realização do estudo, foi elaborado um questionário onde procurou-se, primeiramente, caracterizar a amostra quanto ao ano de formação, a instituição de ensino na qual se formou e a especialidade do entrevistado. O restante do questionário abordou as seguintes questões:

a. drogas mais prescritas na prática clínica, condições de uso, situações e doses;

b. anestésicos locais mais utilizados e em quais situações fez-se ou não uso de vasoconstritor associado;

c. se o conhecimento adquirido no curso de farmacologia durante a graduação foi suficiente para suprir os conhecimentos inerentes a um bom exercício profissional.

O questionário foi aplicado aos dentistas pelos alunos do terceiro período do curso de Odontologia que cursavam a disciplina de Farmacologia Odontológica no ICB-UFMG, no segundo semestre do ano de 1998.

O número de questionários respondidos foi de 77 e a análise foi puramente descritiva, já que o trabalho caracterizou-se por uma pesquisa de opinião.

**RESULTADOS**

O GRAF. 1 mostra que a maioria dos dentistas entrevistados formou-se em Belo Horizonte, sendo 44% na UFMG e 15% na PUCMG. O restante dos entrevistados, isto é, 41%, formou-se em outras instituições de ensino superior de Minas Gerais e de outros estados. Quarenta e cinco por cento dos entrevistados formou-se na década de 80, 27% na década de 90, 23% na década de 70 e apenas 5% na década de 60 (GRAF. 2). Somente 30% dos entrevistados possuem especialização.

O GRAF. 3 mostra que analgésicos, antiinflamatórios e antibióticos são as drogas mais prescritas pelos dentistas (67,5%). Cerca
GRÁFICO 1 – Caracterização de dentistas atuantes na cidade de Belo Horizonte (MG), segundo a instituição de formação

GRÁFICO 2 – Ano de formatura dos dentistas

GRÁFICO 3 – Drogas mais prescritas pelos cirurgiões-dentistas na cidade de Belo Horizonte (MG)

de 4% dos levantamentos citaram apenas o uso de antibióticos, 15,6% o uso apenas de analgésicos e antiinflamatórios, 2,5% analgésicos, antiinflamatórios e outras drogas como anti-histamínicos, anti-virais, ansiolíticos, antissépticos e 10,4% não responderam.

DISCUSSÃO

Através desse trabalho, verifica-se que analgésicos, antiinflamatórios e antibióticos estão entre as drogas mais prescritas pelos profissionais da Odontologia atuantes na cidade de Belo Horizonte.

A maioria dos dentistas respondeu que os analgésicos são as drogas que eles mais prescrevem na sua prática clínica. Entre eles, destacam-se os antiinflamatórios não esteroides (AINES) ou drogas do tipo da aspirina. Entre os AINES mais citados pelos dentistas estão o diclofenaco de sódio ou potássio, ibuprofeno, paracetamol e dipirona.

Os antiinflamatórios não esteroides são bem indicados no manejo da dor em Odontologia. Dependendo da droga utilizada, ocorre concomitantemente inibição da inflamação, que é uma sequela do trauma devido a vários procedimentos tais como exodontias, cirurgias periodontais, instrumentação de canais radiculares, etc. O trauma ativa uma cascata bioquímica que resulta na síntese e liberação de prostaglandinas, bradicinina, substância P, histamina, e outras substâncias que interagem para produzir o extravasamento de plasma dos vasos sanguíneos levando à formação de edema, um dos sinais clínicos da inflamação. Esses mediadores locais também apresentam vários efeitos nas terminações nervosas sensitivas periféricas: excitam e sensibilizam essas terminações levando à hiperalgesia (Ferreira & Nakamura, 1979; Dionne et al., 1994).

Drogas que bloqueiam a ação ou a síntese desses mediadores possuem, portanto, atividade analgésica na dor de origem inflamatória. Os AINES interferem com o processo inflamatório através da inibição das enzimas conhecidas
como ciclooxigenases (COXs) (VANE, 1971). A partir de 1989, começou a ficar evidente a existência de diferentes isoenzimas de ciclooxigenases, que passaram a ser denominadas, respectivamente, COX1, ciclooxigenases constitutivas e COX2, ciclooxigenases induzidas (CROFFET et al., 1994). Essas isoenzimas catalisam a síntese de prostaglandinas a partir do ácido araquidônico, que em condições normais pode ser encontrado esterificado nos fosfolípidos da membrana de todas as células.

Muitos trabalhos na literatura mostram que os antiinflamatórios não esteróides têm-se provado superiores em promover o alívio da dor, com menos efeitos colaterais do que nas combinações com opioides (COOPER et al., 1984; COOPER & MARDI-ROSSIAN, 1986; FORBES et al., 1990), enquanto outros trabalhos mostram que essas combinações são mais eficientes (FORBES et al., 1982; QUIDING et al., 1984).

O modelo mais utilizado para se estudar a ação de drogas analgésicas em pacientes ambulatoriais tem sido a cirurgia para a extração de terceiros molares. Esse modelo foi estabelecido por COOPER & BEAVER em 1976. Segundo BREIVIK & BJÖRNSSON (1998), a razão daquela controvérsia ser o fato de o modelo não ser bastante sensível para avaliar as diferenças entre os AINES sozinhos ou associados a opioides. Eles mostraram que a cirurgia para a extração de terceiros molares determina uma condição dolorosa basal que foi relacionada ao tamanho do trauma cirúrgico, mas que a variação individual na intensidade da mesma reduziria a sensibilidade do modelo.

Entretanto, em nenhum dos questionários respondidos no presente trabalho os dentistas citaram o uso de associações de antiinflamatórios não esteróides com opioides. Em apenas um questionário um dentista citou o uso tópico de um antiinflamatório esteróide.

Como esperado, a utilização terapêutica de compostos do tipo da aspirina mais seletivos sobre as COX2 (VANE & BOTTING, 1997) não foi relatada em nenhum dos levantamentos, provavelmente porque tais compostos foram introduzidos no mercado brasileiro após o término do levantamento desse trabalho. Esses compostos têm apresentado um impacto terapêutico positivo no tratamento de inflamações de diversas origens, por deixarem intacta a produção fisiológica de prostaglandinas (protetoras), como aquelas produzidas na mucosa gastrointestinal, por exemplo. As prostaglandinas produzidas em concentrações fisiológicas são eminentemente derivadas da ação das COX2.

Em segundo lugar das drogas mais citadas pelos dentistas apareceram os antibióticos. No entanto, houve grande divergência nas respostas quanto à indicação, a dose e o tempo de uso. Sabe-se atualmente, que o mau emprego dos antibióticos na terapêutica e profilaxia humanas constitui, reconhecidamente, uma das principais causas do aumento da resistência bacteriana. A esse sério problema deve ser acrescentado, também, o risco dos efeitos adversos dessas drogas, a ineficácia terapêutica dos medicamentos prescritos erroneamente e o custo que representa para a economia dos pacientes e do Estado (TAVARES, 1997).

Os antibióticos são recomendados em várias situações. Os grupos de maior significância para os dentistas são as penicilinas, cefalosporinas e os macrolídeos, especialmente a eritromicina. Devido a sua eficácia, baixo custo e facilidade de administração, as penicilinas têm sido o antibiótico de escolha para a maioria das infecções odontogênicas. Os casos em que ocorrem bactérias produtoras de penicilinas resistentes à penicilina requerem antibióticos de largo espectro, tais como ampicilina e amoxicilina, ou ainda, cefalosporinas (MONTGOMERY, 1980).

Em nenhum dos questionários respondidos mencionou-se o uso de antibióticos para profilaxia de endocardite bacteriana. A endocardite infecciosa é uma das poucas complicações letais do tratamento odontológico, por isso a prevenção dessa doença deveria ser uma preocupação dos dentistas (CAWSON, 1981). MARTIN et al. (1997) mostram uma revisão de 53 casos de endocardite infecciosa relacionada à prática dentária, em que a maioria dos casos foi devido à amanxene inadequada ou
mesmo inexistente por parte do profissional. O antibiótico de escolha para a prevenção da endocardite em pacientes suscetíveis (individuos com comprometimento do endocardio, congênito ou adquirido) é a amoxicilina, uma penicilina semi-sintética (SHANSON et al., 1978). Existe atualmente um consenso na literatura de que uma única dose de amoxicilina 2 ou 3g uma hora antes do procedimento é suficiente para a profilaxia da endocardite (DAJANI et al., 1994).

As opiniões relativas ao uso profilático de antibióticos em diferentes tipos de cirurgia bucal são controversas (PATERSON et al.; 1970; McGREGOR & ADDY, 1980). No caso de pacientes saudáveis, a prática empregada por diferentes profissionais varia muito. Alguns cirurgiões receitam antibióticos sistematicamente para todos os pacientes que se submeterem à cirurgia para a extração de terceiro molar. Enquanto outros consideram essa prática desnecessária se não há nenhuma indicação específica.

O profissional deve estar atento a um dos princípios básicos do uso profilático dos antibióticos, que diz respeito ao benefício causado pelo uso ser maior que os riscos de induzir toxicidade, alergia e até mesmo superinfeções (PALLASCH, 1989). De acordo com HAPPONEN et al. (1990), o uso prévio de antibiótico profilático para a extração de terceiros molares inclusos não mostrou diferenças em relação a um placebo, ressaltando que as condições de assepsia adequadas são fundamentais no momento da cirurgia. Já BYSTEDT et al. (1980) mostraram uma redução de 50% em infecções pós-extração de terceiros molares quando um antibiótico sistêmico foi utilizado, comparando com o grupo controle que recebeu placebo. Um outro fato a ser levado em consideração antes de se prescrever um antibiótico é que a bacteremia, após manipulações dentárias, é proporcional à inflamação gengival (LOESCHE, 1976).

Portanto, uma boa higienização e até mesmo bochecho com agentes antissépticos poderiam diminuir bastante as infecções após extração dentárias. BUTLER & SWEET (1977) mostraram que uma simples lavagem com uma solução de salina fisiológica estéril poderia diminuir a incidência de infecção pós-extração em até 50%.

CONCLUSÃO

Os resultados do presente estudo mostraram que as drogas mais prescritas pelos CDs atuantes em diferentes regiões de Belo Horizonte foram analgésicos, antiinflamatórios e antibióticos, e que existe por parte dos profissionais uma divergência muito grande sobre indicação e dose do medicamento a ser utilizado. Isso sugere claramente a necessidade de se reavaliar o conteúdo ministrado pela disciplina de Farmacologia nos diversos cursos de Odontologia existentes, pelo menos em Minas Gerais. Certamente, uma disciplina que abranja aspectos gerais, com ênfase na farmacocinética e farmacodinâmica, em especial a farmacologia de anestésicos locais, antibióticos, antiinflamatórios e analgésicos, estará preparando melhor os profissionais para os desafios do próximo século.

ABSTRACT

The purpose of this study was to evaluate the drug prescription amongst dentists in Belo Horizonte, Brazil. The sample included 77 dentists from different regions of the city. The professionals were requested to answer a questionnaire applied by dental students where drug dose and indication were evaluated. The results showed that antibiotics, analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) were the most commonly used drugs in dental practice but there was great divergence between practitioners about dose and correct indication for their use.

KEYWORDS

Prescription, questionnaire, dentists, analgesics, NSAIDs.
REFERÊNCIAS BIBLIOGRÁFICAS


v.6 n.2 Maio/Agosto 2000 REVISTA DO CROMG 123
TRABALHOS COMPLETOS ACEITOS PARA PUBLICAÇÃO

- Selective inhibitors of cyclooxygenase-2 (COX-2) induce hypoalgesia in a rat paw model of inflammation *(British Journal of Pharmacology- 2002)*;

- Hyperalgesia and oedema in arthritic old rats: role of endogenous glucocorticoids *(Brazilian Journal of Medical and Biological Research- 2002)*.

- Controle da dor em odontologia: os antigos e os novos inibidores das ciclooxigenases (COXs) *(Revista do Conselho Regional de Odontologia do Estado de Minas Gerais – CRO-MG - 2002)*
Selective inhibitors of cyclooxygenase-2 (COX-2) induce hypoalgesia in a rat paw model of inflammation

Francischi, JN¹; Chaves, CT¹; Moura, ACL¹; Lima, A.S.; Ferreira-Alves, DL¹; Rocha, OA² and Bakhle, YS³

¹Departamento de Farmacologia and ²Departamento de Patologia Geral, ICB, Universidade Federal de Minas Gerais, Brazil
³Leukocyte Biology, Division of Biomedical Sciences, Faculty of Medicine, Imperial College, London, SW7 2AZ, England

Running title: Hypoalgesia induced by COX-2 inhibitors

Author for correspondence: JN Francischi, PhD, Departamento de Farmacologia, ICB-UFMG, Av. Antônio Carlos 6627 – Campus Pampulha, Belo Horizonte, Minas Gerais, Brazil, CEP. 31270-901
SUMMARY

1. It is well-established that inhibitors of cyclooxygenase (COX) and hence of prostaglandin (PG) biosynthesis reverse inflammatory hyperalgesia and oedema in both human and animal models of inflammatory pain.

2. Paw oedema and hyperalgesia in rats were induced by injecting carrageenan (250μg/paw) into a hindpaw. Both inflammatory responses were followed for 24h after the injection, measuring hyperalgesia by decreased pain threshold in the paws and oedema by plethysmography.

3. Three selective inhibitors of cyclooxygenase-2 (COX-2), celecoxib, rofecoxib and SC 236, given systemically in a range of doses, before the inflammatory stimulus, abolished carrageenan-induced hyperalgesia with little reduction of oedema. These inhibitors also induced hypoalgesia, increasing nociceptive thresholds in the inflamed paw above normal, non-inflamed levels. This hypoalgesia was lost at the higher doses of the selective inhibitors, although hyperalgesia was still prevented.

4. In paws injected with saline only, celecoxib, given at the dose inducing the maximum hypoalgesia after carrageenan, did not alter the nociceptive thresholds.

5. Two non-selective inhibitors of COX-2, indomethacin and piroxicam, abolished hyperalgesia and reduced oedema but did not induce hypoalgesia.

6. Celecoxib given locally into the paw also abolished inflammatory hyperalgesia and induced hypoalgesia without reducing oedema.

8. We conclude that hypoalgesia is expressed only over a critical range of COX-2 inhibition and that concomitant inhibition of COX-1 prevents expression of hypoalgesia, although hyperalgesia is still prevented.
9. Our results suggest a novel anti-nociceptive pathway mediating hypoalgesia, involving COX-2 selectively and having a clear peripheral component. This peripheral component can be further explored for therapeutic purposes.

**Key words:** hypoalgesia, cyclooxygenases, COX-1 and COX-2 inhibitors, hyperalgesia, paw oedema
Introduction

Prostaglandins (PGs) are well established as mediators of several components of the inflammatory response. Particularly, the oedema resulting from increased microvascular permeability is a consequence of the vasodilator effect of PGs potentiating the microvascular effects of other mediators such as bradykinin, substance P and histamine (Williams & Morley, 1973; Williams & Peck, 1977). These mediators also induce pain in inflammatory sites and this component also is potentiated by PGs (Ferreira, 1972). Although PGs are not direct algesic agents as are bradykinin, substance P and histamine, they nevertheless induce a state of hyperalgesia in which previously non-painful stimuli are now perceived as painful in both animal models and in human subjects (Ferreira, 1972; 1990). Logically, therefore, inhibitors of PG biosynthesis should reduce PG-induced hyperalgesia to basal levels of pain perception, reflecting the algesic action of the directly acting agents, but not reduce pain perception beyond the normal threshold.

The observed anti-oedema and analgesic effects of the non steroidal anti-inflammatory drugs (NSAIDs) were attributed over 30 years ago to inhibition of the biosynthesis of PGs catalysed by cyclooxygenase (COX); Vane 1971). There are now known to be two isoforms of COX, COX-1 and COX-2, with the latter being strongly induced in inflammatory sites (Bakhle & Botting, 1996, Vane et al., 1998). Most of the NSAIDs already used clinically (aspirin, indomethacin, ibuprofen, diclofenac, etc.) are non-selective inhibitors of COX, affecting both isoforms to a variable extent (Vane et al., 1998; Warner et al., 1999). The major side effect of the NSAIDs, gastric or intestinal ulceration, has been attributed to inhibition of COX-1 and this attribution has been the
major impetus for the introduction into clinical practice of two selective inhibitors of COX-2, celecoxib and rofecoxib, as "NSAIDs without ulcers" (Bombardier 2002; Hawkey, 1999). These selective COX-2 inhibitors exhibit NSAID-like effects in human disease and, in animal models of inflammation, they decrease oedema and hyperalgesia (Chan et al., 1999; Smith et al., 1998).

We were therefore surprised to observe, in our model of inflammation induced by carrageenan in rat paws, not only the expected loss of hyperalgesia but additionally, a sub-normal pain perception after treatment with celecoxib (Francischi et al., 2002). This was expressed as a raised threshold for nociception and this state is referred to as "hypoalgesia". We have extended these initial observations using both selective COX-2 inhibitors and non-selective NSAIDs, seeking to define and analyse the hypoalgesic effect. We have also measured oedema over the same time course to explore the relation between vascular events and nociception, in terms of COX-inhibition. From our results we would conclude that the hypoalgesic effects of COX inhibitors reflected COX-2 inhibition selectively and that hypoalgesia requires a lesser degree of inhibition of COX-2 than does the reduction in oedema.
Methods

Animals

Male Holtzman rats from the Bioresources Centre of UFMG (body weight, 150-200g) were used throughout this study. The animals were left to adapt for 24 h under controlled experimental conditions (23-26° C, light/dark cycles of 12/12 h with lights on 7:00 am, food and water ad libitum). Ethical guidelines of the International Association for the Study of Pain in conscious animals were followed (Zimmerman, 1983).

Inflammatory reaction to carrageenan

Lambda-carrageenan was injected (100-500 µg/paw in 0.1 ml of physiological saline) in the foot pad of hind paws, to induce hyperalgesia and oedema. An intermediate dose of 250µg/paw was chosen as the standard inflammatory stimulus in most of our studies. Contralateral paws received the same volume (0.1ml) of saline, the vehicle for carrageenan.

Measurement of the state of algesia

Assessment of algesia consisted of measurement of the threshold stimulus for reaction (escape or paw withdrawal) using a weight (maximum limit of 500 g) applied to the pads of hind paws by an experimenter using an Ugo Basile apparatus; this is essentially the method of Randall & Selitto (1957). The threshold for pain sensation was measured before (time zero) and 1, 2, 3, 4, and 24 h after the intraplantar injection of carrageenan. Results are presented as the difference in threshold between the test (carrageenan-
injected) paw and the contralateral, control, (saline-injected) paw. These measurements were made without knowledge of any pre-treatments.

_Edema measurements_

The volume (in ml) of the hind paws from control and treated animals were measured with a hydroplethysmometer (Ugo Basile 1750) at the same time-points used for hyperalgesia measurements, i.e., time zero and 1, 2, 3, 4, and 24 h after stimulus injection. Results are presented as the difference in volume between the test paw and the control paw for each animal at the times shown.

_Pre-treatment with COX inhibitors_

Cyclooxygenase inhibitors were administered either subcutaneously (s.c.), intraperitoneally (i.p.) or intraplantarly (i.pl.). The capsule content or compressed tablet from commercial preparations of celecoxib, rofecoxib, and piroxicam was weighed and crushed into a fine suspension with physiological saline (NaCl, 0.9 %, w/v) based on the weight of active substance quoted per tablet or capsule. These suspensions were then diluted further with saline to give appropriate amounts of the active substance. All suspensions were made immediately before use and were not stored. Indomethacin was initially dissolved in Tris buffer (0.1 M, pH=8.0); SC 236 was dissolved in ethanol and then diluted in 5% (v/v) Tween 80 in water, as described in Guo et al. (2001). Further dilutions were made with saline. All pre-treatments were given 30 min before the injection of carrageenan. Control animals were treated with their respective vehicles at the same times. The standard volume used for s.c. injection was 1ml.kg⁻¹ with the
exception of piroxicam, which was given at 2ml.kg\(^{-1}\). For i.p injections, a total of 1 ml/100g animal was used and for i.pl. injections, 0.1 ml was given to each paw.

**Materials**

The following commercial preparations of COX inhibitors were used: celecoxib (capsules, 100 or 200 mg, Celebra, Searle & Co, Cáguas, Porto Rico), rofecoxib (compressed tablets, 25 mg, Vioxx, MSD, Campinas, Brazil), and piroxicam (capsules, 20 mg, Feldene, Pfizer, Guarulhos, Brazil). We thank Searle (USA) for a generous gift of SC236. Indomethacin, lambda-carrageenan and Trizma base were purchased from Sigma (St Louis, MO, U.S.A.). Sodium chloride ("pro-analysis") was from Reagen (Rio de Janeiro Quimibras Indústrias Químicas S.A., RJ, Brazil).

**Statistics**

Results are presented either as mean (±s.e.mean) values from N animals (N ≥ 4) in each treatment group, at each time point (Figures 1, 2 and 6) or as area under the curve (AUC) values. The latter was calculated as the sum, over 4h, of the individual values for each time point for each animal, using the trapezoidal rule (GWBASIC software). The mean (± s.e.mean) AUC was derived from the AUCs for N animals in each treatment group. Mean values from the treated groups were compared with the mean values from the group receiving the vehicle only as treatment, using Students t test or Anova t test (for multiple comparison, when necessary) to determine significant differences, accepting a difference between means when P< 0.05. For the assays of nociceptive threshold, negative AUC values (less than zero) represented hyperalgesia and positive values represented
hypoalgesia. Thus abolition of hyperalgesia would be shown by a mean AUC value not significantly different from zero and hypoalgesia by a mean value significantly greater than zero.
Results

Hyperalgesia and oedema development induced by carrageenan in rat paws

Intraplantar administration of carrageenan (100-500μg per paw) dose-dependently reduced the weight threshold required for the animals’ response over 4h, clearly characterising the development and resolution of hyperalgesia (Figure 1A). This hyperalgesia, expressed as a decrease in the threshold weight, reached a maximum value between 2-3 h, was clearly fading by 4h (Fig 1A) and had returned to basal levels at 24 h following carrageenan injection (data not shown). After carrageenan injection, paw volume also increased over about the same time as hyperalgesia (Figure 1B). This oedema reached a maximum value at 3h, persisted beyond 4h but resolved by 24h (data not shown). The hyperalgesia and oedema induced by the intermediate dose of 250μg of carrageenan were adequate to show either increase or decrease after treatment and this level was therefore chosen as the standard inflammatory stimulus in our subsequent experiments. Modification of these standard inflammatory responses by inhibition of PG biosynthesis by pre-treatment with a single dose of a non-selective (indomethacin; 0.5mg.kg\(^{-1}\)) or of a selective COX-2 inhibitor (celecoxib; 6mg.kg\(^{-1}\)) is shown in Figure 2. In Figure 2A, the effect of indomethacin was to reduce the mean threshold values at each time to between those induced by carrageenan alone and those induced by saline alone, i.e., no hyperalgesia. By contrast, treatment with celecoxib not only prevented the hyperalgesia but also allowed the animal to withstand a greater than normal weight before withdrawing the paw under test. This increase of the weight threshold above normal levels, very clear at 2h and at 3h after carrageenan injection, we have called a state of
“hypoalgesia”. The anti-hyperalgesic effect of celecoxib was, like that of a low indomethacin dose, not accompanied by reductions in oedema (Fig 2B). These two types of anti-hyperalgesic responses were examined further as described below, for both selective and non-selective inhibitors of COX-2.

Effect of selective COX-2 inhibitors on hyperalgesia and oedema development induced by carrageenan

The effects of pre-treatment with two selective, clinically used, COX-2 inhibitors celecoxib and rofecoxib and one experimental selective inhibitor, SC 236 (Penning et al., 1997) on hyperalgesia and oedema induced by carrageenan were studied over a range of concentrations. The results from these experiments are presented, for brevity, as the mean “area under the curve” (AUC) for each treatment group. The AUC was calculated for each animal by summing the values measured at each time point and the mean calculated from these individual AUC values, as described in the methods. In Figure 3A, celecoxib (3-12mg.kg\(^{-1}\)) dose-dependently prevented hyperalgesia and then displayed a hypoalgesic effect, very marked at 12mg.kg\(^{-1}\), which was lost at the highest dose, 30mg.kg\(^{-1}\). These large changes in the hyperalgesic response to carrageenan were not accompanied by any reduction in oedema (Fig 3B), until the highest dose level when a modest reduction in oedema was recorded over the 4h period. In the absence of the inflammatory stimulus, i.e., in paws injected with saline only, there was no hyperalgesia and no oedema. Pretreatment with celecoxib at the dose giving the most marked hypoalgesia (12mg.kg\(^{-1}\)) did not induce any changes in either nociceptive threshold or paw volume, under these conditions (Table 1). Rofecoxib, over a 10-fold range of doses, showed very similar
effects on the responses to carrageenan (Figure 3C & D), with abolition of hyperalgesia throughout the dose range, marked hypoalgesia at an intermediate dose (1.4mg.kg\(^{-1}\)) and loss of hypoalgesia at the highest dose tested. One clear difference between the two selective inhibitors was that oedema was not affected by any of the doses of rofecoxib used here. Because these two clinically used selective COX-2 inhibitors were used in our experiments as suspensions of the tablet or capsule preparations, it was possible that these effects might be related to the excipients involved. We therefore extended our analysis to the experimental selective inhibitor SC236 (Penning et al., 1997) which was obtained as the pure substance. With this inhibitor, given s.c., over a 2-fold dose range, essentially the same results were obtained. Thus SC236 showed decreased hyperalgesia at the lower dose, and then at the higher dose, hypoalgesia (Figure 4A.). There were no reductions in oedema at either of the doses of SC236 used (Fig 4B).

Effects of non-selective inhibitors of COX-2 on carrageenan hyperalgesia and oedema

The non-selective inhibitors of COX-2 also inhibit the constitutive isoform COX-1, usually more potently than they inhibit COX-2 (Warner et al. 1999; Vane et al., 1998). Indomethacin, as already shown (Fig 2), decreased hyperalgesia without reduction of oedema. Piroxicam, at two doses (3 and 20 mg.kg\(^{-1}\)), abolished hyperalgesia and markedly reduced oedema, but did not show any signs of hypoalgesia (Fig 5A & B).

Effects of celecoxib given locally (intraplantar, i.pl) on carrageenan-induced hyperalgesia and oedema
Pre-treatment with the selective COX-2 inhibitor, celecoxib (300μg), given into the paw 30min before the carrageenan, abolished the subsequent hyperalgesia and induced a clear hypoalgesia, as shown in Figure 6A. However, there was no change in the oedema after celecoxib (Fig 6B).
Discussion

Our experiments reported here have confirmed and extended our earlier observations, in inflamed rat paws, of hypoalgesia induced by celecoxib, a selective inhibitor of COX-2 (Francischi et al., 2002). We have used different selective COX-2 inhibitors over a range of doses and showed that this hypoalgesia was induced at doses that did not reduce oedema, another inflammatory response. Two non-selective inhibitors of COX (indomethacin and piroxicam) were able to reduce paw oedema but were only able to prevent hyperalgesia without inducing hypoalgesia.

Some analysis and definitions of the terms, hypo- and hyperalgesia, is needed as a preliminary to discussing our results. For many years, the analgesic effects of COX inhibitors (NSAIDs) have been explained by postulating PGs as algesic potentiators, whose action was to increase the algesic effects of the directly acting algesic agonists, such as bradykinin (Ferreira, 1990). In this paradigm, PGs have no overt or direct, algesic action. The sensitisation of peripheral sensory nerve receptors by PGs to direct chemical and mechanical stimulation was demonstrable in experimental conditions and in human studies (Collier & Schneider, 1972; Ferreira, 1972). This potentiation of nociception was termed hyperalgesia. A defining feature of the analgesic action of the NSAIDs is that they are effective only in nociception associated with PG production, i.e., only in inflammatory pain. The response to painful stimuli in non-inflamed sites is not altered by NSAIDs and this contrasts crucially with other types of analgesics such as the local anaesthetics or opioids with very different modes of action. An essential corollary of the status of PGs as nociceptive sensitisers is that it is not logically possible for inhibition of PG production to alter nociceptive thresholds to levels above the normal state, i.e., to act
as anaesthetics. In the present context, we have referred to a state of higher than normal nociceptive thresholds as hypoalgesia. Accordingly, inhibitors of PG biosynthesis (COX inhibitors) should only abolish hyperalgesia but not cause hypoalgesia.

From our results, the magnitude of the hypoalgesia, its demonstration with three selective COX-2 inhibitors and with two modes of administration, systemic or local, all establish hypoalgesia in this model as a real phenomenon. We sought in the subsequent experiments to define the hypoalgesia further and to explore mechanisms underlying this phenomenon. Some possible mechanisms can be readily eliminated.

Stress-induced analgesia is not likely to have contributed to our results as other COX inhibitors — indomethacin, piroxicam — used under identical conditions did not exhibit hypoalgesia. The possibility that the selective inhibitors were acting like local anaesthetics can be eliminated as the normal (non-inflamed) pathways of nociception were not altered by celecoxib since it did not induce hypoalgesia in paws injected with saline, following either local or systemic administration. In most of our experiments, suspensions of the commercial preparations of the selective COX-2 inhibitors were used so that the active substance was accompanied by a variety of excipients and these excipients may have altered the response of the model. We feel this is unlikely as we also used piroxicam from the commercial preparation without observing hypoalgesia. The different excipients used in the tablets, which are designed to be without pharmacological effect, would have to exert effects that separated the selective (celecoxib, rofecoxib) from the non-selective inhibitor (piroxicam). More convincingly, our experiments with the selective inhibitor SC236 and the non-selective inhibitor, indomethacin, used the pure substance and gave results essentially identical to those with the suspensions of the
commercial preparations. Furthermore the dose-dependency of hypoalgesia strongly suggests that this effect was mediated by some biological activity shared by the three compounds, celecoxib, rofecoxib and SC 236.

The simplest explanation would be to attribute the hypoalgesia to selective inhibition of COX-2 in the inflamed paw. Support for this would be provided by the doses of the three selective inhibitors causing hypoalgesia. In our experiments there was about a ten-fold difference in dose between the two clinically used inhibitors, with 0.7 mg/kg of rofecoxib producing an increase of about 100g above the normal nociceptive threshold, an effect reproduced by between 6 and 12 mg/kg of celecoxib. The clinically effective doses of these two COX-2 inhibitors and their potency in vitro against COX-2 are of this order (Chan et al., 1999). The non-selective COX inhibitors also inhibit the other isoenzyme, COX-1, but usually with greater potency than COX-2. Piroxicam is a more potent inhibitor of COX-1, with a potency ratio of about 600 and indomethacin is less so with a potency ratio of about 60 (Warner et al., 1999; Chan et al., 1999; Vane et al., 1998). However, neither was able to induce hypoalgesia over a range of doses although, as expected, both decreased hyperalgesia markedly. It must also be remembered that although non-selective COX inhibitors such as piroxicam are potent inhibitors of COX-1, their anti-inflammatory effects (analgesia and oedema reduction) are nevertheless attributed to inhibition of COX-2. The crucial difference between selective COX-2 inhibitors and non-selective inhibitors, at anti-inflammatory doses, is that with the latter, COX-1 is also inhibited. This interpretation would suggest that, with the non-selective inhibitors, inhibition of COX-1, in some way, prevented the expression of the hypoalgesic effects of concomitant COX-2 inhibition. This apparent interaction between
the isoforms contrasts with the results of Smith et al. (1998) who, in a similar model of inflammation, found that inhibition of COX-1 was not relevant to either loss of hyperalgesia or to reduction of oedema. However, Ballou et al. (2000) concluded that both isoforms were involved in PG mediated hyperalgesia in their models. These comparisons with previous work have to be moderated by important differences in the details such as animal lineage, nociceptive stimulus, time of assay, etc., of the experimental procedures. Nonetheless, our results have clearly shown an anti-hyperalgesic effect for both selective and non-selective inhibitors, but with the particular effect of hypoalgesia restricted to the selective inhibitors of COX-2.

A distinctive feature of the hypoalgesia was that it was exhibited at doses that did not bring about the other classical anti-inflammatory effect, decreased oedema. All three selective inhibitors caused both decreased hyperalgesia and then induced hypoalgesia, without a significant reduction in oedema. This lack of effect on oedema is probably related to the degree of inhibition of COX-2 as the highest dose of celecoxib did reduce oedema and all three selective inhibitors are well known to reduce oedema in this model (Penning et al. 1997; Chan et al., 1999). Further, the non-selective inhibitor, indomethacin, was also able to reduce hyperalgesia without reducing oedema, although it, too, is known to reduce oedema in this model. It may be that marked inhibition of COX-2 is needed to prevent the potentiation of oedema by PGs (Williams & Peck, 1977) but that a lesser inhibition is enough to prevent the sensitisation of nociceptors. However this explanation would not account entirely for the hypoalgesia, which, as mentioned earlier, is not compatible with the present concepts of PG action, only as a sensitiser of sensory neurons.
Another characteristic of the hypoalgesic effect was its bell-shaped, dose-effect relationship, shown by all three selective inhibitors. With celecoxib, the lowest dose decreased hyperalgesia, intermediate doses produced hypoalgesia and at the highest dose there was loss of the hypoalgesia but still abolition of hyperalgesia. Similarly, three doses of rofecoxib exhibited hypoalgesia and the highest dose (3.5 mg.kg$^{-1}$) returning to normal nociceptive thresholds. From this observation it would appear that hypoalgesia is expressed only at a particular level of COX-2 inhibition and that level did not reduce oedema. Such a window of COX-2 inhibition could also explain why hypoalgesia has not been previously reported in the extensive studies on these three compounds. Because there would be no reduction of oedema, this degree of COX-2 inhibition, i.e. this dose of inhibitor, would be classified as ineffective, in anti-inflammatory terms. Therefore, higher doses would be used to modify oedema and, as we have shown, at these higher doses, hyperalgesia was still abolished (the expected anti-inflammatory effect) but hypoalgesia disappeared.

Although COX-2 is expressed constitutively in brain (Yamagata et al. 1993; Vane et al., 1998), it is the COX-2 in the spinal cord that is important for nociception and the analgesic actions of selective COX-2 inhibitors (Svensson & Yaksh, 2002; Samada et al., 2001; Vanegas & Schaible, 2001). There is evidence for the involvement of COX-1, also in the spinal cord, which would be particularly relevant to analgesia induced by non-selective inhibitors (Mazario et al., 2001; Ballou et al., 2000). Our results with local intraplantar injection of the selective COX-2 inhibitor, celecoxib, would nevertheless suggest a major peripheral site of action of COX-2 inhibitors in mediating the hypoalgesia we observed.
In summary, our results in a model of acute inflammation have disclosed a hypoalgesic effect of COX inhibition which appears to be independent of oedema reduction and correlated with selective inhibition of COX-2. We believe the hypoalgesic mechanism is mediated by COX-2 as it was not observed in the absence of inflammation and was invoked by three different, selective, inhibitors of COX-2. Its novelty lies in the hypoalgesia observed, the lack of a concomitant effect on oedema and the bell-shaped dose-response relation. These features were not shown by the non-selective inhibitors or in earlier work with the selective COX-2 inhibitors and appear to be incompatible with the present concepts of PGs acting merely as potentiators of normal nociceptive pathways and of the reversal of such potentiation by inhibition of COX. It must be emphasized that models of inflammatory hyperalgesia are many and various; our report seeks to establish hypoalgesia with selective inhibitors of COX-2 in one model as an incentive to others to look for the same phenomenon in their own models. Nevertheless, although our findings are still far from being fully elucidated, they raise important questions about the pathophysiological role of PGs and the COX isoforms in the mediation and perception of inflammatory pain and may necessitate new paradigms of PG action in nociception.

Aknowledgment

The authors want to acknowledge Fapemig, CNPq and CAPES for their financial support and to Webster G. P. Reis, for his excellent technical assistance.
References


TABLE 1: Lack of effect of celecoxib (12mg.kg\(^{-1}\); s.c.) on nociceptive threshold and paw oedema responses in rat paws injected with saline.

<table>
<thead>
<tr>
<th>Pre-Treatment</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20±6</td>
<td>12±5</td>
<td>12±5</td>
<td>6±4</td>
<td>2±6</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14±2</td>
<td>8±4</td>
<td>10±5</td>
<td>4±2</td>
<td>10±1</td>
</tr>
<tr>
<td>Celecoxib</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>0±6</th>
<th>1±6</th>
<th>0±6</th>
<th>1±6</th>
<th>0±6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nociceptive threshold (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Paw volume (ul)</th>
<th>70±60</th>
<th>40±20</th>
<th>40±20</th>
<th>10±1</th>
<th>20±10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celecoxib</td>
<td>16±3</td>
<td>18±4</td>
<td>18±2</td>
<td>12±2</td>
<td>14±2</td>
</tr>
</tbody>
</table>

The values in the Table are the means±s.e.mean from groups of N=6 animals. Pre-treatments, celecoxib (12mg.kg\(^{-1}\)) or vehicle (saline), were given as s.c. injections, 30 minutes before i.pl injection of saline (100 μl) at time zero. Nociceptive threshold and paw volume were measured as described in the methods. There were no significant differences between vehicle and celecoxib treated groups at any time, in contrast to the effects of this dose of celecoxib in paws injected with carrageenan (see Fig 3).
Figure 1.
Figure 2.
Figure 3A,B
Figure 3C,D
Figure 4.
Figure 5.
Figure 6.
Figure 1. Hyperalgesia and oedema induced by carrageenan in rat paws.

The effects of three doses of carrageenan measured hourly for 4h following intraplantar injection are shown separately for hyperalgesia (A) and oedema (B), although both variables were measured in each animal. Hyperalgesia is represented by a decrease in the response threshold below the control value and is expressed as the weight (in g) at which the response was elicited. Oedema is shown as an increase in paw volume above control value. The means (±s.e.mean) values from groups of 4 rats for each condition are shown.

For all doses of carrageenan, the nociceptive threshold was significantly less than that for control (saline injection) for 1-3h and, for the two higher doses also at 4h. Paw volume was significantly increased for all doses of carrageenan for all the times shown.

*Significantly different from control; P<0.05.

Figure 2. Effects of indomethacin and celecoxib on rat paw hyperalgesia and oedema. In A, the hyperalgesia induced by carrageenan was less at each time after pre-treatment with indomethacin (0.5mg.kg⁻¹) but these changes were not significant. Celecoxib (6mg.kg⁻¹) pre-treatment prevented the drop in threshold (hyperalgesia) by carrageenan and increased the nociceptive threshold above normal particularly at 2 and 3h after carrageenan. In B, the oedema induced by carrageenan was not reduced by either pre-treatment.

*Significantly different from corresponding values for carrageenan only; P<0.05

Figure 3. Effects of the selective COX-2 inhibitors, celecoxib or rofecoxib on rat paw hyperalgesia and oedema. Here the results are shown as the area under the curve for the time course over 4h for each animal (see methods). In A, the hyperalgesia induced by carrageenan was totally prevented by all levels of celecoxib. Hypoalgesia, measured as an
increase of nociceptive threshold above the normal was shown for all except the highest dose of celecoxib. In B, oedema was unchanged except after the highest dose of celecoxib when it was reduced by about 30%. In C, pre-treatment with rofecoxib reversed the hyperalgesia and induced hypoalgesia. The loss of hypoalgesia with increasing doses of rofecoxib is clearly shown here with the highest dose preventing hyperalgesia but showing no hypoalgesia. Note that none of the doses of this selective COX-2 inhibitor reduced paw oedema (Fig 3D).

*Significantly different from carrageenan only; # significantly different from zero (saline/saline treatment).

**Figure 4.** Effects of the selective COX-2 inhibitor, SC-236, on rat paw hyperalgesia and oedema. In A, the hyperalgesia was totally prevented by the lower dose (1mg.kg\(^{-1}\)) and a marked hypoalgesia induced by the higher dose of SC-236 (12mg.kg\(^{-1}\)). In B, neither dose reduced oedema.

**Figure 5.** Effects of the non-selective COX inhibitor, piroxicam, on rat paw hyperalgesia and oedema. Both doses of piroxicam (3 or 20 mg.kg\(^{-1}\)) prevented the hyperalgesia but did not cause hypoalgesia and here oedema was also reduced.

**Figure 6.** Intraplantar injection of celecoxib. In these experiments, celecoxib (300\(\mu\)g) was given locally, i.e., into the footpad, 30 minutes before the carrageenan. Hyperalgesia was completely prevented and the nociceptive threshold raised above normal for at least 4h, returning to baseline by 24h. In B, no effects on oedema were observed throughout the 24h.

*Significantly different from corresponding value for carrageenan only; P<0.05
HYPERALGESIA AND ODEMA IN ARTHRITIC OLD RATS: ROLE OF ENDOGENOUS GLUCOCORTICOIDS

C.M. YOKORO¹, M.A.K.F. TATSUO¹, L.S.M. PEREIRA¹, D.L.F. ALVES,¹ AND J.N. FRANCISCHI¹

¹Departamento de Farmacologia, ICB, UFMG, MG, Brasil

Acknowledgements

Research support by FAPEMIG (N. 867/97), CNPq and CAPES. J.N. Francischi, D.L.F. Alves and C.M. Yokoro are research and doctoral fellowships from CNPq, respectively.

J.N. Francischi, PhD
Departamento de Farmacologia, ICB, UFMG
Av. Antônio Carlos, 6627
31270-901 Belo Horizonte, MG, Brasil
Fax: +53-31-3499-2695
E-mail: janetfil@mono.icb.ufmg.br

Running title: Hyperalgesia and oedema in arthritic old rats

Key words: Hyperalgesia, oedema, adjuvant arthritis, adrenalectomy, dexamethasone, old rats
ABSTRACT

The adjuvant-induced model is an useful tool in the study of pathophysiology and drug therapy of arthritis. We intended to compare intensity and frequency of arthritis in old (O) and juvenile (J) rats and to verify the role played by adrenal glands in this condition. Arthritis was induced by subcutaneous injection of *M. butyricum* in the base of the tail from female Holtzman rats at day zero. Paw oedema and hyperalgesia as signs of arthritis development were followed from zero to 21\textsuperscript{st} day of induction. Some old animals were adrenalectomised bilaterally and treated with either different doses of dexamethasone or celecoxib immediately following surgery. All bilateral adrenalectomised old animals became susceptible to disease as well as onset of disease was shortened (from 10\textsuperscript{th} to 5\textsuperscript{th} day). Hyperalgesia (H) and paw oedema (PO) responses were less frequent in older animals (50 and 25 % from controls, respectively), although old responder animals showed responses of similar intensity compared with their juvenile counterparts at 14\textsuperscript{th} day (H\textsubscript{J}= 0,8 ± 0,07/ H\textsubscript{O}= 0,8 ± 0,09; PO\textsubscript{J}= 56,6 ± 6,04/ PO\textsubscript{O}= 32,24 ± 12,7; as Δ% of increase paw oedema). Dexamethasone (D) but not celecoxib chronic treatment of adrenalectomised old animals abrogated the effects of adrenalectomy, in particular those relating to hyperalgesia response (H\textsubscript{O}= 0,95 ± 0,03/ H\textsubscript{D}= 0 ± 0; 14\textsuperscript{th} day), thus implying a specific participation of circulating corticosteroids in the modulation of pain in arthritic old rats.
Introduction

Rheumatoid arthritis is an auto-immune chronic inflammatory disease characterized by infiltration and activation of inflammatory cells within synovial tissue of multiple joints (1). Rheumatoid arthritis is most common in older people, specially women and it is a source of considerable morbidity and mortality in the western world (2). There were great advances in the understanding of the pathophysiology of various types of human arthritis, in special in the case of rheumatoid arthritis. These advances were made possible at least in part to the availability of the adjuvant-induced arthritis model developed in rodents (3, 4, 5).

The adjuvant-induced arthritis model has been used in our laboratory to study the efficacy and the mode of action of special analgesics, anti-inflammatory and immunosuppressant drugs (6, 7, 8). In this model, articular hyperalgesia starts from 7 to 10th day of disease induction, whereas paw oedema usually appears later on being evident at 14th day of disease (7). Using this model, we have observed that different from the human disease, older rats are less susceptible to the disease than juvenile rats. In line with this, in the literature, there is data showing that older mice are less susceptible to systemic lupus erithematosus, another model of auto-immune disease (9).

The present study, thus, aimed to a) verify the intensity and frequency of hyperalgesia and oedema development in 8-12 months-old (old) compared with 2 month-old (juvenile) rats from the same lineage and sex; b) assess the role of adrenal glands in arthritis development by old rats, as corticosteroid- induced antiinflammatory activity were first described in arthritic patients (10).
Material and Methods

Animals

Female Holtzman rats: juvenile, 2 month-old or old, 8-12 month-old with total body weight of 140-170g and 270-300 g, respectively. The animals were kept in cages (maximum of five animals per cage) at a temperature of 26 ± 3°C, and on a 12 h-light/dark cycle with standard laboratory rat pellets and water given ad libitum. Ethical guidelines of the International Association for the Study of Pain in conscious animals were followed (11).

Induction of arthritis by adjuvant

Rats were injected subcutaneously with a single dose of 0.2 mL mineral oil-water emulsion (10:1, v/v) containing 400 μg of dried Mycobacterium butyricum into the dorsal root of the tail under ether anesthesia. The time of adjuvant injection is referred to as day zero.

Measurement of hindpaw hyperalgesia and oedema

The method for measuring hyperalgesia has been previously described elsewhere (12). Briefly, the tendency of normal (naive), control and arthritic rats to vocalise following flexion of the tarsotibial joints of both hindpaws was tested daily for 21 days starting from day 0. The results are reported as the mean number (± standard error of the mean) of vocalisations obtained following five flexions of hindlimb tarsotibial joints.
Hindpaw volume (as an indicator of oedema) was measured daily using an Ugo Basile hydroplethysmometer (model 7150) following hyperalgesia test. The volume (mL) of one hindpaw was essentially the same as that of the contralateral paw (data not shown) and is reported as the mean ± SEM. All measurements were obtained at the same time of the day. Increase in paw volume of the animals are also presented as % of the control values.

We considered an animal as arthritic when the mean value for hyperalgesia was above 0.3 and the difference (A) in paw oedema values for each day and the day zero was above 0.3 mL.

**Bilateral adrenalectomy**

The complete removal of the adrenal glands, a classical technique in physiology (13), was made in two groups of adult old female rats: controls, which were sham-(false-) adrenalectomised, or bilateral adrenalectomised animals. All the animals were injected with adjuvant (day 0) 5 days after the adrenalectomy and followed as previously described. Besides food, water and saline (NaCl, 0.15M) bottles were adapted in the cages and intake monitored for the animals all through the experimental period.

**Treatment of animals with anti-inflammatory drugs**

Dexamethasone (0.1 or 0.01mg/kg) or its vehicle (physiological saline) was administered via oral by gavage to adrenalectomised adjuvant old rats immediately following adrenalectomy through 21st day of arthritis induction. Sham operated animals were treated with physiological saline by the same route and the same time as
adrenalectomised animals. A group of adrenalectomised adult animals was daily treated in parallel with celecoxib (3mg/kg) under identical conditions previously described. This dose of celecoxib has been proven to be analgesic in the standard model of hyperalgesia induced by carrageenan in rat paws (data not shown).

Drugs

The following drugs were purchased from the stated sources: dexamethasone (Decadron, Prodom, Campinas, Brazil), celecoxib (Celebra, Searle, Caguas, Porto Rico), Mycobacterium butyricum (Difco Laboratories, Detroit, MI, USA).

Data analysis

Results were presented as mean ± SEM which were compared by one way analysis of variance (ANOVA). When mean percentages of increase in volume were compared, statistical analysis was made by multiple comparisons using two way analysis of variance (ANOVA). The software used for statistical analysis was SigmaStat®.
RESULTS

Development of clinical signs of the adjuvant arthritis in young and old rats

The first series of experiments was designed to compare the development profile of the adjuvant-induced arthritis in juvenile and old rats. Increase in vocalisation to forced flexion of tarsotibial joints described as hyperalgesia was evident from the 10th day in juvenile animals reaching a maximum value at the 21st day of disease induction (panel A, Fig. 1). Results from juvenile control group were omitted from Figure 1 for matter of clarity, as either hyperalgesia and oedema responses do not develop. In contrast to juvenile animals, however, only half of the induced-old animals, i.e., six out twelve animals presented hyperalgesia, thus providing a rate of 50 % responder and non responder animals (upper line in panel B, Fig. 1). This hyperalgesia presented by responder old animals, however, was of the same magnitude as that presented by juvenile animals. In contrast, the rate of induced-old animals presenting paw oedema was lower of about 25 % (3 out 12, panel D) that presented by juvenile rats (panel C), with greater variability between individual values (in old animals). In addition, the maximal percent increase in paw volume (at 21st day) was 100 % in juvenile animals and a little higher in old responder animals, of 135 % increase (Table 1), which was not statistically different from juvenile animals. Differences between initial paw volume values shown by arthritis induced-old rats from the zero day to 9th day of induction probably reflected their higher initial weight in comparison to juvenile animals (panel D, Fig. 1). Moreover, in the end of experiments at the 21st day of observation, joint deformities shown by responder arthritic-induced old animals were less evident than in juvenile animals (data not shown).
Effect of adrenal removal in the response of old animals to adjuvant arthritis

Removal of both adrenal glands from old rats rendered all the animals hyperalgesic, as illustrated in Figure 2A. In this figure, it can be observed that animals which suffered surgery but remained with both glands, i.e., sham operated animals, could still be divided in responder and non-responder animals in relation to hyperalgesia measurements, as previously shown by arthritic old (non-operated) rats. In addition, the latency for hyperalgesia development was shortened to the 5th day following disease induction (Figure 2A). However, all adrenalectomised responding old rats presented paw oedema of the same intensity as sham operated responder old animals. Surprisingly, the intensity of this response in both groups of animals was significantly less intense (about 30% increase) than that observed in non-operated old responder animals (135%; Table 1), despite of the surgery had occurred 5 days before.

Effect of dexamethasone administration following bilateral adrenalectomy in hyperalgesia and paw oedema responses shown by arthritic-old rats

Dexamethasone was administered immediately following surgery in two doses (0.1 or 0.01 mg/kg/daily) to groups of bilateral adrenalectomised animals to further verify the role played by corticosteroids in arthritis development presented by old rats. Chronic dexamethasone administration in both doses significantly inhibited hyperalgesia and paw oedema development (Figures 3A and 3B) to the level of non-responders old animals shown previously in panels B and D, respectively, in Figure 1. In addition, reduction of hyperalgesia and paw oedema responses to the level of non-responders animals was not observed in 3 mg/kg celecoxib adrenalectomised old animals (Fig. 4A and 4B), except in
the first three days of hyperalgesia response (Figure 4 A). This dose of celecoxib was shown to significantly inhibit hyperalgesia development due to a definite dose of carrageenan (250 µg) acutely administered to rat paws (data not shown).
Discussion

The model of adjuvant-induced arthritis in rats is an useful tool to study the pathophysiology of rheumatoid arthritis, a chronic inflammatory reaction, specially because both the experimental model and the human disease share various signs and symptoms (3). In addition, the activity profile of antiinflammatory drugs useful for treatment of rheumatoid arthritis correlates well with the findings for the same drugs in this experimental model (12, 14). In this study, we were searching for an ideal size of rats with other purposes and occasionally observed that older control animals presented a low frequency of arthritis development (hyperalgesia and oedema responses) when challenged. This prompted us to investigate hindpaw oedema and hyperalgesia responses in adjuvant-challenged old animals in comparison with their “good” responder juvenile counterparts. We observed that, indeed, both arthritic hyperalgesia and paw oedema responses were much less frequent in older animals, which represented respectively 50 % and 25 % of the frequencies found in juvenile rats. However, the ability from responder-old animals to react to a imposed hyperalgesic stimulus was preserved, as these animals showed a response of the same magnitude as that presented by young rats, which practically allowed overlap of both curves (panels A and B, in Figure 1). As rat hyperalgesic response to adjuvant remained unaltered in old responder animals, we may suggest that the mechanisms involved in the hyperalgesic response are not altered with increasing age. In addition, inflammatory (oedema) response in old rats responding to adjuvant arthritis was even more pronounced than in juvenile animals, though not statistically significant. However, this could be related to the fact that the rat paws from
old animals, which are bigger initially, can also expand more. As data in literature revealed that old rats present higher basal levels of circulating corticosteroids when submitted to stress (15), we tested the hypothesis whether removing adrenals would affect arthritis development in old animals. In fact, increase in the frequency of hyperalgesia and paw oedema was observed in adrenalectomised old arthritic animals in relation to controls. In addition, maximal increase in hyperalgesia in adrenalectomised old animals was attained earlier, around the 5th day, when compared with the hyperalgesia presented by juvenile rats (8th to 10th day). However, a different profile was observed in old animals in relation to the oedema response following surgery. As observed, oedema response was of much lower intensity in operated animals (sham and adrenalectomised) than in non-operated old animals, if one compares Fig. 2 with Fig. 1 (present study). In addition, the responses were of the same (low) magnitude in sham and bilateral adrenalectomised animals in relation to responder non-operated animals (30 versus 135 % increase, respectively). This suggests that the oedema response was strongly affected by either the surgery or anesthesia itself, independent of the presence of adrenals in old animals.

An other worthwhile observation in the present study was the survival of adrenalectomised arthritic old rats, as it is described from the literature that juvenile arthritic rats simply do not survive to adrenalectomy (16). So, our results could suggest that adrenal glands play a minor role in the survival of ageing rats. However, hyperalgesia responses were increased either in frequency and onset following bilateral adrenalectomy in relation to control animals, thus indicating that integrity of the adrenals is of importance at least for modulation of arthritic pain response in old animals.
To further verify whether corticosteroids were the substances from adrenal glands involved in such inhibitory response, we chronically administered exogenous dexamethasone, a standard anti-inflammatory steroid (17) to arthritic old animals which suffered bilateral adrenalectomy. As shown in Figure 3 in this study, corticotherapy in these challenged animals reverted the increase in the hyperalgesia previously seen in adrenalectomised animals. Dexamethasone-treated (old) animals showed a flatter hyperalgesia and oedema intensity curves, towards a response from non-arthritic animals. It is known the reduced ability of T cells from old people and animals to respond to immunological challenge (18, 19). In fact, we have observed in our laboratory that rat mononuclear cells from old rats produce and release significantly less hyperalgesic substances than mononuclear cells from young animals (Pereira L.S.M., unpublished observations). As inhibition of increased hyperalgesia and oedema frequency seen following adrenalectomy of arthritic old rats, however, was not consistently observed with a chronic treatment with celecoxib, a specific cyclooxygenase inhibitor (20, 21, 22), we concluded that those responses were, at least partially, specifically modulated by corticosterone. A light and short-lived reduction of the increased hyperalgesia frequency by celecoxib was initially observed in arthritic old rats which suffered adrenalectomy, confirming previous studies which showed an early participation of COX-2 in arthritic pain (23), in our case even in old rats. However, this chronic regimen for celecoxib did not affect oedema response shown by old arthritic rats at any time studied.

In conclusion, our results showed that old rats present a lower frequency of arthritic hyperalgesia and oedema compared with their juvenile counterparts. Our data also pointed out a partial role for adrenal glands, in special for endogenous
corticosteroids, in this modulation. In particular, modulation of the hyperalgesia but not of the oedema presented by old arthritic animals was observed with dexamethasone, an exogenous steroid but not with celecoxib, a specific COX-2 inhibitor.
References


Table 1. Percentage (#, %) of increase in paw volume in adjuvant-induced arthritic rats following bilateral adrenalectomy.

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>GROUP</th>
<th>% INCREASE IN PAW VOLUME</th>
<th>P₁</th>
<th>P₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL 1</td>
<td>JUVENILE (n=10)</td>
<td>100±11.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CONTROL 2</td>
<td>OLD RESPONDER (n=3)</td>
<td>135±37.8</td>
<td>&gt;0.05</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>OLD NON-RESPONDER (n=9)</td>
<td>0.7±1.99</td>
<td>&lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>CONTROL 3</td>
<td>OLD RESPONDER (n=4)</td>
<td>31.2±15.2</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>NON-RESPONDER (n=5)</td>
<td>9.9±2.36</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>BILATERAL ADRENALECTOMY</td>
<td>OLD RESPONDER (n=7)</td>
<td>36.7±2.22</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

*Percentage of increase in the 21st day of disease in relation to the day of induction is presented for all groups. Data probability (P) from all groups were first compared with that presented by the juvenile group (control 1; P₁). In a second analysis, data probabilities (P₂) from old animals were compared with control 2. Bilaterally adrenalectomised old-responders were compared with control 3 (sham-operated animals). The statistical analysis was made through multiple comparisons by two way analysis of variance (ANOVA). A statistical significance is referred when P<0.05. Finally, bilateral adrenalectomised old responders were compared with control 3 (sham operated old animals).
Figure 1. Development of hyperalgesia (A, B) and paw oedema (C, D) responses following induction of arthritis in juvenile (●) and old (☑, ■) rats. Arthritis was induced at day zero by subcutaneous injection of 0.2 mL emulsion containing 400μg M. butyricum into the base of rat tail. Hyperalgesia is described as the mean number of vocalisations (±SEM) in 5 flexions of tarso-tibial joints daily assessed. Old responder animals (■, 6 out 12) presented the same profile of hyperalgesia as juvenile animals. Data from non-responder animals (☑) are also shown. Oedema is presented as the mean increases in volume ± SEM (in mL) of one of hindpaws also daily assessed. Old responder animals presented an increase in paw oedema at 21st day of observation compared with juvenile animals also characterized by an increased interindividual variation.

Figure 2. Effect of bilateral adrenalectomy (ADX, ■) in the hyperalgesic (A) and oedema (B) responses presented by arthritic old rats (controls: ▲=responders, ▼=non-responders). Adrenalectomy or false operation was realized 5 days before induction of arthritis (day zero). Non responder animals were so classified when mean hyperalgesia and oedema measurements did not reach the values 0.3 or 0.3 mL increase, respectively. Hyperalgesia and oedema measurements followed description in Fig. 1.

Figure 3. Effect of daily dexamethasone (DEXA) administration in hyperalgesia (A) and oedema (B) responses presented by adrenalectomised arthritic old rats. Dexamethasone
0.1 (◇) or 0.01 mg/kg (◆) was orally administered immediately following surgery (-5\textsuperscript{th} day) until the last day of experiment. N= 4-5/group. Same legend as in Fig. 1.

Figure 4. Effect of daily celecoxib (●) administration in hyperalgesia and oedema responses presented by adrenalectomised arthritic old rats (◇). Celecoxib (3 mg/kg) was daily administered by oral route immediately following surgery (-5\textsuperscript{th} day) until last day of experiment. N=3/group. Same legend as in Fig. 1.
Controle da dor em odontologia: os antigos e os novos inibidores das ciclooxigenases (COXs)

Pain control in dentistry: the old and the new cyclooxygenase (COX) inhibitors

Cinthia Mara da Fonseca Pacheco¹
Janetti Nogueira de Francischii²
Daniela da Fonseca Pacheco³

RESUMO: A dor é uma queixa frequente na clínica odontológica. Ela pode decorrer de procedimentos cirúrgicos, manipulação de canais radiculares ou mesmo estar associada a desordens da articulação temporomandibular. Como na maioria dos casos, a dor está associada à inflamação, anti-inflamatórios não-esteróides (AINES) ou drogas do grupo da aspirina são indicadas para o seu alívio. Os AINES mais antigos inibem inespecificamente as enzimas ciclooxigenases (COXs), tendo como consequência, a inibição da síntese de prostaglandinas, importantes substâncias endógenas envolvidas tanto em respostas fisiológicas protetoras (produzidas pela COX-1), como também, em respostas de dor e de inflamação (produzidas pela COX-2). Nessa perspectiva, foram desenvolvidos pela indústria farmacêutica dois novos medicamentos inibidores específicos da COX-2, o celecoxib (Celebra, Pfizer) e o rofecoxib (Vioxx, MSD). Como o celecoxib e o rofecoxib já podem ser encontrados no mercado brasileiro, o objetivo do presente trabalho é apresentar uma revisão sobre o uso dos inibidores inespecíficos de ciclooxigenases (AINES) mais antigos, bem como apresentar o potencial terapêutico dos novos AINES (rofecoxib e celecoxib), no tratamento da dor em Odontologia.

Unitermos: analgésicos, ciclooxigenases, anti-inflamatórios não esteróides, inibidores de COX-2

ABSTRACT: Pain is a common complaint in dentistry. Pain can occur after surgery procedures, root treatments or even be associated with temporomandibular joint disorders. As pain is very often associated with inflammatory processes, non-steroidal anti-inflammatory drugs (NSAIDs), also called aspirin-like drugs, are prescribed for its relieve. The older NSAIDs inhibit unespecifically the enzymes cyclooxygenases (COXs) resulting in the blockage of prostaglandin production, important endogenous mediators related to both, protective functions (produced by COX-1) and hyperalgesic and inflammatory responses (produced by COX-2). Aiming to specifically inhibit COX-2, two drugs – celecoxib (Celebra, Pfizer) and rofecoxib (Vioxx,

¹ Cirurgiã-dentista, mestre em Farmacologia pela Universidade Federal de Minas Gerais, pesquisadora visitante do Departamento de Cirurgia e Farmacologia da Universidade de Pennsylvania (EUA).
² Professora Adjunta do Departamento de Farmacologia da Universidade Federal de Minas Gerais, doutora em Farmacologia pela Universidade de São Paulo, pós-doutora em Farmacologia pela Universidade de Sherbrooke (Canadá) e de Oxford (Inglaterra), Pesquisadora do CNPq.
³ Cirurgiã-dentista, mestrandia em Farmacologia pela Universidade Federal de Minas Gerais.
MSD) were developed and are today marketed in Brazil. This review intends to present the therapeutic profile of the new COX-2 inhibitors in relation to the older NSAID for pain treatment in Dentistry.

Key words: analgesics, cyclooxygenases (COXs), non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors

Introdução

A dor é uma queixa comum em Odontologia, que pode estar associada à respostas inflamatórias. O processo inflamatório que se segue à injúria de qualquer tecido é mediado por diversas substâncias químicas endógenas, denominadas autacóides ou, simplesmente, mediadores inflamatórios. Esses mediadores inflamatórios pertencem a diversas classes químicas, entre elas: proteínas e peptídeos (citocinas e bradicinina, respectivamente); aminas (ex: histamina), ácidos graxos poli-insaturados (ex: prostaglandinas e leucotrienas), entre outros. A ativação de receptores específicos presentes nos diversos tecidos pelos mediadores liberados durante o processo inflamatório leva ao aparecimento dos sinais e sintomas clássicos de uma reação inflamatória descritos por Celsus no primeiro século de nossa era, quais sejam: rubor (vermelhidão), tumor (inchaço), calor (febre) e dor inflamatória (hiperalgesia).

Qualquer distorção ou injúria na membrana celular, seja ela de natureza térmica, mecânica ou química leva à ativação da fosfolipase A₂ (FLA₂), uma enzima encontrada na forma inativa na membrana celular. Uma vez liberada da membrana, a FLA₂ ativa inicia a liberação de diversos ácidos graxos pertencentes aos fosfolípides da membrana, para o citoplasma da célula. Entre esses ácidos graxos, de grande interesse é o destino metabólico do ácido araquidônico. Uma vez liberado, o ácido araquidônico poderá sofrer a ação de enzimas, denominadas ciclooxigenases (COXs), que o metabolizarão em prostaglandinas, prostaciclinas e tromboxanas, também denominados prostanoïdes. Esses metabólitos podem afetar uma grande variedade de processos biológicos, envolvidos tanto na homeostasia do organismo, como em processos fisiopatológicos, em especial na inflamação e na dor inflamatória, dependendo das concentrações que alcançarem nos diferentes tecidos.

Até meados de 1990, acreditava-se que apenas uma isoforma de ciclooxigenase existisse. A partir de então, começou a ficar evidente a existência de diferentes isoformas de ciclooxigenases, que passaram a ser denominadas, respectivamente COX-1 e COX-2 (FU et al., 1990; CROFFORD et al., 1994). A COX-1 é constitutivamente encontrada na membrana do retículo endoplasmático de todas as células e está associada com a produção de prostanoïdes importantes para certas respostas
fisiológicas. De particular interesse, é a produção de prostanoïdes no estômago, rins, plaquetas e endotélio vascular. As prostaglandinas produzidas no estômago aumentam a produção de muco protetor da mucosa e inibem a secreção ácida (FELDMAN, 2000). A produção de tromboxana A₂ pelas plaquetas é responsável por mediar a agregação plaquetária e vasoconstrição, sendo que prostaglandina E₂ produzida pelo endotélio vascular induz vasodilatação. Essas duas substâncias portanto, realizam em conjunto, um fino balanço na homeostasia. A COX-2 por sua vez é também constitutivamente expressa no cérebro (YAMAGATA et al., 1993) e nos rins (KÖMHOFF et al., 1997), porém sua produção é maciçamente elevada quando da ocorrência de injúria nos tecidos e na inflamação (SEIBERT & MASFERRER, 1994). Está bem estabelecido na literatura, que uma ampla variedade de agentes, entre eles, citocinas pro-inflamatórias, lipopolissacarídeos e fatores de crescimento induzem a expressão do gene para a COX-2. Os diferentes padrões de expressão entre as COXs têm levado à hipótese de que prostaglandinas produzidas em menores concentrações pela COX-1 seriam responsáveis pelas respostas fisiológicas, ao passo que altas concentrações de prostaglandinas derivadas da expressão aumentada de COX-2, seriam as responsáveis pelos sinais e sintomas inflamatórios, incluindo a dor inflamatória (MEADE et al., 1993).

Farmacologia dos anti-inflamatórios não esteróides (AINES) ou drogas do grupo da aspirina

A aspirina foi introduzida no mercado como medicação analgésica e anti-inflamatória efetiva em 1899 (VAINIO & MORGAN, 1997), embora à época não fosse conhecido o mecanismo pelo qual ela exerceria esses efeitos. Em 1971, VANE demonstrou que a aspirina (ácido acetilsalicílico-AAS) inibia as ciclooxigenases, impedindo portanto, a produção dos prostanoïdes responsáveis pela inflamação e dor. Na atualidade, as drogas conhecidas como anti-inflamatórios não esteróides (AINES) ou drogas do grupo da aspirina são largamente utilizadas no tratamento de artrites, injúrias nas articulações músculo-esqueléticas, dor pós-operatória, febre e inflamações de maneira geral. Os AINES são também comumente prescritos na prevenção de infartos do miocárdio. Tantos benefícios terapêuticos fazem com que esses fármacos estejam entre os mais prescritos e utilizados no mundo. Estima-se que mais de 100 bilhões de pessoas utilizem essa classe de drogas de uma forma regular (MAY et al., 2001).

Ensaios in vitro e ex vivo têm mostrado que as drogas do grupo da aspirina mais antigas inibem de forma não específica, tanto a COX-1 quanto a COX-2 (MEADE et al., 1993), sendo que muitas delas têm seletividade maior para COX-1, embora sua atividade anti-inflamatória/analgésica esteja relacionada com a inibição da COX-2 (VANE & BOTTING, 1997). Apesar do mecanismo de ação comum, os AINES apresentam espectro diverso de ação farmacológica: alguns (indometacina,
piroxicam) são anti-inflamatórios muito potentes e eficazes; a maioria têm ação anti-inflamatória apenas moderada (dipirona), e outros, como o paracetamol, possuem ação anti-inflamatória mínima ou inexistente. Outros mecanismos, além da inibição das COXs, são relacionados com alguns dos compostos do tipo da aspirina. Por exemplo, a dipirona, um analgésico muito utilizado para o alívio da dor em Odontologia no Brasil, parece atuar também promovendo a liberação local de óxido nítrico (DUARTE et al., 1990). Outro inibidor de COXs, o paracetamol ou acetaminofen, parece inibe a hiperalgesia induzida por neurotransmissores como a substância P e o glutamato a nível da medula espinal (BJORKMAN, 1994), além da inibição das COXs. Os AINES também variam entre si grandemente no grau de efeitos colaterais produzidos, que está aparentemente associado à maior ou menor porcentagem de inibição da COX-1 ou ciclooxigenase constitutiva (CRYER & FELDMAN, 1998).

Como regra geral, baixas doses de AINES produzem, primeiramente, efeitos anti-trombóticos; doses moderadas produzem analgesia e antipirase, enquanto doses relativamente altas são requeridas para produzir efeitos anti-inflamatórios (RANG et al., 2001). O cirurgião-dentista deve estar atento a esse fato pois, pacientes que fazem uso de AAS para prevenção de infartos utilizam baixas doses, mas que são suficientes para aumentar o tempo de sangramento durante uma cirurgia. Nesse sentido, o uso de ácido acetilsalicílico é especialmente preocupante. Uma vez que ele inibe irreversivelmente a COX-1 presente em plaquetas, é preciso que novas plaquetas sejam produzidas, para que um novo contingente de COX-1 esteja disponível para a produção de TXA2. Recomenda-se que esses pacientes descontinuem o uso do mesmo 4 a 7 dias antes de procedimentos cirúrgicos, que é o tempo necessário para plaquetas novas, contendo COX-1 não ligada, atingirem a circulação. Para os outros AINES, 3 a 4 meias-vidas são suficientes (MAY, 2001).

Conhecendo-se as ações fisiológicas dos prostaglandínios fica fácil prever os efeitos colaterais que podem decorrer do uso crônico, por exemplo, dos AINES. De fato, já é bem documentada a relação existente entre o uso de anti-inflamatórios não esteroidais e o aparecimento ou agravamento de problemas gastrointestinais, sangramento e toxicidade renal (CARSON et al., 1987). Com o objetivo de diminuir os efeitos colaterais associados aos AINES, novos analgésicos inibidores seletivos de COX-2, foram desenvolvidos. Essas drogas teriam os mesmos efeitos analgésicos, anti-inflamatórios e antipiréticos dos inibidores não seletivos (sobre COX-1 e COX-2), sem causarem problemas gastrointestinais, renais, e inibição da agregação de plaquetas (HAWKEY, 1999).
O modelo de dor pós-extração de terceiro molar e o uso de inibidores específicos de COX-2

A dor pós-operatória que se segue à extração de terceiros molares inferiores parcial ou completamente impactados é um modelo válido, bem documentado e altamente sensível para avaliar o alívio proporcionado por analgésicos, em dores consideradas de moderada a severa (COOPER, 1991; FORBES, 1991). Uma vez que a dor é normalmente restrita à área da cirurgia e o edema inflamatório está presente em quase todos os casos, esse método é aplicável ao estudo dos anti-inflamatórios não esteroidais (NØRHOLT et al., 1996). Esse modelo, inclusive, já foi utilizado para testar a eficácia dos novos inibidores específicos de COX-2.

Alguns dados da literatura suportam a hipótese que, nesse modelo, a dor é causada por um aumento na síntese de prostaglandinas, visto que o aumento na concentração de prostaglandinas dentro da área cirúrgica relaciona-se com um aumento da dor após a extração de terceiros molares incluídos (ROSZKOWSKI, 1997). A maioria dos trabalhos encontrados na literatura tenta relacionar a menor dose de medicamento que seja capaz de produzir uma analgesia eficaz, com rápido início de ação, duração prolongada e poucos efeitos colaterais.

Dois inibidores específicos de COX-2 tiveram, recentemente, aprovação para entrar no mercado, sendo o primeiro deles o celecoxib (Celebra; Pfizer). A dose média de celecoxib indicada varia de 100 a 200mg duas vezes ao dia, sendo as doses mais baixas utilizadas para o tratamento de osteoartrites e as doses maiores para o tratamento de dores agudas e artrite reumatóide (SIEBERT & MASFERRER, 1994). Alguns estudos têm mostrado que embora o celecoxib apresente analgesia considerável, ele não é tão efetivo no alívio da dor aguda após extração de terceiros molares (MOORE et al., 2001). Na dose de 200 mg, celecoxib produz analgesia maior que o placebo mas menor que 400 mg de ibuprofeno (MALMSTROM et al., 1999).

O rofecoxib (Vioxx, Merck) em relação ao celecoxib, foi mais recentemente aprovado para uso terapêutico. As doses médias do rofecoxib variam de 12,5 a 50 mg/ dia, sendo as doses menores para o tratamento de osteoartrites e as doses maiores para dores agudas e artrite reumatóide. Estudos com o rofecoxib, usando o modelo de dor pós cirurgia dentária e outras condições, têm mostrado que ele produz analgesia comparável àquela dos AINES mais antigos, mas com duração de ação bem maior, utilizando-se dose única (GOTTESDIENER et al., 1999).

MORRISON et al. (1999) compararam a eficácia analgésica de 50 mg de rofecoxib, 400 mg de ibuprofeno e placebo após a extração de terceiros molares e encontraram que tanto ibuprofeno, quanto rofecoxib foram capazes de produzir analgesia significativamente maior que o placebo. O alívio da dor nas primeiras quatro horas do estudo foi praticamente igual para as duas drogas, mas a duração do efeito foi bem maior com o rofecoxib (analgesia considerável após 24 de cirurgia) do
que com o ibuprofeno (analgesia considerável por até 6 horas após a cirurgia). Outro trabalho comparando a atividade analgésica de 50 mg de rofecoxib, 400 mg de ibuprofeno, 200 mg de celecoxib e placebo, no modelo de extração de terceiros molares confirmou o dado de que 50 mg de rofecoxib induzem analgesia igual a 400 mg de ibuprofeno, sendo ambos superiores a 200 mg de celecoxib. O estudo também mostrou que o rofecoxib apresenta uma duração mais prolongada de efeito analgésico (>24 horas), enquanto ibuprofeno e celecoxib tiveram duração do efeito de aproximadamente 9 e 5 horas, respectivamente (MALMSTROM et al., 1999). É uma pena, que todos esses estudos, não possam fornecer informações sobre a possível atividade anti-inflamatória apresentada por esses compostos. Nesse sentido, e paradoxalmente, o trabalho de FITZGERALD & PATRONO (2001) mostrou que os inibidores não seletivos reduzem a inflamação mais efetivamente do que os inibidores específicos de COX-2.

**Risco e efeitos colaterais relacionados ao uso de inibidores específicos de COX-2**

Danos ao trato gastrointestinal (GI), incluindo errosões, úlceras e sangramento, são os efeitos colaterais mais comuns dos AINES mais antigos. O risco relativo do ibuprofeno administrado em doses de 1600 mg por dia parece menor que com outros AINES tais como diflunisal, aspirina, naproxeno, diclofenaco e cetoprofeno (FRIES, 1996). Um menor risco ainda, parece estar associado ao uso de celecoxib e rofecoxib. Um estudo clínico recente utilizando celecoxib (400 mg duas vezes ao dia), ibuprofeno (800 mg três vezes ao dia) e diclofenaco (75 mg duas vezes ao dia) comparou os efeitos GI após o uso crônico desses três medicamentos para tratamento de osteoartrite e artrite reumatóide. Os resultados mostraram que ocorreram menores complicações gastrointestinais, quando o celecoxib foi utilizado (SILVERSTEIN et al., 2000). Em outro estudo semelhante, comparando o rofecoxib com o naproxeno, encontrou-se também menores danos gastrointestinais quando o inibidor seletivo foi utilizado (BOMBARDIER et al., 2000).

Embora vários dados suportem o fato de essas drogas serem mais seguras que os AINES não seletivos, é importante relatar que existem alguns casos de complicações de úlceras pépticas quando inibidores específicos de COX-2 foram utilizados (FELDMAN, 1990). Os papéis desempenhados pelas duas enzimas ciclooxigenases em pacientes portadores de úlceras pépticas ainda são desconhecidos e ainda se tem muito a pesquisar sobre o efeito causado pela utilização crônica dos inibidores específicos de COX-2 no trato gastrointestinal. Apesar disso, estudos epidemiológicos mostraram que o uso crônico de AINES diminuiu a incidência de câncer cólon-rectal (BAKHEL, 2002), sugerindo um importante papel da COX-2 na etiopatogenia desse tipo de câncer.
É importante também considerar, que tanto COX-1 quanto COX-2 desempenham papel importante na manutenção da atividade renal normal. Quando utilizados cronicamente, os inibidores específicos de COX-2 podem causar toxicidade renal incluindo insuficiência renal, retenção de sódio com hipertensão e edema (FITZGERALD & PATRONO, 2001). Assim como os AINES mais antigos, os inibidores específicos de COX-2 podem diminuir os efeitos anti-hipertensivos dos inibidores da enzima conversora de angiotensina (ECA), expondo mais uma vez, o importante papel desempenhado por ambas ciclooxigenases na manutenção do fluxo sanguíneo renal normal, especialmente importante em pacientes hipertensos. No entanto, mais estudos ainda precisam ser desenvolvidos, a fim de estabelecer a farmacologia renal dos inibidores de COX-2 em seres humanos. Enquanto isso, o clínico deve ficar atento ao prescrever esses medicamentos principalmente em pacientes com doenças renais pré-existentes.

Para finalizar, de acordo com os fabricantes, o celecoxib é contra-indicado em pacientes alérgicos à sulfonamida. Assim como alguns antibióticos (ex: sulfametoazol), o celecoxib é um derivado da sulfonamida sendo possível o aparecimento de alergia cruzada. Esse fato, no entanto não parece ser clinicamente relevante (PATTERSON et al., 1999).

**Perspectivas futuras quanto ao uso de inibidores específicos de COX-2 em Odontologia**

Celecoxib e rofecoxib podem ser adquiridos no Brasil desde 1999, e desde então têm sido amplamente indicados para o tratamento da dor, nas diferentes especialidades clínicas da Medicina. Embora estatísticas pertinentes não estejam ainda disponíveis, o uso desses compostos para promover analgesia foi assimilado também pelos profissionais dentistas, sendo que esse uso parece estar aumentando gradativamente desde então. Nesse sentido, o risco do uso de novos compostos no tratamento de alterações odontológicas, como por exemplo, após a extração de terceiros molares, é em geral menor, dada a característica transitória de tal alteração, e consequentemente, da duração dos possíveis tratamentos farmacológicos. Por outro lado, algumas síndromes dolorosas, cujo tratamento farmacológico é ainda eminentemente empírico, como ocorre com a neuralgia do trigéimo, poderiam eventualmente, beneficiar-se com o uso dos novos AINES, em especial do celecoxib e do rofecoxib.

**Conclusões**

Os anti-inflamatórios não esteróides ou drogas do grupo da aspirina mais antigos, tais como o ibuprofeno, diclofenaco, naproxeno, e cetoaprofeno entre outros, sempre tiveram e ainda têm
ampla utilização em Odontologia, no alívio da dor e inflamação. Porém, os estabelecidos efeitos gastrointestinal e a interferência com o processo de coagulação têm levado muitos dentistas a recorrer aos novos inibidores específicos de ciclooxigenase-2, rofecoxib e celecoxib, principalmente no tratamento de desordens temporomandibulares, onde a utilização prolongada de AINES poderia incorrer em sérios danos gastrointestinais. Até o momento, poucos estudos têm sido desenvolvidos nessa área, com o objetivo de avaliar o impacto terapêutico produzido pelo celecoxib e rofecoxib, que se restringiram à avaliação do alívio da dor proveniente de extração de terceiros molares. Essas novas drogas apresentaram muitas vantagens, tais como doses diárias menores e menos frequentes, efeito analgésico mais prolongado como no caso do rofecoxib, além de analgesia satisfatória sem interferência no processo de coagulação, de grande vantagem em procedimentos cirúrgicos. No entanto, mais estudos são necessários para demonstrar um efeito anti-inflamatório efetivo associado ao uso dos inibidores específicos de COX-2, além de seu efeito analgésico já demonstrado. A possibilidade do uso desses inibidores em condições dolorosas de fisiopatologia desconhecida, em que a terapêutica farmacológica ainda é empírica, como na nevralgia do trigémeo, é discutida.

Tabela: Farmacocinética do ibuprofeno e de inibidores seletivos de COX-2 em dores agudas e na osteoartrite.

<table>
<thead>
<tr>
<th>Nome genérico</th>
<th>Doses agudas</th>
<th>Logia</th>
<th>Meia-vida (t1/2, horas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofeno (Advil, Motrin)</td>
<td>400-600 mg a cada 4-6 horas</td>
<td>400-800 mg 3 vezes ao dia</td>
<td>2</td>
</tr>
<tr>
<td>Celecoxib (Celebra)</td>
<td>Não aplicável</td>
<td>100 mg 2 vezes ao dia</td>
<td>11</td>
</tr>
<tr>
<td>Rofecoxib (Vioxx)</td>
<td>50 mg 1 vez ao dia</td>
<td>12.5-25 mg 1 vez ao dia</td>
<td>17</td>
</tr>
</tbody>
</table>

Fonte: MOORE et al., 2001

Referências Bibliográficas


17. MALMSTROM, K., DANIELS, S., KOTEY, P., DEIDENBERG, B. C., DESJARDINS, P. J. Comparison of rofecoxib and celecoxib, two cyclooxygenase-2 inhibitors, in postoperative dental


26 RESUMOS APRESENTADOS SOB A FORMA DE “POSTERS”, SENDO 15 EM CONGRESSOS INTERNACIONAIS
ACTIVATION OF MELANOCORTIN TYPE 3 RECEPTOR AS A NOVEL MECHANISM FOR ACTH EFFICACY IN GOUTY ARTHRITIS

Stephen J Getting, Roderick J Flower & Mauro Perretti
William Harvey Research Institute, Queen Mary University
London, Charterhouse Square, London, EC1M 6BQ.

Adrenocorticotropic hormone (ACTH) was successfully used to control human gouty arthritis (Gutman & Yu, 1950) but no mechanism of action was identified, besides classical adrenal gland stimulation. We have tested here the hypothesis that a melanocortin receptor (MC-R) could be targeted by ACTH to modulate experimental joint inflammation.

Intact or adrenalectomized male Sprague-Dawley rats (220-270 g body weight) were anesthetized with halothane and injected intra-articular (i.a.) with 1 mg monosodium urate (MSU) crystals. Animal sacrifice occurred at 16 or 96 h post injection and the joint size and arthritis score calculated. Knee joints were then lavaged and neutrophil accumulation measured by staining in Turk’s solution and light microscopy. Specific ELISA were used to quantify interleukin(IL)-1 and IL-6 concentrations. ACTH1-39 alone or with the MC3-R antagonist SHU9119 (10 µg, 9 nmol) (Fan et al., 1997), was given s.c. (20-100 µg, 30 min) or i.a. (1-5 µg). Some naive rats were treated with ACTH1-39 and plasma corticosterone levels measured by radioimmunoassay 2 h. FACS analysis was used to detect MC-R expression on rat knee joint macrophages using rabbit polyclonal antibodies from Santa Cruz (1:50 final dilution) and an anti-goat IgG conjugated to Alexa488™ (Molecular Probes, Leiden, The Netherlands), measuring median fluorescence intensity (MFI) units in the FL1 channel. Values were analysed by ANOVA and Bonferroni test with *P<0.05 taken as significant.

MSU crystals provoked an intense neutrophil accumulation at 16 h (1.93 ± 0.11 x 10^6 joint^-1) inhibited by ACTH given either i.a. (42% at 5 µg) or s.c. (-74% at 100 µg) (n=6, P<0.05, in both cases). Similar degrees of inhibition were observed for arthritis score, joint size and cytokine release. The MC3-R antagonist SHU9119 abrogated these effects. ACTH 100 µg s.c., but not i.a. at 5 µg dose, caused a significant increase in plasma corticosterone levels (ng ml^-1): 33 ± 5, 44 ± 20 and 214 ± 43 for vehicle, i.a. and s.c. ACTH, respectively, (n=5, P<0.05). ACTH (5 µg i.a.) maintained its anti-inflammatory activity also in adrenalectomized rats (n=5, P<0.05), indicating the existence of corticosterone independent mechanism(s). FACS analysis showed that joint macrophages expressed MC3-R (42 ± 2 MFI units, P<0.05, n=4), but MC1-R or MC5-R, with punctuate pattern of localisation as visualised by immunofluorescence. A second injection of MSU crystals (time 72 h) led to an increased neutrophil influx as measured 24 h later. ACTH (5 µg i.a. given only at 72 h) inhibited also this second influx of PMN by 63% (P<0.05, n=6), and this effect was again blocked by co-injection with SHU9119. A similar pattern of inhibition was also determined for joint size and cytokine levels.

In conclusion, this study indicates the existence of a novel molecular mechanism for ACTH therapy, and it suggest that MC3-R agonists could be novel anti-inflammatory drugs.

This work was supported by the Arthritis Research Campaign (grant P0552).

A NEW ANTI-NOCICEPTIVE PATHWAY MEDIATED BY CYCLOOXYGENASE-2 IN RAT PAW INFLAMMATION INDUCED BY CARRAGEEAN

J.N. Francischini, C.T. Chaves, A.S. Lima & Y.S. Bakke1
Dept de Farmacologia, ICB/UFMG, Belo Horizonte, MG, Brazil and 1Leucocyte Biology, Division of Biomedical Sciences, Faculty of Medicine, Imperial College, London, SW7 2AZ.

Inflammation induced by carrageenan in rat hind paws exhibits two characteristic features, oedema and hyperalgesia. Both of these inflammatory responses can be modified by preventing prostaglandin biosynthesis through inhibition of cyclooxygenase (COX) with non-steroidal anti-inflammatory drugs (NSAIDs). There are two isoforms of COX and the NSAIDs are either non-selective or selective inhibitors of COX-2, the isoform induced by inflammatory stimuli (Vane et al., 1998). We have assessed both types of NSAID against the hyperalgesia and oedema induced in rat paws by carrageenan.

Male Holtzmann rats (150-200g) were given carrageenan (250µg/paw). Mechanical hyperalgesia (Randall & Selitto, 1957) and oedema (plethysmography) were measured hourly, for 4h subsequently. Commercial preparations (tablets or capsules) of piroxicam (Feldene, Pfizer, Brazil), a non-selective COX inhibitor, and of celecoxib, (Celebrex, Searle, Puerto Rico), a selective COX-2 inhibitor (Penning et al., 1997), were crushed in isotonic saline to give a fine suspension, containing a dose of NSAID calculated from the amount of active substance in the tablet. Indomethacin (Sigma) was dissolved in buffer and diluted in saline. All NSAIDs were given as subcutaneous injections (1ml/kg), 30 minutes before carrageenan, to groups of at least 4 rats. Carrageenan-induced hyperalgesia (-50±8g mean±s.e.mean); below control threshold, at 3h) was reduced by indomethacin (0.5mg/kg; -20±17g) or piroxicam (3mg/kg; -20±15g). The carrageenan-induced oedema (0.6±0.06ml at 3h) was either not affected (indomethacin, 0.7±0.07ml) or mildly decreased (piroxicam, 0.43±0.03ml; p<0.05; Anova t-test) by these NSAIDs. Celecoxib (3-12mg/kg) successively decreased hyperalgesia and then raised the pain threshold (hypoaesthesia) above that in the test paw (160±50g at 3h: p<0.05; Anova t-test), without any reduction in oedema. At the highest dose used (30mg/kg), celecoxib did reduce oedema at 3h to 0.42±0.06ml; however, at this level, hypoaesthesia was lost although hyperalgesia was still reduced (-34±18g). Celecoxib (12mg/kg) did not modify the pain threshold in (non-inflamed) paws injected only with saline, instead of carrageenan, over the 4h of observation.

From these results we conclude that, although oedema and hyperalgesia were induced together by the same level of carrageenan, hyperalgesia was more sensitive to inhibition of COX. Only selective inhibition of COX-2 raised nociceptive thresholds in the paw above normal levels (hypoaesthesia). This effect was not associated with decreased oedema and was not exhibited in the absence of inflammation.

We thank FAPESP, CNPq and CAPES for support
GERP’2001
2º Congresso Paulista de
Geriatria e Gerontologia
"Consensos e Recomendações"

1º Simpósio das
Ligas de Geriatria

Exposição de Serviços
e Produtos Geriátricos

19 a 22 de abril de 2001
Hotel Transamérica - São Paulo, SP

RESUMOS
### CULTURA DE LECUDICOS MONOCONIDIAIS DE RATOS VELOZES E MENOR DO QUE A DE RATOS JOVENES.

**Pereira, H. T. Antonio C. D. B. R., Mário R. P.**

Serviço de Geriatria do Hospital das Clínicas da Fac. de Medicina da USP

A父親通げ消毒学的、微生物学的、免疫学的、免疫学的免疫学的実験学的試験の結果、動物の若さと老化の関係を考察した。特に老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考慮した時に、老化の影響を考
International Tachykinin Conference

TACHYKININS 2000

Physiology, Fundamental & Clinical Pharmacology, Drug Discovery & Clinical Applications

La Grande Motte, France
October 17-20 2000

PROGRAM and ABSTRACTS
05/01

CYTOSOLIC PHOSPHOLIPASE A2 IS A KEY PLAYER IN THE PRO-INFLAMMATORY RESPONSES OF ASTROCYTOMA CELLS

M. Hernández, L. Fuentes, M. L Nieto and M. Sánchez Crespo
IBGM, Facultad de Medicina, 47005-Valladolid, SPAN

Astrocytes constitute the most abundant cell type in the CNS and respond to stimuli to which neurons are also responsive. The use of astrocytoma cells with well-defined morphological and functional markers has allowed addressing the mechanisms of tissue injury operating in the CNS. On the basis of the effects produced by engaging different types of receptors: muscarinic acetylcholine receptors, thrombin, secreted phospholipases A2 and TNF-α, different transcriptional programs that involve the MAP kinase/cytosolic phospholipase A2 system and the transcription factor NF-kB were described. Activation of cytosolic phospholipase A2 was a constant finding, irrespective of the nature of the agonist used; however, only TNF-α produced COX-2 induction linked to NF-kB activation. Interestingly, whereas both thrombin and secreted phospholipase A2 behaved as mitogenic stimuli, TNF-α enhanced apoptotic cell death. These findings indicate that cytosolic phospholipase A2 activation is involved in different patterns of response to pro-inflammatory agonists.

05/02

PHOSPHATIDYL-SERINE PREVENTS THE SYNTHESIS OF PRO-INFLAMMATORY MOLECULES IN RAT MICROGLIAL CELLS

R. De Simone, M. A Ajmone-Cat and L. Minnigetti
Neurobiology Unit, Laboratory of Pathophysiology, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy

The recognition of apoptotic neurons by microglia is poorly understood, but recent studies suggest that exposure of phosphatidylserine (PS) on dying neuron surface is a crucial event. The interaction of PS with its receptor (PS-R) induces peripheral macrophages to acquire an anti-inflammatory phenotype by preventing the synthesis of inflammatory molecules, but, to date little is known concerning the effect of PS on microglial functions. We have used microglial cultures from neonatal rat brain to investigate the expression of PS-R and the biological consequences of its interaction with the ligand PS. RT-PCR analysis revealed that mRNA for PS-R is expressed at low levels in unstimulated cultures and is upregulated in lipopolysaccharide (LPS) or PS-treated cells. The presence of PS liposomes strongly reduced the release of pro-inflammatory molecules such as nitric oxide, interleukin-1β and tumour necrosis factor α by LPS-activated microglia. The inhibitory activity was specific for PS as it was mimicked by the head group phospho-serine but not by phosphatidyl-choline liposomes. Our data suggest that PS through its receptor modulate macrophagic activation toward an anti-inflammatory phenotype.

05/03

CHRONIC BUT NOT ACUTE ADMINISTRATION OF PROPRANOLOL INHIBITS ARTHRITIS DEVELOPMENT IN RATS; A STUDY USING SYSTEMIC AND INTRACEREBROVENTRICULAR ROUTES

R F Brito, C M Yokoro and JN Franchi
Department of Pharmacology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, CEP 31270-901

Aim: to evaluate the effect of chronic and acute propranolol administration in adjuvant-induced arthritis in rats (n=4-5/group). Results are presented below with % inhibition in brackets.

<table>
<thead>
<tr>
<th>Propranolol, HCl (P) in Triton butter (C)</th>
<th>Hypermegalin</th>
<th>Oedema*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route Dose Schedule</td>
<td>1st day of arthritis (mm:ss)</td>
<td>1st day of arthritis (mm:ss)</td>
</tr>
<tr>
<td>SC</td>
<td>C</td>
<td>0.14 mg day (2x-daily)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.16 mg day (2x-daily)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.16 mg day (2x-daily)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.16 mg day (2x-daily)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.14 mg day (1x-daily)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.14 mg day (1x-daily)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.14 mg day (1x-daily)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.14 mg day (1x-daily)</td>
</tr>
</tbody>
</table>

*Hypermegalin/oedema / in litres / in mm high-crestal joints; oedema increase in haemato volume ml/ml. SP0.05, ANOVA test.

FINANCIAL SUPPORT: FAPESP, CNPQ, CAPES.
130

INFLAMMATORY RESPONSE TO Paracoccidioides brasilienisi STRAINS

Scapolo, S.H.B. & Frechão, D.
Pathology Department, Medical School, UNESP, Botucatu, São Paulo, Brazil

The aim of this study was evaluation of indomethacin treatment on inflammatory response to P. brasilienisi. Swiss male mice were inoculated ip with 10^1 yeasts form of Pb-As, Pb-As and Pb-18 strain, and treated 1x/day, ip with indomethacin (1mg/kg) or distilled (0.1 ml). After 1, 3, and 7 days the following parameters were evaluated: total and differential inflammatory influx, spontaneous H2O2 liberation and cytokine production. The indomethacin treatment promoted: Pb-As - increased mononuclear influx on day 1, and did not affect H2O2 liberation or IL-10 production; Pb-As - increased neutrophils influx, on day 1, and H2O2 on day 7, but did not affect IL-10 production; Pb-18 - did not affect inflammatory influx, increased H2O2 liberation and decreased IL-10 on 3rd day. Taken together, these results suggest that different mechanism mediated by PGs are involved for each Pb strain.

Supported by FAPESP, CNPq.

132

IN VITRO PGE2 AND IL-6 RELEASE FROM EHRlich TUMOR CELLS

Vilela, L.C. & Frechão, D.
Pathology Department, Medical School, UNESP, Botucatu, São Paulo, Brazil

Previous studies in our laboratory demonstrated high level of PGE2 and IL-6 in peritoneal cavity during in vivo Ehrlich ascites tumor (EAT) evolution. We have investigated PGE2 and IL-6 release from EAT cells in vitro. Triplicate of EAT cells suspension were cultured in RPMI 1640 medium, supplemented with 10% FCS, 100 U/ml penicillin, 100 µg gentamicin, and 2mM glutamine, in 5% CO2 at 37°C. PGE2 and IL-6 presence were studied 1x/day for five days. Our investigation showed that EAT cells produce high level of PGE2 during all time studied (around 2000 pg/ml) and IL-6 release just on the 1st day of culture. It seems PGE2 liberation by EAT cells is responsible for progressive Ehrlich tumor growth.

133

CORRELATION BETWEEN EOSINOPHILIA AND INTERLEUKIN-5 (IL-5) LEVELS IN LUNG OF MICE INFECTED WITH Strongyloides venezuelensis

E R Machado,¹ M T Leta,² F F Anibal,² J B A Oliveira², G C Barberà,² A I Medeiros² and L H Facciol²
¹UNICAMP and ²School of Pharmaceutical Sciences of Ribeirão Preto, SP, Brazil

Objective: Investigate the correlation between eosinophilic (EO) numbers and IL-5 levels in lung of S. venezuelensis infected mice. Methods: Mice were s.c. infected with 1500 larva of strains 02 and 49 of S. venezuelensis. Non infected mice were used as control. At days 3, 5, 7, 14 and 21 post infection (pi) the EO was evaluated in BALF and IL-5 levels were detected in lung parenchyma. Results: At days 5, 7, and 14 pi there was an increasing number of EO (peaked at day 5: 142±2 x 10^3/µl and 103±2 x 10^3/µl for strain 02 and 49 respectively). A peak of lung IL-5 was present at day 7 pi (strain 2: 44±11; strain 49: 27±2 pg/ml). Conclusions: EO recruitment was higher with strain 02 than strain 49. Biologically these findings can lead to distinct effects on IL-5 production induced by 02 strain when compared to 49 strain.

Support: CAPES-CNPFAPESP. (facciol)@fcfrp.usp.br

134

SOLUBLE GUANYLATE CYCLASE INHIBITION IN CONTRAST TO NOS-INHIBITION PROTECTS AGAINST TUMOUR NECROSIS FACTOR-INDUCED LETHAL SHOCK

Cavalcanti A, Van Mollie W, Janaeena D, Evererd B, Huang P, Fiers W and Broeckaert P

Dept. of Molecular Biology, VIB, Ghent University, Lodewijkstraat 35, B-9000 Gent, Belgium.

University of Maastricht,²Harvard Medical School

Nitric oxide is the key mediator of hypotension and shock, the dose limiting toxicity of treatment with Tumour Necrosis Factor (TNF), a cytokine also involved in systemic inflammation. We observed that inhibition of soluble guanylate cyclase (sGC) protected mice against the bradycardia, hypotension and lethality induced by TNF while NOS-inhibition could not prevent lethality and rather aggravated toxicity. Interestingly the antiinflammatory activity of TNF was left unimpaired. Using NOS-isoform knockout mice, we furthermore observed that NOS-2 and not NOS-3 is the source of the protective NO dispensable to survive a challenge with TNF. Also, inhibitors of TNF-induced lethality unrelated to this pathway could no longer protect in NOS-2 knockout mice. These results demonstrate that modulation of sGC has its own specificities with distinct consequences from modulation of upstream and downstream molecules.

135

CULTURED MONONUCLEAR CELLS FROM OLD RATS IN PRESENCE OF CARRAGEENIN (CG) INDUCE LESS HYPERALGESIA THAN CELLS FROM JUVENILE RATS

L S M Pereira, M A Resende, MSA Castro and JN Fracessil<br>
Department of Pharmacology, Institute of Biological Sciences, Federal University of Minas Gerais, Av. Antônio Carlos 6627, 31270-901, Belo Horizonte, Minas Gerais, Brazil

The aim of this study was to compare the production of hyperalgesic substances by purified mononuclear cells in culture, from old (24-month old) and juvenile (2-month old) rats. Cells were purified from peripheral blood (98 % purity) and cultured in presence or absence of 2 µg/ml κ-carrageenan (CG) for 2 h. The supernatants were centrifuged and frozen until test. After thawing, supernatants (0.1 ml) were injected intraplantarly in recipient rat paws (n=4-5) and mechanical hyperalgesia measured (Randall-Sellito method, g ± sem) for the next 24 h. Supernatants from old animals induced 40 % less hyperalgesia at 3 h than supernatants from juvenile animals (p<5±5.8, -8±4.4 g respectively).

FAPEMIG, CNPq and Cupen
6ème Congrès Annuel de la Société Française de Pharmacologie

23èmes Journées de Pharmacovigilance

Joint Meeting with Pharmacological Societies of Brazil and Portugal

www.pharmacol-fr.org/rennes2002

8, 9, 10 avril 2002

Rennes

Centre culturel le Triangle
Boulevard de Yougoslavie
35000 Rennes

ABSTRACTS
FORMALIN INDUCES LEUKOCYTE RECRUITMENT TO PERITONEAL CAVITY OF RATS.

J. M. M. SANTOS, J. N. FRANCISCHI

Federal University of Minas Gerais, Dept. of Pharmacology, Av. Antonio Carlos 6627, Belo Horizonte, MG – Brazil.

INTRODUCTION AND GOALS: Formalin injection into small animals is an established model in the study of pain. However, edema development is also a prominent feature following formalin administration to rat paws. The aim of this study was to verify whether leukocyte recruitment would follow formalin injection into rat peritoneal cavity. METHODS: Formalin (F) was prepared from dilution of 37% formaldehyde in sterile physiological saline and intraperitoneally injected (1.0 mL containing 1/10 solution/animal) in Holtzman female rats (weight: 140-170 g; n=3-10). Control (C) animals were injected with same volume of sterile saline. After neck dislocation and exsanguination of animals, leukocyte counts (cell number/mm$^3$ or %) were obtained by light microscopy in Neubauer chamber and stained smears prepared from peritoneal lavage fluid collected at different times following formalin injection (0 – 24 h), respectively. RESULTS: Formalin (0.625-2.5 %) induced a dose- and time-dependent increase in leukocyte number (F$_{4h}$=19.0±1.0×10$^3$, F$_{24h}$=22.0±2.5×10$^3$; C$_{4h}$=13.0±1.0×10$^3$, C$_{24h}$=15.5±1.5×10$^3$; Anova t test, P<0.05). This increase was related to an increase in neutrophil number (Neu) in low concentration formalin at 4 h (Neu: F$_{12h}$=8.3±2.2×10$^3$; C=3.8±0.9×10$^3$) whereas an increase in lymphocyte number (Ly: F$_{2h}$=10.9±2.2×10$^3$; C=3.4±0.6×10$^3$, P<0.05) and a decrease in mast cell number (Mt: F$_{2h}$=0.06±0.06×10$^3$; C=0.4±0.06×10$^3$, P<0.05) were detected at 24 h with the highest concentration of formalin used. Subcutaneous (sc, 2mg/kg) dexamethasone pretreatment (30 min) reduced both neutrophil at 4 h (Dexa=1.5±0.5×10$^3$; C=7.4±1.7×10$^3$) and lymphocyte counts at 24 h (Ly: dexa=0.07±0.04×10$^3$; C=0.034±0.034×10$^3$) without affecting mast cell reduction. Sc pizotifen (5-HT$_2$ antagonist) pre-treatment in an effective anti-edematogenic dose (2mg/kg) did not affect leukocyte counts (P>0.05) after formalin injection. CONCLUSIONS: Formalin induced different profile of leukocyte migration to rat peritoneal cavity, particularly neutrophils in the early inflammatory response (first 4 h) and lymphocytes in the late response (at 24 h), depending on dose and time of stimuli administration. The presence of leukocytes may have derived from cytokine release induced by formalin injection, as it has been inhibited by corticosteroid treatment. In addition, cell recruitment due to formalin injection did not seem to have involved activation of serotonin (5-HT$_2$) receptors.

Cefalotin dose-dependently inhibited cell recruitment due to LPS injection at rat peritoneal cavities. G.B. Menteshe, K.L-M. Malton, and J.N. Francischi. Pharmacological Department, ICB/UFMG, Belo Horizonte, Brazil. CEP 31270-901. Analogous, antipyretic and anti-cedemogeneic activities were already related to use of the specific COX-2 inhibitors, such as for celecoxib. However, data on inhibition of leucocytes recruit by these drugs are still lacking in the literature. The aim of the present study was to verify the inhibitory potential of cefalotin on cell migration induced by LPS (a cell endotoxin, LPS) in rats. Female Holtzman rats (140-180g) were subcutaneously pretreated with 0.3-3.0 mg/kg cefalotin (cele) or 2 mg/kg indomethacin (indo) and 30 rats later were intraperitoneally injected with LPS (451 ± 11.0 · 0.3 μg/site or physiological saline). The peritoneal lavage fluid were collected in Neubauer chambers (celo/dm) and differentiated in stained smears under optical microscopy following 6 of LPS injection (5-6 animals/group). Cell migration was dose-dependently inhibited by cefalotin (Celo=11±8.1±1.59±4×10-9, Ciao=7±5.2±2×10-10) and indomethacin (Indo=12±6.5±0.7x10-7. Ciao=20±6.5±2.0x10-9). ANOVA test, P<0.05, being the beta inhibitory concentration at 6mg/kg cefol in increase in celebrose was the predominant cell inhibited by celecoxib or indomethacin. This is the first demonstration that a specific COX-2 inhibitor (celecoxib) reduced cell migration due to the rank order of agonist potency of celecoxib being BK at 3-field less potent than cefalotin. These results suggest that for this strain and studied site (skinal) neuropeptides, in special cefalotin, play an important role in vascular permeability increase.

Financial support: FAPEMIG, CNPq and CAPES.

P294

Antagonist activity of 6-guanidinohexahydro-2,4-dione (6GHP) and 6-propenyl-
hexahydro-2,4-dione (6P6H)

D.O. Medina, J.C. Cuba, L.D. Indemini, I.C. Souza, C.T. Santana, and P.T. Souza Jr. Pharmacology, Federal University of Mato Grosso, Brazil. Department of Chemistry, Federal University of Alagoas, Department of Chemistry, Federal University of Mato Grosso, Brazil. The antagonistic effect of 6GHP and 6P6H, obtained from the hexane extract of Calophyllum brasiliense Camb. stem bark inhibited the formation of ethanol and indomethacin gastric lesions in mice and rats. From DCMF, were isolated three chromatographic bands, which were identified as 6GHP and 6P6H. This study evaluated the antagonist activity of 6GHP and 6P6H, synthetic analogues of 5,6-dihydroxyhexahydro-2,4-dione, this later a precursor of chondroitin acid on gastric lesions induced by indomethacin (0.05 mg/kg), and ethanol (0.1 ml/100 g). In the control group, indomethacin-induced stomach ulcerations presented median value of 12 (9.19). 6GHP and 6P6H (0.5 mg/kg p.o.) reduced the ulcer index to 5.5 (11.12; p<0.05) and 5.1 (11.15; p<0.05) respectively. With 10 mg/kg of 6GHP and 6P6H, the median values were 6.5 (11.11) and 7.5 (12.12), they were not significantly different from control. The ulcerate area in ethanol-induced gastric lesion was 16±1%. In the control (with 6GHP and 6P6H), the ulcerated areas observed 8±1% and 6±1% respectively, but they were not significantly different from the control. The results indicate that 6GHP and 6P6H retain in their structures antagonist activity only in indomethacin model. Financial support: CNPq.
ENCONTRO CIENTÍFICO

BIOQUÍMICA E IMUNOLOGIA

CIÊNCIAS BIOLÓGICAS, FISIOLOGIA E FARMACOLOGIA

17 A 21 DE JUNHO DE 2002

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

UFMG
Contribuição do estudo da farmacologia da inflamação e dor à pesquisa e ao ensino da graduação e pós-graduação.

J. N. Francischhi,
Departamento de Farmacologia-ICB-UFMG

A questão sobre a importância da pesquisa básica para o desenvolvimento, de quaisquer naturezas, atravessa gerações. Esse assunto se torna sobremaneira relevante, quando se considera que o nosso é um laboratório de pesquisa básica. No laboratório, diferentes modelos experimentais de inflamação e dor são utilizados, com a participação ativa de estudantes de graduação e pós-graduação em seu desenvolvimento. Como objetivos, visamos entender tanto a histopatologia de doenças inflamatórias agudas e crônicas, como também o mecanismo de ação das drogas analgésicas, anti-inflamatórias e imunossupressoras utilizadas clinicamente. Apesar de não contarmos com indústria farmacêutica no país interessada no desenvolvimento de analgésicos/anti-inflamatórios brasileiros, constitui-se um desejo nosso – quixá químico – poder contribuir no desenvolvimento desses medicamentos. Entre os modelos experimentais de inflamação e dor utilizados no laboratório, podemos destacar o modelo de inflamação (edema) e dor induzidos pela formalina, o edema e a hiperalgesia induzidos pela carragenina e a atrofia induzida por adjuvante. A padronização de um novo modelo de inflamação na polpa dental de ratos se encontra também em andamento, que acreditamos, ajudar-nos-á a compreender os mecanismos de inflamação e dor aí localizados, que por mecanismos ainda desconhecidos, culminam com a necrose pulpar. Com esses estudos, foi possível demonstrar novos mecanismos de doenças, como 1) a participação objetiva do sistema nervoso central na histopatologia da atrofia, 2) o mecanismo de ação diferenciado de drogas do tipo da aspirina (dipirona, acetaminofen), 3) mecanismos envolvidos no envelhecimento experimental e humano, e mais recentemente, 4) a descrição de um novo mecanismo, denominado hipoléptico, associado ao uso terapêutico dos inibidores específicos de COX-2 (celecoxib e rofecoxib). Tais mecanismos fomentam importantes bases para a pesquisa atual e futura na área da inflamação e dor, bem como para a formação de estudantes de Graduação, em especial, aqueles destinados à área da Saúde (Medicina, Odontologia, Enfermagem). Além disso, todos os resultados mencionados serviram de base na formação em pesquisa de vários estudantes de Graduação (23), no preparo (5) e defesa de dissertações de Mestrado (4) e teses de Doutorado (6) até o momento, além de várias publicações em revistas científicas nacionais e internacionais.

Suporte financeiro: CNPq, CAPES e FAPEMIG
"Contradições do Envelhecer no Brasil: Tecnologia e Carências".

ANAIIS

2002
XIII CBGG

RIO DE JANEIRO - HOTEL INTERCONTINENTAL
19 A 22 DE JUNHO DE 2002

SOCIEDADE BRASILEIRA DE GERIATRIA E GERONTOLOGIA
Certificados


II Congresso Brasileiro de Gerontologia e Geriatria

II Congresso de Gerontologia e Geriatria da SBB-G-RN
94

A atividade hiperalergética em ratos do sobredosatante da cultura de leucócitos mononucleares de ídios hígidos é menor do que a de adultos jovens.

Leani, S. M. Pereira; Webster, G. P. Reis; Vanderzon, A. Romoldo; Resende, M. A. Janetti; N. Francisch; Departamento de Farmacologia - ICB / Universidade Federal de Minas Gerais-Beato Horioge-MG

Vários fatores parecem contribuir para a "imunoneutrocinia" tanto a nível humoral, quanto a nível celular. Particularmente, a atividade dos linfócitos está aparentemente diminuída e provavelmente relacionada com a diminuição de IL-2 e INF-alfa, que resulta em uma alteração na ativação do sistema imunológico, que pode ser observada em diferentes condições patológicas.

Recentemente, observamos que os macrófagos, que sofrem uma ativação que resulta em uma diminuição na produção de interleucina-1 (IL-1), são capazes de inibir a produção de interleucina-2 (IL-2) e interleucina-3 (IL-3), que são essenciais para a proliferação e diferenciação de linfócitos T.

Conclusões: As observações indicam que a atividade hiperalergética em ratos do sobredosatante da cultura de leucócitos mononucleares de ídios hígidos (SLM) é menor do que a de adultos jovens. Esta ativação pode ser responsável por uma diminuição na ativação do sistema imunológico e, consequentemente, por uma diminuição na produção de interleucina-1 (IL-1), que é essencial para a proliferação e diferenciação de linfócitos T.

99

Hábito de cochilar e qualidade do sono em um grupo de ídios aliviados.

Autores: Maria F. Celioli e Luiz Marçant-Barretto

Departamento de Enfermagem, Universidade Estadual de Campinas, Campinas, São Paulo.

Introdução: A modificações nos hábitos e na qualidade do sono são frequentes nessas condições, mas ainda são incipientes. Entre as modificações relatadas, destaca-se a alteração no sono, que pode ser decorrente de alterações na atividade cerebral, principalmente durante o sono REM.

Os ídios aliviados apresentam um perfil de sono REM ativo, com um aumento na atividade cerebral durante este período.

Conclusões: Os ídios aliviados apresentam um perfil de sono REM ativo, com um aumento na atividade cerebral durante este período. Isso sugere que a atividade cerebral durante o sono REM pode ser maior nos ídios aliviados, o que pode ser um fator contribuinte para a melhora na qualidade do sono.

APOIO: FAPESP, CNPq, CAPES
FeSBE 2001
14.149
OS EFEITOS DO DIAZEPAM NA INFILTRAÇÃO INDUZIDA POR ERADICINENA EM RATOS. Paes-Rêgo, N.; Martôlo, E. J.; Salomão, E.; Ladeira, F.; Barbosa, J. L.; Florentino, R. M. N.; UFFM - USP - SP.

Objetivo: Sabido que o ativo influente com diazepam, estudo de resposta imune não específica, caracterizada pelo processo inflamatório induzido por mediadores químicos. Este trabalho analisou os efeitos do diazepam (Diaz) na resposta inflamatória induzida por mediadores químicos. O objetivo do presente estudo é avaliar a influência do diazepam sobre a resposta imune não específica em ratos.

Métodos e Resultados: Avaliou-se a resposta imune não específica em ratos após o administrar Diaz de diferentes doses (10, 20 e 30 mg/kg, p.o.). A resposta foi avaliada através de um teste de carrapato, em que se observou uma resposta imune não específica em ratos após a administração de Diaz.

Conclusões: O diazepam apresentou efeitos anti-inflamatórios, com redução da resposta imune não específica em ratos após a administração de Diaz.

Apê de financiamento: CNPq, FAPESP

14.150

Objetivo: A nasimuda (N. nucifera) é um anti-inflamatório não esteroidal amplamente utilizado que possui atividade antiflatulente. Neste estudo, efetuamos uma análise da eficácia da N. nucifera sobre a resposta inflamatória induzida por mediadores químicos e diferenciam-se mediadores endógenos como antiflatulente.

Métodos e Resultados: Avaliaram-se a atividade anti-inflamatória da N. nucifera, usando-se uma mistura de mediadores químicos como: histamina, bradicinina e serotonin. A resposta inflamatória foi avaliada através do teste de carrapato, em que se observou uma resposta imune não específica em ratos após a administração de N. nucifera.

Conclusões: A N. nucifera apresentou atividade anti-inflamatória, com redução da resposta imune não específica em ratos após a administração de N. nucifera.

Apê de financiamento: CNPq, FAPESP

14.151

Objetivo: A indução de COX-2 é uma via importante na regulação do ciclo inflamatório. Neste estudo, efetuamos uma análise da eficácia da N. nucifera sobre a resposta inflamatória induzida por mediadores químicos e diferenciam-se mediadores endógenos como antiflatulente.

Métodos e Resultados: Avaliaram-se a atividade anti-inflamatória da N. nucifera, usando-se uma mistura de mediadores químicos como: histamina, bradicinina e serotonin. A resposta inflamatória foi avaliada através do teste de carrapato, em que se observou uma resposta imune não específica em ratos após a administração de N. nucifera.

Conclusões: A N. nucifera apresentou atividade anti-inflamatória, com redução da resposta imune não específica em ratos após a administração de N. nucifera.

Apê de financiamento: CNPq, FAPESP

14.152
LACTONAS SEQUIENTES RIBOSINELAS EM BIA A LEVAN EXCEEDED E LEUCOCITOS POLINUCLEARES: Biiglione, M. P.; L.; Zadale, F.; M.; Nucera, C. V.; Prino, E.; Corradini, F.; Clinica de Estudio e Clinica Integrada, EFOA - MG, Bioquímica, USP - SP, Química Fundamental, UERJ - SP.

Objetivo: Foi testada a capacidade de lactonas sequentias (LS) em inibir a liberação de anión superóxido (O_2^-) por leucócitos polinucleares (PMN) e visando uma utilização como anti-inflamatórios.

Métodos e Resultados: As LS foram isoladas de extrato fracionado de Wunderlichia crassina através de técnicas de purificação e caracterização. A LS foi estudada em relação à sua capacidade de inibir a liberação de anión superóxido (O_2^-) por leucócitos polinucleares (PMN).

Conclusões: As LS mostraram capacidade de inibir a liberação de O_2^- por PMN, sugerindo um potencial uso como anti-inflamatórios.

Apê de financiamento: CNPq, FAPESP

14.153
SUPEROXIDE ANION PRODUCTION BY CIRCULATING LEUCOCYTES INDUCED BY SODIUM AND COX INHIBITORS: Effects of NOX 1 and COX Inhibitors. Musarrat, M., T.; Tabas, S. A.; Franco, P.; Cardoso, L.; Antunes, E.; Deleu, J.; Farmacologia, UNICAMP - SP, Farmacologia, UNICAMP - SP, Farmacologia - ICBI - UFRJ - SP.

Objetivo: O objetivo do presente estudo foi avaliar a produção de superóxido (O_2^-) por leucócitos circulantes em ratos após a administração de inibidores do enzima cicloossidrogenase-1 (COX-1) e cicloossidrogenase-2 (COX-2).

Métodos e Resultados: Avaliou-se a produção de O_2^- por leucócitos circulantes em ratos após a administração de inibidores do enzima cicloossidrogenase-1 (COX-1) e cicloossidrogenase-2 (COX-2).

Conclusões: A administração de COX-1 e COX-2 inibidores apresentou redução significativa na produção de O_2^- por leucócitos circulantes.

Apê de financiamento: CNPq, FAPESP

14.154
DROGAS ANTI-INFLAMATÓRIAS NO EDEMA INDUZIDO PELA FORMALINA EM RATS. Mota, L.; Lisboa, M.; Francisch, J. N. D.; Farmacologia, UFRGS - MG.

Objetivo: O objetivo do presente estudo foi avaliar a eficácia de drogas anti-inflamatórias em inibir o edema induzido pela formalina em ratos.

Métodos e Resultados: Avaliou-se a eficácia de drogas anti-inflamatórias em inibir o edema induzido pela formalina em ratos.

Conclusões: As drogas anti-inflamatórias demonstraram eficácia em inibir o edema induzido pela formalina em ratos.

Apê de financiamento: CNPq, FAPESP
injetados intraperitonealmente com 0,1 ml de diversos sobreviventes. O efeito foi observado por um período de 8 semanas e a medicação foi administrada duas vezes ao dia.

Conclusão: Os resultados mostraram que a medicação é eficaz e pode ser utilizada como tratamento para o câncer gastrointestinal.

**Apêndice financeiro:**

**14.157**

**CAPTACAO LINQUETICA NA RESISTENCIA INFLAMATORIA PERDIDA INDUCIDA POR ADIVINHAR DE FREUND:**

**MÉTODO:**

Focos de Cox-1 e Cox-2 foram evidenciados na produção de prostaglandinas no tecido subcutâneo de células mononucleares induzidas com carragenina.

**Conclusão:**

A CXL e Cox-2 estão envolvidas na produção de prostaglandinas, possivelmente em resposta à carragenina.

**Apêndice financeiro:**

**14.159**

**ATIVIDADE ANTI-INFLAMATÓRIA DA ADENOSINA:**

L. Regard, C. C. Fontes, J. B.; Santos, A. R. D. F.; Farmacologia, USP - SC; Farmácia e Farmacologia, UNIVAG - SC.

**Objetivo:**

A adenosa (AD) e o ATP exercem um papel protetor na resposta inflamatória e na transmissão do sinal (Eur. J. Pharmacol. 317: 1-11, 1998). Com o objetivo de estudar essas ações na inflamação, foram realizados estudos com AD e ATP.

**Conclusão:**

A adenosa (AD) e o ATP exercem um papel protetor na resposta inflamatória e na transmissão do sinal.

**Apêndice financeiro:**

**14.161**

**EFEITO INFLAMATÓRIO DE ANALÓGOS DE TALIDOMIDA APÓS INHALAÇÃO DE LÍPPOSOMAS SUSPENDIDOS:**

M. Balbino, C. S. G.; Farmacologia, USP - SC.

**Objetivo:**

Estudar o efeito de analógos de talidomida em suspenso em líplosomos.

**Conclusão:**

Os analógos de talidomida em suspenso em líplosomos apresentaram efeitos anti-inflamatórios.

**Apêndice financeiro:**

**14.162**

**EFEITO DO TÊMEN EM TRATAMENTO CROMO ADE FARMACIADA NA INFLAMAÇÃO ALÉRGICA PULMONAR EM RATOS:**

R. G. de Oliveira, A. P. Silhol de Anselmio, A. M. J.; Farmacologia, UNIVAG - SC.

**Objetivo:**

A farmacap (TF) é um inibidor mediator da reação de lesão inflamatória (TLR) devido ao uso de AINs. O tratamento da NSAID está associado com aumento na inibição na resposta inflamatória pulmonar (IP).

**Conclusão:**

O uso de farmacap (TF) tem efeito anti-inflamatório na resposta inflamatória pulmonar.

**Apêndice financeiro:**

**Capes, CNPq, CAPES, FAPERJ, FINEP, FUND. JOSE BONHOFER**
14.085
HIGH RATE OF APOPTOSIS WELL CORRELATED WITH REFRACTORYNESS OF DIABETIC MAST CELLS TO ANTIGEN STIMULATION. Barretto, Ede C.; Carvalho, Vile F.; Fuentes, M. S. D.; Marthin, M. A.; Silva, P. M. R. E.; Fisiologia e Farmacodinâmica, FCRUZ-RJ; Farmacologia, UERJ-RJ.

Além de mastocitose de alérgica-défice, os rastros foram observados no tecido linfático e tireoide, retratando a importância e a necessidade de identificar e caracterizar adequadamente esses eventos.

14.086
EFEITO DA DEAMETASONA SOBRE INFLÂMULOS DE EPOCINOLOS E PRODUÇÃO DE EOSTROINA NA CAVI- DAD DIGESTIVA DE CAMUNDONGOS SENSIBILIZA- DOS. Silva, J. P.; Pires, A. A. de A.; Perez, S. A.; Martins, M. A.; Silva, P. M. R. E.; Fisiologia e Farmacodinâmica, FCRUZ-RJ.

Objetivo: Este estudo investigou o efeito da anti-inflamatória no controle da produção de eostroinas e prostaglandinas em crachóide de camundongos sensibilizados com eostroinas.

Métodos e Resultados: Após a aplicação dos eostroinas, houve uma diminuição significativa na produção de prostaglandinas e eostroinas. A utilização da deametasona resultou em uma redução significativa na produção dessas substâncias.

14.087
INFLUÊNCIA DO ESTADO DIABÉTICO SOBRE A REATIVIDADE CUTÂNEA ANAFILÁCTICA EM RATOS. Carvalho, Vile F.; Fuentes, M. S. D.; Barretto, E.; Carvalho, Vile F.; Fuentes, M. S. D.; Marthin, M. A.; Silva, P. M. R. E.; Fisiologia e Farmacodinâmica, FCRUZ-RJ; Farmacologia, UERJ-RJ.

Além de inmunolocalização do antígeno no tecido, a hiperglicemia foi observada em todos os animais.

14.088
O PAPEL DAS GLÂNDULAS ADRENALINAS NA HIPERAL- GÊSIA E EDENA DE RATAS ARTIFICIAIS VELHAS. Yoko, C. M.; Maruyama, U.; Francis, I. D.; Farmacologia, UFMG - MG; Fisiologia e Bioética, UFMG - MG.

Objetivo: Verificar o papel das glândulas adrenais no desenvolvimento de adrena em ratas velhas.

Métodos e Resultados: Analisaram-se a atividade da glândula em diferentes momentos durante a vida de cada rato, incluindo momentos de estresse e de relaxamento.

14.089
MELOXIP TALAMO Sucedido COM O AÇEQUIN DAX 10 mg/kg e ADX 10 mg/kg. Nesse caso, a adiposidade foi reduzida em cerca de 60%.

14.090
EFEITO AGUDO DA LESÃO DO NÚCULO PARA- TRIGENINAL SOBRE A TEMPERATURA CORPORAL DE RATOS BALB/c A. C.; Y.; Souza, G. E. P. D.; Linsdley, C. J.; Biônica, UNIFESP - UF, Biônica, UNIFESP - UF; Física e Química - FC; PUCP - USSP.

Objetivo: O núcleo paratrigeminal (PaT) tem conexões com estruturas bulbares (núcleo reticular lateral, parabraquial e trato solitário) que estão associadas à função de termorregulação corporal. Destaca-se, também, por sua participação em reflexos de controle do tônus muscular e em respostas emocionais.

Métodos e Resultados: A lesão do núcleo paratrigeminal levou a um aumento marcado na temperatura corporal dos ratos. No entanto, a resposta foi mais acentuada em ratas com deslocamento do núcleo paratrigeminal para o lado oposto.

Apêndice: O uso de termorregulação corporal é uma ferramenta útil para estudos sobre o controle do tônus muscular e de outras funções críticas.
Conclusões: O modelo de infeção em células locais é eficaz e permite a coleta do material que pode ser analisado por medidas que favorecem o monitoramento da inflamação através de mediadores químicos que participam ativamente no processo inflamatório.

Apesar de financiamento: CNPq, FAPERJ, SECTAM, FAPESP, UFPA.


Objetivo: Estudar interface entre tumores e células do hospedeiro através da quantificação local de leucócitos e NO em pacientes com tumores ovarianos císticos.

Métodos e Resultados: Foram analisados 62 pacientes com tumores não neoplásicos (51,8% e neoplasias (48,2%), de neoplasias benignas e malignas, respectivamente. De 71,4% das neoplasias malignas, as pacientes são mais frequentes, comparado a 38,6% em caso de neoplasias benignas. Após exame do resto, o líquido foi puncionado, centrifugado e levado à centrifugação e doblecado de eletrodução. Em 59 casos estudados, 36 neoplasias benignas e 51 malignas (1200 g/mL) em sangue. Em 10,6% do total, reflectindo no teste.

Conclusão: Os resultados indicam que a injeção de NO e do efeito de inibição no crescimento tumoral.
14.114

Métodos: Resultados e Conclusão: Não foi provado que LPS induz hiperglicemia e insulinopenia na pre-handamento isolado de monócitos e de monócitos em 250 mg contamia por 2 h foi injetado inteiramente na pata de ratos (n=5) para dosagem de hiperglicemia pelo método de Randoval- Soelting (1957). Hiperglicemia foi detectada nas pata de ratos 24 horas após a injeção do LPS 250 mg contamia por 2 h. O objetivo do presente estudo foi confirmar se os monócitos pulmonares do ratos jovens e velhos induzem a pata de ratos pelo submucosas de monócitos e de células mononucleares de ratos jovens e velhos, respectivamente (p<0.05).

Conclusões: A injeção intrapleural de LPS em ratos jovens e velhos induz hiperglicemia e insulinopenia. CAsas do LPS na pata de ratos, o presente estudo confirma a contribuição dos monócitos pulmonares e de células mononucleares de ratos jovens e velhos, respectivamente (p<0.05).

Apóio financeiro: CAFES, FAPESM, FiPPG

14.115
ROLE OF NITRIC OXIDE IN FEVER AND DEVELOPMENT OF PYRGEN C-TOXICITY TO LPS. Ferreira, M. E. D.; Coelho, M. M.; Peri, I. A.; Raimundo Social - For. Fumegi, M. L.; M. M.; FMA-UCS; MG, Farmacologia - FMRP, USP; Psicologia e Fisiologia - IPCR, USP; SP.

Objective: Pyrogenic tolerance is observed after several challenges with a pyrogenic stimulus. However, the mechanisms involved in its development are not fully characterized. The aim of this study was to investigate whether the expression of inducible nitric oxide synthase (iNOS) and pyrogenic cytokines, such as the tumor necrosis factor (TNF), contributes to the development of pyrogenic tolerance observed after two injections of LPS. Methods and Results: A feco-monitored model was infected with lipopolysaccharide (LPS) (100μg/kg) or saline, followed by a second injection of LPS (50μg/kg) or saline. The animals were divided into four groups: control, saline treated, LPS treated, and LPS treated with the iNOS inhibitor, L-NAME. The results showed a significant increase in the expression of iNOS and TNF in the LPS treated group compared to the control group. Conclusions: The present study provides evidence for the involvement of iNOS and TNF in the development of pyrogenic tolerance. Apoio financeiro: CAFES, FAPESP, FiPPG

14.116
CALCUM SENSITIVE POTASSIUM CHANNELS MAY UNDERLY VESTIBULAR HYPOREJONSULPS. Farias, N. C.; Benele-Mantung, L.; Borges, A. F.; Ferrer, T.; Fuma, G.; Paiva, T. B.; Bieroska, UNIFESP - SP; SP, Bieroska, UNIFESP - SP.

Objective: The enkephalin-independent effect of lipopolysaccharide (LPS) in isolated axons from sponta-
14.162


**Métodos e Resultados:** A atividade antiinflamatória foi avaliada em rato através de teste de coroas abdu-

centes e teste de urina de acúmulo de óxido nitрогênio no fígado. Os animais eram submetidos por via oral (w.o.d) a diferentes doses de 100, 250 e 500 mg/kg do composto. Os resultados foram analisados por via o.D.. Os resultados mostraram que os composta apresentaram atividades antiinflamatórias superiores ou equivalentes às dos composta convencionais. As propriedades antiinflamatórias foram evidentes a doses de 250 mg/kg e superiores. Os resultados estão sendo publicados em uma revista científica. Apoio financeiro: FINEP, CNPq, FAPERJ, PRONEX.

14.163
**EFETOS DO PROPRIANOLOID E RAMOS ARTIFICIAIS. Yokote, C. M.; Brno, R. F.; Franco, C. J. N. D.; Farmacêuticos, UFMG - MG.

**Objetivo:** Avaliar o efeito do propionilol em pacientes submetidos a cirurgias de interrupção do feto (C) e seus efeitos sobre a função renal e hepática.

**Métodos e Resultados:** C: n = 10; G: n = 10. Os animais foram submetidos a cirurgias de interrupção do feto (C) e grupos controlados (G). Os resultados mostraram que os animais do grupo C apresentaram efeitos benéficos sobre a função renal e hepática.

14.165

**Objetivo:** Avaliar o efeito do novo derivado do DOR antiinflamatório na resposta inflamatória.

**Métodos e Resultados:** Utilizaram-se ratos de espécie Wistar, submetidos a cirurgias de interrupção do feto (C) e grupos controlados (G). Os resultados mostraram que os animais do grupo C apresentaram efeitos benéficos sobre a função renal e hepática.

14.166
PAPEL DO OXIDO NÍTRICO NO RS) NA RESPOSTA DESEMOGÊNICA INDUZIDA POR KÖNIGSBAUMER (DI) EM PATA DE CAMUNDONGO. Lenir Vieira Costa, A. C.; Looza Fonte, C. T. D.; Linsa, V. D.; Sobrino, J. Z.; Balleto de Mello, F. V.; Cunha, F. G.; Ribes, R. C.; Farmacêutica, UFRJ - R1; FISIOLOGIA, UCRP - R1; FISIOLOGIA, UCRP - R1; FISIOLOGIA, UFRJ - R1.

**Objetivo:** Avaliar o efeito do novo derivado do DOR antiinflamatório na resposta inflamatória.

**Métodos e Resultados:** Utilizaram-se ratos de espécie Wistar, submetidos a cirurgias de interrupção do feto (C) e grupos controlados (G). Os resultados mostraram que os animais do grupo C apresentaram efeitos benéficos sobre a função renal e hepática.

14.167
**EFEITOS DA ADMINISTRAÇÃO AGUDA E PROLONGADA DE CARBAMAZEPINA EM MODELOS EXPERIMENTAIS DE INFLAMAÇÃO E IMMUNIDADE. Minho, R. J.; FISIOLOGIA, UCRP - R1; FISIOLOGIA, UCRP - R1.

**Objetivo:** Avaliar o efeito do novo derivado do DOR antiinflamatório na resposta inflamatória.

**Métodos e Resultados:** Utilizaram-se ratos de espécie Wistar, submetidos a cirurgias de interrupção do feto (C) e grupos controlados (G). Os resultados mostraram que os animais do grupo C apresentaram efeitos benéficos sobre a função renal e hepática.

Apoio financeiro: CNPq, CAPES.
14.179
DETERMINAÇÃO DA TOXICIDADE AGUDA E ATIVA-
IDADE ANTICONECITIVA DA 2-HIDROXICCAFÉ1,3-
INDANODONA Caffe, O. M.; da Costa e Silva, L. L.;
Gomes, L. M.; Silva, M. T.; Santos, F. R. D.; Silva,
C. L.; Netto-Ferreira, F. C.; Cortez, W. S.; Câncer,
Reimbold, C. F. - Ciências Médicas, UFCR (2004),
1, 3-Indandiona é um resíduo de processamento de
café, que apresenta atividade antimicrobiana, espe-
dido para os microrganismos anaeróbios Gram-
positivos, como Clostridium perfringens, Bacteri-
oides fragilis e Eubacterium. A atividade antimicro-
biana da 1,3-Indandiona foi verificada em cultura de
D. radioderma e D. radiodermis, evidenciando a
atividade antimicrobiana, pelo método de difusão
em disco, para os microrganismos mencionados.
EFEITO ÓPTICO DA 1,3-INDANODONA
EFEITO DE QUEMÌCO-CATERÁGICO E FINTOXO-
CAMA NO EFEITO INFLAMATÓRIO DE INDUZIDOS
FEITOS EM CARCINÓGENOS EM RATS. M. T. D.;
Teixeira Cheunes, C.; Lima, A. S.; Bakthy, Y. S.; Fاما-
cofília, UFC - MG.
Efeitos de diferentes estimuladores de inflamação
em ratos com diferentes níveis de atividade im-
une. Avaliação da resposta inflamatória em ratos
com diferentes níveis de atividade imune, para os
efeitos de diferentes estimuladores de inflama-
ção. Os ratos foram divididos em três grupos:
controle (C), estímulo (E) e estímulo + 1,3-INDANODONA
(C + E). Na avaliação da resposta inflamatória
foi utilizada a técnica de neutrófilos e monócitos,
que foram coletados do sangue periférico por
caça de neutrófilos e monócitos, para os diferentes
níveis de atividade imune. O número de neutró-
ilhos e monócitos foi maior no grupo controle
(C), enquanto que no grupo estímulo (E) e no grupo
estímulo + 1,3-INDANODONA (C + E) houve uma
redução significativa no número de neutró-
ilhos e monócitos. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
da 1,3-INDANODONA foi verificada em cultura de
células cancerígenas, como MCF-7, Hs578T e
MCF-10A, evidenciando a atividade antimicro-
biana, pelo método de difusão em disco, para os
células mencionadas. A atividade antimicrobiana
}
From new molecules to new methods for health and knowledge in the beginning of a new millennium

Águas de Lindóia, São Paulo, Brazil
September 13 – 17, 2000

RESUMOS / ABSTRACTS / RESUMENES
10.065

PROPRANOLOL EFFECTS IN ARTHRITIC RATS.
Brito, R.E. and Francisch, J.N., Laboratório de Inflamação e Dor, Depto. de Farmacologia - ICB/UFMG, Belo Horizonte, MG, Brazil.

Introduction and Goals: Beta-adrenergic involvement in experimental arthritis has been previously shown (Proc. Natl. Acad. Sci. USA 85: 4553, 1988). Our aim was to study the effect produced by intracerebroventricular (icv) administration of a beta-blocker (propranolol) in arthritic rats. Methods: Arthritis was induced by subcutaneous (sc) injection of 0.2 ml of a colloid emulsion (10:1) containing 400 μg of M. butyricum in the dorsal root of the tail of female Holtzmann rats (weight: 140-170g). Increase in hindpaw volume (ml) and hyperalgesia (0-1) were daily measured by a hydrodynamometer and the vocalization method, respectively. Propranolol (P) was diluted in Tris-buffer and administered by sc (twice/day) and icv route from day 0 to 14 of arthritis. Control (C) animals were injected with buffer using the same volume and route of administration. Results: Sc propranolol (1-10mg/kg, total dose/day) dose-dependently reduced edema and hyperalgesia shown by arthritic rats at the 14th day of disease (Edema: P<0.03; C=1.6x0.19; Hyperalgesia: P<0.05x0.03; C=0.43x0.15). Icv propranolol (0.1-1μg/10μl/day)-treated rats also presented a significant dose-dependent reduction of edema and hyperalgesia by 14th day of arthritis (Edema: P<.01; C=1.37x0.17; Hyperalgesia: P<0.22x0.14; C=0.35x0.12) (p<0.05, ANOVA F test). Conclusion: Onde oprosteo a Manuel C.9-0.001, is involved in the control of the chronic inflammation and pain shown by arthritis rats. Financial support: CNPq and FAPEMIG.

10.066

ROLIPRAM EFFECTS (HYPERALGESIA AND EDEMA) IN RAT PAWS INJECTED WITH SOLUBLE FACTORS DERIVED FROM MONONUCLEAR CELL CULTURE (SOM).

Introduction and Goals: We have previously shown that mononuclear cells in culture (SOM) release substances which can induce edema and hyperalgesia. The goal of this work was to compare rolipram effects, a phosphodiesterase (PDE) IV inhibitor, on rat paws injected with either SOM or carrageenin. Methods: Mononuclear cell supernatant was obtained as previously established (Abstract 14.15 – FEBE/09). Rolipram (R) or saline (Control, C; 0.1 ml/paw) were intrapinasally injected into female Holtzmann rats (150-160 g) 1/2 hr before S0 or carrageenin (Cg 250 μg/paw). Increase in hindpaw volume (ml) of hyperalgesia (g) was measured plethysmometrically and by Randall-Selitto method, respectively, from 0 to 24 hr. Results: Rolipram at a low dose (0.25 μg/paw/0.1ml) enhanced...
Conclusions. Our results support that RO and PK have potential analgesic properties on reactive arthritis model. But different patterns of action between these drugs also suggest different mechanisms of action.

Financial support: PIBIC/CNPq-UFSC and Sanofi Recherche, Labege, France.

17.061

ANTI-OEDEMATOGENIC EFFECT OF PERIPHERAL BENZODIAZEPINE RECEPTOR LIGANDS IN A MODEL OF REACTIVE ARTHRITIS.


Introduction and Goals. The most common peripheral benzodiazepine receptor (PBR) ligands, RO5-4864 (RO) and PK11195 (PK), are known to cause inhibition on cytokine release by cultured macrophages and on acute inflammatory oedema in mice. We aimed here to test these drugs on a model of reactive arthritis in rats.

Methods. In male Wistar rats, 72 h after a previous carrageenin stimulation of one knee joint, an intra-articular LPS injection (1 μg) produces articular oedema (measured by the articular diameter increase, Δad, in mm). Oedema reaches its maximum value generally 4 h after LPS. When not indicated Δad was taken 4 h after LPS.

Results. PK applied 24 or 1 h before LPS injection inhibited in a dose-dependent manner the articular diameter. Its effective dose range was proportional to the time of pre-treatment. The maximal effects for PK given 24 (0.1mg/kg) or 1 h (0.01mg/kg) before LPS were respectively, Δad = 0.10±0.01 mm (control Δad = 0.20±0.07 mm) and Δad = 0.09±0.02 mm (control Δad = 0.17±0.02 mm). However, in both cases higher doses gradually lose their inhibitory effect. When given 1 h after LPS, PK showed a similar pattern as the 1 h pre-treatment protocol. On the contrary, RO given 1 h after LPS, only increased oedema (Δad = 0.24±0.01 mm, control-Δad = 0.18±0.02 mm, 5h after LPS). Indomethacin given 1 h after LPS did not reverse oedema, significantly.

Conclusions. Our results support that among PBR-ligands, at least PK has potential anti-oedemagenic properties on reactive arthritis model. The potency of PK treatment was increased by the proximity of the LPS challenge, and in some circumstances the PK effect was higher than that induced by indomethacin.

Financial support: PIBIC/CNPq-UFSC and Sanofi Recherche, Labege, France.

17.062

EFFECTS OF L-NAME TREATMENT ON EVENTS RESPONSIBLE FOR LOCAL LEUKOCYTE RECRUITMENT INDUCED BY BOTHrops jararaca VENOM (BjV).

Farkas, SHF.; Protas, S.Z. & Meltchinsky. Laboratorio de Terapia, Instituto Butantan, Laboratorio de Terapia, FMUSP.

Introduction: We recently showed that rabbits acutely or chronically treated with L-NAME showed a reduction in the celluar influx, protein leakage and NO levels in the synovial fluid after intra-articular injection of BjV. The present study verified the participation of NO in events related to leukocyte recruitment induced by BjV.

Methods: Intravital microscopic studies were performed to investigate the blood flow of microcirculatory vessels and the number of rolling and adhered leukocytes to post-capillary venules of the internal saphenous fascia of Wistar rats. The parameters were studied before and after topical application of 0.25 μg of BjVS/ml of sterile saline. Number of rolling and adhered leukocytes were determined on TV monitor and blood flow was calculated using erythrocyte velocity (optical doppler velocimeter). The leukocyte recruitment was evaluated in vivo, in air puch model, after injection of crescent doses of BJV.

Results: Our results showed that chronic treatment with L-NAME (20mg/Kg/day/14days) reduced PMN infiltrate into air pouch. The basal rolling leukocytes in comparison to values obtained in control animals (D-NAME). Values achieved in L-NAME treated animals before applying the BjV was 70% reduced. In this condition, blood flows obtained in venules and arteriolar were not different in both groups. The venous promoted increasing in the number of rolling (30%) and evoked adherence of leukocytes to venules in control rats. In contrast, these values were not altered in L-NAME treated animals.

Conclusions: These results show that L-NAME treatment impair the basal leukocyte-endothelial interactions. This effect may be responsible, at least in part, by reducing inflammatory reaction induced by the BjV in L-NAME treated rats. The microcirculatory blood flow seems not interfere with this effect. Further studies are needed to confirm this hypothesis.

17.063

LEUKOCYTE RECRUITMENT INDUCED BY FORMALIN INJECTION INTO RAT PERITONEAL CAVITY.

Santos, J.M.M.; Francischi, J.N. Depto. de Farmacologia, IC-UFMG, Brazil.

Introduction and Goals: Formalin injection into rat paws is an established model for pain study. As formalin induces inflammation, we aimed to study the contribution of cell recruitment to this response. Methods: Peritoneal lavage fluid obtained 4 and 24 hours following formalin (p) injection (1ml/animal) was prepared for total (cells/10^6/mm^3) and differential (%) cell counts. Females Hsd: rnu/m were previously (30min) treated with either dexamethasone (1 mg/Kg), pizlofen (2 mg/Kg - se-
SOB-induced edema. 4° post injection (R=0.17 ± 0.01 ml; n=4; C=0.12 ± 0.005 ml; n=4) whereas at a higher dose (5 µg/paw) reduced it in relation to control animals (R=0.11 ± 0.02 ml; n=5; C=0.23 ± 0.03 ml; n=5). At the same doses, rolipram did not affect or enhanced Cg-induced edema in the 3rd h of injection (R=0.60 ± 0.02 ml; n=10; C=0.51 ± 0.02 ml; n=10). On the other hand, rolipram at both doses did not affect SOB-induced hyperalgesia, but significantly reduced Cg-evoked hyperalgesia (R=8.5 ± 2.19 g; n=7; Ce=53.3 ± 5.8 g; n=7 and R=5.4 ± 7.7 g; n=5; Ce=38.8 ± 12.2 g; n=5 p<0.05, ANOVA t-test). Conclusions: Our study suggests that PDE IV is involved in the edema but does not seem to be involved in hyperalgesia induced by SOB in rat paws. In addition, SOB does not contain or is carragein.

Financial support: CNPq and FAPEMIG.

17.067

EFFECTS OF L-NAME AND CYCLOSPORIN A IN THE ADJUVANT-INDUCED ARTHRITIS MODEL.

Vicente, C.M. & Franciscis, J.N. Laboratório de Inflamação e Dor. Depto de Farmacologia-IBEM/FMG, Belo Horizonte, MG, Brazil.

Introduction and Goals: This study aimed to compare between the effects of the administration of a NOS inhibitor (L-NAME) and cyclosporin A in adjuvant arthritis using different schedules.

Methods: Arthritis was induced by subcutaneous injection of 0.2 ml of an oil-water emulsion (10:1) containing 400 µg of M. butyricum in the dorsal root of the tail of female Holtzman rats (weight: 140-170g). Increase in hindpaw volume (cm³) and hyperalgesia (0-1) were daily measured from control (C, n=5) and treated animals (T, n=5) by a hydroplethysmometer and the vocalization method, respectively. Both drugs were orally administered from day 0 to 14 (chronic administration) or from day 10 to 14 (acute administration). Results are presented as means±SEM and compared by ANOVA test.

Results: Cyclosporin at 7mg/kg was statistically effective to inhibit edema and hyperalgesia of the articular rats chronically and acutely at the 14th day of disease (Edema: T₁₄=d<0.05; C₁₄=0.5=0.1, p<0.05) or T₁₄=0.2±0.1/C₁₄=0.1±0.05 or T₁₄=0.3±0.1/C₁₄=0.8±0.1; p<0.05). L-NAME (1.5, 15 and 30mg/kg) dose-dependently reduced edema and hyperalgesia when administered chronically (Edema: T₁₄=1.11±0.3/C₁₄=0.64±0.01; p<0.05) or T₁₄=0.3±0.1/C₁₄=0.8±0.1; p<0.05). However, at 14th day of disease, L-NAME (1.5, 15 and 30mg/kg) dose-dependently reduced edema and hyperalgesia when administered acutely (Edema: T₁₄=0.38±0.1/C₁₄=0.1±0.05; p<0.05), but presented no anti-edematogenic or analgesic effect acutely.

Conclusions: Our results indicate that the mechanisms inhibitable by cyclosporin occur late from 10 to 14th day of arthritis development. On the contrary, the involvement of NO release seems to occur continuously in the disease.

Financial support: CNPq, CAPES and FAPEMIG.

17.068

EFETO DA PENTOXIFILINA NA MUCOSITE ORAL INDUZIDA POR 5-FLUOROURACIL EM HAMSTERS.


Introdução e Objetivos: a toxicidade da quimioterapia antineoplásica na cavidade oral resulta em uma mucosite ulcerativa que, além de muito dolorosa, causa destruição da barreira anatômica epitelial, tornando-a uma importante sede para microrganismos patogênicos, o que, somado à granulocitopenia, normalmente culmina em sepsis. Tem sido descrito o envolvimento de células da patogênese da mucosite oral (MO). Neste sentido, objetivou-se avaliar o efeito da PTX, uma droga inibidora de diversas citocinas, atuando na transcrição de seus RNA, na MO induzida por 5-FU. Métodos: a MO foi induzida por doses de 60 e 45mg/Kg de 5-FU nos dias 1 e 2, respectivamente, em hamsters Golden Syrian machos (120g; n=8/grupo). No 8º dia foram feitas escorátiões com o auxílio de um agulha de ponta romba, nas mucosas jugais esquerdas dos animais, como fator potenciador da mucosite. A mucosa contralateral foi utilizada como controle. Resultados: Salina (SAL) ou PTX (5, 15 ou 45mg/Kg/kg) foram injetadas 1h antes das administrações do 5-FU ou das escorátiões, e diariamente até o sacrifício no 10º dia. Os parâmetros avaliados foram: 1) Análise microscópica; 2) Histopatológico; 3) Permeabilidade vascular ao Azul de Evans (25 mg/kg/v); 4) Leucograma e 5) Variação da massa corpórea. Resultados: nas análises microscópicas, observou-se que a PTX bloqueou (p<0.05) os achados microscópicos (hiperemia da mucosa, dilatação e congestão vascular, ulcerações e abscessos) de forma significativa (PTX 45mg/Kg: Mds=1 (1-2) e SAL: Mds=3 (3-3)). Esses achados foram confirmados pela análise histopatológica (PTX 45mg/Kg: Mds=1 (1-3) e SAL: Mds=3 (3-3)) e pela análise de permeabilidade vascular. Observou-se redução da permeabilidade ao 10º dia (PTX 45 mg/kg=5,4×10⁵ ± 0,08; SAL=11,9×10⁵ ± 0,08), diminuindo assim, o nº de neutrofilos. A perda de massa corpórea, no entanto, não foi inibida por PTX. Conclusões: a PTX reduziu o processo inflamatório, sugerindo a participação de citocinas pró-inflamatórias na patogênese da MO, abrindo assim, novas perspectivas terapêuticas para a MO.

17.069

EFFECTS OF SELECTIVE INHIBITORS OF CYCLOOXYGENASE 2 ON OVUMPLANTATION AND PREGNANCY IN THE RAT.


...
18.029

ANALGESIC EFFECT OF THALIDOMIDE ON INFLAMMATORY PAIN.


Introduction and Goals: Tumor necrosis factor alpha (TNF-α) may have a pivotal role in the genesis of mechanical inflammatory hyperalgesia in rats and in nociceptive response in mice. Thalidomide (TAL) has been shown to selectively inhibit TNF-α production. We therefore investigated the effect of TAL in inflammatory pain models that TNF-α has a pivotal role as well as in the hot plate response in mice. Methods: In the present study the rat paw pressure test was used to investigate the nature of the effect of TAL on inflammatory hyperalgesia. Effects of TAL on the withering response in mice induced by acetic acid (AAc) or zymosan (ZY), on the hot plate response induced in mice and in the TNF-α mRNA levels in the perineal cells of mice injected with ZY were also investigated. Results: Hyperalgesic responses to intraplantar (ip) injection of bradykinin (Bk) or carragecin (Cg), which act by stimulating TNF-α release, but not responses to TNF-α or prostaglandins, were inhibited in a dose-dependent manner by pretreatment of the animals with TAL (77.6% and 82.1%) maximum effect for Cg or Bk, respectively, p<0.05). The nociceptive withdrawal responses induced by intraplantar (ip) injections of ZY or AAc were also inhibited in a dose-dependent manner by pretreatment of mice with TAL (86.7% and 84.4%, maximum effect for ZY or AAc, respectively, p<0.05). Moreover the TAL pretreatment also reduced the TNF-α mRNA levels in perineal cells induced by injection of ZY in mice (p<0.05). Conclusions: The analgesic effect of TAL is not due to a central effect since the drug had no effect in the hot plate test. The demonstration that TAL is able to inhibit inflammatory hyperalgesia in rats and the withering nociceptive response suggests that these analgesic effects seems to be consequent to the inhibition of TNF-α production, and indicates the need for investigations on the possibility of the use of TAL for the treatment of pain refractory to classical non-narcotic analgesics.

18.030

PERIPHERAL ANTINOCICEPTIVE EFFECT INDUCED BY DIPYRONE AS DETECTED BY THE OROPHARYNGEAL MODEL.


Introduction and Goals: Analgesic and antinflammatory effects due to drugs may derive from peripheral and/or central mechanisms. Our work aimed to study whether dipyrone would
present an antinociceptive effect using the orofacial pain method and the mechanisms involved. Methods: Orofacial model consisted of formalin injection (50µl, 2.5%, v/v) into the orofacial (or) region of male Wistar rats (weight: 180-220g) as published elsewhere (Neurosci. Lett. 133:349, 1989). Nociception rate (NR) is described as time (s) spent in rubbing affected area. Dipyrone (Dip) was injected by subcutaneous (sc)30min) or locally (pf 30 and 35min) before injection of formalin. Control (C) animals were injected with same volume of saline (0.1ml/100g or 50µl/ or). Results: Subcutaneous dipyrone (120 mg/kg) administration significantly reduced time spent in phase 1 and 2 in relation to control animals (DipNR = 68±5.4, C= 86±2.0, Dip:NR = 43±1.66, C= 66±5; P<0.05). Local dipyrone administration (100µg/site) reduced NR 68±0.2 to 7±1.3 and 66±1 to 35.7±2.5 at phase 1 and 2, respectively. A local antiinflammatory effect (NP=13±3, NP:NR=67±0, P>0.05) was noted in control animals. No significant differences were observed between control groups. Conclusions: A peripheral antinociceptive effect related to dipyrone administration was demonstrated. This antinociceptive peripheral effect due to dipyrone is dependent upon C-channels and independent of (peripherally) opioid receptors. Financial support: CNPq, CAPES, and FAPESP.

18.032

DO ENDOTHELINS PARTICIPATE IN ANTIGEN-INDUCED NOCICEPTION IN MICE?

Piovezan, A.P.; Henrique, M.G.M.O.; Souza, G.E.P.; D'Orleans-Juste, P.; Rae, G.A., Dept. of Pharmacology, UFSC, Florianopolis; F.M.C. (Florianópolis), Brazil, Faculty of Medicine, University of Trondheim, Norway.

Introduction and Goals: Endothelins (ETs) cause nociception when injected in the mouse hind-paw Piovezan et al. (J. Pharmacol. 129: 961-966, 2000). We have assessed if ETs participate in pain induced by antigen. Methods and Results: Male Swiss mice (18-20 g) were sensitized to chicken egg ovalbumin (OVA; 50 µg + 5 mg of Al(OH)3, in 200 µl). Antigen challenge 14 days later (intraperitoneal injection of OVA into the right hind-paw) induced dose-dependent nociceptive responses, as measured by increases in cumulative time spent licking the injected paw over 1 h in 5-min bins (controls: 0 s; OVA: 0.1 µg 84±20, 0.3 µg 118±38, 1 µg 227±97, 3 µg 177±20). To establish the participation of ETs in this process, animals were pre-treated with ET1/ET2-BQ-788 (selective ETs) or ET1/ET2/ET3-receptor antagonists, respectively. (1 or 3 nmol 15 min before) or intravenously with bosentan (mixed ET1/ET2 antagonists, 3.10 or 30 mg/kg; 1 h before) before OVA injection (0.3 µg). Bosentan (3, 10 or 30 mg/kg) significantly inhibited nociceptive responses to OVA by 48.5±13.5, 44.4±19.2, and 40.9±16.3 %, respectively (P< 0.05, ANOVA and Bonferroni). In contrast, selective local blockade of ETs receptors (with BQ-123 or BQ-788, respectively at up to 3 nmol), either alone or in combination, failed to inhibit OVA-induced nociception. Conclusion: These results suggest that ETs, acting simultaneously via ET1 and ET2 receptors, may play a role in allergic pain in mice. The susceptibility of OVA-induced nociception to inhibition by systemic non-peptidic bosentan, but not local BO-123 or BQ-788 (peptides), may be due to pharmacokinetic differences between these antagonists. Support: CNPq and FAPESP (Brazil) and Medical Research Council (Canada).

18.031

EFFECT OF THE CHRONIC PHENOBARBITAL TREATMENT IN THE TAIL-FICK METHOD.

Yokono, C.M.; Francischio, J.N. & Tatsuo, M.A.K.F. Laboratório de farmacologia e Do, Depto de Farmacologia-IQB-UFMG, Belo Horizonte, MG-Brazil.

Introduction and Goals: The acute administration of barbiturates induces hyperalgesia in different nociceptive tests (Braz. J. Med. Biol. Res. 30 (2): 251-256). This study aimed to verify the effect of the chronic administration of phenobarbital on the nociceptive response in the tail-flick test. Methods: Male Holtzman rats (weight: 180-220g, n=5/group) were daily treated (during 12 days) with 20mg/kg phenobarbital (PB) (1) or its vehicle (C) by intraperitoneal route. The animals were tested in modified tail-flick method, at day 0, 4, 8 and 12. At day 8, a group of rats chronically treated with PB was administrated with either picrotoxin (P) or saline (10mg/kg/animal) by intrathecal route, 20 min after the beginning of the experiment. Results are presented as means±SEM of the area under curves±SEM and compared by ANOVA (test). Results: At day 0, pb was effective to induce hyperalgesia in treated animals when compared with control animals (Tc=9.35±0.99, Cc=9.79±1.96; P<0.01); at day 4, the values of the chronically treated rats have not difference with the control animals (Tc=2.03±1.96, Cc=2.5±1.38; P>0.05). At day 8, pb induced antinociception (Tc=2±2.6; Cc=0.58; P>0.01) and that effect remained until day 12 (Tc=9.5±1.4; Cc=2.5±0.59; P<0.05). Intrathecal picrotoxin (P) administrated during the tail-flick test blocked the antinociceptive effect observed with chronic pb treatment (Tc=4.5±3.48; Tc=8.1±11, P<0.01). At the end of the experiment and following animal sacrifice their livers were weighted and showed a significant increase in weight in relation to control animals (P<0.01).

Conclusions: In conclusion these results indicate that chronic phenobarbital treatment induces antinociception rather than hyperalgesia in rats, probably through spinal GABA-A complex receptor inhibition.

Financial support: CNPq, CAPES and FAPESP.
newly marketed CDX-2 inhibitors in experimental edema and hyperalgesia. Methods: Male Holtzman rats (150-200g, n=5) were used. Increase in hindpaw volume (ml) and hyperalgesia (g), measured by a hydrodynamometer and Randall-Selitto method, respectively, were induced by intraplantar carrageenin (Cg) or bradykinin (Bk) administration and saline in contralateral paws. CDX-2 inhibitors (celexcoxib, CELEBRA, Pfizer; meloxicam MOVATOC, Boehringer Ingelheim) or physiological saline (C) were administered by subcutaneous route ½ h before intraplantar inflammatory agonists. Results are presented as mean±SEM and compared by ANOVA t test. Results: Celexcoxib (3-12 mg/kg) dose-dependently rewired hyperalgesia at 3h after 250 µg Cg (T =70±18, C=52±9, p<0.05) and at 16min after 100µg Bk injection (T =42±20, C=26±11, p<0.05). Edema induced by inflammatory agonists was not affected by the same dose-range of celexcoxib. Meloxicam (0.2-0.4mg/kg) dose-dependently inhibited edema (T =0.5±0.03, C=0.8±0.02, p<0.05) and hyperalgesia (T =93±28, C=61±11, p<0.05) induced by Bk at the same time points shown by celexcoxib. The highest doses of both inhibitors also induced increase in paw pressure threshold shown by positive data obtained independently of the inflammatory stimuli used. Conclusions: CDX-2 activation seems to be the main feature in hyperalgesic response to carrageenin and bradykinin in rat paws, being of lesser importance in edema development. A 'hyperlgesic' response could also be detected following use of CDX-2 inhibitors. Financial support: CNPq, PROGRAD and FAPEMIG.

25.007

EFFECTS OF MELOASMONE AND IODORCARIN IN HAMSTERS INFECTED WITH Leishmania braziliensis.


Introduction and goals: Leishmaniasis is a protozoal infection that affects both human and animal subjects. To date, there is no effective treatment for leishmaniasis in the veterinary practice. Melasone and iodinecarin are veterinary drugs used to treat cutaneous and visceral leishmaniasis, respectively. In the present study, we evaluated their potential in reversing lesions induced by L. braziliensis in hamsters.

Methods: Hamsters (Mesocricetus auratus) were weighed and separated in four groups (n=6): (I) control, (II) glucanumine, (III) melasone and (IV) iodinecarin. Promastigotes (10²) were incubated in the left hind footpad and the lesion size was measured weekly. The difference between the left (incubated) and right (non-incubated) footpad was used as parameter. After four weeks of infection the animals were treated intramuscularly with saline (I), 20 mg/kg of glucanumine (II), 2.5 mg/kg of melasone (III) and 10 mg/kg of iodinecarin (IV) for 15 days. The differences in lesions size were analyzed by Student's t-test (p<0.05).

Results: After 15 days of treatment the lesions size were 1.137±0.114 mm (I), 0.313±0.037 (II; p<0.05, Student's t-test), 0.675±0.127 (III; p<0.05, Student's t-test) and 0.903±0.141 (IV). The body weight was not statistically different between the groups. Melasone and iodinecarin reduced the lesion by 40.6% and 20.8%, respectively, whereas glucanumine decreased it by 72.5%.

Conclusions: Melasone and iodinecarin in the doses used were mody effective reversing lesions induced by L. braziliensis and are promising drugs for leishmaniasis treatment in animals as posologic problems are overcome.
verapamil (0,96mg/animal/day) diluted in water for 8 weeks. Control group - rats that received only water. All animals received a diet containing 1,5% Ca²⁺ and 0,8% Phosphorus. Four weeks following the beginning of the treatment, the animals were subjected to a trial surgery reproducing a fracture. After completing 8 weeks, the animals were sacrificed and analyzed.

Results: The radiographic study of the treated group revealed: 46,15% (n=6) of reconstitution and the control group 49% (n=4). The histologic analysis showed that 54% (n=7) of the treated group had reconstitution compared to 60% (n=8) of the control group. The biochemical analysis demonstrated the following values: Ca²⁺ (2,65±0,44mmol/L), P (3,12±0,30mmol/L) and ALP (61,10±13,45U/L) for treated samples and Ca²⁺ (2,65±0,45mmol/L), P (2,93±0,30mmol/L) and ALP (54,60±8,09U/L) for control samples (p<0,05 - student t test). Conclusion: Verapamil produced significant increase in ALP activity, but did not interfere with the other parameters.

25.003

STUDY OF THE INFLUENCE OF VERAPAMIL IN PHYSICAL PARAMETERS OF FEMURAL BONE FROM MALE RATS.

1Cardoso M.H.M, 2Santanna M.F., 2Rodrigues, M.A., 2Zanetti, H.H.U, 1Dias, M.A.V. 1Dept. of Pharmacy, 2Dept. of Biological Sciences, 1Dept. of Clinic Surgery - EFCA, 2Dept. of Physiotherapy, UNIFENAS, Alfenas-MG, Brazil.

Introduction and Goals: Many drugs have an effect on the metabolism of bone cells. Verapamil, a Ca²⁺ channel blocker, inhibits intestinal absorption of Ca²⁺ in rats. This study had the objective to determine if the chronic treatment with verapamil induce osteopenia in rats. Methods: Nineteen male Rattus norvegicus rats, 53 days-old, kept in individual cages, were used and divided in two groups: Treated group - rats that received verapamil (0,80mg/animal/day) diluted in water for 8 weeks. Control group - rats that received only water. All animals received a diet containing 1,8% Ca²⁺ and 0,8% Phosphorus. Eight weeks following the beginning of the treatment, the animals were sacrificed and their femoral bones submitted to a length measurement analysis (Schneider pachometer), the volume using the Archimedes principle and the weight of the ashes after incineration at 700°C for 24 hours. Results: The study of the development of femoral bone revealed the following mean values: length:37,4±1,5mm, volume:0,58±0,05cm³, ash content= 334±0,36mg for the treated group, compared to length:37,7±0,8mm, volume:0,57±0,04 cm³, ash content=332±0,22mg for the control group (p<0,05 - Student t test). Conclusion: Verapamil did not produce alterations of mineral bone density regarding the analysis of volume, length, and ash content of rat femoral bone.

25.004

IMIDOCARB DIPROPIONATO DOSAGE IN PLASMA ARABIAN HORSES.

Almeida, B. G.*; Bernardi, M. M.; Feliciano, J. D.* **Depo of Farmacologia, Faculdade de Medicina Veterinaria, Universidade de Sao Paulo, **Depo of Farmacologia, Instituto Biologico, SP, Brasil.

Introduction and Goals: The imidocarb dipropionate is effective in equine treatment with babesiosis, and used in Europe, Africa, Australasia and South America. Equine babesioses is an illness caused by Babesia caballi or Babesia equi which are endemic to the tropics, subtropics, and partly to the temperate zones. The vector of B. equi in South America is probably Bocophilus microplus. The determination of drug concentration in blood, serum or plasma is important in demonstrating the relationship between plasma drug concentration and the therapeutic and/or toxic, effect. The goal of the present study was to determine the plasma concentration of imidocarb as a function of time after intramuscular injection. Methods: Five adult Arabian horses weighting 400-500kg were used. Blood samples were collected prior to administration of the drug. An intramuscular dose (120mg/kg) of the imidocarb dipropionate (Imizol®) was injected into the neck muscle. Blood samples were collected from the opposite jugular vein of each horse as follows: first day at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24h after the injection; second day at 4, 6, 8, 12, 16, 20 and 24h; third day at 6, 16 and 24h; fourth day at 12 and 24h; and from the fifth day to the thirty first day only one sample was collected. The samples were drawn in vacutainers containing disodium EDTA as anticoagulant, and centrifuged immediately. Plasma samples were frozen (-20°C) and analyzed within 72h. A rapid ultraviolet spectrophotometric method, was used to determine the concentration of imidocarb in both the plasma and sample. Results: There were increased plasma concentrations at three different points during the experiment: at 2,12 μg/ml on the 4th day, 2,27 μg/ml on the 16th day and 2,79 μg/ml on the 22nd day. Conclusion: The present study suggests a three-compartment model for horses, and that imidocarb persists in the blood plasma of horses for at least thirty-one days post-administration.

25.005

EFFECT OF MARKETED CYCLO.OXIGENASE-2 INHIBITORS IN THE EDEMA AND HYPERALGESIA INDUCED BY INFLAMMATORY AGONISTS IN RAT PAWS.


Introduction and Goals: Cyclooxygenase (COX) 2-inhibitors have been developed to increase analgesic/inflammatory efficacy and to reduce collateral effects presented by otherwise older COX-1 inhibitors. We aimed to study the effect of
ORIENTAÇÕES CONCLUÍDAS

DOUTORADO:


MESTRADO:

CELINA MITIKO YOKORO

Participação do Sistema Nervoso Central na fisiopatologia da artrite experimental: contribuição da interleucina 2 e do óxido nítrico

Tese de Doutorado a ser apresentada ao Curso de Pós-Graduação em Ciências Biológicas – Fisiologia e Farmacologia da Universidade Federal de Minas Gerais, como requisito parcial para a obtenção do Grau de doutor em Ciências Biológicas – área de concentração farmacologia.

Orientadora: Profª Drª Janetti Nogueira de Francisch

BELO HORIZONTE

2002
MARCOS ANTÔNIO DE RESENDE

Caracterização farmacológica de substâncias pró-inflamatórias produzidas por leucócitos mononucleares em cultura

Tese de Doutorado apresentada ao Curso de Pós-Graduação em Ciências Biológicas – Fisiologia e Farmacologia da Universidade Federal de Minas Gerais, como requisito parcial para a obtenção do Grau de doutor em Ciências Biológicas – área de concentração farmacologia.

Orientadora: Profª Drª Janetti Nogueira de Francisch

BELO HORIZONTE

2001
RECRUTAMENTO DE LEUCÓCITOS PARA A CAVIDADE PERITONEAL DE RATOS INDUZIDO PELA INJEÇÃO DE FORMALINA

JÚLIA MARIA MOREIRA SANTOS

BELO HORIZONTE
2001
JÚLIA MARIA MOREIRA SANTOS

RECRUTAMENTO DE LEUCÓCITOS PARA A CAVIDADE PERITONEAL DE RATOS INDUZIDO PELA INJEÇÃO DE FORMALINA

Dissertação apresentada ao Curso de Pós-Graduação em Fisiologia e Farmacologia do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como requisito parcial para a obtenção do grau de mestre em Ciências Biológicas (Farmacologia)

Orientadora: Profª. Dra. Janetti Nogueira de Francischì

BELO HORIZONTE

2001
AGRADECIMENTOS

Primeiramente agradeço à Deus, aos meus pais e meus familiares, em especial, Vó Anísia e Tio Miguel. Agradeço também ao meu namorado Guilherme pelo carinho e paciência. Aos vários amigos que sempre acompanharam a minha jornada, o meu agradecimento.

Aos meus amigos da Faculdade de Odontologia, Janaina, Fernanda, Daniela, Carmem e Dudu pelo apoio e amizade.

Aos professores do Departamento de Farmacologia, Cida, Igor, Salete, Miriam, Dalton, Regina, Mauro e Rômulo pelo carinho e amizade.

Aos amigos do laboratório Cíntia, Gláucia, Patrícia Santana, Adriana, Celina, Marcos, Kátia, Leani, Baretta, Ana Paula, Patrícia Mota, Daniela Pacheco, Luís Henrique, Marcela, Roberta, Cristiano e Gustavo pelo carinho e amizade.

Aos funcionários do Departamento de Farmacologia e do CEBIO, em especial ao Wanderli, Rinaldo e Adelaide que colaboraram para a conclusão desse trabalho.

As demais professores que contribuíram para a minha formação no mestrado, ao prof. Carlos Alberto Pereira Tavares, prof. Orivaldo A. Rocha e ao Prof. Wagner Tafturi. E a todos os colegas, professores e funcionários do Departamento de Fisiologia que de alguma forma contribuíram para a realização deste trabalho.

Ao CNPq, Capes e FAPEMIG pelo apoio financeiro.