Título: Lesões hepáticas por tetracloreto de carbono: ultraestrutura

Pesquisador responsável: Virginia Hora Rios Leite

Processo CDS 1305/98
Data de início e término do auxílio: 31/05/2001 a 05/2003

Universidade Federal de Minas Gerais

Relatório Final
Sumário

Resumo .................................................................................................................. 03
Palavras chave ................................................................................................. 03
Introdução ........................................................................................................... 04
Histórico ............................................................................................................ 04
Etapas executadas no projeto ........................................................................... 05
Apresentação e discussão dos principais resultados obtidos .................. 06
Referências bibliográficas ................................................................................ 10
Responsabilidade ............................................................................................ 11
Resumo

Cameron e Karunaratne (1936), descreveram alterações hepáticas na intoxicação por tetracloreto de carbono (CCl₄). Após este, raros trabalhos tiveram enfoque morfológico. Da década de 70 em diante, o modelo da intoxicação pelo CCl₄ foi usado com ênfase no estudo do desenvolvimento da fibrose hepática e na avaliação de potencialização de lesão ou proteção hepática induzidas pelo uso de outras substâncias administradas pré ou pós intoxicação. Aqui, valoriza-se a histologia com ênfase na evolução das lesões. Estuda-se fígado de ratas com 12, 24 e 48 h após 2, 4, 8, 16 e 23 doses subcutâneas de CCl₄. Observa-se que as lesões são dose-dependentes e mais precoces com as doses subseqüentes; exacerbam-se às 24 h e regredem com 48 h. Degeneração gordurosa é inicial e dominante 12 h após 2, 4 e 8 doses; degeneração hidrópica, necrose e apoptose coexistem com a degeneração gordurosa e se intensificam com o aumento do número de doses. Regeneração hepatocitária ocorre sempre às 48 h e se intensifica com o aumento do número de doses; pleomorfismo nuclear ocorre a partir da 8ª dose. Nódulos regenerativos aparecem a partir da 16ª dose. Com o aumento do número de doses surge neoformação conjuntiva formando septos que posteriormente definem nódulos. Nos septos há neocapilarização, proliferação de ductos e apoptose de hepatócitos e de células ductais. Também ocorre apoptose de hepatócitos adjacentes aos septos. Corpúsculos citoplasmáticos aparecem a partir de 4 doses e aumentam em número com as doses subseqüentes. Múltiplas doses de CCl₄ conduzem à cirrose. Neste trabalho constata-se a ocorrência de apoptose e sua variação temporal e topográfica durante a intoxicação assim como a existência de corpúsculos citoplasmáticos até então não considerados por nenhum autor.

Palavras chave: tetracloreto de carbono, fígado, cirrose
Introdução

A pesquisa sobre os efeitos tóxicos do tetracloreto de carbono (CCl₄) se concentrrou nas décadas de 20 e 30. Fiessinger et al. (1922) foram os primeiros pesquisadores a comentar sobre a possível relação tóxica do CCl₄ quando da exposição de seres humanos aos vapores dessa substância. Cameron e Karunaratne (1936) reproduziram em ratos a intoxicação pelo CCl₄ e descreveram as alterações histológicas hepáticas em tempos definidos pós-intoxicação, porém não estabeleceram correlação dinâmica entre as lesões. Após essa época raros trabalhos enfocaram as alterações hepáticas vistas ao microscópio óptico, seja para confirmar ou para complementar as descrições iniciais. Após os anos 70, o modelo murino de intoxicação pelo CCl₄ foi usado com ênfase no estudo do desenvolvimento da cirrose hepática e na avaliação de potencialização de lesão ou proteção hepática induzidas pelo uso de outras substâncias administradas pré ou pós-intoxicação. Poucos são os trabalhos científicos que descreveram as lesões hepáticas pelo CCl₄ e raros os que enfocam de forma sistematizada estas lesões com ênfase na evolução das mesmas.

Neste trabalho o objetivo é estudar a intoxicação hepática por CCl₄ em ratas, com administração de múltiplas doses do tóxico, avaliando-se as lesões hepáticas agudas e crônicas produzidas, no que se refere à evolução morfológica, em diferentes tempos após a aplicação das doses.

Justifica-se a execução da pesquisa, pelo acima descrito e porque, no rato são produzidas lesões semelhantes às encontradas no homem além do que, este modelo permite o aprendizado e o ensinamento dos processos patológicos básicos estudados em patologia (alterações degenerativas, de morte celular e intersticiais, transtornos da circulação, do crescimento e diferenciação celular, inflamações e pigmentações).

Acrecenta-se que esta pesquisa terá continuidade. Estudo morfométrico das lesões em desenvolvimento/involução constitui etapa programada.

Histórico

O CCl₄ foi utilizado como hepatotóxico, experimentalmente, por Fiessinger et al. 1922 ao suspeitar da relação entre o contato humano com o CCl₄ e o desenvolvimento de processos patológicos hepáticos. Moon (1934), em trabalho de revisão, fez coletânea do que foi descrito pelos autores até então; havia sido produzido cirrose hepática experimental, em cães, coelhos e em camundongos por

Várias cepa de ratos (albino, Sprague-Dawley, Wister, Long-Evans, Fischer, Holzman) tem se prestado bem à reprodução de lesões hepáticas agudas e crônicas.

Da década de 70 em diante, os pesquisadores desviaram a atenção para o estudo dos vários componentes do fígado, isoladamente, tanto em condições de normalidade quanto patológicas. Para essa finalidade, o tratamento de células mantidas em cultura e a intoxicação de animais de laboratório com CCl₄ tem sido usado. No entanto, pouca contribuição científica foi dada, na caracterização histológica do que foi descrito pelos autores, inicialmente. Na verdade, na atualidade, não há relato de nenhum trabalho científico que descreva de forma sistematizada as lesões hepáticas produzidas pelo CCl₄, mesmo considerando-se que a forma crônica da doença no roedor, cirrose hepática, se assemelha à humana em muitos aspectos morfológicos, clínicos e laboratoriais (Proctor & Chatamra, 1982; Martinez-Hernandez 1985).

Etapas executadas no projeto

Na execução do projeto fez-se: acasalamento das matrizes de ratas, obtenção de crias, separação machos das fêmeas e composição em grupos de até seis animais por gaiola, num total de oitenta ratas; atualização da revisão bibliográfica e leitura de trabalhos científicos; administração do CCl₄, sacrifício dos animais após diferente número de doses do tóxico; colheita das amostras e processamento do material para microscopia óptica e eletrônica; microtomia e colorações para microscopia óptica; ultramicrometria de cortes semi e ultrafinais, contraste eletrônico, observação e documentação fotográfica; análise das lâminas e elétron-micrografias; redação do/s trabalho/s científico/s e apresentação em seminário e congresso.

Encerrado este projeto, a pesquisa terá continuidade porque o material colhido e armazenado está reservado à execução de outros trabalhos. Na próxima etapa será feito estudo morfométrico das lesões em desenvolvimento/involução.
Apresentação e discussão dos principais resultados obtidos

As ratas foram sacrificadas em tempos diferentes após a administração de número variável de doses e os resultados foram:

Após 2 doses de CCl₄

12 h (Grupo I), o exame histológico mostrou arquitetura lobular preservada. As lesões ocorreram na zona 3 do ácino hepático, se extendendo à zona 2. Ocorreram: degeneração gordurosa, lesão predominante; muitos hepatócitos baloniformes, intensamente tumefetos, com citoplasma de aspecto rendilhado e núcleos em posição central, situados nas adjacências da veia central; raros e diminutos focos de necrose hepatocitária associados a infiltrado inflamatório de neutrófilos e, numerosos hepatócitos em apoptose. Mitoses hepatocitárias, ocasionais, ocorreram em áreas preservadas.

24 h (Grupo II), a arquitetura lobular manteve-se preservada. As lesões eram nas zonas 3 e 2 constatando-se: moderada degeneração gordurosa; pequeno número de hepatócitos baloniformes situados à distância da veia central; aumento do número de focos de necrose hepatocitária, com frequência confluentes, associados a neutrófilos e, hepatócitos apoptóticos em quantidade superior à vista no Grupo I. Mitoses hepatocitárias, raras, ocorreram em áreas preservadas.

48 h (Grupo III) a arquitetura lobular manteve-se preservada. As lesões ocorreram só na zona 3 observando-se: discreta degeneração gordurosa; raros hepatócitos baloniformes situados à distância da veia central; escassos focos de necrose hepatocitária e, hepatócitos em apoptose em quantidade inferior à vista no Grupo II. Mitoses em hepatócitos foram raras, como no Grupo II.

Após 4 doses de CCl₄

12 h (Grupo IV), a arquitetura lobular se manteve preservada. As lesões eram nas zonas 3 e 2, ocorrendo: intensa degeneração gordurosa; hepatócitos baloniformes (semelhantes aos do Grupo I, porém situados na zona 2); poucos e diminutos focos de necrose hepatocitária associados a infiltrado inflamatório neutrofílico e, aumento do número de hepatócitos apoptóticos, se comparado ao Grupo I. Pequeno número de hepatócitos em mitose ocorreram em áreas preservadas e na transição com áreas lesadas. Observou-se alguns macrófagos na zona 3 contendo pigmento acastanhado.

24 h (Grupo V), a arquitetura lobular esteve preservada. As lesões se mantiveram nas zonas 3 e 2 constatando-se: intensa degeneração gordurosa; hepatócitos baloniformes (semelhantes aos do Grupo I, irregularmente distribuídos nas duas zonas); aumento do número de focos de necrose hepatocitária se comparado ao Grupo IV, frequentemente confluentes, associados a neutrófilos; hepatócitos em apoptose, em quantidade superior à do Grupo IV e raros hepatócitos com corpúsculos citoplasmáticos eosinofílicos, único ou múltiplos, arredondados ou ovalados, de contornos nítidos e tamanhos e intensidade de eosinofilia variáveis.
Hepatócitos em mitose ocorreram como no Grupo IV. Ocorreram macrófagos na zona 3 à semelhança do Grupo IV. Até este tempo pós-intoxicação, não foram notadas alterações nas colorações para fibras reticulares (prata amoniaca de Gomori) e colágeno I (tricôrmeo de Masson).

48 h (Grupo VI) a arquitetura lobular manteve-se preservada. As lesões ocorreram nas zonas 3 e 2, observando-se: moderada degeneração gordurosa; hepatócitos baloniformes (semelhantes aos do Grupo V); redução do número de focos de necrose hepatocitária e de hepatócitos em apoptose ao se comparar com o Grupo V, porém, superior ao Grupo III; raras hepatócitos com corpúsculos citoplasmáticos, como no Grupo V e, pequenas células fusiformes (fibroblastos-like), numerosas, situadas nas zonas 3 e 2. Mitoses hepatocitárias foram mais frequentes que nos grupos anteriores ocorrendo nas mesmas áreas. Macrófagos na zona 3 foram vistos como no Grupo V. Houve discreto aumento da quantidade de fibras reticulares e colágeno I nas zonas 3 e 2.

Após 8 doses de CCl₄

12 h (Grupo VII), a arquitetura lobular estava alterada. As lesões comprometiam as zonas 3 e 2 e em áreas, se estendiam às veias centrais de outros lóbulos. Caracterizaram-se por: intensa degeneração gordurosa; hepatócitos baloniformes (situados na transição área lesada/ preservada); múltiplos e confluentes focos de necrose hepatocitária formando ponte entre veias centrais e associados a infiltrado de neutrófilos; hepatócitos em apoptose em quantidade superior ao Grupo IV; aumento do número de hepatócitos com corpúsculos citoplasmáticos (superior aos Grupos V e VI) e, aumento do número de células fusiformes, situadas nas zonas 3 e 2 (se comparado ao Grupo VI). Hepatócitos em mitose, binucleados, poliplóides e com núcleos pleomórficos ocorreram nas áreas preservadas e na transição destas com as lesadas. Aumento do número de macrófagos na zona 3 (comparado aos Grupos IV e V). Fibras reticulares e colágenas formavam delicados feixes intra e interlobulares, subvertendo a arquitetura lobular. Além disto, foi evidente a proliferação de células ductais na periferia dos lóbulos assim como nos feixes de tecido conjuntivo.

24 h (Grupo VIII), a arquitetura lobular manteve-se alterada. Degeneração gordurosa, hepatócitos baloniformes, focos de necrose hepatocitária, hepatócitos em apoptose, hepatócitos com corpúsculos citoplasmáticos e células fusiformes, lesões consideradas no Grupo VII, ocuparam as mesmas zonas, porém foram mais intensas e extensas. Hepatócitos em mitose, binucleados, poliplóides e com núcleos pleomórficos ocorreram nas mesmas áreas e em mesma frequência do Grupo VII. Macrófagos de situação na zona 3, à semelhança do Grupo VII, foram observados. Fibras reticulares e colágenas, como no Grupo VII, subvertiam a arquitetura lobular. Similar ao Grupo VII, ocorreu proliferação de células ductais.

48 h (Grupo IX), a arquitetura lobular se manteve alterada. Degeneração gordurosa, hepatócitos baloniformes, focos de necrose hepatocitária, hepatócitos em apoptose, hepatócitos com corpúsculos citoplasmáticos e células fusiformes,
ocorreram nas zonas 3 e 2; foram lesões mais discretas que as do Grupo VIII e mais intensas que as do VI. Hepatócitos em mitose, binucleados, poliplóides e com núcleos pleomórficos ocorreram em frequência e topografia semelhantes à observada nos Grupos VII e VIII. A disposição das fibras reticulares e colágenas, assim como a proliferação das células ductais, foram idênticas às dos Grupos VII e VIII.

**Após 16 doses de CCl4**

12 h (Grupo X), feixes espessos de tecido conjuntivo formavam septos que se insinuavam nos lóbulos hepático definindo nódulos, que isolavam número variável de hepatócitos, segmentos de lóbulos ou lóbulos inteiros. Nos septos observou-se: intensa proliferação de células do epitélio ductal, isoladas ou agrupadas, com frequência formando pequenos ductos; grande número de hepatócitos em apoptose e de outros com corpúsculos citoplasmáticos eosinofílicos; neoformação de capilares e, macrófagos contendo pigmento acastanhado. Nos nódulos existiam proliferação de células ductais e de ductos, hepatócitos em apoptose e hepatócitos com corpúsculos citoplasmáticos eosinofílicos, localizados preferentemente na periferia, portanto, nas adjacências dos septos; também ocorreram, em intensidade variável de nódulo para nódulo, degeneração gordurosa, hepatócitos baloniformes, focos de necrose hepatocitária, hepatócitos em mitose, binucleados, poliplóides ou com núcleos pleomórficos e macrófagos com pigmento acastanhado. Vários dos nódulos mostravam predominantemente regeneração hepatocitária sendo denominados de nódulos regenerativos. Fibras reticulares e colágenas intra e inter lobulares evidenciavam a subversão da arquitetura lobular.

24 h (Grupo XI), as lesões foram semelhantes às do Grupo X, porém, diferente deste grupo, ocorreu intensa degeneração gordurosa.

48 h (Grupo XII), as lesões foram semelhantes às do Grupo X.

**Após 23 doses de CCl4**

12 h (Grupo XIII), o aspecto histológico foi semelhante ao do Grupo X, no entanto, aqui, os septos eram mais espessos, os nódulos apresentavam forma anular e os nódulos regenerativos foram mais frequentes; as colorações para fibras reticulares e colágenas ressaltaram estes achados.

24 h (Grupo XIV), o aspecto histológico neste grupo foi muito semelhante ao do Grupo XIII.

48 h (Grupo XV), o aspecto histológico neste grupo foi semelhante ao dos Grupos XIII e XIV, porém mitoses ocorreram em abundância.

Na avaliação do material desta pesquisa discute-se que 2, 4 e 8 doses de CCl4 produzem no fígado, lesões degenerativas e de morte celular 12 e 24h após a administração do tóxico e regressão das lesões, 48h após. Nossos achados após 2 doses do tóxico foram semelhantes aos de Cameron e Karunaratne (1936) em ratos intoxicados com apenas 1 dose de 0,05-0,1 cc e sacrificados no período de 1h a 14
dias após. Diferente de Cameron e Karunaratne (1936), nesta pesquisa foram também examinadas ratas sacrificadas após 4, 8, 16 e 23 doses. Nos grupos de 16 e 23 doses, com o desenvolvimento da cirrose, o isolamento do parênquima pelos septos fibrosos pode ter contribuído para o aspecto difuso e semelhante das lesões nos tempos estudados, ao contrário das variações temporais observadas nos grupos de 2, 4 e 8 doses. Constatou-se também que a cada dose administrada, as lesões eram mais extensas e intensas nos intervalos de tempo estudados. Estes achados ainda não foram considerados em trabalhos anteriores. O achado inicial de minúsculos focos de necrose observados 12 h após 2 doses, assim como o agravamento das lesões, com aumento da extensão da área acometida e do número de focos de necrose após 24 h, corroboram o raciocínio de que há estreita relação entre dose e tempo de atuação do tóxico. Quarenta e oito horas após, as lesões tendem a regredir.

A administração de múltiplas doses de CCl₄ com o intervalo de tempo executado, culminou no aparecimento de cirrose. Cameron e Karunaratne (1936) observaram que ratos intoxicados cronicamente com doses de 0,25 cc a cada 10 dias não desenvolviam cirrose. Concluiu-se que nas células baliniformes predominando na área de transição. Uma vez instituída a cirrose, as células baliniformes se distribuíam uniformemente em todo o parênquima.

Além da ação tóxica sobre o hepatócito, o CCl₄ estimula a proliferação celular e o reparo tissular (Cameron e Karunaratne, 1936; Rao et al. 1997). Rao et al. (1997) concluíram que 36h após a administração de CCl₄ o dano celular é máximo e o reparo tissular ineficiente. Entretanto a progressão ou a reversão das lesões foi dose-dependente, ou seja, doses menores podem ter seus efeitos revertidos enquanto doses maiores tendem a causar lesões progressivas que podem inclusive levar o animal à morte. Com doses maiores o dano é mais precoce e se estende à zona periportal.

As mitoses eram abundantes 48h pós intoxicação, ocorrendo nos hepatócitos e nas células ductais. Esses achados corroboraram Sutton e Spurgeon (1966). As mitoses são tentativas de restabelecimento da integridade do órgão após as lesões induzidas pelo CCl₄.

A apoptose foi fenômeno muito encontrado, sendo comum 12 h após a administração das doses. Pareceu sempre haver exacerbação 24 h após e tendência à regressão com 48 h. Após instalada a subversão da arquitetura, apoptose foi comum no interior dos nódulos assim como na periferia dos mesmos, ou seja, na proximidade dos septos conjuntivos. Cameron e Karunaratne (1936) não descreveram esta lesão assim como nada referiram que se pudesse hoje considerar ser apoptose. Shi et al. (1998) exploraram a possibilidade de que apoptose pudesse também contribuir como fenômeno de morte celular por CCl₄. Estes autores, ao re-
examinarem a lesão hepática em ratos, identificaram células em apoptose no intervalo de 3 a 96 h após única dose intraperitoneal (0,3 ml/kg), atingindo o pico 6 h após a dose. Nesta pesquisa comprova-se esta variação, corroborando os achados de Shi et al. (1998). Adicionalmente, neste trabalho, observou-se que com o aumento do número de doses (4 e 8 doses), o fenômeno de apoptose tornou-se numericamente mais frequente e que, após instituída a cirrose hepática, ela ocorria também nas adjacências dos septos de fibrose.

A presença de corpúsculos citoplasmáticos eosinofílicos, de aspecto homogêneo e vítreo, vistos a partir de 4 doses, não foi considerada por nenhum outro autor no modelo de intoxicação por CCl₄.

Quarenta e oito h após a administração de 4 doses evidenciou-se aumento da quantidade de fibras reticulares e colágenas na zona 3 com extensão à zona 1; estes achados corroboraram os de Rubin et al (1963) ao estudarem ratos intoxicados com dose correspondente a 50% da utilizada aqui. Takahara et al (1988) constataram proliferação de células de Ito e aumento da produção de colágeno tipo III, 48 a 72 h após única dose de CCl₄.

Septos fibrosos apareceram segundo Rubin et al (1963) com 80 a 95 dias de intoxicação (20 e 27 doses), enquanto a cirrose hepática morfologicamente definida só se estabeleceu após 27 doses. Para Takahara et al (1988) septos fibrosos definindo nódulos se tornaram evidentes após 24 doses. Estes aspectos foram diferentes dos achados deste trabalho no qual se encontrou septos conjuntivos após 8 doses de CCl₄ (30 dias de intoxicação) e cirrose estabelecida com 16 doses do tóxico (60 dias).

Assim, evolutivamente as lesões hepáticas na intoxicação por CCl₄ são dosedependentes e mais precoces com as doses subsequentes; exacerbam-se às 24 h e regredem com 48 h. Descreve-se a ocorrência de apoptose e de corpúsculos citoplasmáticos.

Referências bibliográficas


Cameron GR, Karunaratne WAE: Carbon tetrachloride cirrhosis in relation to liver regeneration. J Path Bact 1936, 42: 1-21


Moon VH: Experimental cirrhosis in relation to human cirrhosis. Arch Path 1934, 18:381-424

McGee JO'D, Patrick RS: The role of perisinusoidal cells in hepatic fibrogenesis. Lab Invest 1972, 26: 429-440


Responsabilidade

Profa Virginia Hora Rios Leite
Departamento de Anatomia Patológica
Histology of Liver Injury in Carbon-Tetrachloride-Treated Rats: a Redescription


*Departamento de Anatomia Patológica da Faculdade de Medicina da Universidade Federal de Minas Gerais. Av. Alfredo Balena, 190 - 5º andar. 30130-100 Belo Horizonte, MG Brazil

Address for Correspondence
Virginia Hora Rios Leite (MD PhD)
Faculdade de Medicina
Universidade Federal de Minas Gerais
Av. Alfredo Balena, 190 – 5º andar
30130-100 Belo Horizonte – MG
Brazil
E-mail: horarios@medicina.ufmg.br

Running title
Liver Injury in Carbon-Tetrachloride: a Redescription
Summary

Few authors have focused on the histological appearance of the hepatic injury following CCl₄ intoxication. In this study, the histopathology and evolution of the CCl₄-induced liver lesions in rats was re-examined within 12, 24 and 48h of the administration of 2, 4, 8, 16 and 23 doses of 0.1ml/100g CCl₄. In addition to the classical lesions previously described, hepatocytes undergoing apoptosis were seen in all rats used in our experiment, their frequency increasing proportionately to the number of doses administered; in the cirrhotic stage, they occurred at the periphery of the nodules and particularly in the septa. The apoptotic (ballooned) hepatocytes varied in topography up to 8 doses of CCl₄, depending on the time interval after administration; in hepatic cirrhosis, although still present, their distribution in the lobule was diffuse. Hepatocytes with eosinophilic cytoplasmic bodies, single or multiple, round or oval-shaped, with sharp contours and variable degree and extent of eosinophilia, appeared after 4 doses of CCl₄ and were progressively more numerous as the number of doses increased; in hepatic cirrhosis, they occurred preferably in the septa and at the periphery of the nodules. The presence of these cytoplasmic bodies has not so far been reported in CCl₄ intoxication.

Keywords

Liver, Carbon-Tetrachloride, Pathology, Rat.
Introduction

Carbon tetrachloride (CCl₄) is a nonflammable, highly toxic liquid compound of the group of halogenated aliphatic hydrocarbons. It was extensively used in the past in the treatment of helminthic infections and is nowadays employed for separating metal alloys, extracting animal and vegetable fats (from bones and seeds), chemical bleaching, admixture with soaps, dissolving latex, and as a constituent of fire extinguishers, lacquers and varnishes.

Much research was carried out in the twenties and thirties on the toxic effects of CCl₄. Fiessinger et al. (1922) were the first authors to comment on the possible toxic role of CCl₄ upon the exposure of human beings to fumes of this compound and experimental dates in mice indicated CCl₄ as responsible for the intoxication. Cameron and Karunaratne (1936) reproduced CCl₄ intoxication in rats and described the histological changes in the liver at various intervals after the administration of a single dose of CCl₄ and at a single interval (24h) after multiple doses. After this, rare histological studies on the hepatic changes in CCl₄ intoxication were conducted, adding practically nothing to the findings of Cameron and Karunaratne (1936). After the seventies, the murine model of CCl₄ intoxication was widely used in studying the development of fibrogenesis in hepatic cirrhosis. Today, the murine model is used in evaluating the potentialization of the hepatic injury or protection induced by the administration of other substances simultaneously with or after CCl₄ exposure.
Shi et al. (1998) re-examined the liver injury induced in rats after a single dose of CCl₄ and explored the possibility that apoptosis may also contribute to its pathogenesis. Thus, it is today important to re-examine the histology of CCl₄-induced hepatic lesions, as this may provide parameters for current and future propositions of experiments with CCl₄.

Our object in this research is to examine the hepatic injury evoked in rats by the administration of multiple doses of CCl₄ and evaluate the liver histopathology at different time intervals after injection of the doses, with emphasis on the evolution of the lesions.

**Materials and Methods**

Fifty-seven female Holtzman rats aged three to six months with free access to water and chow were used. Six animals that did not receive mineral oil served as controls. Of these, three were sacrificed at the beginning of the experiment and the others 20 weeks later. Six other rats received a subcutaneous dose of 0.1ml/100g body weight of mineral oil. Three of these animals were sacrificed 48h after a single dose and the others 48h after the last of 12 doses of mineral oil. CCl₄ (50% vol/vol in mineral oil) was administered subcutaneously in doses of 0.1ml/100g body weight twice weekly. Groups of 3 animals were sacrificed by an anesthesia with ether at intervals of 12, 24 and 48h after 2, 4, 8, 16 and 23 doses (Groups I to XV). Food was withheld from all rats for 16h before sacrifice. Portions of liver were fixed
in formalin and stained with hematoxylin-eosin, Gomori’s ammoniacal silver, and Masson’s trichromic stain.

Results

Macroscopic examination
In the control animals and in Group I, the liver had a smooth, wine-color surface. In all other Groups (II to XV), the liver appeared pale and, as the number of doses increased, gradually became slightly yellowish in color, with a granular to nodular surface (Figure 1) containing connective adhesions between the lobules of the liver and/or between the liver and other organs or structures in the abdominal cavity (intestine, omentum, diaphragm).

Histological examination
No histological changes were seen in the liver of the control animals.

2 doses of CCl₄
At 12h (Group I): The lobular architecture was preserved. The lesions occurred in the centrilobular zone, extending towards the periphery of the intermediate zone. In these areas, the histological changes included: fatty degeneration, the dominant lesion; many ballooned hepatocytes, intensely swollen, with a reticulated cytoplasm and centralized nuclei, located near the central vein; rare, minute foci of hepatocyte necrosis associated with neutrophil inflammatory infiltrate; and
numerous hepatocytes undergoing apoptosis. Occasionally, mitotic hepatocytes were seen in preserved areas (Figures 2-5).

**At 24h (Group II):** The lobular architecture was normal. The lesions were located in the centrilocular and intermediate zones and consisted of: moderate fatty degeneration; a small number of ballooned hepatocytes located at a distance from the central vein; an increase in the number of foci of hepatocyte necrosis, often confluent, associated with neutrophils, and of apoptotic hepatocytes, as compared with Group I. Rare hepatocyte mitoses occurred in the preserved areas (Figures 6 and 7).

**At 48h (Group III):** The lesions were limited to the centrilocular zone. They included: discrete fatty degeneration, rare ballooned hepatocytes located at a distance from the central vein; scanty foci of hepatocyte necrosis; and a larger number of apoptotic hepatocytes than that seen in Group II. As in Group II, hepatocyte mitoses were rare.

**4 doses of CCl₄**

**At 12h (Group IV):** The normal architectural pattern of the liver was maintained. The lesions were located in the centrilocular and intermediate zones: They consisted of intense fatty degeneration; ballooned hepatocytes (located in the intermediate portion of the lobule); rare, minute foci of hepatocyte necrosis associated with neutrophil inflammatory infiltrate; a larger number of apoptotic hepatocytes, as compared with Group I; rare hepatocytes with eosinophilic
cytoplasmic bodies, single or multiple, round or oval-shaped, with sharp contours and variable degree and extent of eosinophilia; and numerous, small fusiform cells located in the centrlobular and intermediate zone of the lobules (Figures 8 and 9). A small number of hepatocytes undergoing apoptosis was seen in the unaffected areas and in the transitional zones between these and the injured areas. A few macrophages containing a brownish pigment appeared in the centrlobular region. There was a discrete increase in the number of reticular and collagen type I fibers in the centrlobular and intermediate zones, as stained with Gomori’s ammoniacal silver and Masson’s trichromic stain.

**At 24h** (Group V): The lobular architecture was normal. The lesions remained in the centrlobular and intermediate zones. They included: intense fatty degeneration; irregularly distributed ballooned hepatocytes; a larger number, as compared with Group IV, of foci of hepatocyte necrosis, often confluent, associated with neutrophils (Figures 10 and 11); more numerous apoptotic hepatocytes than in Group IV; rare hepatocytes with eosinophilic cytoplasmic bodies and fusiform cells, both as in Group IV. Hepatocytes undergoing mitosis, centrlobular macrophages, and reticular and collagen fibers occurred as frequently as in Group IV.

**At 48h** (Group VI): The lesions were located in the centrlobular and intermediate zones, comprising: moderate fatty degeneration; irregularly distributed ballooned hepatocytes; foci of hepatocyte necrosis and apoptotic hepatocytes in a smaller number than in Group V, but more numerous than in Group III; rare hepatocytes
with eosinophilic cytoplasmic bodies and fusiform cells, both as seen in Groups IV and V. Hepatocyte mitoses, though occurring in the same areas, were more frequent than in the previous groups. The reticular and collagen type I fibers remained much as in Groups IV and V.

8 doses of CCl₄

At 12h (Group VII): The lobular architecture was altered. The lesions affected the centriloculobular and intermediate zones, extending, in some areas, towards the periphery of the lobules. They comprised: intense fatty degeneration, ballooned hepatocytes (located in the injured area/preserved area transitional zone); multiple, confluent foci of hepatocyte necrosis associated with neutrophil infiltrate, forming bridges between the central veins; hepatocytes undergoing mitosis in a larger number than that seen in Group IV; hepatocytes with cytoplasmic bodies and fusiform cells in a larger number than that observed in the previous groups. Binucleation, polyploidy and nuclear polymorphism, in addition to mitoses, occurred in the preserved areas and in the transitional zone between these and the damaged areas. Reticular and collagen fibers formed delicate, intra- and interlobular bundles, subverting the lobular architecture. Also evident was the proliferation of bile-duct cells at the periphery of the lobules, as well as in the bundles of connective tissue. Macrophages containing a brownish pigment were seen in the centriloculobular portion and in the bundles of connective tissue (Figures 12-19).
At 24h (Group VIII): The lobular architecture remained altered. The lesions were the most intense of all groups, affecting the centrilobular, intermediate and periportal zones, with only a small band of periportal hepatocytes being spared. They included: fatty degeneration; ballooned hepatocytes (irregularly distributed); foci of hepatocyte necrosis; apoptotic hepatocytes; and hepatocytes with cytoplasmic bodies and fusiform cells. In addition to mitoses, binucleation, polyploidy and nuclear polymorphism occurred in the same areas and as frequently as in Group VII. As in Group VII, reticular and collagen fibers subverted the lobular architecture. Also as in Group VII, proliferating bile-duct cells and macrophages were present.

At 48h (Group IX): Fatty degeneration, ballooned hepatocytes, foci of hepatocyte necrosis, hepatocytes undergoing apoptosis, and hepatocytes with cytoplasmic bodies and fusiform cells were seen in the centrilobular, intermediate and periportal zones. The lesions in this group were not as intense as in Group VIII. In addition to mitoses, binucleation, polyploidy and nuclear polymorphism were seen in the same areas and as frequently as in Groups VII and VIII. The arrangement of reticular and collagen fibers, as well as the proliferation of bile-duct cells, were similar to those observed in Groups VII and VIII. As in these two groups, macrophages were present.

16 doses of CCl₄

At 12 and 24h (Groups X and XI, respectively): The lesions included thick bundles of connective tissue forming septa that penetrated the liver lobules, defining
nODULES that isolated a variable number of hepatocytes, segments of the lobule or the entire lobule. In the septa, there was intense proliferation of bile-duct cells, isolated or in groups, often forming small ducts. A large number of apoptotic hepatocytes and hepatocytes with eosinophilic cytoplasmic bodies, in addition to newly-formed capillaries and macrophages containing a brownish pigment, were also present in the septa (Figures 20-22). In the nodules, there was intense proliferation of bile-duct cells and ducts, in addition to apoptotic hepatocytes and hepatocytes with eosinophilic cytoplasmic bodies, both located preferably at the periphery of the lobule, near the septa. Also seen in the nodules, varying in intensity from one nodule to another, were fatty degeneration, ballooned hepatocytes, foci of hepatocyte necrosis, hepatocytes undergoing mitosis (binucleation; polyploidy or polymorphism), and macrophages containing a brownish pigment. Several of the nodules, referred to as regenerative nodules, showed predominantly hepatocyte regeneration. Intra- and interlobular reticular and collagen fibers evidenced subversion of the lobular architecture. The histological appearance of the liver is that of cirrhosis.

At 48h (Group XII): The lesions were similar to those of Groups X and XI, except that the mitoses were more frequent.

23 doses of CCl₄

At 12 and 24h (Groups XIII and XIV, respectively): The histological appearance was similar to that of Group X, except that the septa were thicker, the nodules had a ring-like shape, and the regenerative nodules were more numerous. The stain
used for reticular and collagen fibers enhanced these findings. The histological appearance of the organ is that of cirrhosis (Figures 23-26).

At 48h (Group XV): The histological appearance of the liver in this Group was similar to that observed in Groups XIII and XIV, except that the mitoses were abundant.

Discussion

The results of this research permit the following evaluation of the evolution of CCl₄-evoked lesions. Fatty degeneration was the initial and dominant lesion at 12h after 2 doses. Fatty degeneration, ballooned hepatocytes, necrosis and apoptosis were more evident at 12h after 4 doses and still more marked after 8 doses than at 12h after 2 and 4 doses, respectively. The intensity and extent of these lesions increased progressively up to 24h after the injection of 2, 4 and 8 doses; at 48h, they decreased in intensity and extent after the injection of 2 doses, only in intensity after 4 and 8 doses, and showed no further regression at 48h after the injection of 16 and 23 doses. At 12h, the ballooned hepatocytes had a different topography from that seen at 24 and 48h after 2, 4 and 8 doses; after 16 and 23 doses, the distribution of these cells was irregular. Fusiform cells were increasingly more frequent, in the injured areas, after 4 and 8 doses. Mitoses were more numerous at 48h after 2, 4 and 8 doses, as well as at 12 and 24h after 4 doses. At
binucleation, polyplody and polymorphism were observed in the preserved areas and in the transitional zones between these and the injured areas. The lobular architecture was preserved after 2 and 4 doses, with only a discrete increase in the connective fibers after 4 doses. After 8 doses, intra- and interlobular reticular and collagen fibers formed delicate bundles, subverting the lobular architecture. After 16 and 23 doses, bundles of connective tissue, increasingly thicker as the number of doses increased, formed septa that defined nodules in the parenchyma (hepatic cirrhosis); various nodules showed predominantly hepatocyte regeneration (regenerative nodules). After 23 doses, the nodules had a ring-like shape. Hepatocytes undergoing apoptosis were seen as early as 12h after 2 doses, and were progressively more frequent as the number of doses increased; in hepatic cirrhosis, they occurred preferably in the septa and at the periphery of the nodules. Cytoplasmic bodies appeared after 4 doses of CCl₄ and their frequency increased proportionately to the number of doses; in hepatic cirrhosis, they occurred preferably in the septa and at the periphery of the nodules. Macrophages were seen, after 4 doses, in the centrilobular portion of the lobule; after 8 doses, in the centrilobular zone and in the bundles of connective tissue; and after 16 and 23 doses, predominantly in the bundles. Proliferation of bile-duct cells at the periphery of the lobules and in the bundles of connective tissue was observed from 8 doses onwards; after 16 and 23 doses, bile-duct cells were frequent in the nodules and particularly in the septa. Newly-formed capillaries were also common in the septa.

Our findings confirm the existence of the lesions reported in the rat by Cameron and Karunaratne (1936) at various time intervals after the administration of a single
dose of CCl₄ and 24h after multiple doses. From the description of these authors (Cameron and Karunaratne, 1936), we understand that at 24h after a single dose of CCl₄ there are extensive degenerative and necrotic changes, followed by complete restoration within 7 to 14 days. In our study, using the same dose size and route of administration of CCl₄, but evaluating the liver at different time intervals (12, 24 and 48h) after multiple doses, we found exacerbation at 24h and regression of the lesions at 48h after 2, 4 and 8 doses, which corroborates the histological description of Cameron and Karunaratne (1936). After 16 and 23 doses, no aggravation or regression of the lesions was observed, the lesions being indistinctly diffuse and severe. Adding to the description referred to above, this study also showed that the lesions developed and aggravated more quickly and took longer to regress, proportionately to the number of doses injected (whether 2, 4 or 8). These observations had not been previously reported. In hepatic cirrhosis, in addition to the toxic action of CCl₄, isolation of the hepatic parenchyma by fibrous septa also contributed to the unchanged appearance (diffuse and severe) of the lesions in the cirrhotic stage (after 16 and 23 doses), contrarily to the time-related variations observed after the injection of up to 8 doses. Thus, this experiment—which used a regular dose size and time interval between doses—allows us to conclude that the largest number of doses leads to the greatest severity of the lesions, as well as to the greatest tendency towards non-regression of the lesions, until hepatic cirrhosis sets on, after which the lesions will remain severe.
In this experiment, multiple doses of CCl₄ culminated in the development of cirrhosis. One of the conditions considered by Cameron and Karunaratne (1936) as essential for the production of cirrhosis by CCl₄ in rats is the prolonged use of a toxic dose, with a short time interval between the doses, to prevent complete repair of the lesions produced by the previous dose. In our study, by observing the evolution of the lesions after the administration of a defined number of doses, which was not done by the above referred authors, it was possible to conclude that after each dose administered the lesions gradually become more intense and show less regression, leading to cirrhosis.

Apoptosis, with distinct variations in frequency and topography, was the feature seen in all rats in our experiment. In the lengthy description of Cameron and Karunaratne (1936), other than a brief mention of “liver cells close to the central veins, with a homogenous appearance, staining deep pink with eosin and often with pyknotic or completely degenerated nuclei” 3h after a single dose of CCl₄, no reference is made to what might today be considered as apoptosis. Shi et al. (1998), re-examining the hepatic injury evoked by CCl₄, identified apoptosis within 3 to 72h, reaching a maximum at 6h, after a single intraperitoneal injection of 0.3 ml/kg of CCl₄, and explored the possible role of apoptosis in the development of CCl₄-induced liver injury. The present work, using multiple doses of 0.1ml/100g and sacrifice of the rats within different times after CCl₄ administration, demonstrates the occurrence of apoptosis as a lesion induced by CCl₄, even when cirrhogenic doses are used.
In our research, ballooned hepatocytes were present both before and after the onset of cirrhosis. In the pre-cirrhotic stage, however, these cells had a clear distribution pattern in the lobule that varied according to the number of $\text{CCL}_4$ doses injected and the time interval after $\text{CCL}_4$ administration. Since Cameron and Karunaratne (1936), ballooned hepatocytes, located initially in the centrilobular region, around the central vein, and later on at random locations, have been reported by several authors, who considered them as cells undergoing hydropic degeneration. Shi et al. (1998), on the other hand, obtained experimental evidence that ballooned hepatocytes are cells undergoing apoptosis. This would explain why, in our research, ballooned hepatocytes were seen in different regions of the liver lobules, according to the time interval after $\text{CCL}_4$ administration.

Eosinophilic cytoplasmic bodies, homogeneous and vitreous, proportionate in frequency to the number of $\text{CCL}_4$ doses, occurring in hepatic cirrhosis preferably in the septa and at the periphery of the nodules, have not been considered by any other author in the $\text{CCL}_4$ intoxication module, and thus deserve further investigation.

That hepatocyte, bile-duct cell and connective tissue proliferation is part of the tissue repair mechanism that follows $\text{CCL}_4$-induced death cell has been demonstrated by several researchers from Cameron and Karunaratne (1936) to Rao et al. (1997), in trying to determine the relationship between $\text{CCL}_4$ dose, liver injury and tissue repair, as well as the progression or reversal of the lesions, depending on the dose size and number of times administered.
In this work, CCl₄-induced hepatic injury was redescribed at defined time intervals following administration of the toxin, with emphasis on the evolution of the lesions. It was found that hepatocyte apoptosis varied in frequency and topography during the intoxication. Also demonstrated was the existence of cytoplasmic bodies, a finding that no other author has yet reported in CCl₄ intoxication.

References


Legends

Figure 1. Macroscopic appearance of rat liver after 23 doses of CCl₄; liver with nodular surface.

Figures 2-5. 12h after 2 doses of CCl₄. Fatty degeneration is the dominant lesion (Fig. 2) x63. Intensely swollen, balloononed hepatocytes with a reticulated cytoplasm and centralized nuclei (Fig. 3) are located close to the central vein (Figs. 4 and 5). An hepatocyte is undergoing apoptosis (arrow) (Fig. 5). Figs. 3 and 5 x400; Fig. 4 x160.

Figures 6 and 7. 24h after 2 doses of CCl₄. Lesions in the centrilobular and intermediate regions of liver lobules (Fig. 6). x63. Foci of hepatocyte necrosis associated with neutrophils (arrows) (Fig. 7). x160.

Figures 8 and 9. At 12h after 4 doses of CCl₄. Lesions affecting the centrilobular and intermediate zones, with balloononed hepatocytes in the intermediate region of the lobule (Fig. 8) x63. Foci of hepatocyte necrosis associated with neutrophilic inflammatory infiltrate (arrows) and numerous small fusiform cells located in the centrilobular and intermediate zones of the lobules (arrow heads) (Fig. 9). x160.
Figures 10 and 11. 24h after 4 doses of CCl₄. Ballooned hepatocytes (irregularly distributed), foci of hepatocyte necrosis, often confluent, associated with neutrophils. x160.

Figures 12-15. 12h after 8 doses of CCl₄. Lesions in the centrilobular and intermediate zones (Fig. 12) and ballooned hepatocytes (located in the injured area/preserved area transitional zone) (Figs. 12 and 13). Lesions extending towards the periphery of the lobules (Fig. 14). Confluent foci of hepatocyte necrosis (arrow) (Fig. 15). Fig. 12 x63; Figs. 13-15 x160.

Figures 16-19. 12h after 8 doses of CCl₄. Hepatocytes undergoing apoptosis (arrows) and fusiform cells (arrow heads); intra- and interlobular presence of delicate bundles subverting the lobular architecture (Fig. 16) x160. Hepatocytes undergoing apoptosis (Fig. 17). Hepatocytes with cytoplasmic bodies (Figs. 18 and 19). Figs. 17-19. x400.

Figures 20-22. 12h after 16 doses of CCl₄. A bundle of connective tissue forms a septum in which there are newly-formed capillaries (Fig. 20) and proliferating bile-duct cells and ducts (Fig. 22). An hepatocyte is undergoing apoptosis in the septum (Fig. 21). Figs. 20 and 21 x160; Fig. 22 x400.

Figures 23-26. 12h after 23 doses of CCl₄. The histological appearance of the liver is that of cirrhosis. Bundles of connective tissue form septa defining nodules that isolate a variable number of hepatocytes, segments of the lobule or the entire
lobule (Fig. 23). Hepatocytes undergoing apoptosis in the septa and at the periphery of nodules (arrow) (Fig. 24). In the septa, in addition to newly-formed capillaries, there is proliferation of bile duct epithelial cells forming ducts (Fig. 25). Regenerative nodules (arrows) (Fig. 26). Figs. 23 and 26 x63; Figs. 24 and 25 x160.
Carbon tetrachloride-induced liver injury in rats: cytoplasmic inclusion bodies detected in hepatocytes

Bordoni LS; CP; Coelho, ACP; Veloso SG; Berenstein CK; Leite VHR

Departamento de Anatomia Patológica da Faculdade de Medicina da Universidade Federal de Minas Gerais, Av. Alfredo Balena, 190 - 5º. andar - 30130-100, Belo Horizonte, MG.

Key Words: Carbon tetrachloride, inclusion bodies, hepatocyte

Introduction

It is well established that carbon tetrachloride (CCL₄) is a typical hepatotoxin causing centrilobular necrosis and the liver injury model in rats is well documented (Fiessinger et al. (1922) Cameron and Karunaratne (1936). Much research was carried out in the twenties and thirties on the toxic effects of CCL₄ (___). Cameron and Karunaratne (1936) minuciosamente described the histological changes in liver at various intervals after the administration of a single dose of CCL₄ and at a single interval (24h) after multiple doses. After this, rare histological studies on the hepatic changes in CCL₄ intoxication were conducted, adding practically nothing to the findings of Cameron and Karunaratne (1936). As a result of liver cell damage occurs, hydropic degeneration, apoptosis, necrosis, fibrosis, and the Kupffer and fat-storing cells become activated (___). It has not been reported whether....

No reference exist about cytoplasmic inclusion bodies detected in hepatocytes in CCL₄-induced liver injury. So, among all CCL₄ injury, we can notice the occurrence of cytoplasmic, eosinophilic, homogeneous, vitreous bodies. In cirrhosis they occur in hepatocytes from nodules and in that isolated from septa. Therefore, among the anatomical pathological findings in CCL₄
intoxication, these cytoplasmic bodies have not been described before. In this study a light and electron microscopy approach of these cytoplasmic bodies will be done.

Materials and Methods

Three to five-months-old female, Holtzman rats, weighing 200 to 220 g (n=18) were supplied ad libitum with water and commercial pelleted diet. The rats were randomized into six groups of three animals, five of them treated with CCl₄ (0.1 ml/100g body weight, subcutaneous route) dissolved in equal volume of corn oil, twice weekly. When necessary, the doses of CCl₄ were readapted to the body weight. After 12 h of fasting, the rats were sacrificed by an anesthesia with ether at 48 h after 2, 4, 8, 16 and 24 doses. Liver right lobe samples with 0.3 cm thickness were fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin, Masson trichrome, Gomori’s ammoniacal silver, and PAS with and without diastase. Simultaneously, small fragments of liver were fixed in 3%glutaraldehyde, cacodylate buffer at pH 7.2 for 2 h, postfixed in osmium tetroxide, dehydrated in graded alcohol solutions and then, included in epon. PAS with diastase semithin sections were examined for identification of cytoplasmic bodies; ultrathin sections of areas with this bodies were made using diamond knife. The sections were contrasted with uranyl acetate and lead citrate, and examined in electron microscopy (EM 9-S2 Zeiss).

Results

After 23 doses of CCl₄, the liver appeared pale and slightly yellowish in color, with granular to nodular surface, containing adhesions between lobes and with abdominal organs or structures (intestine, omentum, diaphragm).

The histological appearance of cirrhosis was evident such as, thick bundles of connective tissue, defined nodules isolating a variable number of hepatocytes, segments of lobules, or entire lobule. The cytoplasmic bodies were identified as eosinophilic (variable degree and extent of eosinophilia), vitreous, homogeneous, single or multiple, round or oval in shape, sharp contour, PAS positive diastasis resistant. They were finding in hepatocytes of nodules, and in that one isolated from septa.
At electron microscopy, the cytoplasmic bodies had a granular aspect, moderate electron density, and sharp-defined contour or not, in continuity with other cytoplasmic components. The cisterns of agranular and granular endoplasmic reticulum were dilated. In the granular endoplasmic reticulum (GER) there was a similar substance of that one observed in cytoplasmic bodies.

Discussion

Positive PAS staining after diastase treatment identify glicoproteins ( ) and the hepatocitic inclusions are likely to contain this kind of material.

The ultrastructural similar aspect of cytoplasmic bodies and GER content, confirm the histological feature of cytoplastic inclusion, and allow to establish a relationship between protein storage in GER cisterns without secretion. De Lellis et al. (1972) observed that the cisterns of GER were dilated and contain microfibrillar substance, morphologically similar to the one in cytoplasmic bodies of alpha-1-antitrypsin deficiency.

The CCl₄ injury is caused by CCl₃ metabolite. Free radical and hydrogen peroxide are responsably for unsaturated lipids peroxidation from membranes system, with possible conversion of others molecules to secondary free radical and cellular damaged enhancement. This causes Ca²⁺ release from mitochondria and endoplasmic reticulum to cytosol, in the same way that allow the Ca²⁺ uptake from extra-cellular middle. The injury means to interfere in Ca²⁺ pumps that prevent the intracellular increase of the ion.

It grows up a metabolic disturbance due to elevation Ca²⁺ concentration, and decreased K⁺, enzymes, and coenzymes in cytoplasm. The granular and agranular endoplasmic reticulum, at aggression, accumulates protein and become enlarged. These protein might result from enhanced production and/or deficient excretion induced by CCl₄ stored in cytosol.

Pariente et al. (1981) identify in liver of patients with alcoholic cirrhosis and PIM phenotype (without alpha-1-antitrypsin deficiency), PAS-positive diastase-resistant cytoplasmic bodies. The immunofluorescence for A₁AT was positive in some samples and negative in others. For these authors inclusion bodies in the cytoplasm of hepatocytes — even containing A₁AT- do not permit the hepatic lesions to be ascribed to A₁AT deficiency. It may correspond to
glycoprotein storage due to chronic hepatic injury. Lezzeni et al. (1997) demonstrated in hepatic explants, from patients with different causes of cirrhosis, cytoplasmic globules in 10% of samples. The globules were variably sized, round to oval, with a smooth, well defined contour seen on the H&E or PAS-D-stained sections. Immunohistochemical studies demonstrated finally granular AT activity positive material dispersed in the cytoplasm of many of the hepatocytes. These globules were in association with a variety of AT phenotype but 25% of the cases had a normal PiMM phenotype. The authors considered the possibility of AT globules, in patients with a normal PiMM phenotype might constitute as a nonspecific reaction of hepatocyte to injury, with subsequent intracytoplasmic accumulation of AT.

Deutsch et al. (1994) discuss the fact that PAS positive cytoplasmic bodies were observed in individuals with others livers diseases (like hepatic tumors associated with oral contraceptive, hepatomas and alcoholic cirrhosis). In these cases, the elevation of alpha-1-antitrypsin synthesis exceeds the excretion capacity of hepatocytes.

So, the presence of lesions like that described above doesn't permit to attribute them exclusively to alpha-1-antitrypsin deficiency.

Qizilbash et al. (1983) in a study of 500 autopsies, found 27 cases of PAS- positive diastase-resistant (inclusion bodies/globules). They classify the inclusion bodies in: a) type 1, localized in nodules periphery, always associated to cirrhosis or discrete fibrosis; b) type 2, localized in centrilobular areas of liver, without relationship with hepatic disease, peripherally to regions with ischemic and necrotic alterations. The two types had similar morphology, but were always associated to different situations. In none of the cases did type 1 and type 2 globules co-exist in the same section. Pariente et al. (1981) e lezzeni et al. (1997) observed that the cytoplasmic bodies were found mainly in regenerative nodules periphery, and more frequently in hepatocytes in contact with fibrosis. In the samples of this research, PAS-positive diastase resistant cytoplasmic bodies were found in hepatocytes from nodules and in those isolated from septa in all examined animals, what reinforce the descriptions of related authors above.

Scotto et al. (1975), by ultrastructural exam, established that the PAS-positive storage, found in hepatocytes in alpha-1-antitrypsin deficiency, resulted
from accumulated substance in agranular and granular endoplasmic reticulum, especially in the first, and the Golgi apparatus were unaltered. Jeppssom et al. (1975) established a narrow biochemistry similarity between serum and hepatic antitrypsin, contrasting with analysis of carbohydrates of protein, which revealed a great deficiency in hepatic antitrypsin glycosilation. There was completely lack of sialic acid and relative deficiency of others carbohydrates. Eriksson et al. (1975) established that the joined substance in cytoplasmic bodies were alpha-1-antitrypsin without sialic acid. They suggested that insufficient addition of sialic acid to the hepatic protein lead to an elevated insolubility and a great tendency to form aggregates. Kelly et al. (1979) suggest that the PiS protein may had some degree of insolubility due to a similar molecular change but in smaller amplitude then PiZ. This could lead to precipitation in endoplasmic reticulum in an undefined glycosilation state, resulting in low serum levels of protein. Recent studies (Carrell & Lomas, 2002; Lomas & Mahadeva, 2002; Mahadeva et al, 2002), have shown that endoplasmic reticulum retention of mutant A1AT results from a blockage of its processing and secretion. The retained A1AT aggregates are seen in the endoplasmic reticulum of hepatocytes as inclusions bodies periodic acid-Schiff staining.

In our samples, the injury by CCl4 to cellular membranes and organelles could lead to a impaired excretion of proteins by cell, due to physical properties alterations, like low solubility and great tendency to aggregation. Baraona et al. (1977) showed that cellular aggression, like that caused by alcohol could damage the protein secretion mechanisms by microtubules and enhance your production. This could result in an intracellular storage of these proteins. So, if the alpha-1-antitrypsin formation exceed the injured hepatocyte capacity of excretion, there will be retention and formation of aggregates in granular endoplasmic reticulum and cytoplasm, forming the cytoplasmic bodies.

References


Figures 1 and 2 - Light microscopy of hepatocytes showing globular immature and mature inclusion bodies, respectively. HE

Figures 3 and 4 - Electron microscopy of globular immature and mature inclusion bodies in hepatic cell cytoplasm, respectively. X 10240
XXIV CONGRESSO BRASILEIRO DE PATOLOGIA

SUPLEMENTO CIENTÍFICO
Trabalhos Concorrentes a Premiação
Trabalhos Científicos para Apresentação Oral
Trabalhos Científicos Apresentados como Pôsteres

volume 39 • número 1 • janeiro/fevereiro/março 2003

Uma publicação conjunta das sociedades: SBPC/ML (Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial), SBP (Sociedade Brasileira de Patologia) e SBC (Sociedade Brasileira de Citopatologia)
ULTRASTRUCTURE OF HEPATOCYTE INCLUSION BODIES AFTER CCl4 TOXICITY IN THE RAT
Departamento de Anatomia Patológica da Faculdade de Medicina da Universidade Federal de Minas Gerais, Belo Horizonte, MG.

Introduction: Carbon tetrachloride (CCl4) is a well-known hepatotoxic agent, which is widely used in mechanism studies of hepatic injury. The occurrence of cytoplasmic inclusion bodies has not been reported in any studies involving CCl4 intoxication.

Objective: Re-examine the chronic liver injury produced by CCl4.

Methodology: The drug was administered by sc route (0,1ml 100g) twice a week. The livers were examined 12, 24 and 48h after 23 doses of CCl4. One-mm3 samples were fixed in 3% glutaraldehyde and semithin sections were stained with toluidine blue and examined under electron microscopy.

Results: Eosinophilic, viretes, PAS-positive, diastase-resistant globular inclusions were found in hepatocytes predominantly peripheral in location, although some were located within the cytoplasmic nodules, showing immature and 40 mature aspects. Granular electron-dense material was identified in dilated rough endoplasmic reticulum and also free in hepatic cell cytoplasm. So, beyond CCl4 injuries known (fatty degeneration, ballooned hepatocytes, centrilobular necrosis, apoptotic hepatocytes, cirrhosis), it is necessary to emphasize the occurrence of these bodies. Similar globules have been described in -1-antitrypsin deficiency, a model for a congenital disease. Conclusion: The chronic CCl4 action on liver membrane system could change the organelle function and secrete causing cytoplasmic inclusion bodies formation.

Apóia: FAPESP.

DISSEMINAÇÃO ENDOMÍTRIAL DO ADENOCARCINOMA COLORRETA: EXPRESSÃO DAS CITOCÓPATAS 7, 19 E 20
PANNAIN, V.L.; CAROLI-BOTTINO A.; SILVEIRA V.G.; JUNIOR E.D.; RIBEIRO J.
Hospital Universitário Clementino Fraga Filho UFRJ Rio de Janeiro RJ


Apóia: FAPEA.

ACETRETINA ACARRETANDO HEPATITE AGUDA FULMINANTE EM PACIENTE COM PSORIASE
PAIXÃO, ANP; CHANG, D MARTINELLI, ALC.; RAMALHO, LNZ; ZUCOLOTO, S.
Departamento de Anatomia Patológica, Hospital das Clínicas de Ribeirão Preto, FMUSP-USP

Introdução: Algumas drogas utilizadas na terapêutica de doenças dermatológicas crônicas apresentam conhecido potencial hepatotóxico. Entretanto, as alterações hepáticas costumam ser leves e reversíveis após a suspensão do medicamento. Dados clínicos e demográficos: Paciente feminina, 39 anos, em tratamento de psoriase há seis meses com Acitretina (Neobilag®) 25 mg/dia evoluiu há 20 dias com icterícia, colapso, hipofusão e posterior insuficiência hepática e óbito. Métodos diagnósticos: Eleição acometida de enzimas hepáticas (AST e ALT) e aumento de bilirubinas. Achados microscópicos: A autópsia: paciente icterícia, fígado amolecido, pesando 899 g, com extensas áreas de necrose, nefrose colônica, impregnação de bilirubina em meninges e encéfalo, edema cerebral, petequias em miocárdio e músculos gástricos, hemorragia mesentérica, extensa lesões cutâneas planto-metacarpal, disseminadas. Achados microscópicos: hepatócitos apotóticos, extensas áreas de necrose, lagos bilares e infiltrado inflamatório mononuclear com distribuição lobular, compatível com hepatite aguda fulminante. Comentários: A Acitretina, reindicação derivada da vitamina A, ainda não havia sido relacionada à hepatite fulminante no caso de tratamento por psoriase, devido ao, portanto, monitorizar rigorosamente a função hepática nestes casos.

Apóia: FAPEA.

INSULINOMA: APRESENTAÇÃO DE CASO
FERRO, MC; BARCELLOS, CRC; VIEIRA, AEF; ZAMPIERI, M.

Introdução: Os insulinosomas são neoplasias das células B do pâncreas, cujo diagnóstico é baseado no quadro clínico (hipoglicemia, hiperinsulinemia, complicações微). São raros e podem ocorrer em qualquer idade, com maior incidência em mulheres. Sua diferença clínica é exata (1:200 a 1:000). Embora ocorra em qualquer idade, é mais frequente nos pacientes acima de 30 anos. Dados Clínicos: Paciente do sexo feminino, 43 anos, há três meses apresentava 3 a 4 crises hipoglicêmicas por semana, principalmente em jejum, caracterizadas por sintomas de neuroglicopenia e que melharam com administração de glicose intravenosa. Exame físico: IMC 35,5 Kg/m2. Restante sem alterações. Foi internado e instituída dieta fracionada, sem açúcar livre, com melhora clínica, porém com alguns episódios hipoglicêmicos. Através de arteriografia, localização no nível com 3 cm de diâmetro, no corpo pâncreas, que foi encerado. Após a cirurgia os níveis de insulina, pró-insulina e insulina C retomaram ao normal. Achados Microscópicos: Nódulo de 3x2x0,8cm, branco acinzentado e lobulado, coro indistinta, com cortes mostraram áreas acinzentadas na região central. Achados Microscópicos: Proliferação de células pequenas epitélioides, com arranjo cordonal e trabecular. O exame histoquímico revelou positividade para insulina e cromogranina, negatividade para glucagon. Comentários: Houve boa evolução clínica da paciente desde a cirurgia, além da retirada do insulinosoma, neste caso o tamanho da lesão também diminuiu, pois na maioria dos casos mede 1,5cm.
ULTRASTRUCTURE OF HEPATOCYTE INCLUSION BODIES AFTER CCl₄ INTOXICATION IN THE RAT

BORDONI, L.S.; COELHO, A.C.P.; VELOSO, S.G.; BERENSTEIN, C.K.; LEITE, V.H.R.
Departamento de Anatomia Patológica da Faculdade de Medicina da Universidade Federal de Minas Gerais, Belo Horizonte - Minas Gerais.

Carbon tetrachloride (CCl₄) is a well-known hepatotoxic agent, which is widely used in mechanism studies of hepatic injury. Therefore, no reference exist about cytoplasmic inclusion bodies detected in hepatocytes in CCl₄-induced liver injury. So, the authors intend to re-examine the chronic liver injury produced by CCl₄ and a light and electron microscopy approach of these bodies will be done.

The drug was administered to Holtzman female rats by subcutaneous route (0.1mL/100g body weight) twice a week. The livers were examined 12, 24 and 48h after 23 doses of CCl₄. One-mm² samples were fixed in 3% glutaraldehyde and semithin sections were stained with toluidine blue and examined under electron microscopy. PAS after diastase semithin sections were examined for identification of cytoplasmic bodies and ultrathin sections of areas with this bodies were made using diamond knife.

After 23 doses of CCl₄, the liver appeared pale and slightly yellowish in color, with granular to nodular surface containing adhesions between lobes and with abdominal organs or structures (see image 1). The histological appearance of cirrhosis was evident such as thick bundles of connective tissue, defined nodules isolating a variable number of hepatocytes, segments of lobules or entire lobules (see image 2).

The cytoplasmic bodies were identified as eosinophilic (variable degree of eosinophilia), vitreous, homogeneous, single or multiple, round or oval in sharp, sharp contour, PAS-positive-diastase resistant, showing immature and mature aspects. They were found in hepatocytes of nodules and in that one isolated from septa (see images 3, 4 and 5).

So, beyond CCl₄ injuries known (fatty degeneration, ballooned hepatocytes, centrilobular necrosis, apoptotic hepatocytes, cirrhosis), it is necessary to emphasize the occurrence of these bodies.

REFERENCES:

ULTRASTRUCTURE OF HEPATOCYTE INCLUSION BODIES AFTER CCl₄ INTOXICATION IN THE RAT

BORDONI, L.S.; COELHO, A.C.P.; VELOSO, S.G.; BERENSTEIN, C.K.; LEITE, V.H.R.

Depart° de Anatomia Patológica da Faculdade de Medicina da Universidade Federal de Minas Gerais, Belo Horizonte, MG.

Carbon tetrachloride (CCl₄) is a well-known hepatotoxic agent, which is widely used in mechanism studies of hepatic injury. The occurrence of cytoplasmic inclusion bodies has not been reported in any studies involving CCl₄ intoxication. Re-examine the chronic liver injury produced by CCl₄. The drug was administered by sc route (0.1ml/100g) twice a week. The livers were examined 12, 24 and 48h after 23 doses of CCl₄. One-mm³ samples were fixed in 3% glutaraldehyde and semithin sections were stained with toluidine blue and examined under electron microscopy. Eosinophilic, vitreous, PAS-positive, diastase-resistant globular inclusions were found in hepatocytes predominantly peripheral in location, although some were located within the cirrhotic nodules, showing immature and mature aspects. Granular electron-dense material was identified in dilated rough endoplasmic reticulum and also free in hepatic cell cytoplasm. So, beyond CCl₄ injuries known (fatty degeneration, ballooned hepatocytes, centrilobular necrosis, apoptotic hepatocytes, cirrhosis), it is necessary to emphasize the occurrence of these bodies. Similar globules have been described in α₁-antitrypsin deficiency, a model for conformational disease. The chronic CCl₄ action to cell membrane system could change the organellae function and secretion causing cytoplasmic inclusion bodies formation.
Apoio: FAPEMIG (CDS 1305/98)