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Regenerative Ability of Hepatocytes is Inhibited in Early Stages of Liver Fibrosis

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Summary

Female Wistar rats were given three doses of carbon tetrachloride, 10.4 mmol/kg of body weight. The doses were administered within 16 days and another 16 days were allowed to pass before partial (37 %) hepatectomy was done. The liver showed very mild fibrosis at that time. DNA synthesis (measured by ^{3}H-thymidine incorporation) was decreased by 53 % and mitotic activity of hepatocytes was decreased by 56 % when compared to olive oil-pretreated partially hepatectomized controls. The results show that the mitotic potential of hepatocytes in early stages of liver fibrosis is impaired which may influence the course of the disease.

Key words:
Carbon tetrachloride – Liver fibrosis – Liver regeneration – Hepatocyte mitosis

Introduction

Centrilobular necrosis of liver tissue caused by a single dose of carbon tetrachloride is followed by a proliferation of liver cells and the liver appears normal 14 days after injury (Cameron and Karunaratne 1936). The rise in the level of plasma aminotransferases indicating liver damage is followed by an increase in the activity of liver DNA synthesizing enzymes (Nakata *et al.* 1985). Both parenchymal, littoral and bile duct cells respond by division to the injury (Rubin *et al.* 1963, Sutton and Spurgeon 1966). When the damage is repeated, connective tissue septa are formed, with fibres radiating from both the central regions and the portal tracts. Cirrhosis develops in the course of 3 to 5 months (Rubin *et al.* 1963, Pérez Tamayo 1983).

The ability of liver tissue to regenerate may influence the development of fibrosis and cirrhosis. We have therefore examined if the mitotic potential of hepatocytes in injured liver is unchanged. Mild fibrosis was induced in rats by three doses of CCl_4 and after a rest period of 16 days, the rats were subjected to 37 % hepatectomy. The removal of one third of liver tissue evokes a reproducible

regenerative response (Bucher and Swaffield 1964). We found that the wave of DNA synthesis and mitoses that follows the operation was reduced by more than one half in rats with mild liver fibrosis.

Material and Methods

Female Wistar rats were used when they weighed 200–220 g. They were given 3 doses of 10.4 mmol CCl_4/kg of body weight by gastric intubation at 8-day intervals. Carbon tetrachloride was mixed with two volumes of olive oil. Control rats were given olive oil only. Partial hepatectomy was done as described by Higgins and Anderson (1931) and the median liver lobe representing 37 % of liver weight was removed 16 days after the third CCl_4 dosage. Some rats underwent laparotomy only. The rats were killed at different times after surgery. The rats used for the determination of the specific activity of liver DNA were given 7.4 MBq of [methyl-^{3}H]thymidine/kg intravenously 1 h before death. [Methyl-^{3}H]thymidine, specific activity 1.48 TBq/mmol, was obtained from ÚVVVR, Prague. The rats used for the determination of mitotic index of hepatocytes were given 2 mg of colchicine (Fluka, Buchs) per kg of body weight 5–6 h before death. Colchicine was dissolved in saline and injected intravenously.

The median liver lobes obtained at surgery and the left lateral liver lobes obtained at the time of killing were fixed in 10 % formalin. Paraffin-embedded sections were stained with haematoxylin-eosin, with van Gieson stain or they were impregnated with silver. Metaphase nuclei per 1 000 hepatocytes were counted in colchicine-treated rats.

A portion of the left lateral liver lobe was homogenized in cold citric acid (100 mmol/l). The fraction of cell nuclei was hydrolyzed in 5 % perchloric acid at 70 °C for 30 min. DNA content was determined in the extract with diphenylamine reagent (Burton 1956) and radioactivity was counted in an aliquot part after adding Triton-toluene scintillation liquid.

Results

The state of liver tissue was examined histologically 16 days after the third dosage of carbon tetrachloride. Haematoxylin-eosin staining revealed the presence of single macrophages in the centres of liver lobules, containing brownish pigment, possibly a consequence of the resorption of dead hepatocytes. Gömöri impregnation revealed scarring in the central regions of the liver lobules. Proliferating bile ducts were observed in the periportal regions. They were surrounded by reticulin fibres and by mild inflammatory infiltration. Some portal spaces were dilated. Fig. 1 (see Plate 2) shows a newly formed band of connective tissue with capillaries and a bile duct. Branches of reticulin fibres spread from the band radially into the surrounding parenchyma. The reticulin fibres could be detected by silver impregnation but not by van Gieson stain.

The incorporation of ^{3}H-thymidine into liver DNA increased after the removal of 37 % of liver tissue. The maximum was achieved at 42 h when the specific activity of liver DNA was about tenfold higher than that in sham-operated rats. The incorporation was lower in the rats pretreated with CCl_4 than in the olive oil-pretreated rats at all time points examined within 6 days after the operation. It returned to normal by the end of this period (Fig. 2). Mitotic index of hepatocytes was determined 48 h after 37 % hepatectomy. It was by 56 % lower in the rats pretreated with CCl_4 than in the rats that received olive oil only (Table 1).

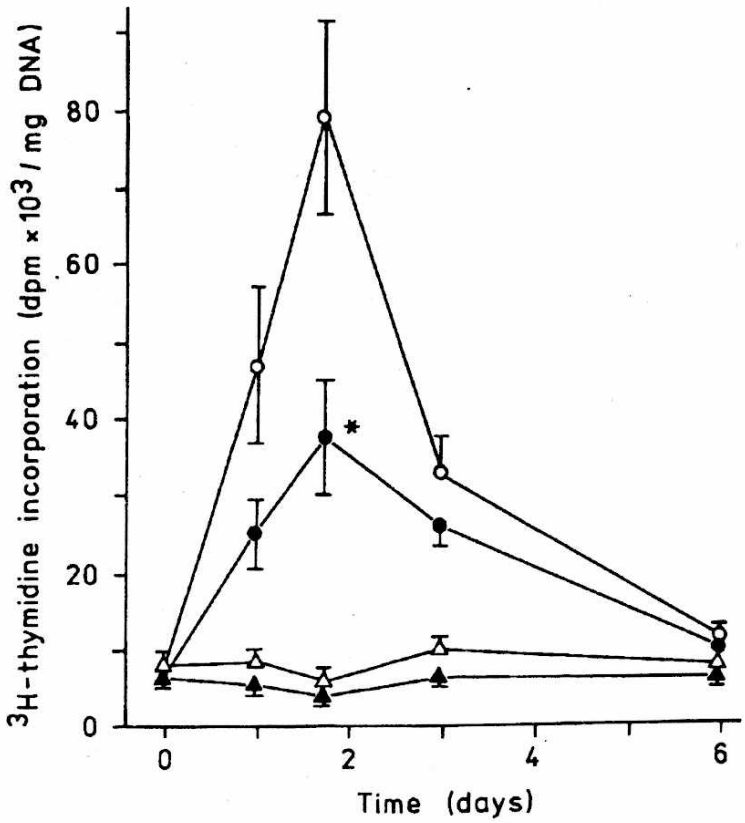

Fig. 2
The effect of CCl_4-pretreatment on ^{3}H-thymidine incorporation into liver DNA at different times after 37 % hepatectomy. Olive oil-pretreated rats (open symbols), CCl_4-pretreated rats (full symbols), laparotomy (triangles, n=5–6), partial hepatectomy (circles, n=8). Means ± S.E.M. Asterisk indicates statistical significance ($P<0.02$) of the difference between oil- and CCl_4-pretreated partially hepatectomized rats. All results obtained in partially hepatectomized rats were significantly different from the results in laparotomized rats at 24, 42 and 72 h.

Table 1
Influence of carbon tetrachloride pretreatment on hepatocyte mitoses after 37 % hepatectomy

Pretreatment	Operation	Number of rats tested	Number of metaphase nuclei
olive oil	37 % hepatectomy	14	9.07 ± 1.67[a]
CCl_4	37 % hepatectomy	14	4.00 ± 1.54[a,b]
olive oil	sham operation	5	0.40 ± 0.24[b]

Means ± S.E.M. a – $P<0.05$ when compared to sham operated rats, b – $P<0.05$ when compared to olive oil-pretreated hepatectomized rats

Discussion

Mild pathological changes induced in rat liver by three doses of 10.4 mmol CCl_4/kg of body weight caused more than a 50 % reduction of ^{3}H-thymidine incorporation into liver DNA and of mitotic counts in liver regenerating after 37 % hepatectomy. Liver regeneration after one-third hepatectomy is slow. Total DNA content that was reduced by the operation to 63 % of the original value, increased during 6 days of the experiment to 75 %. The differences in the DNA content between CCl_4- and olive oil-pretreated rats were not significant. They were probably influenced by the amount of DNA present in the proliferating bile ducts, in inflammatory cells and in fibroblasts present in the fibrotic liver. The weight of the liver remnant also was not influenced significantly. However, labelled thymidine incorporation and hepatocyte mitoses indicated that liver cell proliferation was impaired at the early stage of liver fibrosis.

The regenerative response of cirrhotic liver to another dose of CCl_4 was studied previously by Leevy *et al.* (1962). On the basis of autoradiographic analysis they concluded that DNA synthesis was of the same magnitude in inactive cirrhosis after reinjury as in normal rats receiving their first injury with CCl_4. In a similar experiment Panduro *et al.* (1988) observed only an insignificant difference in labelled thymidine incorporation into liver DNA of rats with advanced fibrosis when compared to controls.

In contrast to these results we found that regenerative potential of hepatocytes was decreased at the beginning of liver fibrosis. The cells did not respond adequately to a proliferation stimulus provided by one-third hepatectomy. The biosynthesis of collagen and of other connective tissue components is the central event in cirrhosis development (Pérez Tamayo 1983). Our results show that the progression of the disease and the gradual decrease of parenchyma to connective tissue ratio in the liver may be facilitated by reduced mitotic ability of hepatocytes, at least at the beginning of the process.

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Plate 2 – Kanta and Chlumská

Fig. 1
Liver of a CCl_4-treated rat 16 days after the third dosage. A connective tissue band with blood vessels and a bile duct originating in the portobiliary space in the left. Reticulin fibres branch off the connective tissue band and run through Disse spaces along sinusoids (x 180, Gömöri stain).

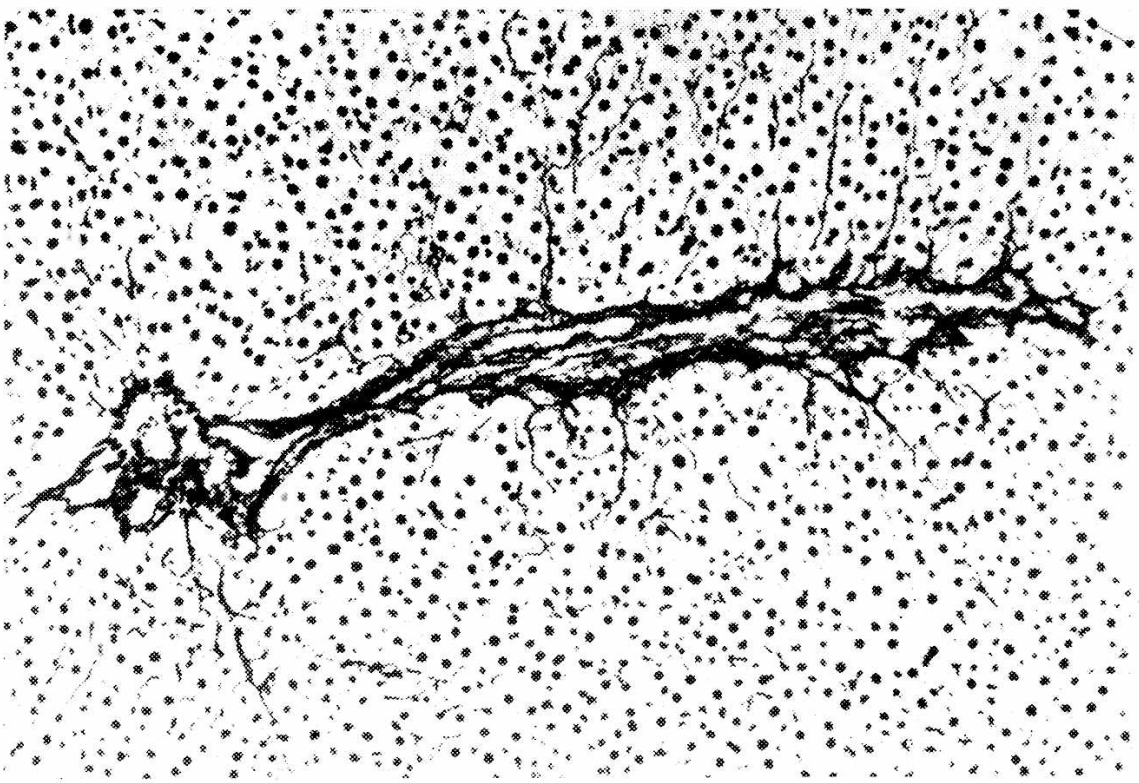

Plate 1 – Thesleff

Fig. 10
High endocytic activity in the endplate region of denervated muscle fibres. a) 12-days denervated mouse hemidiaphragm 2 h after an intravenous injection of horseradish peroxidase. Arrow denotes segments with high endocytotic activity which occur in the endplate region of the denervated muscle. Bar 2 mm. b) Longitudinal section of 14-days denervated mouse tibialis anterior muscle 2 h after a similar injection of horseradish peroxidase. Peroxidase staining appears with a spindle-like distribution in one of the fibres. Bar = 100 μm. c) Transverse section from a muscle similar to b). One fibre contains peroxidase staining with a ring-like distribution. Bar = 50 μm (by courtesy of R. Libelius and S. Tagerud).