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RAPID COMMUNICATION

Hepatocyte Proliferation in Silicotic Rat Liver After Partial Hepatectomy

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Summary
Liver silicosis was induced in rats by an intravenous injection of silica particles. The presence of silicotic granulomas in the liver lowered the initial response of hepatocytes to partial (37 %) hepatectomy and slowed down the decline in the number of dividing cells 6 days after the stimulus, but did not affect significantly the total number of mitotic cells.

Key words
Liver silicosis – Liver regeneration – Hepatocyte mitoses

Liver fibrosis was shown to be accompanied by decreased regenerative ability of hepatocytes. Liver regeneration following carbon tetrachloride application to rats is reduced when the animals were pretreated with a single dose of the toxin. The effects of the pretreatment last for at least 40 days (Kanta *et al.* 1992). Liver damage caused by three subsequent CCl_4 doses impaired the proliferative response of hepatocytes to partial hepatectomy that was performed 16 days later (Kanta and Chlumská 1991).

Liver silicosis can be induced in rats by intravenous administration of silica particles smaller than 5 μm. Macrophage aggregation can be observed in the liver a few hours after the injection and granulomas develop a few weeks later (Kanta *et al.* 1986). In the present study, we have examined whether liver regeneration after partial hepatectomy is influenced by the presence of silicotic granulomas in liver tissue. In a similar study performed in mice, Zucoloto *et al.* (1990) found that fibrosis induced by *Schistosoma mansoni* strongly inhibited hepatocyte labelling with tritiated thymidine after 30 % hepatectomy.

Female Wistar rats (Research Institute for Pharmacy and Biochemistry, Konárovice, body weight 180–200 g) were given an injection of silica particles into the tail vein (Regional Hygienic Station, Ostrava, diameter smaller than 5 μm, 40 mg/kg of body weight). Silica was suspended in saline, control rats received saline alone. The median liver lobe representing 37 % of liver tissue was removed 5 weeks later (Higgins and Anderson 1931). Some rats underwent laparotomy only. The rats were killed at various intervals after surgery (see Figures). Rats in Experiment 1 were given colchicine (Fluka, Buchs) in a dose of 2.2 mg/kg of body weight 5 hours before killing. The tissue was fixed in 10 % formaldehyde. Paraffin-embedded sections were stained with haematoxylin-eosin and the numbers of metaphase nuclei per 1000 hepatocytes were determined. Fig. 1 shows that the maximum number of metaphase nuclei in the liver of rats receiving saline was observed 30 h after partial hepatectomy and it was decreasing during 6 days of the study. The number of metaphase nuclei in rats pretreated with silica reached only 62 % of the values found in the controls at 30 h after partial hepatectomx but it decreased much more slowly. It was equal to 262 % of the control values 144 h after the surgery. Silicotic granulomas had no effect on the number of mitotic hepatocytes in intact and sham-operated rats (not shown).

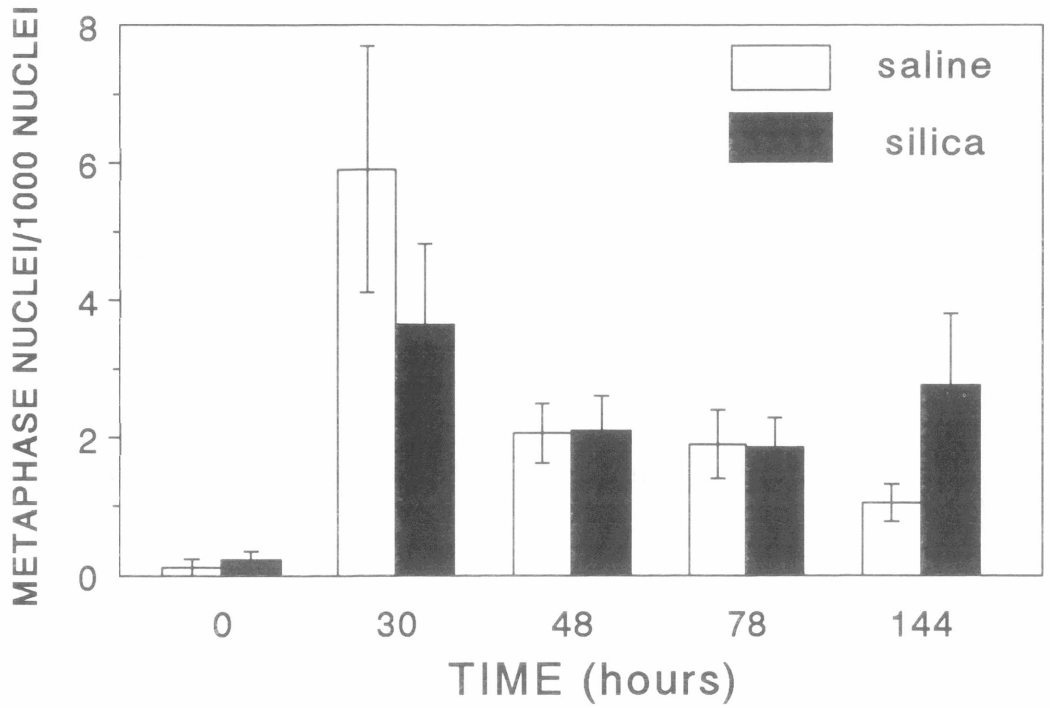

Fig. 1
The influence of silicosis on hepatocyte mitoses after 37 % hepatectomy. Means ± S.E.M. (10 rats in each group, 2000 cells counted in each liver).

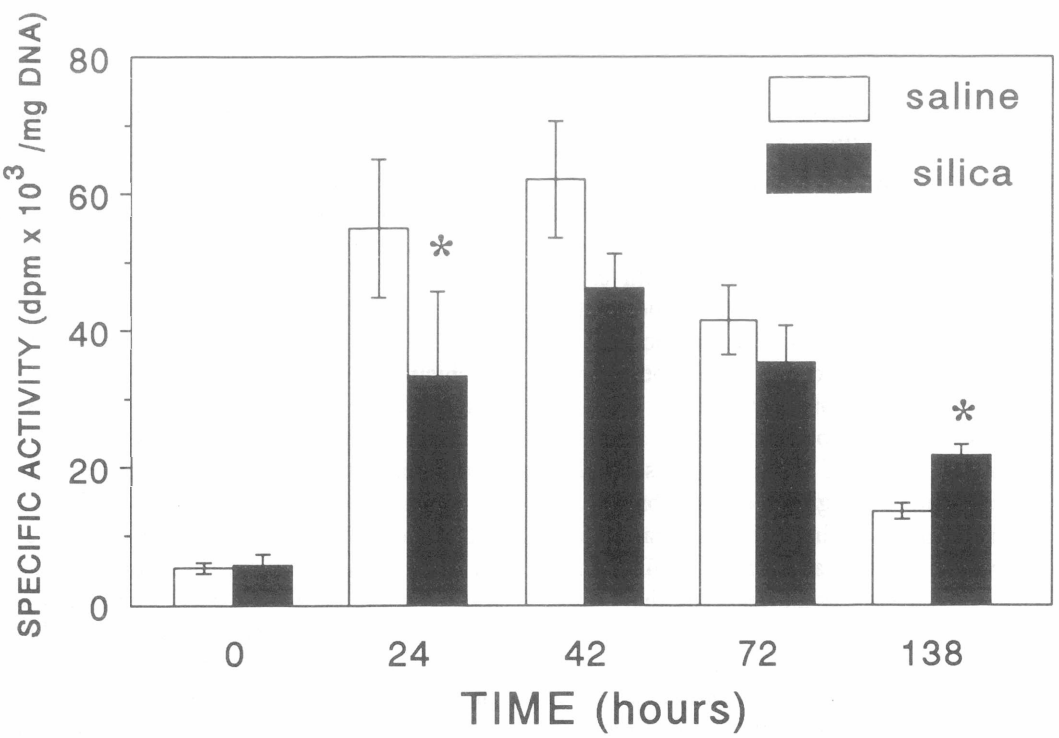

Fig. 2
The influence of silicosis on ^{3}H-thymidine incorporation into liver DNA after 37 % hepatectomy. Means ± S.E.M. (8–9 rats in each group). Asterisks denote statistically significant differences ($p<0.05$).

Rats in Experiment 2 received 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 ÚVVR, Prague. 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. Fig. 2 shows that the specific activity of DNA in the silicotic livers reached only 61 % and 74 % of control values 24 and 42 h after partial hepatectomy, but it was 167 % of the control value at 138 h after the surgery.

The results were evaluated statistically by the Mann-Whitney test. Both experiments gave similar results suggesting that liver regeneration is slightly retarded by the presence of silicotic granulomas.

Various types of liver injury were found to affect liver regeneration negatively (Kanta and Chlumská 1991, Zucoloto *et al.* 1990). Mice respond to 30 % hepatectomy with a burst of cell division and the peak of hepatocyte labelling is found 48 h after the operation. This peak was suppressed almost to zero when mice were infected with *Schistosoma mansoni* 6 months previously (Zucoloto *et al.* 1990). The granulomas found in schistosomiasis result from the response of T-lymphocytes to an immunological stimulus while silicotic granulomas are of the "foreign body" type and macrophages play the main role in their formation. The degree of fibrosis in liver infected with *Schistosoma mansoni* is much larger than that in the silicotic liver. Morcos *et al.* (1985) found the eightfold increase in collagen content in schistosomiasis while Kanta *et al.* (1986) observed only a 1.6 fold increase in collagen content per gram of liver tissue. Collagen deposits in Disse spaces and in interhepatocellular locations such as observed in chronic schistosomiasis by Grimaud and Borojevic (1980) may affect hepatocyte proliferation negatively. Different cytokines secreted by the cells composing the granulomas may also affect hepatocyte proliferation differently. Liver silicosis, at least in its early stage, influences the course of liver regeneration but not its extent.

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Reprint Requests

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