Effects of dosage and cadmium pretreatment on the binding of cadmium in rat bile

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Summary. The effects of dosage and of cadmium pretreatment on the binding of cadmium in rat bile were studied. With increasing dose a higher cumulative biliary excretion of Cd was observed and a higher percentage of the Cd was excreted in a low-molecular-weight form. On the other hand, after cadmium pretreatment, a decrease in the cumulative biliary excretion of cadmium was observed but a greater percentage of that excreted into the bile was bound to high molecular weight compounds.

Within the last few years some data of considerable importance on the biliary excretion of cadmium have been obtained. Biliary excretion of Cd increases with increasing dose of Cd administered[1-4]. Cadmium in bile is bound both to high- and low-molecular weight bile components[1,5].

Cd pretreatment decreases the toxicity of the subsequently administered toxic dose of cadmium[6]. In rats pretreated with Cd higher uptake of Cd in the liver and lower excretion via urine and feces, as well as lower biliary excretion, were found[7-9]. Cadmium pretreatment induces formation of metallothionein in the liver thus increasing the binding capacity of the liver for Cd[7,9,10]. In this study we examined the effect of the dose and Cd pretreatment on the biliary excretion of Cd and binding of Cd in rat bile.

Material and methods. Female Wistar rats (mean weight 200 g) were used in the experiment. Cannulation of bile duct and collection of bile were performed in the same way as described earlier[11]. $CdCl_2$ was administered i.v. in the doses 0.6, 1.25 and 2.625 mg of Cd/kg b.wt. In experiments both the radioisotope ^{115m}Cd and the stable isotope of Cd were used. In the 1st case the bile samples were measured in the well-type scintillation counter Tesla. The concentration of the stable isotope of Cd was determined with a Varian-Techtron Atomic Absorption Spectrophotometer model AA-4[5].

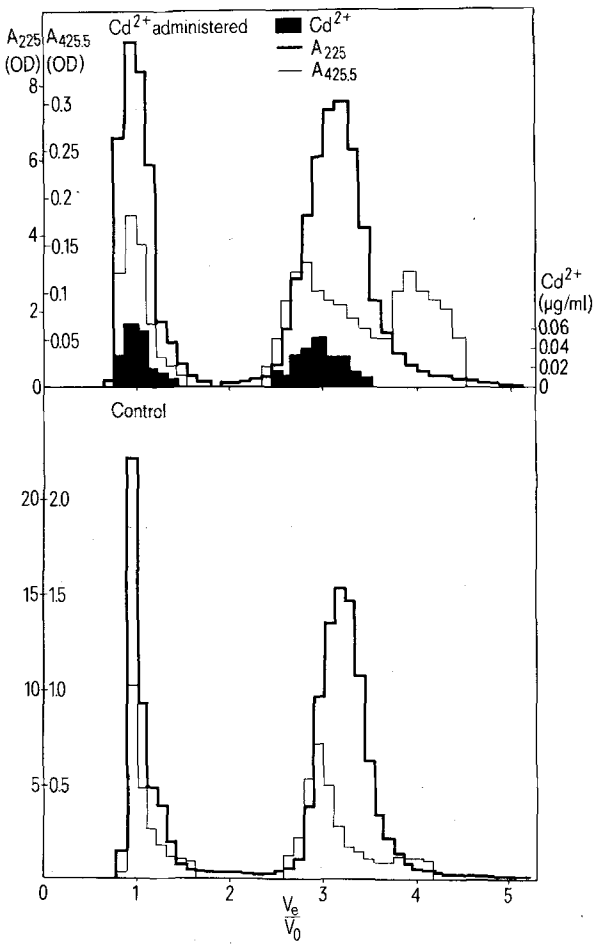
Effects of dose and Cd pretreatment on the binding of Cd in rat bile

Dose (mg of Cd/kg b.wt)	Fraction I	Fraction II	Cumulative biliary excretion per 5 h (percent of the administered dose)
0.6*	55.8 (43.0-68.6)	44.2 (31.4-57.0)	4.8 ±1.7
0.6 PRE-B	85.3 (82.5-88.2)	14.7 (11.8-17.5)	0.03±0.01
1.25*	9.3 (8.1-10.2)	90.7 (88.1-92.6)	5.6 ±0.9
1.25 PRE-A	83.5 (80.6-86.4)	16.5 (13.6-19.4)	0.03±0.02
2.625*	3.1	96.9	12.1 ±2.5
2.625 PRE-A	87.2 (84.4-90.0)	12.8 (10.0-15.6)	0.07±0.01

The bile, collected 0-3 h after i.v. administration of $CdCl_2$, was fractionated using column chromatography (Sephadex G-100). Fraction I (V_e/V_0 at 'void volume'), Fraction II (V_e/V_0 at 2.8-3.4). The conditions of the chromatographic separation are in the legend to the figure. PRE-A. The rats were pretreated with 2 s.c. doses of $CdCl_2$ (2.5 mg of Cd/kg b.wt) at 48-h-intervals. After further 5 days the rats were given a single i.v. injection of $CdCl_2$ in the doses of 1.25 or 2.625 mg of Cd/kg b.wt. PRE-B. The rats were pretreated with single i.v. doses of $CdCl_2$ (0.6 mg of Cd/kg b.wt). After 20 h have elapsed, the 2nd i.v. dose of $CdCl_2$ (0.6 mg of Cd/kg b.wt) was administered. * Without any pretreatment. The values shown in table are expressed in percentage of the total quantity of Cd found in both fractions I and II (means and limit values from 1-3 chromatographic fractionations). In case of cumulative biliary excretion of Cd-means and 95% confidence intervals for means; number of rats: 6-8.

Cd pretreatment: a) The rats under study (20) were pretreated with 2 s.c. doses of $CdCl_2$ (2.5 mg of Cd/kg b.wt) at 48 h intervals. 5 days later the rats were given a single i.v. injection of $CdCl_2$ in the following doses: 1.25 mg or 2.625 mg of Cd/kg b.wt. b) The 2nd group of rats (6) were pretreated with a single i.v. injection of $CdCl_2$ (0.6 mg of Cd/kg b.wt). 20 h later a 2nd i.v. injection of $CdCl_2$ (0.6 mg of Cd/kg b.wt) was given.

The bile, collected 0-3 h after i.v. administration of $CdCl_2$, was fractionated using column chromatography on Sephadex G-100 to obtain a metal-binding pattern of bile



Chromatography on Sephadex G-100 of the bile collected during the 1st 3 h after i.v. injection of 0.6 mg of Cd^{2+}/kg, given as $CdCl_2$, in 1 ml saline solution. Elution curves were plotted in absorbance values measured at 225 nm (——), 425.5 nm (——) and in Cd^{2+} concentration µg/ml (black). 108 µg of freeze-dried bile were dissolved in 1.0-1.5 ml of formate buffer (0.1 M, pH 7.4) and applied to a Sephadex column. Fractions of 3.7-4.7 ml were collected at a flow rate of 6.3 ml/h[5].

components in the same way as described earlier[5]. The cadmium content determined in the high-molecular weight fraction I (V_e/V_o at 'void volume') was compared with that of cadmium found in the low-molecular weight fraction II (V_e/V_o 2.8–3.4). The results were expressed as a percentage of the total quantity of cadmium found in both fractions.

Results and discussion. The figure shows a typical result of column chromatography on Sephadex G-100 of the bile samples collected during the 1st 3 h after i.v. administration of 0.6 mg of Cd/kg b.wt[5]. Cd^{2+} cations were eluated both at 'void volume' (fraction I) and in the fraction with V_e/V_o around 3 (fraction II). The quantity of cadmium in both fractions was approximately the same.

In the table the amounts of Cd found in both fractions I and II in relation to the dose administered and to Cd pretreatment are shown. Results are expressed in percentage of the total quantity of cadmium found in both fractions after chromatographic fractionation. The table clearly shows that the cadmium content of the low-molecular weight fraction II increases when higher doses of Cd are administered. On the contrary Cd pretreatment increases Cd content in the high-molecular weight fraction I. These results are in a good conformity with cumulative biliary excretion of Cd: with increasing dose of Cd administered, cumulative biliary excretion of Cd increases. It may be therefore said that cumulative biliary excretion of Cd correlates with Cd content in the low-molecular weight fraction II. In case of Cd pretreatment the Cd content in the fraction II decreases, and similarly there is a decrease in the biliary excretion of Cd. After i.v. injection of the dose of 2 mg of Cd/kg b.wt, more than 98% of the cadmium in bile samples was associated with a low-molecular weight compound, with molecular weight less than 4000 (probably Cd-glutathione complex)[1]. After administration the dose of 2.625 mg Cd/kg b.wt we found 96.9% of Cd in fraction II (table). According to Cherian and Vostal[1] the low cumulative biliary excretion of Cd and the higher percentage of Cd accumulation in the liver in rats given low doses suggest that liver initially has a high affinity for cadmium and therefore only a small percentage of Cd is transported to the bile. With higher doses of Cd the cadmium binding sites in the liver may be saturated and more Cd is available for biliary excretion. In this case Cd might form a complex with low-molecular weight compounds in the bile.

Cadmium pretreatment induces formation of metallothionein in the liver resulting in higher retention of Cd in the liver since the Cd-metallothionein complex is only poorly excreted in the bile[7]. In rats pretreated with Cd we found the higher portion of Cd in bile in the high-molecular weight fraction I. The nature of the macromolecules binding Cd in the bile has not yet been identified. It seems that there exists a correlation between the binding capacity of liver for cadmium, the cumulative biliary excretion and the binding of Cd in the bile.

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Does an excess in liver proline increase the accumulation of collagen induced by carbon tetrachloride?

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Summary. A 20-fold, diet-induced increase in liver proline does not result in an increased accumulation of hydroxyproline following chronic carbon tetrachloride administration.

Collagen accumulation is the hallmark of cirrhosis resulting from a variety of different etiologies. It has been shown that collagen accumulation induced by carbon tetrachloride (CCl_4), ethanol and schistosomiasis, both in animals and in humans, is correlated with the levels of free proline in the liver[2–4]. Two possible explanations are conceivable for this correlation: a) that the amount of proline in the liver controls the amount of collagen synthesized or b) that the correlation between the increase in collagen and proline is an epiphenomenon in which proline levels are not causally related to actual collagen synthesis. This distinction, besides its interest in relation to the mechanisms that control collagen synthesis, may have clinical implications in cirrhosis since proline levels in the liver, and thus fibrogenesis, could be modified through manipulations in dietary proline.

In the present experiments we have increased the amount of proline in the diet of animals administered carbon tetrachloride and we have determined its effect on liver proline levels and on collagen levels, measured chemically as hydroxyproline.

Materials and methods. Male Wistar rats weighing 150 g (Canadian Breeding Laboratories, Ottawa, Canada) were divided into 6 groups with 4–5 animals per group: I. CCl_4 – high proline diet; II. CCl_4 – normal chow diet; III. Control (corn oil instead of CCl_4) – high proline diet; IV. Control – normal chow; V. CCl_4 – high alanine diet; VI. Control – high alanine diet.

The high proline diet consisted of a standard rat Purina Chow containing an added 30% l-proline. Animals consumed this diet ad libitum. The high alanine diet served as a control for a non-specific effect of ureogenesis. An excess of aminoacids, through an increased urea synthesis, has been shown to sensitize the liver to hypoxia[5], a condition that might accentuate the CCl_4-induced hepatotoxicity. Therefore, we carried the 2 extra groups of animals in which proline was replaced equimolarly by alanine, an aminoacid known to induce a very high degree of ureogenesis and of necrosis after hypoxia[5].

Carbon tetrachloride was prepared in a 1:1 solution in corn oil and injected s.c. at a dose of 1 ml CCl_4 kg/b.wt, 3 times weekly. Diet manipulation and CCl_4 administration were