Selective Uptake of Cadmium by the Parenchymal Cells of Liver

Kelvin CAIN and David N. SKILLETER

M.R.C. Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road,
Carshalton, Surrey SM5 4EF, U.K.

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The uptake of cadmium *in vivo* into parenchymal and non-parenchymal (sinusoidal) cells of the liver was studied by a cell-separation technique. Liver parenchymal cells accumulate cadmium more readily than do non-parenchymal cells and synthesize metallothionein. It is proposed that cadmium uptake and metabolism from injected CdCl_2 is restricted almost exclusively to the liver parenchymal cells.

Cadmium is a highly toxic element that is accumulated in the liver and kidneys of mammals (Friberg *et al.*, 1974). Irrespective of the route of administration, the liver initially accumulates most of the cadmium dose (for review see Friberg *et al.*, 1974), and acute liver damage will result if the dose is high enough. Sub-toxic exposure to cadmium results in the synthesis of the metal-binding protein metallothionein in both the liver and kidney (for review see Cherian & Goyer, 1978), and this protein has been proposed to provide a detoxifying mechanism for cadmium and other heavy metals and also to function in the homeostasis of the essential Zn^{2+} and Cu^{2+} ions.

It is not known whether or not cadmium uptake and metallothionein synthesis are specific for a particular cell type in the liver. Evidence from histological studies (Hoffman *et al.*, 1975) showing that cadmium-induced damage is predominantly to the liver parenchyma suggests that the cadmium is localized in the parenchymal cells and not the non-parenchymal (sinusoidal) cells. The present paper describes the use of a cell-separation technique to determine the cell specificity of cadmium uptake and metallothionein synthesis in the liver.

Materials and Methods

Materials

Carrier-free $^{109}\text{CdCl}_2$ (specific radioactivity 8.51 MBq/ml, $<1\mu\text{g}$ of Cd^{2+} /ml) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were obtained from sources detailed previously (Cain & Holt, 1979; Skilleter & Price, 1978).

Animal injections and cell-purification techniques

Female rats (200 g body wt.) were injected as described in Table 1. Unless otherwise indicated the

injection solution was made up in iso-osmotic saline (0.9% NaCl) containing the required dose of Cd^{2+} as $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ and labelled with $^{109}\text{CdCl}_2$. At the indicated times animals were anaesthetized with 7.5 mg of Sagatal/100 g body wt., and liver parenchymal and non-parenchymal cells were prepared by the method of Seglen (1973) as described previously (Skilleter & Price, 1978). Briefly, the method uses a collagenase-perfusion technique to produce a dispersed liver preparation known as the initial cell suspension. The initial cell suspension is then subjected to various centrifugations to produce four fractions: (1) parenchymal-cell suspension (containing less than 2% non-parenchymal cells); (2) a non-parenchymal-cell suspension completely free of parenchymal cells; (3) a 50 g supernatant that is largely broken parenchymal cells and some non-parenchymal cells; (4) a 500 g supernatant that is a cell-free supernatant containing cell debris and any soluble proteins released by cell damage. Damage to the plasma membrane was determined by the Trypan Blue-exclusion test, and cell numbers were estimated in a Neubauer counting chamber.

Metallothionein identification

Cytosol fractions were prepared from the cells by homogenization in 10 mM-Tris/HCl buffer, pH 8.0, in a Potter–Elvehjem homogenizer and centrifuging at 40 000 rev./min (100 000 g, r_{av} 5.8 cm) in a Beckman 65 rotor for 1 h. The resulting supernatant (cytosol) was then analysed by Sephadex G-75 gel filtration and D-52 DEAE-cellulose anion-exchange chromatography as previously described (Cain & Holt, 1979).

Analytical techniques

The $^{109}\text{Cd}^{2+}$ radioactivity was counted in a Packard model 5330 Auto Gamma spectrometer, with a sample of the injection solution as a reference

standard. Alternatively, total Cd^{2+} was analysed by atomic absorption by spraying a small volume (approx. 0.5 ml) of the eluate directly into the air/acetylene flame of a Perkin-Elmer 460 spectrophotometer.

Results and Discussion

Expt. A of Table 1 shows the distribution of $^{109}\text{Cd}^{2+}$ in the various liver cell fractions after the injection of a sub-lethal dose (LD_{50} dose = 1.77 mg of Cd^{2+}/kg body wt.; Samarawickrama & Webb, 1979) of 0.25 mg of Cd^{2+}/kg body wt. Over 50% of the injected dose was found in the dispersed liver preparation (initial cell suspension) 30 min after injection, a finding that agrees with previous studies (Decker *et al.*, 1957; Perry & Erlanger, 1971) showing the rapid clearance of cadmium from the blood and concomitant liver uptake. In the initial cell suspension 51% of the $^{109}\text{Cd}^{2+}$ present was associated with the parenchymal-cell fraction, a value that corresponds to an estimated 92% of the hepatic burden of the metal being located in the liver parenchyma. Most of the remaining $^{109}\text{Cd}^{2+}$ in the initial

cell suspension (45%) was found in the 50 g and 500 g supernatants, and this probably represents the cadmium liberated from damaged cells. In contrast, only 0.3% of the $^{109}\text{Cd}^{2+}$ present in the initial cell suspension was found in the purified non-parenchymal-cell fraction. However, the cell cadmium content of this cell fraction was found to be about one-sixth that of the parenchymal cells. This would correspond to only 8% of the total metal in the liver being in the non-parenchymal cells (Table 1, Expt. A). The possibility that $^{109}\text{Cd}^{2+}$ was washed out of the liver cells during perfusion was eliminated, since analysis of the perfusion fluids showed a virtual absence of radioactivity.

It is significant that the sub-lethal dose of 0.25 mg of Cd^{2+}/kg body wt. produced a marked increase in cell damage. It is possible that, if this occurred in the non-parenchymal cells, this would release the cadmium and thereby account for the very low cadmium content. Expt. B of Table 1 was designed to test this hypothesis and shows that after a tracer dose of ^{109}Cd the radioactivity of the parenchymal cells was about 10 times that of the non-parenchymal cells. Also, the estimated proportion of total liver

Table 1. Cadmium uptake into liver parenchymal and non-parenchymal cells

Three experiments are shown in the Table in which each rat received ^{109}Cd -labelled CdCl_2 followed by the subsequent liver-cell separation and purification procedures described in the Materials and Methods section. In Expt. A the rat received an intravenous injection of a sub- LD_{50} dose of cadmium (0.25 mg of Cd^{2+}/kg) followed 30 min later by anaesthesia and liver-cell separation. In Expt. B the rat was treated as in Expt. A except that a tracer dose of $^{109}\text{CdCl}_2$ (3.2 ng) was used. In Expt. C the rat received two subcutaneous injections of 1.5 mg of Cd^{2+}/kg on alternate days and was then left for 1 week before the liver-cell separation techniques were performed.

Cell preparation	Total ^{109}Cd [c.p.m. (%)]	Cd content of cell fraction (c.p.m./ 10^6 cells)	Cells excluding Trypan Blue (%)*	Estimated Cd in total liver (%)†
Expt. A				
Initial cell suspension	109 900 (100)			
Parenchymal	56 105 (51)	118	55	92
50 g supernatant	41 860 (38)			
Non-parenchymal	400 (0.3)	20	100	8
500 g supernatant	8260 (7)			
Expt. B				
Initial cell suspension	243 800 (100)			
Parenchymal	150 240 (61)	428	80	95
50 g supernatant	49 000 (20)			
Non-parenchymal	1015 (0.4)	41	100	5
500 g supernatant	27 825 (11)			
Expt. C				
Initial cell suspension	13 546 000 (100)			
Parenchymal	717 950 (53)	1191	77	93
50 g supernatant	353 070 (26)			
Non-parenchymal	4800 (0.3)	160	100	7
500 g supernatant	286 794 (21)			

* Normal Trypan Blue exclusion of our preparations is routinely about 70–80% for parenchymal cells.

† Values calculated assuming that liver comprises approx. 65% parenchymal and 35% non-parenchymal cells (Blouin *et al.*, 1977).

cadmium in the latter cells had fallen to 5%. Moreover, the percentage of undamaged parenchymal cells is equivalent to control values (80%), and, as expected after this non-toxic dose, the cadmium content of the 50g supernatant fraction relative to Expt. A is low. It is therefore probable that the very low radioactivity associated with the non-parenchymal cells represents either a relatively poor uptake of cadmium by these cells or alternatively residual contamination brought about by the separation procedures. Evidence that the former alternative is correct comes from preliminary experiments (D. N. Skilleter, unpublished work) showing that in culture parenchymal cells are much more efficient at accumulating $^{109}\text{Cd}^{2+}$ than are non-parenchymal (Kupffer) cells.

These experiments demonstrate that after an acute dose of cadmium the metal is taken up predominantly by the parenchymal cells. However, as mentioned in the introduction, chronic exposure to cadmium leads to the induction of metallothionein synthesis. Furthermore, there is evidence that cadmium is transferred from the liver to the kidney throughout the duration of exposure (Gunn & Gould, 1957). There is also evidence of cadmium redistribution within the liver (Berlin & Ullberg, 1963), and it may be that the non-parenchymal cells because of their phagocytic properties could be implicated in this process. However, Expt. C of Table 1 shows that even in rats injected 1 week before the cell-separation procedures there is still no evidence of any increased cadmium content of the non-parenchymal cells. At this stage most of the cadmium would be bound to metallothionein and hence be non-toxic, and this is reflected in the higher proportion of undamaged cells (77%). Fractionation of the parenchymal cells revealed that 74% of the cellular cadmium was in the cytosol. Gel filtration of the cytosol (Fig. 1a) showed that 91% of the radioactivity was eluted at a V_e/V_0 ratio of 1.8–2.0. The peak had a high 254 nm absorbance, and on anion-exchange chromatography showed two major peaks (Fig. 1b). The chromatographic properties of the soluble cadmium-binding protein isolated from the parenchymal cells are all characteristic of metallothionein isolated from whole liver (Cherian & Goyer, 1978), and it may be concluded that the parenchymal cells selectively take up cadmium from the blood and synthesize liver metallothionein.

Similar chromatographic procedures were carried out on the non-parenchymal cells. However, because of the very low yield it was only possible to employ an analytical Sephadex G-75 column, and this revealed the presence of a single peak that was eluted at a V_e/V_0 ratio of approx. 1.5. It was not possible to determine whether or not this was a metallothionein.

The mechanisms of cadmium uptake into the

Fig. 1. Characterization of $^{109}\text{Cd}^{2+}$ -binding protein isolated from the cytosol of parenchymal cells

A cell cytosol fraction was prepared as described in the Materials and Methods section from parenchymal cells isolated from two rats that had received cadmium as described in Expt. C of Table 1. A 4 ml portion was chromatographed on an analytical Sephadex G-75 column (80 cm × 1.5 cm) (fraction volume 2.3 ml) and the result is shown in (a). A further 15 ml was chromatographed on a preparative Sephadex G-75 column (80 cm × 2.5 cm) (fraction volume 4.9 ml) and in this case the eluate fractions were analysed for Cd^{2+} by flame atomic absorption spectrometry. The resulting fractions corresponding to a V_e/V_0 ratio of 1.8–2.0 (i.e. the metallothionein fraction) were pooled and run on to a D-52 DEAE-cellulose anion-exchange column (30 cm × 1.5 cm) equilibrated with 10 mM-Tris-HCl buffer, pH 8.0. The column was then eluted with a linear gradient of 10–200 mM-Tris/HCl buffer, pH 8.0 (400 ml, fraction volume 2.9 ml), and the result is shown in (b).

liver are poorly understood. Experiments with liver parenchymal cells in culture (Failla *et al.*, 1979) have indicated that cadmium influx is temperature- and concentration-dependent, characteristics similar to those reported for zinc uptake into these cells (Failla & Cousins, 1978). It is possible therefore that cadmium is transported into the parenchymal cell by the zinc carrier. The poor cadmium uptake

into the non-parenchymal cells may therefore indicate that these cells do not have a zinc-uptake mechanism as efficient as that found in the parenchymal cells.

It is noteworthy that other reticuloendothelial-type cells such as peritoneal macrophages are also inefficient at taking up cadmium (Cox & Waters, 1978), and, although they do possess an endogenous protein similar to metallothionein, there is no evidence of synthesis *de novo* on exposure to cadmium. Metallothionein synthesis therefore may be related to a cell's ability to take up cadmium.

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