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Transfer of liver cadmium to the kidney after aflatoxin induced liver damage

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Cadmium exposure in man and animals leads to the synthesis of metallothionein in the liver and kidney, an increased concentration of the metalloprotein in the cell and the subsequent storage of the metal [1]. Metallothionein (MT) synthesis has been proposed to be a protective mechanism for detoxifying cadmium [2]. However, several workers [3–5] have shown that injected metallothionein is filtered and reabsorbed by the kidney and subsequently is acutely toxic to the kidneys. The biological half-time of Cd^{2+} in the liver is less than that in the kidney and at (or after) exposure there is apparently a slow transfer of the cation from the former organ to the latter [6, 7]. Nordberg *et al.* [8] have proposed that the transfer occurs through the release of MT from the liver and its transport in the blood to the kidney. It is possible that liver damage in cadmium-exposed animals (or man) could lead to the rapid transfer of large amounts of MT to the kidney and thus to renal dysfunction even though the concentration of cadmium in this target organ remains below the generally accepted critical level of 200 p.p.m. Although Webb and Etienne [5] were unable to detect such a redistribution after the administration of the hepatotoxins CCl_4 and retrorsine to cadmium-exposed rats, the work described herein shows that transfer of cadmium to the kidney does occur after liver damage is induced with aflatoxin. The implications of this finding to the toxicity of cadmium are discussed.

To produce high hepatic contents of cadmium, male Fischer rats (200 ± 10 g) were injected subcutaneously with three doses on alternate days of 1 mg Cd^{2+}/kg body wt, followed by three doses of 2.5 mg Cd^{2+}/kg body wt. Control animals were treated in the same manner except that they received equivalent volumes of saline (0.1 ml/100g body wt). The animals were then left for 1 month to recover from the injection regime.

Liver damage was initiated by an intra-peritoneal (i.p.) injection of aflatoxin B_1 (0.5 mg/kg body wt, Makor chemicals, Jerusalem, Israel) as a solution (0.5 ml/kg body wt) in spectroscopic grade dimethylsulphoxide (BDH Chemicals Ltd., Poole, Dorset, U.K.). Control animals received equivalent volumes of dimethylsulphoxide (DMSO). Aflatoxin is a potent hepatotoxin [9] and the dose used in this experiment has been shown to produce a marked periportal necrosis, with bile duct proliferation and oval cell infiltration within 48 hr of the injection (Dr. G. E. Neal, personal communication). Consequently, the four groups of animals (i.e. Group 1, Cd/DMSO; Group 2, Cd/aflatoxin; Group 3, saline/aflatoxin; Group 4, DMSO) were killed 48 hr after the administration of aflatoxin, saline or DMSO. The livers and kidneys were removed, weighed and small aliquots taken for metal determination and histology. The remaining tissue was frozen in liquid N_2 and then stored at −20°. The cadmium–metallothionein content of the frozen tissues was determined, as previously described [10], by preparing a 100,000 g supernatant of the tissue and separating the metallothionein on a Sephadex G-75 column. (Pharmacia Ltd., London, U.K.).

Cd^{2+}, Zn^{2+} and Cu^{2+} were assayed by atomic absorption spectroscopy using a Perkin–Elmer 460 spectrophotometer. Tissue aliquots were digested with a mixture of "Aristar" perchloric and nitric acid (1:4) as described [10] before redissolving in 5% HCl and analysing for metals. Column fractions were analysed directly. Samples for histology were fixed in formol alcohol and mounted in paraffin wax. Sections were cut at 5 μm and stained with either haemotoxylin–eosin or PAS.

Aflatoxin produced a characteristic histological damage in the livers of both the aflatoxin and Cd/aflatoxin animals. However, the extent of the lesions in the Cd/aflatoxin was significantly less than with aflatoxin alone, and it may be that the cadmium treatment has some protective effect against aflatoxin. The cadmium concentration in the liver after the aflatoxin treatment was unaffected but the total cadmium content was reduced by approximately 240 μg (Table 1). There was also an accompanying marked liver weight decrease, with the Cd/aflatoxin group showing a 3 *g* (25 per cent) decrease in liver weight with respect to the Cd/DMSO group (Table 1) and the saline/aflatoxin group showing a (30 per cent) decrease with respect to the DMSO control.

The metal contents of the livers from the Cd/DMSO and Cd/aflatoxin are shown in Table 1. In terms of Cd^{2+} concentration there is no significant difference; however, the total Cd^{2+} content of the liver is decreased by approximately 240 μg (i.e. a 15 per cent decrease in the liver burden) and thereby reflects the decrease in liver weight. There is an even greater effect on the zinc (31 per cent decrease) and copper content (51 per cent).

The loss of Cd^{2+} from the livers of the Cd/aflatoxin animals is paralleled by an increase in the Cd^{2+} content of the kidneys. Histologically, aflatoxin produced no significant damage in the kidneys and the organ weights were similar; consequently, both the Cd^{2+} concentration and total Cd^{2+} content were increased by 42 and 36 per cent respectively. There was little or no change in the copper and zinc content.

It is known that approximately 80 per cent of the cadmium burden in the liver and kidneys of Cd^{2+} exposed rats is bound to the inducible protein, metallothionein. In Table 2 the metallothionein content of the liver and kidneys from the treated groups are shown. There is a clear increase in the kidney levels of the Cd/aflatoxin treated compared to the Cd/DMSO group and this is paralleled by a decreased liver content in the Cd/DMSO group.

These results show that the liver damage produced by aflatoxin leads to a decreased liver weight and loss of Cd^{2+}, Zn^{2+} and Cu^{2+}. In the case of the Cd^{2+}, approximately 240 μg is lost from the liver, whereas the kidney gains 100 μg. If it is assumed that aflatoxin only affects the liver Cd^{2+} burden, then the kidneys have accumulated 41 per cent of the liberated cadmium. This would indicate that the cadmium was in a form which was filtered and reabsorbed in the kidneys with high efficiency. Studies on the uptake of cadmium from the blood in the tissues have shown that inorganic forms of Cd^{2+} are normally taken up predominantly by the liver. However, the simultaneous injection of a chelating agent and a cadmium salt results in the kidney and not the liver taking up the metal (see ref. 11 for review). The molar ratio of chelating agent to Cd^{2+} to produce this redistribution to the kidney is very large; for example, Kennedy [12] used cysteine/Cd ratios of 250 to produce cadmium uptake and damage in the kidneys. In contrast, McGivern and Mason [13] demonstrated that Cd^{2+}/chelating agent complexes at a 1:1 ratio

Table 1. The effect of aflatoxin-induced liver damage on the Cd^{2+}, Zn^{2+} and Cu^{2+} content of the liver and kidneys from cadmium-pretreated rats

Treatment	Organ wt (g)	Metal concentration (μg/g)			Total metal (μg)		
		Cd^{2+}	Zn^{2+}	Cu^{2+}	Cd^{2+}	Zn^{2+}	Cu^{2+}
Liver							
Cd/DMSO	11.8 ± 0.4 (N=4)	119.9 ± 6.4 (N=4)	62.6 ± 2.2 (N=4)	6.5 ± 0.2 (N=4)	1418.3 ± 103.3 (N=4)	739.3 ± 35.9 (N=4)	77.2 ± 3.5 (N=4)
Cd/aflatoxin	8.8 ‖ 0.3§ (N=4)	134.6 ± 13.4(ns)‖ (N=4)	52.7 ± 5.0‡ (N=4)	4.5 ± 0.3§ (N=4)	1175.7 ± 74.5† (N=4)	460.9 ± 28.3§ (N=4)	39.4 ± 3.3§ (N=4)
Saline/aflatoxin	7.5 ± 0.4 (N=4)	0 (N=4)	18.5 ± 0.5 (N=4)	9.4 ± 0.4 (N=4)	0 (N=4)	139.2 ± 9.3 (N=4)	70.4 ± 5.0 (N=4)
DMSO	10.7 ± 0.1 (N=4)	0 (N=3)	29.68 ± 1.3 (N=3)	3.97 ± 0.5 (N=3)	0 (N=3)	318 ± 14.5 (N=3)	42.8 ± 5.4 (N=3)
Kidney							
Cd/DMSO	2.2 ± 0.02 (N=3)	129.8 ± 4.5 (N=3)	28.1 ± 0.2 (N=3)	27.2 ± 0.8 (N=3)	279.7 ± 9.2 (N=3)	60.5 ± 0.5 (N=3)	58.7 ± 1.4 (N=3)
Cd/aflatoxin	2.1 ± 0.04‡ (N=4)	185.2 ± 5.9§ (N=4)	26.8 ± 1.8(ns) (N=4)	35.4 ± 4.2‡ (N=4)	379.9 ± 13.2§ (N=4)	55.1 ± 4.3ns (N=4)	72.4 ± 7.8‡ (N=4)
Saline/aflatoxin	2.0 ± 0.04 (N=4)	0 (N=4)	26.5 ± 0.3 (N=4)	7.1 ± 0.02 (N=4)	0 (N=4)	47.5 ± 5.2 (N=4)	14.6 ± 0.6 (N=4)
DMSO	1.9 ± 0.04 (N=4)	0 (N=3)	25.2 ± 1.4 (N=3)	10.28 ± 0.3 (N=3)	0 (N=3)	47.6 ± 1.4 (N=3)	19.5 ± 0.3 (N=3)

* Metal determinations were carried out on duplicate tissue samples taken from the excised tissues, immediately after killing. The procedures for digestion of the tissues by wet ashing and the subsequent metal analysis by atomic absorption are described in the text. The results are expressed as means ± S.E., n = number of animals.

† $P < 0.05$ for comparison between Cd/aflatoxin and Cd/DMSO groups.

‡ $P < 0.01$ for comparison between Cd/aflatoxin and Cd/DMSO groups.

§ $P < 0.005$ for comparison between Cd/aflatoxin and Cd/DMSO groups.

‖ ns = no significant difference between groups.

Table 2. The effect of aflatoxin-induced liver damage on the Cd–metallothionein content of the liver and kidneys from cadmium-treated rats*

Treatment	Metallothionein concentration Cd(μg/g)	Zn(μg/g)	Cu(μg/g)	Total metallothionein Cd(μg)	Zn(μg)	Cu(μg)	Cd/Zn (mole/mole)	Cd/Cu (mole/mole)
Liver								
Cd/DMSO	71.0 ± 3.5 (N=4)	19.7 ± 1.0 (N=4)	1.6 ± 0.3 (N=4)	839.7 ± 55.5 (N=4)	232.8 ± 16.3 (N=4)	18.5 ± 3.5 (N=4)	2.1	25.6
Cd/aflatoxin	79.5 ± 5.1ns§ (N=4)	16.2 ± 1.6 (N=4)	0.7 ± 0.1† (N=4)	696.84 ± 18.9† (N=4)	139.1 ± 7.5‡ (N=4)	6.2 ± 1.1† (N=4)	2.9	64.2
Saline/aflatoxin	0 (N=4)	0.35 ± 0.05 (N=4)	0.5 ± 0.01 (N=4)	0 (N=4)	2.64 ± 0.4 (N=4)	3.7 ± 0.2 (N=4)	—	—
Kidney								
Cd/DMSO	97.7 ± 7.3 (N=3)	8.0 ± 0.6 (N=3)	14.1 ± 1.0 (N=3)	210.4 ± 13.9 (N=3)	17.2 ± 1.5 (N=3)	31.4 ± 2.0 (N=3)	7.1	3.8
Cd/aflatoxin	136.6 ± 6.3† (N=4)	8.1 ± 1.3ns (N=4)	17.9 ± 2.6ns (N=4)	279.8 ± 10.3† (N=4)	15.7 ± 2.6ns (N=4)	36.8 ± 5.3ns (N=4)	9.8 (N=4)	4.3
Saline/aflatoxin	0 (N=4)	6.0 ± 0.4 (N=4)	0.95 (N=4)	0	11.7 ± 0.8	1.8 ± 0.5	—	—

* Metallothionein was isolated by Sephadex G-75 gel filtration as described in the text. The metallothionein calculations are based upon the amount of metalloprotein found in the 100,000 *g* supernatant fraction (cytosol). No correction has been carried out for that proportion of the cytosol which has been trapped in the nuclei and mitochondrial pellets. Thus in this case 70 per cent of the tissue Cd^{2+} was associated with metallothionein and this is therefore an underestimation of the total cellular content of metallothionein.

† $P < 0.05$ for comparison between Cd/aflatoxin and Cd/DMSO groups.

‡ $P < 0.005$ for comparison between Cd/aflatoxin and Cd/DMSO groups.

§ ns = no significant difference between groups.

were taken up by the liver and not preferentially by the kidney. It is unlikely, therefore, that the liver–kidney transfer of cadmium demonstrated in this work is due to the release of Cd/cysteine type complexes from the necrotic liver cells. It is more probable that Cd–metallothionein is released (from the necrotic liver cells) and is subsequently filtered and reabsorbed by the kidney. It has been proposed [5] that the renotoxic effect of Cd–metallothionein is due to the lysosomal degradation of the protein releasing the toxic Cd^{2+}. The lack of kidney damage observed in the cadmium/aflatoxin animals may be due to the fact that the endogenous thionein can bind any Cd^{2+} released from the metallothionein which has been reabsorbed by the kidney and thereby prevent a toxic effect.

In summary, aflatoxin-induced liver damage produces in cadmium-treated rats a loss of hepatic cadmium concomitant with an increase in renal cadmium levels.

It remains to be established whether or not this rapid cadmium transfer is a common effect produced by hepatotoxins in general or is peculiar to aflatoxin-induced liver damage. It is therefore of interest that a recent study has suggested that the induction of zinc–metallothionein protects against CCl_4-induced liver damage [14]. This finding may explain why Webb and Etienne [5] did not produce a cadmium transfer with CCl_4 or retrorsine and the protective effect observed in this study. Currently, other hepatotoxins, particularly those used in industry, e.g. CS_2 and beryllium, are being investigated. If transfer is a general response to such agents, clinically this could be important not only to those individuals currently exposed to cadmium but also to those who have been previously exposed. Thus, for example, an individual may have been industrially exposed to cadmium without any obvious symptoms. At a later stage the individual may suffer hepatic injury (such as alcohol-induced cirrhosis), resulting in a subsequent transfer of cadmium to the kidney, which if it reached the critical concentration (200 p.p.m.) would lead to renal damage.

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Effect of 5-hydroxytryptamine on free amino acid composition of stomach and plasma, and on protein synthesis in the stomach of rats

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5-hydroxytryptamine (5-HT) is distributed throughout the body and is known to be involved in regulating many physiological processes. In stomach and duodenum, where it is present in large amounts, the amine is thought to play a role in the regulation of gastric secretory activities [1–3]. Besides this, the amine is found to affect protein synthesis in gastrointestinal tissues. We have recently demonstrated that a single injection of 5-HT markedly reduces amino acid incorporation into total protein of the stomach, small intestine and colon *in vivo* [4, 5]. The mechanism of inhibitory action of 5-HT is, at present, unknown. However, our earlier observation of higher acid-soluble radioactivity in the stomach during the period of lower incorporation after 5-HT injection [5] indicates that the 5-HT-mediated inhibition of protein synthesis in the stomach is not due to diminished uptake of the precursor amino acid by the tissue. To determine further whether 5-HT would affect the amino acid pool, the concentration of free amino acids in both plasma and stomach was analyzed following a single injection of the amine. The incorporation of [^{3}H]-leucine into total protein of the stomach and the specific radioactivity of the precursor pool were also measured after 5-HT injection. We observed that a single injection of 5-HT caused a marked (60.5 per cent) reduction in [^{3}H]-leucine incorporation into total protein of the stomach *in vivo*, with no change in specific radioactivity of the precursor amino acid. Furthermore, 1 hr after 5-HT administration, the concentrations of free amino acids in plasma (excepting aspartic acid) were decreased, whereas in the stomach they were found to be increased (except for aspartic acid, proline, threonine and glutamic acid) when compared with the corresponding control.

Adult male Wistar rats (200–250 g) were fasted for 24 hr before they were injected (i.p.) with either 0.9% NaCl (saline) or 20 mg/kg 5-hydroxytryptamine creatinine sulfate (Sigma Chemical Co., MO) in saline, and were decapitated