Inhibition of Hepatic Drug Metabolizing Enzyme by Cadmium in Mice

by TAKEMI YOSHIDA, YOKO ITO, YASUO SUZUKI
Pharmaceutical Institute, Tohoku University
Aobayama, Sendai 980, Japan
and MITSURU UCHIYAMA
National Institute of Hygienic Sciences
1-18-1, Kamiyoga, Setagaya, Tokyo, Japan

Recently evidence has been reported that the administration of some metals impaired hepatic drug metabolizing enzymes in animals (RIBEIRO 1970; TEPHLY and HIBBELN 1971; SCOPPA et al. 1973; UNGAR and CLAUSEN 1973; HADLEY et al. 1974). UNGAR and CLAUSEN(1973) have shown that the inhibition of hepatic drug metabolizing enzyme by cadmium is well related to the accumulated content of cadmium in mouse liver when measured 4 days after treatment with cadmium nitrate. Other studies have demonstrated that the administration of cadmium to rats or mice induced cadmium-binding protein, terminated "metallothionein" (NORDBERG et al. 1971; SHAIKH and LUCIS 1972; PIOTROWSKI et al. 1974). Based on these observations, the present investigations were designed to examine in detail the effect of cadmium on hepatic drug metabolizing enzymes and to determine whether metallothionein is involved in the protection of the impaired activity of these enzymes by the administration of cadmium.

EXPERIMENTALS

Animals and treatment: Male ddY mice weighing 22-25 g were used. Cadmium chloride in different concentrations, dissolved in 0.9% NaCl, was administered to mice. Mice were sacrificed at the different times indicated in the figure and tables.

Preparation of liver and analyses: Livers were rapidly excised and homogenized in 1.15% KCl in Potter-Elvehjem homogenizer with a Teflon pestle. The homogenates were centrifuged at 9,000 x g for 30 min. When microsomes were harvested, livers were perfused with cold 1.15% KCl, homogenized and centrifuged as described above. Microsomes were obtained by the cenrifugation of the 9,000 x g supernatant at 105,000 x g for 60 min. The microsomal pellets were then washed once with 1.15% KCl and suspended in 0.1 M phosphate buffer(pH 7.0) so that 1 ml contained 2 mg of microsomal protein.

Aminopyrine N-demethylation and aniline hydroxylation were determined by 9,000 x g supernatant fraction according to the methods of COCHIN and AXELROD(1959) and

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 4 © 1976 by Springer-Verlag New York Inc.

KATO and GILLETTE(1965), respectively. Microsomal cytochrome P-450 content was determined by the method of OMURA and SATO(1964). Protein content in 9,000 x g supernatant and in microsomal fraction was estimated by the methods of GORNALL and BARDAWILL(1949) and LOWRY et al. (1951), respectively.

RESULTS AND DISCUSSION

Fig. 1 shows the time course of the activity of aminopyrine N-demethylation after the administration of cadmium chloride(3 mg/kg) to mice. Administration of cadmium to mice significantly inhibited the activity of aminopyrine N-demethylation in 24 hr and 48 hr, but the activity returned nearly to control values in 72 hr. The duration of the inhibiting effect of cadmium on hepatic drug metabolizing enzymes in mice differed somewhat from that observed by other investigators. UNGAR and CLAUSEN (1973) determined p-nitroanisole O-demethylation activity 4 days after the administration of cadmium nitrate and found a significant decrease in the activity. On the other hand, HADLEY et al.(1974) reported that the potentiation of hexobarbital sleeping times was weak in mice when observed 2 days after the administration of cadmium acetate. It is unclear why such differences occurred.

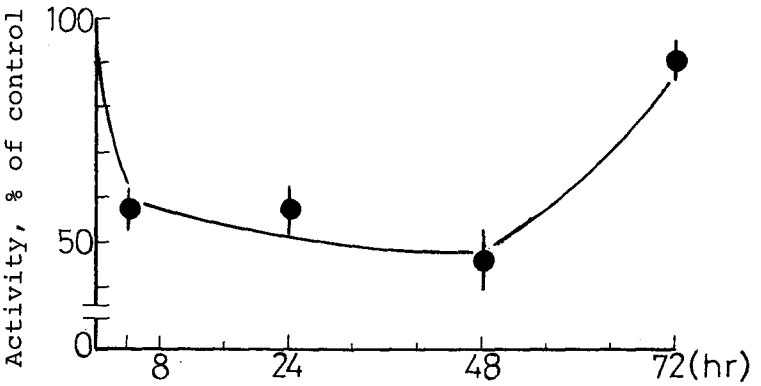

Fig. 1 Time course of aminopyrine N-demethylation activity after the administration of cadmium chloride(3 mg/kg)

In further studies, the 48-hr interval after cadmium administration was chosen for examination, since the potentiation of the inhibition of aminopyrine N-demethylation was maximal, in our experiment, at this time period. Table I shows the dose-related changes in hepatic drug metabolizing enzymes, both aminopyrine N-demethylation and aniline hydroxylation.

TABLE I

Dose(mg/kg)	Aminopyrine N-demethylation	Aniline hydroxylation
Control	13.75 ± 0.16	7.93 ± 0.03
Cadmium chloride		
1	10.56 ± 0.48	6.07 ± 0.28
3	6.54 ± 0.53	3.74 ± 0.25
5	3.31 ± 0.27	2.97 ± 0.22

Enzyme activities are expressed as the mean ± S.E. of the metabolites produced in nmoles/mg protein/30 min. Each value was obtained from 6 to 10 mice.

The intensity of the inhibition of drug metabolizing enzymes by cadmium was dose-dependent, suggesting that the inhibition was a result of a direct action of cadmium upon the drug metabolizing enzymes.

It has been shown that mice exposed to low levels of cadmium survive longer than untreated mice when exposed to a higher dose of metal(YOSHIKAWA 1970, 1973). An experiment was undertaken to demonstrate whether this phenomenon could also be observed at the step of enzyme system(Table II).

TABLE II

Pre-dose (mg/kg)	Challenging dose(mg/kg)	Aminopyrine N-demethylation	Aniline hydroxylation	Cytochrome P-450
Control	Control	17.50 ± 2.28	6.69 ± 0.44	0.746
0.5	5.0	10.14 ± 1.54	1.56 ± 0.22	0.396
1.0	5.0	14.80 ± 0.09	3.08 ± 0.24	-
3.0	5.0	16.48 ± 0.03	6.38 ± 0.30	0.761

24 hr after pre-dosed with cadmium chloride, mice were injected intraperitoneally with the challenging dose. Mice were sacrificed 48 hr after the challenging dose. Enzyme activities are expressed in nmoles/mg protein/30 min. Microsomal cytochrome P-450 content was determined by microsomes prepared from three pooled livers and expressed as the mean of two independent determinations in nmole/mg protein.

Table II suggests that the inhibition of drug metabolizing enzymes by cadmium was due to the decrease in cytochrome P-450 content as observed in rats by HADLEY et al. (1974). The mechanism for the decrease in cytochrome P-450 content by cadmium has not been clarified. SCOPPA et al. (1973) suggested that the impaired activity of hepatic δ-aminolevulinic acid(ALA) dehydratase by lead-poisoning may have resulted in a decreased synthesis of cytochrome P-450. However, this mechanism is not fully applicable to the case of cadmium, since hepatic δ-ALA dehydratase activity was reduce only 20% by the administration of cadmium chloride when compared to that of the control. It is

possible, however, that the impaired δ-ALA dehydratase activity by cadmium may contribute to some extent to the decrease in cytochrome P-450 content.

As can be also seen in Table II, the inhibition of hepatic drug metabolizing enzymes and the decrease in cytochrome P-450 content by the challenging dose were diminished when mice were pre-dosed with cadmium chloride. The degree of the protection against the challenging dose was corelated with the pre-dose levels. The effect of the challenging dose on hepatic drug metabolizing enzymes and cytochrome P-450 content was completely abolished when mice were pre-dosed with 3 mg/kg dose level of cadmium chloride. This phenomenon ceased within 7 days, since the protective effect could not be observed when the interval between pre-dose and the challenging dose was 7 days.

It is of interest to note that when mice were pre-exposed to cadmium, protection against the later higher doses could be observed at the step of enzyme system. Still higher pre-dose levels are, however, needed to effect the profound protection of the inhibition of drug metabolizing enzyme system by the challenging dose than that observed in lethality test by YOSHIKAWA(1970, 1973)

At the present stage of knowledge, it is considered that metallothionein may be involved in the biological protection of animal against the cadmium poisoning. Further studies, however, will be needed to clarify whether metallothionein contributed to the present results.

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