Nutritional Sciences 9(1), February, 2006, pp.20-28
ⓒ 2006 The Korean Nutrition Society

Influence of Dietary Zinc, Copper and Cadmium Levels on Rat Liver Aryl Sulfotransferase IV Activity

Keun Hee Chung[§], David P. Ringer[1] and Kyung Ok Shin

Department of Food and Nutrition, Sahmyook University, Seoul 139-742, Korea
[1]American Cancer Society, Atlanta, GA 30329, U.S.A

Aryl sulfotransferase (AST) IV is a liver enzyme involved in detoxication and has been shown to be susceptible to down regulation by a number of hepatotoxic xenobiotics. Studies presented here to investigate the ability of biological and non-biological divalent metal cations on AST IV activity showed that AST IV was strongly inhibited following *in vitro* or *in vivo* exposure to Zn(II), Cu(II) or Cd(II). It was found that 0.025~2.5 uM of these metal ions were sufficient to cause 50% of inhibition *in vitro* in purified AST IV and 0.25~25 uM of these metal ions in liver cytosolic fractions. For the *in vivo* study, 1,000 mg Cu(II)/kg, 2,000 mg Zn(II)/kg or 250 mg Cd(II)/kg was added to individual diets and administered to three (3) groups of rats over a 7 week period. The Cu(II)-supplemented diet produced no apparent change in rat growth rate and resulted in 30-fold increase in liver cytotolic Cu(II) levels, suggesting that elevated levels of Cu(II) ion in the liver were responsible for the loss of AST IV activity. In contrast, the Zn (II)-supplemented diet caused a decrease in rat growth rates and resulted in zero increase in liver Zn(II) levels, which suggested an indirect inhibition mechanism was caused by Zn(II) in the liver. Rats were fed the Cd-supplemented diet also displayed a decrease in growth rate with little or no change in liver Cu(II) or Zn(II) levels. When the liver cytosols of rats from the metal ion diets were immunochemically analyzed for the AST IV and albumin contents, no significant changes were observed in albumin levels. However, AST IV contents in the cytosols of rats fed the Zn(II)-supplemented diets showed a slight decrease in amount. These results showed that AST IV activity *in vitro* and *in vivo* can be inhibited by Cu(II), Zn(II), and Cd(II) by apparently different mechanisms. The immediate response to a Zn injection showed a decrease in AST IV activity but not in the AST IV content in liver cytosol. These mechanisms appeared to involve direct actions of the metal ion on AST IV activity and indirect actions affecting AST IV amount.

Key words: Aryl sulfotransferase IV, Copper, Cadmium, Zinc, Liver cytosol

Received December 14, 2005; Revised February 2, 2006; Accepted February 9, 2006

INTRODUCTION

An important functions of the liver is to detoxify blood borne xenobiotics by way of enzymatic conjugation to polar water-soluble metabolites. In some instances, the enzymatic conjugations may lead to both detoxication as well as to the production of highly toxic metabolites capable of causing genotoxic and cytotoxic damage. One enzyme involved in such conjugation reaction is aryl sulfotransferase (AST) IV. Specifically, AST IV has been reported[1-11] to be one of the primary enzymatic activities in the liver responsible for generating bioactivated, autogenic metabolites of various arylamine hepatocarcinogens, such as 2AAF. In addition, AST IV activity has been shown to display an unusual susceptibility to inhibition *in vivo* during the dietary administration of a number of known hepatocarcinogens.[12-14] Recently, the ability of AST IV activity to be modulated during hepatocarcinogenesis by 2AAF in the diet has been suggested to have a role in liver cancer development.[30] It has been reported that a rat liver sulfotransferase with structural properties similar to AST IV and able to utilize N-OH-2AAF as an acceptor in sulfate monoster formation was inhibited by various divalent cations, including Zn(II) and Cu(II) cations.[15] Zinc and copper divalent cations have been well characterized as essential to biological functions of living organisms[16] and to play a role in the regulation of some proteins.[17,18] Furthermore, it has been well established that the amounts of these metal cations must be maintained within narrow limits if functional integrity of living organisms is to remain unimpaired.[16] In order to assess

§ To whom correspondence should be addressed.
(E-mail : khchung@syu.ac.kr)

the possible effects of these metals on AST IV activity, studies were conducted *in vitro* and *in vivo* with divalent cations of zinc and copper as well as cadmium, a known antagonist of zinc (II) and copper (II) and animal toxin.[19-29] The results of these studies indicated that (a) purified AST IV and AST IV in liver cytotols were readily inhibited *in vitro* by the addition of zinc (II) and copper (II) and cadmium (II) ions, (b) AST IV activity in the cytosols of rats fed diets supplemented with the zinc (II), copper (II) and cadmium (II) ions or rats injected intraperitoneally with these metal ions were also significantly inhibited, and (c) the mechanism of metal ion inhibition on AST IV activity may involve both decreases in enzyme specific activity and down regulation of enzyme expression.

MATERIALS AND METHODS

1. Animal and Dietary Protocols

Male Sprague-Dawley rats (8 weeks, SASCO, Inc., Omaha, NE) were fed one of five diets for 7 weeks. The diets contained a zinc test diet (#23566) purchased from U.S. Biochemical Corp. (Cleveland, OH) supplemented as described below:

1) Control diet, containing 10 mg Cu (II)/kg diet and 50 mg Zn (II)/kg diet;
2) Zinc (II)-deficient diet, containing 10 mg Cu (II) /kg diet and < 2 mg Zn (II)/kg diet;
3) Zinc (II)-supplement diet, containing 10 mg Cu (II) /kg diet and 2,000 mg Zn (II)/kg diet;
4) Copper-supplement diet, contai/kg diet and 50 mg Zn (II)/kg diet;
5) Cadmium-supplement diet, containing 10 mg Cu (II) /kg diet and 50 mg Zn (II)/kg diet and 250 mg Cd (II) /kg diet

Eight rats were placed on each of diets 3, 4 and 5 while 29 rats were given diet 1 and 24 rats diet 2. After 7 weeks, one-half of control and one-half of zinc-deficient rats were injected once intraperitoneally (i.p. with 20 mg Zn/kg body weight). All rats were housed in stainless steel cages and fed 15 g of assigned diet/rat/day throughout the experimental period.

2. Chemicals

p-Nitrophenylsulfate, PAP, Zinc acetate, cupric sulfate and cadmium chloride were purchased from Sigma Chemical Co. (St. Louis, MO). The N-OH-2AAF was supplied by Dr. Robert A Froyd (Associate Member, OMRF, Oklahoma City, Oklahoma). 2-Naphthol was purchased from Aldrich Chemical Co. (Milwaukee, WI). All solvents and acids were analytical grade and were obtained from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). PAPS was purchased from Dr. Sanford Singer, Dept. of Chemistry, University of Dayton, Dayton, OH. Purified AST IV and antisera to AST IV were prepared as previously described by Ringer *et al.*.[30] Rabbbit anti-serum to albumin was purchased from Cappell Co., Westchester, PA.

3. Preparation of Rat Liver Cytosols

For the preparation of rat liver cytosols, rats were killed by cervical dislocation and their livers were removed and homogenized in a Potter-Elvehjem homogenizer with 3 vol. of 25 M sucrose. Post-microsomal supernatant was obtained by centrifugation at 100,000 g for 60 min. The upper lipid layer of the post-microsomal supernatant fraction was removed, and the remaining supernatant was collected and protein concentration was determined according to the method of Lowery *et al.*.[31]

4. Sulfotransferase Assays

N-OH-2AAF sulfotransferase activity in liver cytosols was measured as described by Mulder and Meerman.[8] In this assay, p-nitrophenylsulfate was used as a sulfate donor in the phenol sulfotransferase catalyzed conversion of PAP to PAPS. The rate of sulfation was monitored spectrophotometrically by following the accumulation of p-nitrophenol (450 nm) that paralleled the formation of the N-OH-AAF sulfate ester. A typical reaction mixture contained in 0.5 mL: 100 mM Tris (pH 8.0), 10 mM p-nitrophenylsulfate, 20 uM PAP, 0.5 mM N-OH-AAF, 5% (v/v) ethanol, and 300 μg protein from cytosol. The reaction was initiated by the addition of protein and the absorbancy at 405 nm was continuously monitored with a Gilford 250 spectrophotometer during a 10-min incubation at 31 ℃. Sulfotransferase activity was expressed as nmoles of p-nitrophenol released per min per mgcytosol protein.

In some experiments, 2-naphthol was used as substrate to measure the activity of sulfotransferase.[32] The standard assay for AST IV was conducted at pH 5.5 with 0.25 mM 2-naphthol as acceptor substrate. Incubations were terminated after 3 min at 37 ℃ and the product was determined with methylene blue. The enzyme activity was expressed as the nmol of p-nitrophenol formed-nitrophenylsulfate/min /mg cytosol protein.

5. Immunodetection(Western Blot) of AST Ⅳ and Albumin

The analysis of liver cytosols for AST Ⅳ and albumin content was performed by electrophoresis, electroblotting, and immunochemical detection with antibodies to AST Ⅳ and albumin as previously described by Ringer *et al.*.[30]

Briefly, 4 μg cytosolic fraction was elctrophoresed using a Phast System single-dimension 10~15% gradient polyacrylamide gel with SDS-buffer strips, Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ). Proteins from the Phast system gels were then electrophoretically transferred from the gels to nitrocellulose paper with the aid of Trans-Blot apparatus (model 250, Bio-Rad, Richmond, CA). The nitrocellulose was then immunochemically developed by the initial 2-h incubation with antiserum to AST Ⅳ (diluted 1:1,000), of antiserum to albumin (diluted 1:10,000), followed by binding of peroxidate-conjugated anti-rabbit IgG as the secondary antibody. Positive signals on the filter paper were generated by a peroxidase-catalyzed reaction between hydrogen peroxide and 4-chloro-1-naphthol.

6. Metal Analysis

Glassware used in the analysis for metals were cleansed in 3.2 N nitric acid (for 24 h) and rinsed at least five times with distilled deionized water for analyses of cytosol and tissues. Samples (1 mL of cytosol and 1 g of tissues) were placed in 5 mL Erlenmeyer flasks and 2~4 mL of 12 N nitric acid was added. Samples were digested (100 ℃) and then evaporated and diluted with distilled deionized water to appropriate volumes. Trace element levels were determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 306, Norwalk, CT).

7. Serum GOT Assay

Serum GOT activity in rat serum was determined using a Sigma GOT kit (cat. #505, Sigma Chem. Co., St. Louis, MO). Measurements were made with a Gilford 250 spectrophotometer, by Karmen's method.[33] Serum GOT activity was expressed in I.U. (25 ℃), where I.U. equaled the amount of enzyme which convert 1uM of substrate per min per liter of serum.

RESULTS

1. In Vitro Inhibition of AST Ⅳ Activity by Zinc (Ⅱ), Copper (Ⅱ), and Cadmium (Ⅱ)

The ability of Zn (Ⅱ), Cu (Ⅱ) and Cd (Ⅱ) to inhibit the sulfotransferase activity of AST Ⅳ was assessed both for the purified enzyme and rat liver cytosolic fraction. As shown in Fig. 1A, all 3 metal ions were effective inhibitors of purified AST Ⅳ, producing 50% inhibitions over a relatively narrow, 0.025~2.5 uM, concentration range. A similar pattern of sulfotransferase inhibition by the metal cations was observed for rat liver cytosols, Fig. 1B, but required higher concentrations of metal ions for 50% inhibition, i.e., 0.25~25 uM. The order of magnitude for higher metal ion range required for the inhibition of cytosolic sulfotransferase activity probably reflects the relative effect of ion loss through binding interaction with other proteins in the cytosol. Although liver cytosols contain a number of aryl sulfotransferase, it was possible to distinguish AST Ⅳ by its activity as in Fig. 1B by measuring its activity toward 2-naphthol at pH 5.5 (Sekura /Jakoby). These conditions were used in subsequent studies to monitor the cytosolic AST Ⅳ activity of livers from rats exposed to the metal ions in their diets.

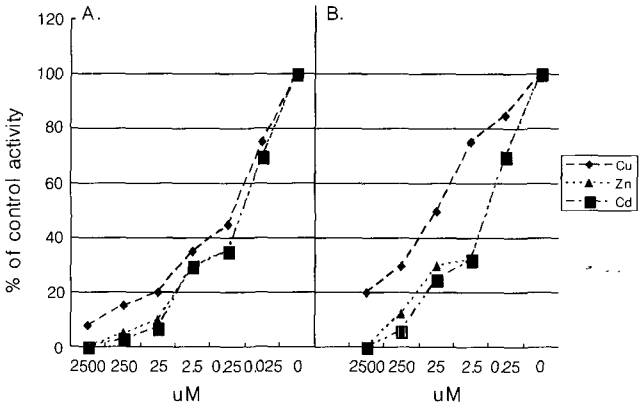

Fig. 1 Inhibition of AST Ⅳ activity by the divalent cations of Zn, Cu and Cd (A), Purified AST Ⅳ and (B), liver cytosol.
Purified AST Ⅳ and rat liver cytosols were prepared as described in Materials and Methods. PAPS-dependent sulfotrnasferase activity was determined by the 2-naphthol assay. The results are expressed relative to the control without addition of the metal ions.

2. Effect of Zinc (Ⅱ) and Copper (Ⅱ) Accumulation in Liver on Rat Growth Rates and Serum GOT Levels

Rats were fed 5 test diets of varying zinc (Ⅱ), copper (Ⅱ) and cadmium (Ⅱ) compositions (see Materials and Methods) for 7 weeks. Whole liver tissue samples and liver cytosolic fractions were obtained at the end of the 7 weeks from each animal, then zinc (Ⅱ) and copper (Ⅱ) accumulations were determined by atomic absorption spectroscopy, as shown in Table 1. As can be seen, the only diet which significantly altered tissue or cytosol copper (Ⅱ) levels was the Cu-supplemented diet. In response

to a 100-fold increase in dietary copper, liver tissue and cytosols showed 40- and 30-fold increases in copper (II), respectively. In contrast, zinc (II) accumulation in liver tissue and cytosol was little affected by the administration of a diet with 40-fold increase of zinc (II) or a zinc-deficient diet containing <4% the zinc in the control diet. However, zinc (II) levels in liver samples did significantly increase over control levels when the diet was supplemented with 250 μg cadmium/g diet. Cadmium (II) had no effect on copper (II) accumulation in the same liver samples.

Remarkable changes in rat body and liver growth rates were observed during the 7-week period of test diet administration. The Zn-deficient, Zn-supplemented, and Cd-supplemented diets caused 17~20% decreases in rat body weights (Table 1) and 24~36% decrease in rat liver weights (Table 1) while the Cu-supplemented diet had no effect on rat growth rates. Among the rats fed the diets causing reduced growth rates, only the decreases for the rats fed the Zn-deficient diet appeared to be proportionate and resulted in values for liver as % of rat body weight that matched values for rats fed control diet (Table 1). Both Zn-supplement and Cd-supplement diets caused disproportionately greater decreases in liver weights than body weights and produced 18~19% lower values for liver as % of rat body weight.

The serum GOT levels for each group of rats were also determined at the end of the 7 weeks. As shown in Table 2, the diets caused little or no elevation in serum GOT values, with only the Zn-deficient diet producing a small but significant 2-fold increase. These results indicated that while some of the test diets did affect rat body and liver growth rates, there was little if any acute toxicity caused by the diets.

Table 2. General effects of test diets on rat growth rate

	Liver weight (A)	Body weight (B)	Liver /Body weight (C)	SGOT (D)
Control	10.8±0.6	315.0± 15.2[a)]	0.034	6.7±2.0
Zn-deficient	8.2±0.3	258.0± 2.4[b)]	0.032	15.8±4.2
Cu 1,000	10.1±0.1	309.3± 13.8[a)]	0.033	9.0±2.7
Zn 2,000	6.9±0.3	251.0±11.39[b)]	0.027	13.0±8.9
Cd 250	7.2±0.4	261.3± 5.7[b)]	0.028	5.9±1.9

(A), liver weight; (B), rat body weight; (C), liver as % of rat weight; (D), SGOT activities of rats.
Rats were fed individual diets for 7 weeks as described in Methods and Materials. Each bar represents the average of four rats. The SEM is indicated in the Fig., Zn-, zinc-deficient diet; Zn, zinc-supplement diet (2,000 mg Zn (II)/kg diet); Cu, copper-supplement diet (1,000 mg Cu (II)/kg diet); Cd, cadmium-supplement diet (250 mg Cd (II)/kg diet).
Data were analyzed statistically using the SPSS program.
The values with different small letters in the same column are significantly different ($p<0.05$).

Table 1. General effects of test diets on rat growth, body weight, liver weight, metal accumulation

Diet and supplement	amount added	Average weight(g) at 7 weeks		Metal accumulation			
				Liver		Cytosol	
		Body	Liver	Cu	Zn	Cu	Zn
	(μg/diet)			(μg/g fresh tissue)		(μg/mL cytosol)	
Control, zinc test		315.0±15.2[a)]	10.8±0.6	2.6± 0.2[a)]	29.1±1.5	1.1± 0.2[a)]	3.2±0.1
Zn	50						
Cd	0						
Zinc-deficient		258.0± 2.4[b)]	8.2±0.3	3.8± 0.4	29.8±0.8	1.0± 0.2	2.6±0.2
Zn	<2						
Cd	0						
Zinc-supplement		251.0±11.3[b)]	6.9±0.3	3.3± 0.5	30.5±1.5	1.2± 0.2	3.4±0.3
Zn	2,000						
Cd	0						
Copper-supplement		309.3±13.8[a)]	10.1±0.1	127.2±59.0[b)]	29.6±0.8	28.1± 9.7[b)]	3.4±0.2
Cu	1,000						
Zn	50						
Cd	0						
Cadmium-supplement		261.3± 5.7[b)]	7.2±0.4	1.9± 0.3	40.8±2.3	1.0± 0.1	5.8±0.4
Zn	50						
Cd	250						

Rats were fed individual diets for 7 weeks as decribed in Methods and Materials.
Zn-, zinc-deficient diet; Zn, zinc-supplement diet (200 mg Zn (II)/kg diet); Cu, copper-supplement diet (1,000 mg Cu (II)/kg diet); Cd, cadmium-supplement diet (250 mg Cd (II)/kg diet).
Data were analyzed statistically using the SPSS program. The values with different small letters in the same column are significantly different ($p<0.05$).

3. Sulfotransferase Activity of Liver Cytosols from Rats Fed Diets with Differing Metal Ion Contents

Cytosolic fractions were isolated from the livers of rats following completion of 7 weeks on metal test diets and evaluated for levels of sulfotransferase activity using 2-naphthol and N-OH-2AAF as substrates. As shown in Fig. 2, relative to values for cytosols from rats fed control diet, rats administered the diets supplemented with zinc (Ⅱ), copper (Ⅱ) or cadmium (Ⅱ) caused a 40~50% decrease in the activity towards 2-naphthol, while rats received the Zn-deficient diet showed a 30% increase.

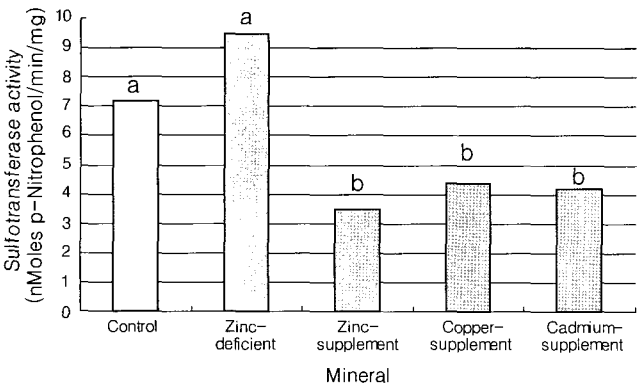

Fig. 2 Liver cytosolic AST Ⅳ activity of the livers from rats fed diet with differing metal ion contents. AST Ⅳ activity was assayed using 2-naphthol as substrate. Each bar represents the mean and S.E.M. for three to five determination for four individual samples. The value with different small letters on the bars are significantly different ($p<0.05$). AST Ⅳ activity was assayed using N-OH-2AAF as substrate as described in Materials and Methods. Each bar represents the average of duplicate determinations performed on a cytosol pool containing 1mg each from 4 different cytosols.

When cytosols were also evaluated for sulfotransferase activity utilizing N-OH-2AAF as a substrate, the pattern of activity was essentially identical to that seen with 2-naphthol. Sulfotransferase activity in liver cytosols toward N-OH-2AAF has been reported to be primarily an activity of AST Ⅳ[30] and further supports the 2-naphthol results indicating that the losses in cytosolic sulfotransferase activity reflects a loss in AST Ⅳ activity.

4. Effect of a Single Acute Dose of Zinc (Ⅱ) on Liver Sulfotransferase Activity

In order to determine whether AST Ⅳ activity was sensitive to inhibition by a rapid increase in zinc levels, a single i.p. dose of 20 μg Zn (Ⅱ)/g B.W. was administered to rats fed either control diet or the Zn-deficient diet. As shown in Fig. 3 and 4, during the following 6 hrs post-injection, there was large and rapid accumulation of zinc (Ⅱ) in the liver tissues and cytosolic fractions of rats from both dietary protocols. Interestingly, accumulations of zinc (Ⅱ) in the rats fed Zn-deficient diet lagged somewhat behind those fed control diet. Measurement of liver sulfotransferase activities toward 2-naphthol and N-OH-2AAF, as in Fig. 5, showed that the activity was decreased as zinc was accumulated in the liver. Determination of serum GOT values, as shown in Table 2, for the rats in these experiments indicated that at 6 hrs post-injection of zinc, rats fed control diet went from 8 to 110 I.U./mL while rats fed the Zn-deficient diet went from 19 to 62 I.U./mL (data not shown). These results suggest considerably more cytotoxicity occurred with the i.p. injection of zinc than occurred during the chronic administration of the Zn-supplement diet (see Fig. 2 and Fig. 5), and raise the possibility that lost activity may not simply resulted from the elevated levels of zinc (Ⅱ) in the liver.

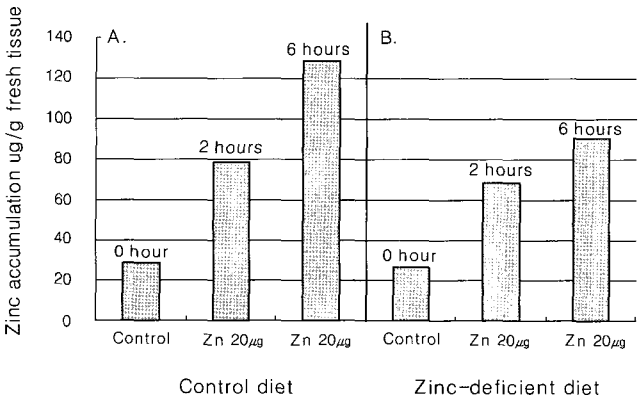

Fig. 3 Zinc accumulation in the liver fresh tissue in i.p. injected rats (Zn 20 μg/g. B.W.)
A; Control diet, B; Zinc-deficient diet

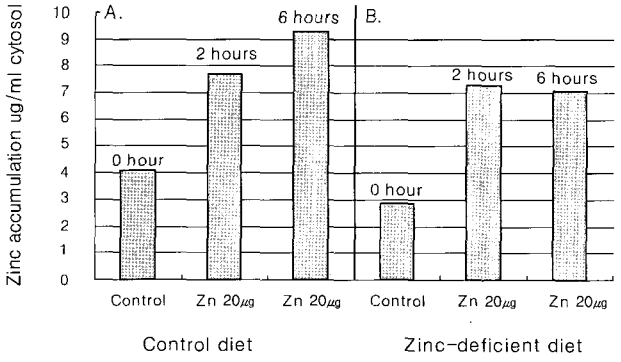

Fig. 4 Relative liver cytosolic zinc level in i.p. injected rats(Zn 20 μg/g. B.W.)
A; Control diet, B; Zinc-deficient diet

5. Comparison of the Relative AST Ⅳ and Albumin Content in the Liver Cytosols of Rats Administered Diets with Varying Metal Ion Contents

To gain further insight into mechanisms responsible for the loss of AST Ⅳ activity, pooled liver cytosols from each test diet group were immunochemically evaluated for relative AST Ⅳ content. As shown in Fig. 6a, Western-blot immunochemical analysis indicated that a qualitative pattern of altered AST Ⅳ content was existed which paralleled the previous pattern of altered AST Ⅳ activity caused by the test diets (see Fig. 5).

On a quantitative basis, the immunochemical data suggested that only a portion of the metal ion-mediated losses in AST Ⅳ activity could be attributed to a lowering of AST Ⅳ cytosolic content. When liver albumin levels were similarly determined for the same cytosolic pools, as in Fig. 6b, no differences in albumin levels were observed for any of the metal ion test diets, suggesting a selectivity

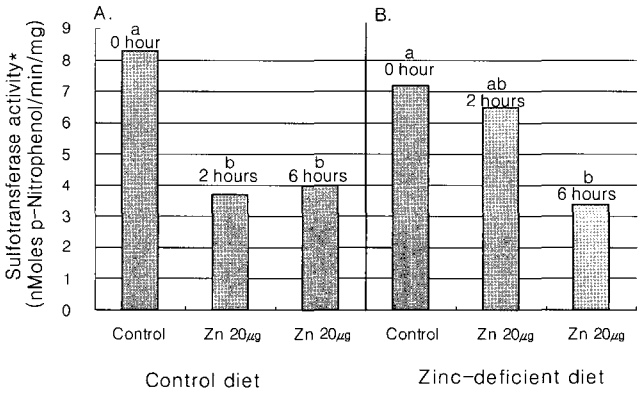

Fig. 5 Liver cytosolic AST Ⅳ activity of rats following i.p. injection of 20 μg Zn(Ⅱ)/g B.W.

A; Control diet, B; Zinc-deficient diet
AST Ⅳ activity was assayed using 2-naphthol as substrate. Liver cytosol were prepared from the rats injected with water (control) or with zinc (20 μg/g B.W.). Each bar is the average of two or three determinations for four individual samples. Values with different small letters are significantly different at 5% level.

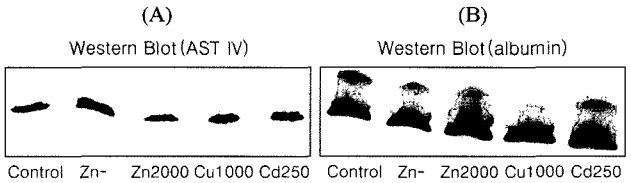

Fig. 6 Immunochemical analysis (Western Blot) of relative AST Ⅳ and albumin contents of liver cytosolic fractions from rats on diets with varied amounts of metal ions.

Western-blot immunodetections of AST Ⅳ (A) and albumin (B) levels were performed as described in Materials and Methods on liver cytosols from rats fed control diet (Control), Zn-deficient diet (Zn-), Zn-supplemented diet (Zn 2,000), Cu-supplemented diet (Cu 1,000), or Cd-supplemented diet (Cd 250).
Data represent the pattern observed for duplicate determination on the pooled cytosolic samples described in Fig. 2.

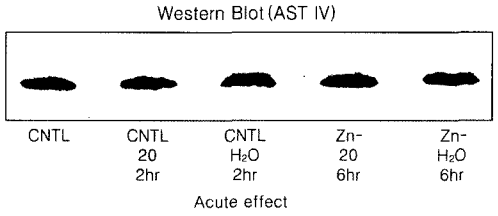

Fig. 7 Immunochemical analysis (Western-blot) of relative AST Ⅳ of liver cytosolic fractions in i. p. injected rats (Zn 20 μg/g. B.W.) fed control and zinc-deficient diet.

Western-blot immunodetections of AST Ⅳ were performed as described in Materials and Methods.

for metal ion action on AST Ⅳ. Also, as shown in Fig. 7, the acute Zinc i.p. injection did not alter the contents of AST Ⅳ contents of cytosolic fractions

DISCUSSION

AST Ⅳ is a liver phenol sulfotransferase known to have broad substrate specificity[32] and to be involved in the bioactivation of various chemical carcinogens.[2,5] Previous reports have shown that AST Ⅳ activity can be down modulated by xenobiotics and genotoxics in the liver.[12,13] The identification of compounds which alter the activity of detoxication enzymes, such as AST Ⅳ, and understanding the mechanisms involved are important components in the process of assessing the overall biological response to toxic chemicals and designing ways to overcome or prevent toxic damages. Results reported here demonstrate for the first time that chronic administration of diets to rats which contain elevated levels of zinc, copper or cadmium divalent cations cause a loss of liver AST Ⅳ activity. Furthermore, results are presented which suggest that mechanisms involving (a) inhibition of AST Ⅳ activity by metal ions, and (b) a metal ion-mediated decrease in AST Ⅳ abundance are responsible for the loss in activity.

Wu and Straub[15] demonstrated that zinc (Ⅱ) and copper (Ⅱ) were strongly inhibitory at very low concentrations to a partially purified liver sulfotransferase with activity for N-OH-2AAF. In this study, similar inhibitions by zinc (Ⅱ) and copper (Ⅱ) were observed for purified AST Ⅳ, and liver sulfotransferase suggested to be the principal liver N-OH-2AAF sulfotransferase activity. Although the sensitivity to metal ion inhibition for AST Ⅳ (Fig. 1) appeared to be approximately 10-fold greater than reported by Wu and Straub,[15] it is not known whether these 2 enzymes are one in the same or isoenzymes. The inhibition of AST Ⅳ to cadmium is the first report of sulfotransferase

sensitivity to cadmium (Ⅱ). Cadmium (Ⅱ) is a non-biological transition metal with physicochemical properties similar to zinc(Ⅱ) and copper(Ⅱ) allowing it to interfere with normal biological roles of zinc (Ⅱ) and copper (Ⅱ). Zinc (Ⅱ), copper (Ⅱ) and cadmium (Ⅱ) were also shown to inhibit AST Ⅳ activity when added to rat liver cytosolic fractions (Fig. 1). This provided a basis for the monitoring of changes in AST Ⅳ activity following *in vivo* accumulations of the metals in rat liver. When rats were administered diets supplemented with copper (Ⅱ) (1,000 mg/kg diet), liver cytosolic fractions showed a marked increase in copper ion concentrations and a corresponding 50% decrease in AST Ⅳ activity (Table 1 and Fig. 2). The levels of copper (Ⅱ) in the cytosol corresponded to concentration in excess of 400 uM and were well above the levels needed for direct inhibition of AST Ⅳ activity *in vitro* (see Fig. 1). Furthermore, the copper supplemented diet neither caused a change in rat body or liver growth rates, nor in rat serum GOT levels. Taken together, these suggest that copper (Ⅱ) ion lowering of liver AST Ⅳ activity in rats fed the copper supplemented diet probably reflects direct inhibition of activity by nearly 30-fold elevation of copper (Ⅱ) ion in liver cytosols.

In contrast, rats fed the zinc-supplemented diet (2,000 mg/kg diet) showed essentially no change in liver cytosolic zinc (Ⅱ) ion concentration, yet also caused a >50% loss of cytosolic AST Ⅳ activity. Also in contrast to copper (Ⅱ), the zinc-supplemented diet caused a small but significant reduction in rat body and liver growth rates (Table 1). While this suggested a retarding of rat growth and development by elevated zinc (Ⅱ), the observations showing that liver 0.027% of rat body weight values and serum GOT values were normal (Table 2), indicated that the liver was not a specific target for zinc (Ⅱ) toxicity. Moreover, when a zinc-deficient diet (<2 mg Zn (Ⅱ)/kg diet) was similarly administered to rats, rat growth rates were again retarded, serum GOT levels slightly (2-fold) elevated, but no significant changes were observed in liver cytosolic AST Ⅳ activity nor in zinc (Ⅱ) accumulation. This supports the above conclusion suggesting that zinc-related changes in rat growth rate do not correlate with subsequent changes in AST Ⅳ activity. Of further interest, the 7 week administration of the zinc-deficient and zinc-supplemented diets caused essentially no change relative to control diet in liver accumulation of zinc (Ⅱ) (Table 1), indicating that the liver maintained tight regulation on dietary uptake of zinc. However, zinc (Ⅱ) levels in the liver could be rapidly elevated 2-4 fold upon i.p. injection of 20 mg Zn (Ⅱ)/kg B.W. in rats previously maintained on control or zinc-deficient diets (Fig. 4), causing a 50% drop in cytosolic fraction AST Ⅳ activity by 6 hours post i.p. injection. While this suggests that elevated levels of zinc (Ⅱ) in the liver can result in a lowering of AST Ⅳ activity, caution is required since the i.p. injection of zinc (Ⅱ) also resulted in 10-fold elevation of rat serum GOT levels (data not shown).

When rats were administered diets supplemented with cadmium (Ⅱ) (250 mg/kg diet), liver AST Ⅳ activities were again lowered to approximately 50% of control liver levels. The cadmium-supplemented diet also appeared to cause a decrease in rat body and liver growth rates, but unlike the zinc diets, to have a proportionately greater effect on liver growth rates (Table 1). Interestingly, the cadmium-supplemented diet also caused a 2-fold increase in liver zinc (Ⅱ) accumulation while not changing liver copper levels. Cadmium (Ⅱ) has previously been reported to cause a slight increase in liver zinc accumulations although the mechanism is not known.[34] While it is possible that the cadmium-mediated elevation of liver zinc (Ⅱ) levels may account for the loss in AST Ⅳ activity, levels of cadmium (Ⅱ) in the liver, not determined in this study, must also be considered as a possible cause.

Among other mechanisms by which the metal ion-supplemented diets can cause lowering of liver AST Ⅳ activity is the metal-mediated decrease in liver AST Ⅳ content. Immunochemical evaluation of the relative levels of AST Ⅳ in liver cytosolic fractions revealed that while the copper-supplemented, cadmium-supplemented and zinc-deficient diets did not cause significant lowerings of AST Ⅳ, both zinc-supplemented diets produced a relative lowering of cytosolic AST Ⅳ (Fig. 6). The fact that the decreased enzyme amount did not quantitatively parallel the activity losses may reflect technical differences between measurements of AST Ⅳ activity and amount, or may indicate that lowering of AST Ⅳ activity results from a combination of inhibited AST Ⅳ activity and decrease in enzyme amount. Immunochemical determination of albumin levels in the same samples showed no metal diet-mediated changes in albumin content, suggesting an absence of general translational problems in the tissues and supporting a selective effect on AST Ⅳ.

These results showed that losses in liver AST Ⅳ activity result from either *in vitro* or *in vivo* exposure to zinc (Ⅱ), copper (Ⅱ), and cadmium (Ⅱ). While the *in vitro* results and copper-supplemented diet results indicate that the metal ions can act directly to cause the inhibition of AST Ⅳ activity, the diets supplemented with zinc (Ⅱ) and cadmium (Ⅱ) suggest other mechanisms, such as those that able to cause a decrease in AST Ⅳ amount

in the liver, must also be involved. Elucidation of these mechanisms and assessments of the effect of metal ions on AST Ⅳ function as xenobiotic detoxifying pathway will require further research.

Literature Cited

1) Blanck A, Wicksell L, Eriksson LC. Sulfation of N-hydroxy-2-acetylaminofluene in normal and modular liver from male Wistar rats. *Toxicol Pathol* 15:103-105, 1987
2) Debaum JR, Miller EC, Miller JA. N-hydroxy-2-acetylaminofluorene sulfotransferase:its probable role in carcinogenesis and ptrtein-(methion-S-yl)-binding in rat liver. *Cancer Res* 30:577-595, 1970
3) Debaum JR, Rowley JY, Miller FC, Miller JA. Sulfotransferase activation of N-hydroxy-2-acetylamino-fluorene in rodents liver susceptible and resistant to this carsinogen. *Proc Soc Exp Biol Med* 129:268-273, 1968
4) Irving CC. Comparative toxicity of N-hydroxy-2-acetylaminofluorene in several strain of rats. *Cancer Res* 35:2959-2961, 1975
5) King CM, Phillips B. Enzyme-catalized reactions of the carcinogen N-hydroxy-2-flutoenylavetamide with nucleic acid. *Science* 159:1351-1353, 1968
6) Lai C-C, Miller JA, Miller EC, Liem A. N-Sulfooxy-2-aminoflurene is the major ultimate electrophilic and carcinogenic metabolite of N-hydroxy-2-acetyl-aminoflurene in the livers of infant male C5BL/6J*C3H/HeJF1 (BGC3F1)mice. *Carcinogenesis* 6:1037-1045, 1985
7) Meerman JHN, Mulder GJ. Prevention of the hepatoxic action of N-hydroxy-2-acetylaminofluorene in the rats by inhibition of N-O-sulfation by pentachlorophenol. *Life Sci* 28:2361-2365, 1981
8) Mulder GI, Meerman JHN. Sulfation and gluconidation as competing pathways in the metabolism of hydroxamiv acids: the role of N, O-sulfonation in chemical carcinogensis of aromatic amines. *Environ Health Perspect* 49:27-32, 1983
9) Meerman JHN, van Doorn ABD, Muller GJ. Inhibition of sulfate conjugation of N-hydroxy-2-acetyl-aminofluorene in isolated perfused rat liver and in the rat *in vivo* by pentachlorophenol and low sulfate. *Cancer Res* 40:3772-3779, 1980
10) Weisberger JH, Yamamoto RS, Williams GM, Gramtham PH, Matsushima T, Weisburger EK. On the sulfate ester of N-hydroxy-N-fluorenylacetamide as key ultimate hepacarcinogen in the rat. *Cancer Res* 32:491-500, 1972
11) Yamamoto RS, Glass RM, Frankel HH, Weisburger EK, Weisburger JH. Inhibition of the toxicity and carcinogenicity of N-2-fluorenylacetamide by acetamide. *Toxicol Appl Pharmacol* 13:108-117, 1968
12) Ringer DP, Kampschmidt K, Jing RL, Jackson S, Kizer DE. Rapid decrease in N-hydroxy-2-acetylaminolfuorene sulfotransferase activity of liver cytosols from rats fed carcinogen. *Biochem Pharmacol* 32:315-319. 1983.
13) Ringer DP, Norton TR. Further characterization of the ability of hepatocarcinogens to lower rat liver aryl sulfotransferase activity. *Carcinogenesis* 8:1749-1752, 1987
14) Ringer DP, Norton TR, Kizer DE. Effect of sulfotranferase inhibitors on the 2-acetylaminofluotene-mediated lowering of rat liver N-hydroxy-2-acetylaminofluorene sulfotransferase activity. *Biochem Pharmacol* 34: 3383-3386, 1985
15) Wu SG, Straub KD. Purification and characterization of N-hydroxy-2-acetylaminofluorene sulfotransferase from rat liver. *J Biol Chem* 251:6529-6536, 1976
16) Underwood EJ. Trace elements in human and animal nutrition. pp.95, Academic Press, New York, 1977
17) Bremmer I, Hoekstra WG, Davis NT, Young BW. Effect of zinc status of rats on the synthesis and degration of copper-induced metallothioneins. *Biochem J* 174:883-892, 1978
18) Rucker R, Tinker D. The role of Nutrition in gene expression: A fertile field for the application of molecular biology. *J Nutr* 116:177-189, 1986
19) Smith SE, Larson EJ. Zinc toxicity in rats and antagonistic effects of copper and silver. *J Biol Chem* 163:294-298, 1946
20) Van Reen R. Effects of excessive dietary zinc in the rat and the interrelationship with copper. *Arch Biochem Biophys* 46:337-342, 1953
21) Hill CH, Starch B, Payne WL, Barber CW. *in vivo* interactions of cadmium with copper, zinc, and iron. *J Nutr* 80:227-235, 1963
22) Powell SD, Miller G, Morton JD, Clifton CM. Influence of dietary cadmium level and supplemental zinc on cadmium toxicity in bovine. *J Nutr* 84:205-214, 1960
23) Matrone G, Hartman RH, Clawson AJ. Studies of manganese-iron antagonism in the nutrition of rabbits and baby pigs. *J Nutr* 67:309-317, 1959
24) Supplee WC. Production of zinc deficiency in turkey poults by dietary cadmium. *Poult Sci* 40:827-828, 1961
25) Hill CH. Reversal of selenium toxicity in chicks by mercury, copper and cadmium. *J Nutr* 104:593-598, 1974
26) Peterson RP, Jensen LS. Interrelationships of dietary silver with copperin the chick. *Poult Sci* 54:771-775, 1975
27) Sheroeder HA, Mitchener M, Nason AP. Life-term effects of nickel in rats:Survival, tumors. Interaction with trace elements and tissue levels. *J Nutr* 104:239-243, 1974
28) Thomson A, Hansard SL, Bell MC. The influence of aluminumand zinc upon the absorption and retention of calcium and phosphorus in lambs. *J Anim Sci* 18:187-192, 1959
29) Van Compen DR. Copper interference with the interstinal absorption of zinc by rats. *J Nutr* 97:104-108, 1970
30) Ringer DP, Norton TR, Boyd HW. 2-Acetylaminofluorene-mediated alterationin the level of liver arylsulfotransferase Ⅳ during rat hepatocarcinogenesis. *Cancer Res* 50:5301-5307, 1990
31) Lowery OH, Rosebrough NJ, Farr AL, Randall RJ. Protein determination with the folin phenol reagent. *J Biol Chem* 193:265-275. 1951

32) Sekura RD, Jakoby WB. Aryl Sulfotransferase Ⅳ from rat liver. *Arch Biochem Biophys* 211:352-359, 1981
33) Karmen A. A note on the spectrometric assay of glutamic-oxalacetic transaminase in human blood serum. *J Clin Invest* 34(1):131-133, 1955
34) Chung K, Romero N, Tinker D, Leen C, Amemiya K, Rucker R. Role of copper in the regulation and accumulation of superoxide dismutase and metallothionein in rat liver. *J Nutr* 118:859-864, 1988