LOW ACTIVITY OF ALDEHYDE DEHYDROGENASE (ALDH) IN LIVER OF *SUNCUS MURINUS*: POSSIBLE EXPLANATION FOR HIGH SENSITIVITY TO ALCOHOL

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ABSTRACT — *Suncus murinus* (suncus) is an animal species highly sensitive to ethanol (Lin, S.-C., Saito, H., Yohro, T. and Shiga, J., J. Toxicol. Env. Health, 18: 575-587, 1986). To elucidate the mechanism involved in sensitivity to alcohol, we investigated the properties of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the suncus in comparison with the rat.

The activities of ALDH in microsomes and mitochondria of suncus livers were lower than those in rat livers, although the activity of cytosolic ADH was not different between the suncus and the rat.

We propose that the high sensitivity of the suncus to alcohol can be accounted for, at least in part, by the low activity of ALDH in suncus liver.

KEY WORDS : Alcohol dehydrogenase, Aldehyde dehydrogenase, Liver, Microsomes, Mitochondria, Suncus

INTRODUCTION

House musk shrew, *Suncus murinus* (Suncus), is an animal species which belongs to the family of *Soricidae* of the order of *Insectivora*. This species is small in body size and extremely nervous. The *Insectivora* are regarded as the direct ancestors of the primates in the phylogenetic tree. Recently, the suncus has been introduced as a novel experimental animal, since this animal can induce vomiting when vibration or several agents including anti-cancer drugs are given. Thus, the suncus has been developed and used as an experimental animal for emesis research (Ueno *et al.*, 1987 and 1988). However, there is little information on this species with respect to drug-metabolizing

enzymes (Mizokami *et al.*, 1988; Miura *et al.*, 1991; Nakura *et al.*, 1992, 1994, 1995). Characterization of suncus drug-metabolizing enzymes is an essential step in evaluating the suncus as an experimental animal model for toxicological and pharmacological research. In previous studies, we found that the activities of major drug-metabolizing enzymes such as cytochrome P-450, glutathione S-transferase, UDP-glucuronyltransferase, flavin-containing monooxygenase, arylhydrocarbon hydroxylase and N-acetyltransferase (NAT) in suncus livers were lower than in rat livers (Nakura *et al.*, 1994). In our previous studies, we also found that NAT and O-acetyltransferase (OAT) activities in the suncus could not be detected either *in vivo* or *in vitro* (Nakura *et al.*, 1995). The con-

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tent of cytochrome P450IIE (CYP2E) in suncus liver decreased when the suncus was fasted (Nakura *et al.*, 1992).

It has been reported that acute hepatotoxicity was induced in the suncus even by a small amount of ethanol (Lin *et al.*, 1986). After a single oral administration of a small amount of ethanol, the gallbladder became enlarged and dark blue in color. The levels of GOT and GPT were elevated in proportion to the dose of ethanol (Lin *et al.*, 1986). Ethanol is known to be metabolized mainly by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Thus, it seemed possible to assume that the hepatic disorder induced by ethanol was caused by deficiency or insufficiency of ADH and/or ALDH activities. To assess this possibility, we measured the activities of ADH and ALDH in suncus liver, compared with rat livers. The comparison between suncus and rat liver ALDH raised the possibility that the high sensitivity of the suncus to alcohol was due to poor ALDH activity in suncus livers.

MATERIALS AND METHODS

Animals

Male suncus (6 weeks old) were purchased from the Central Institute for Experimental Animals (Kanagawa, Japan). Male Sprague-Dawley (SD) rats (6 weeks old) were from Nippon SLC (Shizuoka, Japan). They were maintained in air-conditioned quarters with 12 hr light-dark cycles. Suncus and rats were given laboratory chow (Clea Japan, Tokyo, Japan) and water *ad lib*.

Subcellular fractionation

Subcellular fractions were prepared by the method of Koivula and Koivusalo (1975) with minor modifications. Briefly, livers from male suncus and rats were removed immediately after decapitation, and put into an ice-cold 0.25 M sucrose solution. All further steps were carried out at 4°C. The livers were homogenized in 0.25 M sucrose solution in a homogenizer to yield a 10% (w/v) homogenate. This was centrifuged at 900 x g for 10 min, and the resultant pellet was washed once with a 0.25 M sucrose solution, and then resuspended in 7 ml of 0.01

M sodium-potassium phosphate buffer (pH 7.4). This fraction was termed the nuclear fraction. The combined supernatant was centrifuged at 5000 x g for 10 min and the pellet containing mitochondria was washed once with 10 ml of 0.25 M sucrose solution and resuspended in 4 ml of 0.01 M sodium-potassium phosphate buffer (pH 7.4). This fraction was termed the mitochondrial fraction. The combined postmitochondrial supernatant was centrifuged at 10,000 x g for 10 min. The pellet was washed once with a 0.25 M sucrose solution and resuspended in 4 ml of 0.01 M sodium-potassium phosphate buffer (pH 7.4). This fraction was termed the lysosomal fraction. The microsomal fraction was sedimented by centrifuging the post-lysosomal supernatant at 105,000 x g for 60 min. The clear supernatant was designated as the cytosolic fraction. After rinsing the final tight pellet with the 0.25 M sucrose solution, this was resuspended in 4 ml of 0.01 M sodium-potassium phosphate buffer (pH 7.4).

Assay for enzyme activity

ADH activity was assayed spectrophotometrically as follows. The reaction mixture consisted of 0.06 M glycine-HCl buffer (pH 10) containing a desired amount of the cytosolic fraction, 0.5% Triton X-100, 0.5 mM NAD⁺ and 12.5 mM ethanol in a final volume of 1 ml. After preincubation without ethanol at 25°C for 3 min, the incubation was started by the addition of ethanol (12.5 mM). The incubations were carried out at 25°C for 6 min. The reduction of NAD was measured at 340 nm due to the formation of NADH.

To assay the activity of ALDH, the reaction mixture consisted of a 0.05 M sodium-potassium phosphate buffer (pH 7.4) containing a desired amount of a subcellular fraction, 0.05 M pyrazole, 0.5% Triton X-100, 3 mM MgCl₂, 1 mM NAD⁺ and 0.5 mM acetaldehyde. After preincubation at 25°C for 3 min, the reaction was started by addition of acetaldehyde. NAD reduction was measured at 340 nm for 6 min.

RESULTS AND DISCUSSION

It has been reported that the suncus is highly sensitive to ethanol, showing hepatotoxicity

after intake of ethanol at 1 ml/kg (Lin *et al.*, 1986).

To clarify the mechanisms involved in the high sensitivity of the suncus to ethanol, the activities of ADH and ALDH in livers were examined. Ethanol is metabolized as follows: (i) ethanol is converted to acetaldehyde by ADH using NAD as a coenzyme and (ii) acetaldehyde is dehydrogenated by ALDH to yield acetic acid. To account for the high sensitivity of the suncus to alcohol, we set forth a hypothesis that the suncus possessed poor activities of ADH and/or ALDH in the liver. Fig. 1 shows the activities of ADH in suncus and rat liver. The activity of ADH in liver cytosols was not different between the suncus and rats. The other sub-cellular fractions from livers of both suncus and rats did not show any detectable activity of ADH (data not shown).

On the other hand, microsomal, mitochondrial and cytosolic fractions of livers from both the suncus and rats showed considerable ALDH activities (Fig. 2), while the activities were not detectable in nuclear and lysosomal fractions (data not shown). In microsomal and mitochondrial fractions, ALDH activity in rats was higher than that in the suncus, although cytosolic ALDH activity in rats was almost the same as that in the suncus. It has been proposed that ALDH is classified by its cellular distribution into three isozymes. ALDH1 is in cytosols, ALDH2 in mitochondria and ALDH3 in microsomes. Thus, we concluded that the activities of ALDH2 and ALDH3 in suncus livers were lower than in rat livers. Many Japanese and

Mongolians who are discomforted by drinking even a small amount of alcohol possess the mutation of the ALDH2 gene. Because of the mutation, they have a poor capacity to oxidize acetaldehyde, resulting in the accumulation of acetaldehyde in the blood when they drink alcohol (Harada *et al.*, 1991). It is of interest that suncus liver also possesses a low activity of ALDH2.

Calculating microsomal and mitochondrial ALDH activities on the basis of liver weight, the total activities of ALDH in the suncus were only about 20-30% the level seen in rats. There were no differences in cytosolic ALDH and ADH activities per 1 g of liver between the suncus and

Fig.1. Alcohol dehydrogenase activity in hepatic cytosols. Reduction of NAD associated with the dehydrogenation of ethanol was measured. Open circle: suncus, closed circle: rat. All values represent mean $\pm$ S. D. (n=5).

Fig.2. Aldehyde dehydrogenase activity of hepatic (A) microsomes, (B) mitochondria and (C) cytosols. Reduction of NAD associated with the dehydrogenation of acetaldehyde was measured. Open circle: suncus, closed circle: rat. All values represent mean $\pm$ S. D. (n=5).

rats (Table I). This result indicates that the metabolic capacity of suncus livers to oxidize acetaldehyde was less than that in rat livers.

The reaction kinetics of ALDH in liver microsomes and mitochondria of the suncus and rats were examined. The results are shown in Fig. 3. K_m and V_{max} values for microsomal ALDH in the suncus were $46.1 \mu\text{M}$ and $19.8 \mu\text{mol/min/mg}$ protein, while the values in rats were $53.1 \mu\text{M}$ and $56.7 \mu\text{mol/min/mg}$ protein, respectively. The hepatic clearance (V_{max}/K_m) values with respect to microsomal ALDH were 0.43 and 1.07 for the suncus and rats, respectively. The difference seen in V_{max} values may be one of the causes of differences in the sensitivity to alcohol between the suncus and rats. The K_m and V_{max} values of mitochondrial ALDH were $16.5 \mu\text{M}$ and $18.4 \mu\text{mol/min/mg}$ protein in the suncus and $30.7 \mu\text{M}$ and $32.4 \mu\text{mol/min/mg}$

protein in rats, respectively. Both the K_m and V_{max} values for suncus mitochondrial ALDH were lower than those for rat mitochondrial ALDH. Thus, the hepatic clearance (V_{max}/K_m) values with respect to mitochondrial ALDH were calculated to be 1.12 and 1.06 for the suncus and rats, respectively.

Since the toxicity of alcohol is generally known to be caused by acetaldehyde, we examined the activities of ADH as an acetaldehyde-forming enzyme and ALDH as an acetaldehyde-detoxifying enzyme. As far as we examined, we found a clear difference in microsomal and mitochondrial ALDHs, but essentially no differences in ADH and cytosolic ALDH between the suncus and rats. Thus, we tentatively propose that the low capacity of microsomal and mitochondrial ALDHs may account, at least in part, for the high toxicity of alcohol in the suncus.

Table 1. Activities of aldehyde dehydrogenase and alcohol dehydrogenase in hepatic subcellular fractions.

Species	ALDH activity			ADH activity
	Microsomes	Mitochondria	Cytosols	Cytosols
	$(\mu\text{mol of generated NADH/min/g liver})$			
Rats	1.03 ± 0.19	0.44 ± 0.06	0.25 ± 0.03	276 ± 34
Suncus	$0.19 \pm 0.08^*$	$0.13 \pm 0.05^*$	0.27 ± 0.04	280 ± 14

All values represent mean $\pm$ S.D. ($n=5$).

Significantly different from rats ; $*p < 0.01$.

Fig.3. Lineweaver-Burk plots of aldehyde dehydrogenase in hepatic microsomes and mitochondria. The incubation mixture consisted of a 0.05 M sodium-phosphate buffer (pH 7.4) containing 0.1 mg protein of microsomes or mitochondria, 0.05 M pyrazole, 0.5% Triton X-100, 3 mM MgCl_2 , 1 mM NAD^+ and a varying concentration of acetaldehyde. Open circle, suncus; closed circle, rat. All values represent means of five animals.

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