EFFECT OF METHYLMERCURY ON THE ETHANOL ELIMINATION FROM THE BLOOD AND THE ACTIVITY OF ALCOHOL DEHYDROGENASE

Yoshio OHMIYA and Kengo NAKAI
Department of Pharmacology, Akita University School of Medicine, Akita 010, Japan
Accepted April 11, 1977

Abstract—In an attempt to assess the effects of methylmercury on ethanol metabolism, Sprague-Dawley rats were treated with a daily dose (10 mg/kg i.p.) of methylmercuric chloride for 2 consecutive days and given a test dose (0.4 g/kg i.v.) of ethanol 24 hr after the last treatment. Blood ethanol levels were measured using gas chromatography by the direct introduction of blood samples into the sample vaporizing apparatus attached to the chromatograph. While treatment with methylmercury elicited a slight retardation in the ethanol elimination from the blood during 30 to 90 min, methylmercury did not essentially alter ethanol metabolism. There was no significant change in hepatic alcohol dehydrogenase activity of methylmercury-treated rats. By contrast, the activity of alcohol dehydrogenase purified from liver or yeast was remarkably inhibited by methylmercury and the type of inhibition proved to be non-competitive. Moreover, the inhibited activity was reactivated easily by sulfhydryl agents. From these results, it is conceivable that methylmercury has little influence on ethanol metabolism in vivo because of its non-specific binding with sulfhydryl groups in the organism.

It is well known that it is the liver and kidney which retain the highest concentrations of mercury after treatment with methylmercury (1). Nevertheless, interest has focused on the dramatic neurotoxic effect of methylmercury and the hepatotoxic effect has been somewhat neglected. Recently, an increasing number of data regarding the hepatotoxicity have been reported. Methylmercury-treated rats exhibited decreased hepatic microsomal mixed-function oxidase activity, which correlated with the prolongation of hexobarbital sleeping time (2, 3). An inhibitory effect of methylmercury was also shown on liver mitochondrial oxidase and dehydrogenase activities (4). Adenyl cyclase localized in the liver plasma membrane proved to be extremely sensitive to methylmercury, and HgCl₂ and p-chloromercuribenzoate were next in order of sensitivity (5). Moreover, methylmercury is considered to affect the stability of the hepatic lysosomal membrane (6). Apparently there is little documentation concerning the effects of methylmercury on the activity of cytoplasmic enzymes.

Ethanol is metabolized principally in the liver and the rate-limiting enzyme is thought to be alcohol dehydrogenase (ADH) which is localized in the cytoplasm (7). Coldwell and Platonow (8) studied the effect of methylmercury on the rate of disappearance of ethanol from the blood and found a highly significant increase in blood ethanol levels and a faster disappearance rate. In their experiments, swine were given methylmercury and ethanol concurrently injected intracardially and intraperitoneally. Since their experimental con-
ditions were rather extraordinary, a generalization of the effects of methylmercury on ethanol metabolism may not be feasible.

Using a different methodology, we attempted to clarify the effects of methylmercury on ethanol metabolism.

MATERIALS AND METHODS

Chemicals and abbreviations used

Methylmercuric chloride (MMC) (Wako Pure Chemical Ind.), yeast alcohol dehydrogenase (ADH-Y) (Seikagaku Kogyo), horse liver alcohol dehydrogenase (ADH-L) (Boehringer Mannheim Corp.), nicotinamide adenine dinucleotide (NAD) (Tokyo Kasei), reduced glutathione (GSH) (Boehringer Mannheim Corp.), oxidized glutathione (GSSG) (Sigma Chemical Co.), thiamine and thiamine tetrahydrofurfuryl disulfide (TTFD) (Takeda Chemical Ind.), and cysteine and cystine (Wako Pure Chemical Ind.). All other chemicals were obtained commercially and used without further purification.

Animals

Female Sprague-Dawley rats weighing about 250 g were used.

Treatments

MMC was dissolved in olive oil at the concentration of 1% and given i.p. to animals in a daily dose of 10 mg/kg for 2 successive days. Olive oil alone was given as a vehicle control. A test dose (0.4 g/kg) of ethanol was given i.v. as a 40% (w/v) solution containing 1.4 M glucose 24 hr after the last administration of MMC.

Ethanol elimination from the blood

About 0.2 ml of blood samples were drawn at various time-intervals through a cannula inserted into the carotid artery of rats. Blood ethanol levels were determined using gas-chromatography by the direct injection of 1-5 μl of blood into the sample vaporizing apparatus attached to the gaschromatograph. In principle, the method described by Yokota et al. (9) for inhalation anesthetics was used.

Liver sampling and subcellular fractionation

The rats were exsanguinated from the carotid artery. The livers were quickly removed, weighed, and homogenized in 9 volumes of ice cold 1.15% KCl solution. The homogenate was centrifuged at 105,000 g for 2 hr in an ultracentrifuge and a clear supernatant was obtained. Protein content was determined by the method of Lowry et al. (10).

ADH activity assay

ADH activity was estimated by the method of Bonnichsen and Brink (11) with slight modification: the reaction mixture contained 3 ml of 0.1 M glycine-NaOH buffer (pH 9.6), 0.1 ml of liver supernatant or purified enzyme solution, and 0.1 ml of 95% ethanol. The blank contained 0.1 ml of distilled water in place of ethanol. To each was added 0.1 ml of 1% NAD solution and NADH formation was measured at 340 nm in a recording spectrophotometer for a 3 min period. Activity was expressed as nmoles of NADH/min/g liver.
or mg protein. Effect of MMC on purified ADH activity was studied by addition of 50 μl of MMC solution containing 5 mM Na₂CO₃ to the reaction mixture. Sulfhydryl (SH) and disulfide (SS) agents were also added to the reaction mixture as 50 μl of water solution about 5 min after MMC. When the concentration of NAD or ethanol was varied, the concentration of its reaction partner was kept constant.

RESULTS

Effect of MMC on ethanol elimination from the blood

Time courses of mean blood ethanol levels following an intravenous injection of a test dose (0.4 g/kg) are depicted graphically in Fig. 1. Treatment with MMC decreased slightly the rate of ethanol elimination from the blood during 30 to 90 min. Thereafter, no significant difference was observed between control and MMC-treated groups. Under identical experimental conditions, treatment with pyrazole, a potent ADH inhibitor produced a marked retardation in ethanol disappearance.

Fig. 1. Time course of blood ethanol level in rats. A test dose (0.4 g/kg) of ethanol was given i.v. to control (— ● —) and MMC-treated (— ○ —) rats. A daily dose (10 mg/kg) of MMC was given i.p. for 2 consecutive days and 24 hr before ethanol administration. Each point represents the mean of 6 or more experiments and vertical bars indicate S.E.M. Significant differences from control are denoted by *P < 0.05.

Effect of MMC on hepatic ADH activity

Since it is generally accepted that the principal enzyme responsible for ethanol oxidation is hepatic ADH, effect of treatment of rats with MMC on the ADH activity was studied. Fig. 2 presents a comparison of hepatic ADH activity of MMC-treated rats to that of controls. There were no significant differences in the activities whether expressed as per g liver or per
Inhibition of purified ADH activity by MMC

In vitro inhibition of purified ADH activity by MMC is shown in Fig. 3. When molecular weight of yeast alcohol dehydrogenase (ADH-Y, right) was assumed to be 150,000 (12) and liver alcohol dehydrogenase (ADH-L, left) 73,000 (13), the enzyme concentrations used were equivalent to 0.1 μM and 0.2 μM respectively. Under the present experimental conditions in which the activities were estimated as approximately 100 nmoles of NADH/
min/reaction cuvette, the concentrations of MMC required for 50% inhibition were 1.3 µM for ADH-Y and 3.0 µM for ADH-L. As a matter of course, the inhibition rate by a given concentration of MMC increased with decreasing amounts of enzymes (data not included).

**Type of inhibition by MMC**

As is evident from the double reciprocal plots illustrated in Fig. 4, the type of inhibition

---

**Fig. 4.** Inhibition of ADH-L activity by MMC: double reciprocal plot. The constant NAD concentration was 450 µM for varying concentrations of ethanol from 0.16 to 1.25 mM (upper) and the constant ethanol concentration was 615 mM for varying concentrations of NAD from 3.3 to 26.6 µM (lower).

**Fig. 5.** Reactivation of MMC-induced inhibition of ADH-L activity by SH agents. SH or SS agents were added about 5 min after addition of 25 µM of MMC to the reaction mixture as described in Fig. 3 and the reaction was initiated by addition of 1% NAD solution.
was shown to be essentially non-competitive, whether with respect to ethanol (upper) or NAD (lower). It was clear that MMC bound with many sites other than the enzymatic active ones.

Reactivation of MMC-inhibited ADH activity by SH agents

In the experiments in which ADH activity was inhibited by near 100% by 25 μM of MMC, the extent which SH agents reactivated the activity was determined and results are shown in Fig. 5. With GSH in a concentration of 100 μM the inhibited activity was recovered completely whereas the corresponding SS agent GSSG had no effect. 2-Mercaptoethanol exhibited a lesser ameliorative effect than GSH (Fig. 5, left). Similarly, cysteine and thiamine were found to reactivate MMC-inhibited ADH activity, whereas SS agents cystine and TTFD had no effect (Fig. 5, right).

DISCUSSION

The present results concerning the effect of MMC on the ethanol elimination from the blood are in striking contrast to those reported by Coldwell and Platonow (8). These authors found a faster disappearance rate of ethanol induced by the simultaneous administration of intracardial methylmercuric acetate. However, the injurious influence on the organism of methylmercury in a toxic dose develops after a period of at least hours or days, as demonstrated by behavioral changes (14), functional and structural disturbances of the nervous system (15, 16) and reduction of amino acid incorporation into proteins (17). In our experiments, ethanol was administered i.v. to intoxicated rats usually with loss in body weight and abnormal gait.

A slight retardation in elimination of ethanol from the blood was observed in MMC-treated rats within the first 90 min (Fig. 1). Taking into account the lack of decrease in hepatic ADH activity (Fig. 2), the retardation was probably due to factors other than the reduction of metabolic capacity. Numerous studies of ethanol metabolism have been carried out on the assumption that the rate of ethanol disappearance from the blood reflects the rate of ethanol metabolism. However, the disappearance rate can also be changed by other factors such as alteration in the distribution of body water, distribution of the blood flow among various tissues, and peripheral circulatory flow rates (18). On the other hand, the possibility has been discussed that the capillary damage due to acute mercury poisoning results in a loss of protein or fluid through the vessel walls and a decrease in the circulatory volume (19).

Ethanol, which is principally metabolized by ADH, is also oxidized in vitro by a microsomal ethanol oxidizing system (MEOS). However, there has been no agreement regarding the significance of MEOS in ethanol metabolism in vivo. In preliminary work in our laboratory, we found that the marked reduction of MEOS activity induced by CCl₄ did not affect the rate of ethanol elimination from the whole body of mice. Thus it would appear that when the ADH activity is intact, further systems are not required for ethanol metabolism.

In the present experiment, MMC was shown to have a potent inhibitory effect on the activity of purified ADH obtained from yeast or liver (Fig. 3) despite the lack of inhibition of hepatic ADH activity with the MMC treatment (Fig. 2). This difference between in
*vitro* and *in vivo* effects of MMC can be explained by the non-specific and reversible binding of MMC with SH groups of ADH, as suggested by the essentially non-competitive type of inhibition (Fig. 4) and easy reactivation of the inhibited activity by SH agents (Fig. 5). Since mercurials are characterized by the chemical affinity for SH groups and ADH is known to be a SH enzyme, MMC-induced inhibition of ADH activity is considered to result from the SH blockade. It seems likely that MMC binds with SH groups other than those of hepatic ADH, however, the SH contents were not determined in the present work. Pekkanen and Sandholm (20) reported that treatment of rats with 58 mg Hg/kg of methylmercury for 15 days elicited a statistically significant but slight (20%) decrease in the number of SH groups in the brain and liver. The results obtained herein suggest that MMC has little effect on ethanol metabolism *in vivo* because of its non-specific binding with SH groups in the organism.

_Acknowledgement:_ We gratefully acknowledge the technical assistance of Miss R. Abe.

**REFERENCES**

1) **IVERSON, F., DOWNIE, R.H., PAUL, C. AND TRENHOLM, H.L.**: _Toxicol. appl. Pharmacol._ 24, 545 (1973)

2) **ALVARES, A.P., LEIGH, S., COHN, J. AND KAPPAS, A.**: _J. exp. Med._ 135, 1406 (1972)

3) **LUCIER, G.W., MATTHEWS, H.B., BRUBAKER, P.E., KLEIN, R. AND McDaniel, O.S.**: _Mol. Pharmacol._ 9, 237 (1973)

4) **MAGNVAL, R., BATTI, R. AND THIESSARD, J.**: _Experientia_ 31, 406 (1975)

5) **STORM, D.R. AND GUNSLER, R.P.**: _Nature_ 250, 778 (1974)

6) **PEKKANEN, T.J.**: _Acta vet. scand._ 12, 523 (1971)

7) **HAWKINS, R.D. AND KALANT, H.**: _Pharmacol. Rev._ 24, 67 (1972)

8) **COLDWELL, B.B. AND PLATONOW, N.**: _Toxicol. appl. Pharmacol._ 14, 368 (1969)

9) **YOKOTA, T., HITOMI, Y., OHITA, K. AND KOSAKA, F.**: _Anesthesiology_ 28, 1064 (1967)

10) **LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. AND RANDALL, R.J.**: _J. biol. Chem._ 193, 265 (1951)

11) **BONNICHSEN, R.K. AND BRINK, N.G.**: _Methods in Enzymology_. Edited by COLOWICK, S.P. AND KAPLAN, N.O., Vol. I, p. 495, Academic Press, New York (1955)

12) **HAYES, J.E. AND VEILICK, S.F.**: _J. biol. Chem._ 207, 225 (1954)

13) **THEORELL, H. AND BONNICHSEN, R.K.**: _Acta chem. scand._ 5, 1105 (1951)

14) **POST, E.M., YANG, M.G., KING, J.A. AND SANGER, V.L.**: _Proc. Soc. exp. Biol. Med._ 143, 1113 (1973)

15) **Cavanaugh, J.B. AND CHEN, F.C.K.**: _Acta neuropathol._ 19, 208 (1971)

16) **KLEIN, R., HERMAN, S.P., BRUBAKER, P.E., LUCIER, G.W. AND KRIGRMAN, M.R.**: _Arch. Pathol._ 93, 408 (1972)

17) **Cavanaugh, J.B. AND CHEN, F.C.K.**: _Acta neuropathol._ 19, 216 (1971)

18) **KALANT, H.**: _The Biology of Alcoholism_. Edited by KISSEN, B. AND BIGLIERI, H., p. 1, Plenum Press, New York (1971)

19) **HARVEY, S.C.**: _The Pharmacological Basis of Therapeutics_, 5th Ed., Edited by GOODMAN, L.S. AND GILMAN, A., p. 936, MacMillan Publishing Co., New York (1975)

20) **PEKKANEN, T.J. AND SANDHOLM, M.**: _Acta vet. scand._ 12, 551 (1971)