Chem. Pharm. Bull. 30(4)1437—1442(1982)

Lipid Peroxidation stimulated by Mercuric Chloride and Its Relation to the Toxicity

Masanori Yonaha,*,[a] Yuko Ohbayashi,[a] Takamichi Ichinose,[b] and Masaru Sagai[b]

Hokkaido Institute of Pharmaceutical Sciences,[a] 7-1, Katsuraoka-cho, Otaru, 047-02, Japan and The National Institute for Environmental Studies,[b] P.O. Tsukuba, Ibaraki 305, Japan

(Received September 10, 1981)

The toxicity of mercuric chloride (MC) in mice, measured in terms of the single dose lethal to 50 percent of the animals after 12 h, was enhanced by prior exposure to a vitamin E-deficient diet and by pretreatment with diethyl maleate, and was diminished by pretreatment with *N*,*N*′-diphenyl-*p*-phenylenediamine. Lipid peroxidation as determined by measurement of the pentane content in expired gases of rats showed a dose-dependent increase 12 h after the subcutaneous injection of MC. In the kidney of rats 12 h after a 4 mg/kg dose of MC, formation of thiobarbituric acid (TBA)-reactive substances was greatly increased. Slight increases of pentane in expired gases and of TBA-reactive substances in the kidney of rats at earlier times after a 2 mg/kg dose of MC were also seen compared to the control. After injection of MC (2 or 4 mg/kg), glutathione was decreased in the kidney at 12 h.

The urinary excretions of alkaline phosphatase (ALP) and leucine aminopeptidase (LAP) were markedly increased 6 h after a 4 mg/kg dose of MC, and at a dose of 2 mg/kg the excretion of LAP, but not that of ALP, was also elevated at 12 h after MC.

These results indicate that lipid peroxidation may be partly responsible for the acute-toxic effect of MC, which involves renal damage.

Keywords——mercuric chloride; mercuric chloride toxicity; renal damage; lipid peroxidation; thiobarbituric acid-reactive substances; vitamin E; glutathione; urinary enzyme

Introduction

A possible role of lipid peroxidation has been reported in the pathogenesis of renal necrosis in choline-deficient rats,[1] lung lesions due to paraquat toxicity,[2] carbon tetrachloride toxicity,[3] and ethanol-induced liver injury.[4] Recently, we have demonstrated diene conjugation absorption in the microsomal lipids and the increased production of thiobarbituric acid (TBA)-reactive substances in the kidney of rats given mercuric chloride (MC),[5] but whether stimulated lipid peroxidation is associated with the manifestation of renal damage by MC was not clarified. On the other hand, Arstila *et al.*[6] reported that *in vitro* lipid peroxidation of kidney microsomes induced a highly characteristic sequence of morphological changes, including a change similar to the tubular aggregation found in the kidney after MC poisoning by Gritzka *et al.*[7]

The ability of a lipid-soluble antioxidant, vitamin E, to provide protection against the toxicity of methylmercuric chloride has been demonstrated in terms of improvement of the survival rate of Japanese quails[8] and the growth rate of rats,[9] and development of nerve fibers, glial cells, and fibroblasts from cerebellum tissue in culture.[10] This efficacy of vitamin E against mercury toxicity raises the possibility that lipid peroxidation *in vivo* is induced by mercury, and may be partly involved in the toxic effect of mercury. Martin *et al.*[11] reported that rats reared for a long time on diets deficient in vitamin E and containing 20% lard exhibited a progressive necrosis of the proximal convoluted tubules.

The present experiments were conducted to determine whether lipid peroxidation stimulated by MC is involved in the manifestation of toxicity and kidney damage by MC.

Experimental

Animals——Male rats (8—9 weeks old) of the Wistar strain, kept on a standard laboratory diet, rat chow MF (Oriental Yeast Co., Ltd.), were used. MC ($HgCl_2$, Wako Pure Chemical Industries, Ltd.) was dissolved in saline, and injected subcutaneously into rats at a single dose of 2 mg or 4 mg/kg. Male mice of ddY strain (8 weeks old) were used to determine the dose lethal to 50 percent of the animals at 72 h and TBA-reactive substances in the kidney 24 h after a subcutaneous injection of MC. Mice were intraperitoneally treated with diethyl maleate (DEM) 1.2 ml/kg in corn oil at 30 min or with *N*,*N*′-diphenyl-*p*-phenylenediamine (DPPD) 600 mg/kg in corn oil at each of the time periods (48 h, 24 h, and 40 min) before MC. Other mice were reared on a standard laboratory diet (vitamin E 100 mg/kg diet, Oriental Yeast Co., Ltd.), diet deficient in vitamin E (vitamin E 8 mg/kg diet, Oriental Yeast Co., Ltd.), or diet with excess vitamin E (vitamin E 1000 mg/kg added to vitamin E-deficient diets) for 5 weeks (to 3 weeks-old mice).

Lipid Peroxidation *in Vivo*——Rats were anesthetized with ether and exsanguinated by cutting the femoral artery and vein. Kidneys were quickly excised and homogenized in 0.05 M Tris-KCl (0.14 M) buffer (pH 7.4), containing 3 mM EDTA. Mice were sacrificed by decapitation, and the kidneys were homogenized in the same manner as for preparation of rat kidney homogenates. The kidney homogenates obtained were used for the determination of TBA-reactive substances as described by Uchiyama *et al.*[12] Amounts of TBA-reactive substances were expressed in terms of nmol malondialdehyde (MDA)/mg protein or calculated as percent of the control without MC injection. Gas chromatographic analysis of hydrocarbon gases in the breath of rats was conducted by the method of Dillard *et al.*[13] Pentane in expired gases was calculated as pmol pentane expired/100 g body weight/min, and then expressed in terms of percent of the control without MC injection.

Assay——The content of glutathione in the kidney was estimated by the spectrofluorometric method described by Cohn *et al.*[14] Urine of rats was obtained at 6, 12, and 24 h after MC, and the activities of alkaline phosphatase (ALP) and leucine aminopeptidase (LAP) were determined by the use of Alkaline Phospha K-Test Wako (Wako Pure Chemical Ins., Ltd.) and by the method of Amador *et al.*,[15] respectively. Protein was measured by the method of Lowry *et al.*[16]

Results

Hydrocarbons in Expired Gases of Rats after MC Injection

Hydrocarbon gases in the breath of rats were determined at 4, 12, 24, and 48 h after a 2 mg/kg dose of MC and at 6, 12, 24, and 48 h after a 4 mg/kg dose of MC. No increase of

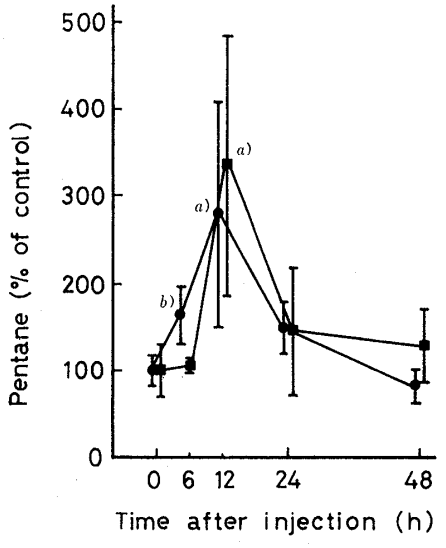

Fig. 1. Pentane in the Breath of Rats injected with MC

Each point represents the mean ± standard deviation from five to six rats. —●—, 2 mg MC/kg; —■—, 4 mg MC/kg.
a) Significant difference, $p<0.05$. *b*) Significant difference, $p<0.01$.

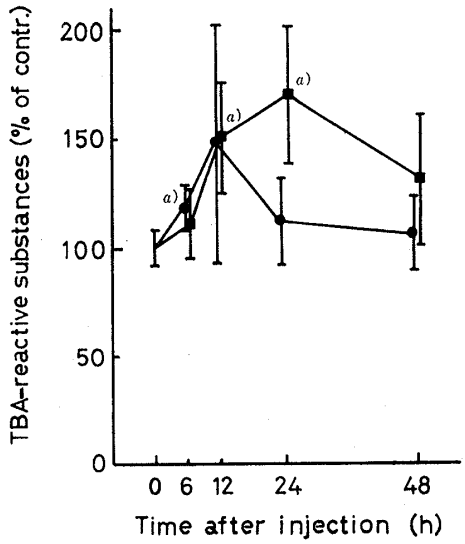

Fig. 2. TBA-Reactive Substances in the Kidney of Rats injected with MC

Each point represents the mean ± standard deviation from five rats. —●—, 2 mg MC/kg; —■—, 4 mg MC/kg.
a) Significant difference, $p<0.01$.

ethane production was seen during the experimental period. Pentane production was significantly increased 12 h after MC injection, then returned to the normal level (Fig. 1). A dose-response relationship between the amount of MC injected and the amount of pentane expired 12 h after the injection was observed ($r=0.768$, $p<0.001$).

TBA-Reactive Substances and Glutathione in the Kidney

TBA-reactive substances in the kidney of rats were significantly increased from 12 to 24 h after MC injection (4 mg/kg), and were slightly increased at only 6 h after injection of a 2 mg/kg dose of MC (Fig. 2). Glutathione in the kidney of rats was markedly decreased at 12 h after injection of MC (2 mg/kg), then returned to normal (Fig. 3).

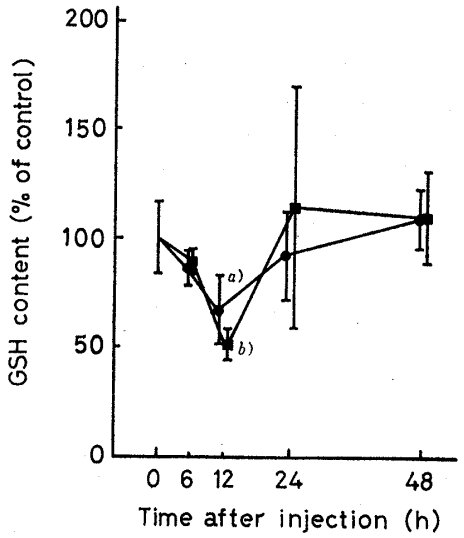

Fig. 3. Glutathione in the Kidney of Rats injected with MC

Each point represents the mean ± standard deviation from five rats. —●—, 2 mg MC/kg; —■—, 4 mg MC/kg.
a) Significant difference, $p<0.01$. *b*) Significant difference, $p<0.001$.

Urinary Excretion of ALP and LAP in Rats after MC Injection

The activities of both ALP and LAP in the urine were markedly increased at 6 h after a 4 mg/kg dose of MC (Fig. 4). After a 2 mg/kg dose of MC, the urinary excretion of LAP was elevated at 12 h, but that of ALP did not significantly increase through 24 h.

Effects of DEM, DPPD, and Vitamin E on the Toxicity of MC to Mice

LD_{50} of MC to mice was enhanced by pretreatment with DEM, a glutathione-depleting agent, and by prior exposure to a diet deficient in vitamin E, and was reduced by pretreatment

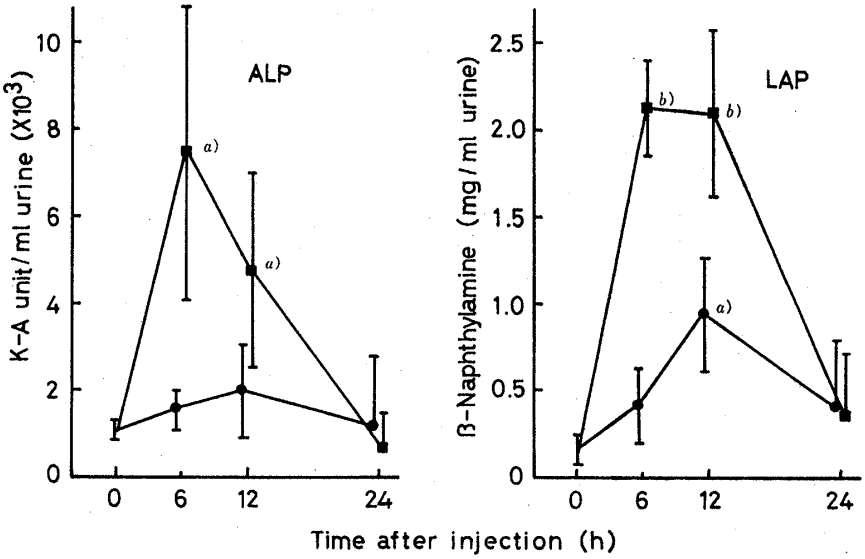

Fig. 4. Urinary Excretion of ALP and LAP by Rats injected with MC

Each point represents the mean ± standard deviation from three to six rats. —●— 2 mg MC/kg: —■— 4 mg MC/kg.
a) Significant difference, $p<0.05$. *b*) Significant difference, $p<0.01$.

Table I. LD_{50} of MC in Mice after Various Pretreatments

Pretreatment	LD_{50} (mg Hg/kg)	(95% C.L.)	Potency ratio
Control (corn oil)	8.6	(7.2—10.2)	—
DEM	1.8	(1.2—2.6)	4.78[a]
Control (corn oil)	8.5	(7.0—10.3)	—
DPPD	10.5	(9.3—11.9)	0.81[a]
Control	7.8	(6.4—9.5)	—
Vitamin E-deficient diet	6.1	(5.0—7.4)	1.28[a]
Vitamin E-excess diet	7.9	(6.7—9.3)	0.99

a) Significant difference from control, $p<0.05$.

Table II. Effect of MC on the Production of TBA-reactive Substances in Mouse Kidneys after Pretreatment with Vitamin E-deficient or Vitamin E-excess Diet

Pretreatment	nmol MDA/mg protein	% of control
Vitamin E-deficient diet		
Control (5) (saline)	0.35±0.06	—
MC (5)	0.64±0.04	182.9±11.4[b]
Vitamin E-excess diet		
Control (4) (saline)	0.24±0.07	—
MC (4)	0.39±0.02[a]	162.5±8.3[c]

The kidneys were analyzed 24 h after the subcutaneous injection of MC (4 mg/kg). The values in parentheses are the numbers of animals. Each value is the mean± standard deviation.

a) Significant difference from the group fed vitamin E-deficient diet, $p< 0.001$.
b) Significant difference from control, $p<0.001$.
c) Significant difference from control, $p<0.05$.

with an antioxidant, DPPD. On the other hand, no change of LD_{50} of MC was found after prior exposure to a diet with excess vitamin E (Table I). Increased formation of TBA-reactive substances was also observed in the kidney at 24 h after the injection of MC (4 mg/kg) into mice, which had been fed a diet deficient in vitamin E or with excess vitamin E (Table II). Prior exposure to a diet with excess vitamin E reduced the formation of TBA-reactive substances in the kidney of mice stimulated by MC in comparison with that in the kidney of mice exposed to a diet deficient in vitamin E.

Discussion

Antioxidants have a protective effect against some tissue injuries in whose pathogenesis lipid peroxidation may be involved. Gallagher[17] showed that the antioxidants α-tocopheryl acetate, sodium selenite, and DPPD afforded protection to rats against carbon tetrachloride-induced liver injury. Di Luzio[18] suggested that the protective actions of vitamin E and DPPD on carbon tetrachloride-induced liver injury are due to their function as antioxidants *in vivo*. DPPD but not vitamine E also reduced the incidence and severity of the renal lesions in choline-deficient rats.[1] MC toxicity in mice was reduced by pretreatment with DPPD, and enhanced by prior exposure to a diet deficient in vitamin E. Formation of TBA-reactive substances in the kidney of mice fed a diet deficient in vitamin E was elevated by MC injection . Furthermore, mice pretreated with DEM were more susceptible to MC toxicity than non-treated mice. These observations suggest that MC toxicity may be partly mediated through lipid peroxidation. On the other hand, high levels of vitamin E did not have any protective effect against MC toxicity, and could not completely protect against the increase of TBA-reactive substances in the kidney after MC.

The exhalation of hydrocarbon gases by animals provides a means to detect lipid peroxidation *in vivo*. Ethane production was shown to be characteristic of lipid peroxidation *in vivo*.[19,20] Recently, pentane was shown to be an even more sensitive index of lipid peroxidation in rats fed a vitamin E-deficient diet[13] and injected with some halomethanes.[21] Pentane production showed a dose-response relationship at 12 h after MC injection, while no increase of ethane production was seen after treatment with MC. Dumelin *et al.*[22] reported that pentane and ethane were the predominant short-chain hydrocarbon gases to arise during iron-catalyzed decomposition of linoleic and arachidonic acid hydroperoxides, and linolenic acid hydroperoxides, respectively. Production of pentane and TBA-reactive substanecs was increased by injection of MC. The results show that lipid peroxidation was stimulated by MC in rats, in agreement with the previous report.[5] The main origin of expired pentane following treatment of rats with MC is probably the kidney, since formation of TBA-reactive substances was increased in the kidney but not in the liver (data not shown). In the present study, glutathione was depressed in the kidney at 12 h after MC injection. The tissue should be more susceptible lipid peroxidation as a result of a depression of glutathione.

ALP and LAP are the most characteristic enzymes of renal tissue, which is affected early and most severely by MC. A significant increase of urinary ALP during 24 h after the subcutaneous injection of MC at a dose of 7.4 μmol (2 mg)/kg[23] or 1 mg/kg[24] has been reported. In terms of the enzyme activity per ml urine, no significant increase of urinary ALP was seen during 24 h after MC (2 mg/kg) in the current investigation. However, in a preliminary experiment the activity of ALP in the first 24 h urine after MC (2 mg/kg) was significantly increased. The discrepancy of the results due to the different expression of the enzyme activity is based on the diuretic effect of MC. Planas-Bohne[25] reported that LAP was more drastically affected than ALP after MC. In fact, urinary LAP was increased 12 h after MC (2 mg/kg). At a 4 mg/kg dose of MC the urinary excretion of ALP and LAP was dramatically increased at 6 h. Pentane exhalation and TBA-reactive substances in the kidney of rats were markedly increased at 12 h after MC (4 mg/kg). These results suggest that lipid peroxidation stimulated by MC may play an important role in renal damage in MC intoxication.

Acknowledgement This work was supported in part by a grant for Environmental Preservation Research from the Environmental Agency of Japan.

References and Notes

1) A.J. Monserrat, A.K. Ghoshal, W.S. Hartroft, and E.A. Porta, *Amer. J. Pathol.*, **55**, 163 (1969).
2) J.S. Bus, S.D. Aust, and J.E. Gibson, "Biochemical Mechanism of Paraquat Toxicity," ed. by A.P. Autor, Academic Press, New York, 1977, p. 157.
3) R.O. Recknagel and A.K. Ghoshal, *Lab. Invest.*, **15**, 132 (1966).
4) N.R. Di Luzio and A.D. Hartman, *Federation Proc.*, **26**, 1436 (1967).
5) M. Yonaha, E. Itoh, Y. Ohbayashi, and M. Uchiyama, *Res. Commun. Chem. Pathol. Pharmacol.*, **28**, 105 (1980).
6) A.U. Arstila, M.A. Smith, and B.F. Trump, *Science*, **175**, 530 (1972).
7) T.D. Gritzka and B.F. Trump, *Amer. J. Pathol.*, **52**, 1225 (1968).
8) S.D. Welsh and J.H. Soares, *Nutr. Rep. Int.*, **13**, 43 (1976).
9) H. Harada, K. Ito, K. Ebato, M. Takeuchi, T. Amemiya, J. Yamanobe, S. Suzuki, and T. Totani, *Ann. Rep. Tokyo Metr. Res. Lab. P.H.*, **26**, 123 (1975).
10) M. Kasuya, *Toxicol. Appl. Pharmacol.*, **32**, 347 (1975).
11) A.J.P. Martin and T. Moore, *J. Hyg.*, **39**, 643 (1939).
12) M. Uchiyama and M. Mihara, *Anal. Biochem.*, **86**, 271 (1978).
13) J.C. Dillard, E.E. Dumelin, and A.L. Tappel, *Lipids*, **12**, 109 (1977).
14) V.H. Cohn and J. Lyle, *Anal. Biochem.*, **14**, 434 (1966).
15) E. Amador, W.E.C. Wacker, "Methods of Biochemical Analysis," Vol. 13, ed by D. Glick, John Wiley and Sons Inc., New York, 1974, p. 313.
16) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
17) C.H. Gallagher, Austral. *J. Exp. Biol.*, **40**, 241 (1962).
18) N.R. Di Luzio, *Federation Proc.*, **32**, 1875 (1973).

19) C.A. Riely, G. Cohen, and M. Lieberman, *Science*, **183**, 208 (1974).
20) D.G. Hafeman and W.G. Hoekstra, *J. Nutr.*, **107**, 656 (1977).
21) M. Sagai and A.L. Tappel, *Toxicol. Appl. Pharmacol.*, **49**, 283 (1979).
22) E.E. Dumelin and A.L. Tappel, *Lipids*, **12**, 894 (1977).
23) E.A. Lock and J. Ishmael, *Toxicol. Appl. Pharmacol.*, **50**, 67 (1979).
24) W.E. Stroo and J.B. Hook, *Toxicol. Appl. Pharmacol.*, **39**, 423 (1977).
25) F. Planas-Bohne, "Clinical Chemistry and Chemical Toxicology of Metals," ed. by S.S. Brown, Elsevier, North-Holland Biomedical Press, 1977, p. 119.