EFFECTS OF CARBON MONOXIDE EXPOSURE ON THE DISTRIBUTION OF METHYLMERCURY IN MICE

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Abstract.....Effects of long-term exposure to sublethal concentration (300-350 ppm) of carbon monoxide (CO) on the distribution of methylmercury (MeHg) in the blood and organs of mice were examined using 6th week-old ICR mice of both sexes. Firstly, female mice were exposed to CO immediately after single ip injection of CH_3HgCl (1 mg/kg). At the earliest stage, brain mercury level was higher in CO mice than in control mice, while blood mercury level was lower in CO mice than in control mice. There were indications that compensatory hemoconcentration and resultant increase of mercury levels in the blood, brain and liver occurred in CO mice by the 8th day of CO exposure. Mercury in the blood, brain, liver and kidney decreased more rapidly in CO mice than in control mice for a short period after hemoconcentration had occurred. Secondly, male mice were pre-exposed to CO for 7 days, received single ip injection of CH_3HgCl (1 mg/kg) and were re-exposed to CO for an additional 21 days. Hemoconcentration, increased mercury levels in the blood, brain and liver were observed in CO mice. Thirdly, male mice were pre-exposed to CO for 7 days, administered po with CH_3HgCl (2 mg/kg) and re-exposed to CO for 24 hr. Mercury levels in the blood, brain and liver but not the kidneys were higher in CO mice than in control mice. The relationships between hemoconcentration and MeHg distribution in vertebrates were discussed.

Key words : methylmercury, distribution, carbon monoxide, mouse.

INTRODUCTION

Organ distribution of methylmercury (MeHg) changes according to various factors such as animal species (Nordberg and Skerfving, 1972), strains of mouse (Miller and Csonka, 1968; Doi and Kobayashi, 1982), dietary protein content (Landry et al, 1979), biliary obstruction (Norseth and Clarkson, 1971) and coadministration of thiol containing chemicals (Berlin et al, 1965; Clarkson et al, 1973; Alexander and Aaseth, 1982).

Contribution of hereditary factors to the distribution of MeHg has also been elucidated (Doi and Kobayashi, 1982; Doi et al, 1983; Doi and Tagawa, 1983). These authors revealed that the number of cysteinyl residues in a hemoglobin (Hb) molecule and amino acid sequence of the Hb molecule determine the affinity of Hb for MeHg and consequently determine the blood mercury concentration following MeHg administration.

The largest part of MeHg in the blood is bound to erythrocytes (RBC) irrespective of the species of animals (Nordberg and Skerfving, 1972). In rat RBC, ethyl- and methyl-mercury compounds are bound mostly to Hb (Takeda et al, 1968; Garcia et al, 1974), while in rabbit and human RBC more than 50% of MeHg is bound to a low molecular weight substance which was assumed to be glutathione (Naganuma and Imura, 1979; Naganuma et al, 1980). Therefore changes in RBC count and Hb concentration in the blood might affect the MeHg concentration in the blood. As is well known, hemoconcentration occurs following long-term exposure to sublethal concentration of carbon monoxide (CO) (Jones et al, 1971; Penny et al, 1979; Thomas and Penny, 1977). This report deals with the effects of continuous CO exposure on the organ distribution of MeHg through compensatory reaction in hematopoiesis against CO.

MATERIALS AND METHODS

Animals: ICR strain mice of both sexes were used at 6 weeks after birth in the experiments. These mice were supplied from Charles River Japan, Inc. Tokyo, at 5 weeks of age and were kept at the Animal Experiment Center of Asahikawa Medical College until use.

MeHg administration and CO exposure: MeHg was administered ip at a dose of 1 mg/kg as an aqueous solution of CH_3HgCl (Wako Pure Chemical Industries, Inc. Osaka) at a concentration of 100 $\mu\text{g/ml}$ and containing 0.85% NaCl and 0.5% L-cysteine in the first and second experiments. In the third experiment, MeHg was administered po with a gastric tube at a dose of 2 mg/kg as the same aqueous solution at the same concentration and with the same constituents.

CO exposure took place in a transparent plastic chamber with a size of $46 \times 56 \times 33$ (H) cm and 8 mm thickness. Two stainless steel wire cages with a size of $22 \times 30 \times 22$ (H) cm were put in a chamber and each cage held less than 18 mice at a time. Another plastic chamber of the same size was used for control mice. CO exposed mice were supplied with a mixture of CO gas and oil-free compressed air at a rate of 5 L/min per a chamber and control mice with oil-free compressed air at the same rate. Commercial grade 1:4 mixture of CO and nitrogen gas was used as the source of CO gas, and CO was given at the concentration of 300 ppm in the first experiment and 350 ppm in the second and third experiments. CO concentration in the chamber was monitored twice a day with Kitagawa colorimetric CO detector tubes (5-1,000 ppm range) and a Komyo 100 ml syringe.

In the first experiment, 60 female mice were injected ip with MeHg solution, and one half of the mice were exposed to CO for 29 days immediately after MeHg injection. The other half of the mice were kept in the control chamber for the same period.

In the second experiment, 35 male mice were exposed to CO for 7 days prior to MeHg administration and were injected ip with MeHg solution on the 8th day of CO exposure. CO exposure was continued for an additional 21 days after MeHg injection. A control of 35 males were kept in the control chamber and treated the same except for the CO exposure.

In the third experiment, 7 male mice were exposed to CO for 7 days prior to MeHg administration and were administered with MeHg solution po on the 8th day of CO exposure. These mice were exposed to CO for an additional 24 hr after MeHg administration. A control of 9 males were treated the same except for the CO exposure.

Chambers were opened and exposure was interrupted for approximately 15 min once a day to clean the waste in the cages and to supply mouse pellets and drinking water. All mice were supplied with commercial mouse pellets and tap water *ad libitum*. Temperature was kept in the range of 23 to 27°C and relative humidity in the range of 65 to 75% in the chambers.

Autopsy and mercury determination in the organs: Five to 7 mice of each group were bled from the femoral artery under anesthesia with pentobarbital (20 mg/kg) at various time intervals, and the brain, liver and kidneys were excised without perfusion. Total mercury analysis of the blood, brain, liver and kidneys was performed by the method described previously (Doi and Kobayashi, 1982).

RESULTS

Experiment 1.

Body weight and physical activity : No statistically significant difference was observed in body weight between CO exposed mice and control mice, though mean body weight of CO exposed mice was larger than that of control mice from 3 days after MeHg injection (Fig. 1).

The physical activity and food intake of the mice were greatly reduced during the first few days of CO exposure. Thereafter, the food intake for the CO exposed mice became greater than that of the control mice.

Hematologic changes : The ratio of wet to dry weight of whole blood decreased significantly in CO mice, though it decreased slightly in control mice (Fig. 2). The difference in wet to dry weight ratio between CO exposed mice and control mice was statistically significant from the 8th day to the end of the experimental period.

Mercury concentration in the organs : Whole blood : Mercury concentration in the blood was lower in CO exposed mice than that in control mice on the first and third day after MeHg injection. On the 8th day, mean blood mercury concentration in CO mice exceeded the value of control mice (Fig. 3).

Brain : On the first day after MeHg injection, mean mercury concentration in the brain was higher in CO mice than that of control mice in spite of the fact that mean

Fig. 1. Effects of CO exposure on the body-weight of female mice (Mean $\pm$ SD).

Fig. 2. Effects of CO exposure on the wet/dry weight ratio of whole blood in female mice (Mean $\pm$ SD).

* : statistically significant at $p < 0.05$.

Carbon monoxide effect on methylmercury distribution

Fig. 3. Effects of CO exposure on the distribution of methylmercury in female mice : Blood, Liver.

blood mercury level was lower in CO mice than in control mice (Fig. 4).

Liver : Changes of liver mercury concentration were similar to those found in blood mercury concentration. Mean liver mercury concentration in CO mice was lower than that of control mice on the first day, and was higher than that of control mice on the 8th day after MeHg injection (Fig. 3).

Kidney : Mean kidney mercury concentration was lower in CO mice than that in control mice throughout the experimental period (Fig. 4).

Mercury concentration in the blood, brain, liver and kidney decreased more rapidly in CO mice than in control mice during the short period from 8 to 14 days after MeHg injection, but the rapid decrease of mercury in CO mice was not observed thereafter.

Experiment 2

Hematologic changes: Hemoconcentration was found in CO mice from the 8th day after CO exposure to the end of experiment. The differences in RBC count, hematocrit (Ht) and Hb concentration were statistically significant between the two groups of mice (Fig. 5).

Mercury concentration in the organs : Whole blood : Mean blood mercury concentration was higher in CO mice than in control mice throughout the period after MeHg injection, though the difference between two groups of mice was not statistically significant at any stage of time course (Fig. 6).

Fig. 4. Effects of CO exposure on the distribution of methylmercury in female mice : Brain, Kidney.

Fig. 5. Hematologic changes in male mice exposed to CO : Erythrocytes, hematocrit and hemoglobin (Mean $\pm$ SD). All values of erythrocytes, hematocrit and hemoglobin in CO mice (■) are significantly ($p < 0.01$) higher than those in control mice (□).

Carbon monoxide effect on methylmercury distribution

Fig. 6. Effects of CO exposure on the distribution of methylmercury in male mice : Blood, Liver, Kidney.

Brain: Mean brain mercury concentration was significantly higher in CO mice than in control mice from the first to 7th day after MeHg injection, but there was no difference at the later stages (Fig. 7).

Liver: Mean liver mercury concentration was higher in CO mice than in control mice on the first and third day after MeHg injection, but was not different after the 7th day (Fig. 6).

Kidney: No difference was found in kidney mercury concentration between CO and control mice (Fig. 6).

Mercury concentration in the liver decreased more rapidly in CO mice than in control mice except the later stages of CO exposure (Fig. 6). Similarly, brain mercury concentration decreased more rapidly in CO mice than in control mice during the period from the third to 14th day after MeHg injection (Fig. 7).

Experiment 3

The results in Exp. 3 were shown in Fig. 8. Hematocrit and Hb concentration were significantly higher in CO mice than in control mice. Mean mercury concentrations in the blood, brain and liver were higher in CO mice than in control mice, though the differences were not statistically significant. Mean kidney mercury concentration was lower in CO mice than in control mice as found in Exp. 1.

* : statistically significant at $p < 0.05$.

Fig. 7. Effects of CO exposure on the distribution of methylmercury in male mice : Brain.

Fig. 8. Effects of CO exposure on blood constituents and distribution of methylmercury in male mice pre-exposed to CO and perorally administered with CH₃HgCl.

* : values for CO mice are significantly higher than those for control mice at $p < 0.05$.

Vertical bars in the figure show the averages for each group of mice. △ : control mice, ▲ : CO exposed mice.

DISCUSSION

The major toxic effects of CO appears in the central nervous system owing to the formation of CO-Hb and to resultant hypoxia. Some compensatory reactions against CO have been described in animals exposed for long periods to sublethal concentration of CO. Cardiovascular changes such as increased cardiac output and increased heart rate were demonstrated (Paulson et al, 1973; Doblar et al, 1977; Pitt et al, 1979; Penny et al, 1979). Increased cerebral blood flow is also an important compensatory reaction against sublethal concentration of CO (Paulson et al, 1973; Doblar et al, 1977; Pitt et al, 1979). These changes appeared in the early stages of CO exposure and were proportional to CO-Hb concentration. In the later stages, increased RBC count and increased Hb concentration are major compensatory reactions in the hematopoietic system (Jones et al, 1972; Thomas and Penny, 1977; Penny et al, 1979).

Increased cerebral blood flow in the early stages of CO exposure is considered to be a possible cause of higher brain mercury concentration in CO exposed mice. Increased mercury levels in the blood, brain and liver on the 8th day after MeHg injection in Exp. 1 and in the early stages after MeHg injection in Exp. 2 can be explained by compensatory increase of RBC and Hb in CO exposed mice. Higher mercury concentrations in the blood, brain and liver in CO exposed mice in Exp. 3 might also be a result of compensatory hemoconcentration by CO exposure.

An exceedingly high affinity of sulfhydryl groups for mercurials has been well known (Rothstein, 1973), and especially Hbs have the highest affinity for alkylmercury compounds as compared to that of the other proteins such as albumin, globulin and thionein (Hughes, 1957; Takeda et al, 1968; Chen et al, 1973; Fang and Fallin, 1976). It seems to be reasonable to consider that the increased Hb accelerates the intestinal absorption and transport of MeHg to and from organs.

The kinetic curve of mercury concentration showed a biphasic profile in the blood, brain and kidney of CO mice in Exp. 1 and in the brain and liver of CO mice in Exp. 2. The biphasic elimination curves have been reported in guinea pigs (Iverson et al, 1973) and in seals (Tillander, 1969). It appeared to be biphasic in some strains of mice (Doi and Kobayashi, 1982), while a single phase elimination has been reported (Östlund, 1969; Suzuki, 1969). Mercury concentration decreased more rapidly in the blood and organs of CO mice during the short period after the increase of Hb concentration in CO mice in Exp. 1 and 2. Some factors are considered as the causes of rapid decrease of mercury in CO mice. The role of Hb concentration was mentioned above. Increased body weight in CO mice might play a role as one of the causes, though statistically significant difference was not found in the body weight between CO and control mice. In the previous study, significant negative correlations were observed between the mercury concentrations in the blood and organs and the body weight of MeHg injected mice (Doi and Kobayashi, 1982). Those negative correlations might be considered as the results of redistribution and dilution of mercury in the increased body mass of the mice.

Different classes of binding sites for MeHg might be one of the causes of the biphasic kinetic curve. Doi and Tagawa (1983) revealed that Hbs of various species of animals have at least two classes of binding sites for MeHg in their molecular structures with a few exceptions. Primary sites involved cysteinyl residues at the outside of $\alpha_1\beta_1$ contact junction and cysteinyl residues in the junction, and secondary sites were consisted only of cysteinyl residues in the junction. Primary sites had the binding constants approximately 10 to 100 times higher than those of secondary sites (Doi and Tagawa, 1983).

Biotransformation of MeHg in the mice and incorporation of MeHg into the fur might also be the possible causes of the biphasic kinetic curve of organ mercury concentration.

Mean kidney mercury concentration was lower in CO mice than in control mice in Exp. 1 and 3. Changes in transport and biotransformation of MeHg owing to CO exposure are assumed to be the possible causes of this phenomenon, though further elucidation should be necessary.

It is a well known fact that mercury levels in hair and blood are always higher in men than in women in the Japanese adult population (Doi and Ui, 1975), and also that anemia is common among Japanese females (Miyata, 1966; Uchida et al, 1970; Ishihara, 1971; Nakamura et al, 1978). It is possible that low mercury levels common in Japanese females are the results of anemia.

Derban (1974) reported on an outbreak of food poisoning due to alkylmercury fungicide in Ghana and he described that the sufferer's nutritional status and the prevalent endemic diseases in the community, i. e. malaria, schistosomiasis, ancylostomiasis, ascariasis and malnutrition, may have affected their susceptibility to the mercurial. Further investigations will be necessary on the roles of these acquired factors in the toxicology of MeHg.

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REFERENCES

- Alexander, J. and Aaseth, J. (1982) : Organ distribution and cellular uptake of methyl mercury in the rat as influenced by the intra- and extracellular glutathione concentration. *Biochem. Pharmacol.*, **31**, 685-690.
- Berlin, M., Jerksell, L. -G. and Nordberg, G. (1965) : Accelerated uptake of mercury by brain caused by 2, 3-dimercaptopropanol (BAL) after injection into the mouse of a methylmercury compound. *Acta Pharmacol. Toxicol.*, **23**, 312-320.
- Chen, R. W., Ganther, H. E. and Hoekstra, W. G. (1973) : Studies on the binding of methyl-

Carbon monoxide effect on methylmercury distribution

- mercury by thionein. *Biochem. Biophys. Res. Commun.*, **51**, 383-390.
- Clarkson, T. W., Small, H. and Norseth, T. (1973): Excretion and absorption of methyl mercury after polythiol resin treatment. *Arch. Environ. Health.*, **26**, 173-176.
- Derban, L. K. A. (1974): Outbreak of food poisoning due to alkylmercury fungicide. *Arch. Environ. Health.*, **28**, 49-52.
- Doblar, D. D., Santiago, T. V. and Edelman, N. H. (1977): Correlation between ventilatory and cerebrovascular responses to inhalation of CO. *J. Appl. Physiol.*, **43**, 456-462.
- Doi, R. and Kobayashi, T. (1982): Organ distribution and biological half-time of methylmercury in four strains of mice. *Japan. J. Exp. Med.*, **52**, 307-314.
- Doi, R. and Tagawa, M. (1983): A study on the biochemical and biological behavior of methylmercury. *Toxicol. Appl. Pharmacol.*, **69**, 407-416.
- Doi, R., Tagawa, M., Tanaka, H. and Nakaya, K. (1983): Hereditary analysis of the strain difference of methylmercury distribution in mice. *Toxicol. Appl. Pharmacol.*, **69**, 400-406.
- Doi, R. and Ui, J. (1975): The distribution of mercury in fish and its form of occurrence. In *Heavy Metals in the Aquatic Environment* (P. A. Krenkel, ed.), p. 197-222, Pergamon Press, Oxford.
- Fang, S. C. and Fallin, E. (1976): The binding of various mercurial compounds to serum proteins. *Bull. Environ. Contam. Toxicol.*, **15**, 110-117.
- Garcia, J. D., Yang, M. G., Wang, J. H. C. and Belo, P. S. (1974): Carbon-mercury bond cleavage in blood of rats fed methyl mercuric chloride. *Proc. Soc. Exp. Biol. Med.*, **146**, 66-70.
- Hughes, W. L. (1957): A physicochemical rationale for the biological activity of mercury and its compounds. *Ann. N. Y. Acad. Sci.*, **65**, 454-460.
- Ishihara, T. (1970): Hematological and nutritional survey in a sea side community. *Japan. J. Public Health*, **18**, 1181-1187.
- Iverson, F., Downie, R. H., Paul, C. and Trenholm, H. L. (1973): Methyl mercury: Acute toxicity, tissue distribution and decay profiles in the guinea pig. *Toxicol. Appl. Pharmacol.*, **24**, 545-554.
- Jones, R. A., Strickland, J. A., Stunkard, J. A. and Siegel, J. (1971): Effects on experimental animals of long-term inhalation exposure to carbon monoxide. *Toxicol. Appl. Pharmacol.*, **19**, 46-53.
- Landry, T. D., Doherty, R. A. and Gates, A. H. (1979): Effects of three diets on mercury excretion after methylmercury administration. *Bull. Environ. Contam. Toxicol.*, **22**, 151-158.
- Miller, V. L. and Csonka, E. (1968): Mercury retention in two strains of mice. *Toxicol. Appl. Pharmacol.*, **13**, 207-211.
- Miyata, S. (1966): Studies on the etiologic agents of the anemia tendency of the Japanese. I. Researches on some inhabitants group. *J. Japan. Soc. Intern. Med.*, **55**, 865-871.
- Naganuma, A. and Imura, N. (1979): Methylmercury binds to a low molecular weight substance in rabbit and human erythrocytes. *Toxicol. Appl. Pharmacol.*, **47**, 613-616.
- Naganuma, A., Koyama, Y. and Imura, N. (1980): Behavior of methylmercury in mammalian erythrocytes. *Toxicol. Appl. Pharmacol.*, **54**, 405-410.
- Nakamura, M., Yoshimoto, S., Asano, Y., Katsuno, S. and Sakamoto, K. (1978): An epidemiological study of anemia of farmer's wives in a rural area of Hyogo prefecture. Part 1. *Japan.*

- J. Publ. Health., **25**, 119-126.
- Nordberg, G. F. and Skerfving, S. (1972) : Metabolism. In *Mercury in the Environment* (L. Friberg and D. Fostal, eds), p. 29-91, CRC Press, Cleveland, Ohio.
- Norseth, T. and Clarkson, T. W. (1971) : Intestinal transport of ^{203}Hg -labeled methyl mercury chloride. *Arch. Environ. Health.*, **22**, 568-577.
- Östlund, K. (1969) : Studies on the metabolism of methyl mercury and dimethyl mercury in mice. *Acta Pharmacol. Toxicol.*, **27**, Suppl. 1, 1-132.
- Paulson, O. B., Parving, H-H., Olsen, J. and Skinhoj. E. (1973) : Influence of carbon monoxide and of hemodilution on cerebral blood flow and blood gases in man. *J. Appl. Physiol.*, **35**, 111-116.
- Penny, D. G., Sodt, P. C. and Cutilletta, A. (1979) : Cardiodynamic changes during prolonged carbon monoxide exposure in the rat. *Toxicol. Appl. Pharmacol.*, **50**, 213-218.
- Pitt, B. R., Radford, E. P., Gurtner, G. H. and Traystman, R. J. (1979) : Interaction of carbon monoxide and cyanide on cerebral circulation and metabolism. *Arch. Environ. Health.*, **34**, 354-359.
- Rothstein, A. (1973) : Mercaptans, the biological targets for mercurials. In *Mercury, Mercurials and Mercaptans* (M. W. Miller and T. W. Clarkson, eds), p. 68-95, C. C. Thomas, Springfield, Illinois.
- Suzuki, T. (1969) : Neurological symptoms from concentration of mercury in the brain. In *Chemical Fallout* (M. W. Miller and G. G. Berg, eds), p. 245-257, C. C. Thomas, Springfield, Illinois.
- Takeda, Y., Kunugi, T., Terao, T. and Ukita, T. (1968) : Mercury compounds in the blood of rats treated with ethylmercuric chloride. *Toxicol. Appl. Pharmacol.*, **13**, 165-173.
- Thomas, M. F. and Penny, D. G. (1977) : Hematologic responses to carbon monoxide and altitude : a comparative study. *J. Appl. Physiol.*, **43**, 365-369.
- Tillander, M. (1969) : Studies on excretion rates of organic mercury compound in seal and fish. cited from Clarkson, T. W. (1972) : Recent advances in the toxicology of mercury with emphasis on the alkylmercurials. *CRC Crit. Rev. Toxicol.*, **1**, 203-234.
- Uchida, A., Tohdo, M., Ogura, K., Uchida, F., Kaneko, I. Ito, S. and Yanagisawa, R. (1970) : A study on anemia in farm women. *J. Publ. Health Pract.*, **34**, 152-159.