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FORMATION AND POSSIBLE ROLE OF BIS(METHYLMERCURIC) SELENIDE IN RATS TREATED WITH METHYLMERCURY AND SELENITE

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Abstract—The metabolic fate of methylmercury after administration of [^{203}Hg]-methylmercuric chloride in combination with sodium selenite was investigated in rats. Whole body autoradiography and radioassay showed that administration of selenite decreased the mercury concentration in the liver and kidney, and increased that in the brain. The rapid changes of methylmercury concentration in the tissues after selenite injection were accompanied by increases in mercury extractable with benzene at neutral pH. The maximum levels of benzene-extractable mercury in the blood, kidney and liver were attained 30 min after selenite injection and were 30, 23 and 8 percent, respectively, of the total mercury. Thin-layer chromatography showed that the benzene-extractable mercury was a complex of methylmercury with selenium, bis(methylmercuric) selenide. These findings indicate that selenite alters the distribution of methylmercury in the tissues by formation of a diffusible complex with methylmercury, bis(methylmercuric) selenide.

It is generally accepted that selenium compounds have protective effects against the toxicity of methylmercury [1–4], but the mechanism of action is unknown.

Recently, Sumino *et al.* [5] reported that, although methylmercury bound to protein could not be extracted with benzene at neutral pH, it became extractable with benzene in the presence of selenite and blood or tissue homogenate. Naganuma and Imura [6] demonstrated that this phenomenon was associated with formation of a complex of methylmercury and selenium, bis(methylmercuric) selenide (BMS). In previous studies on the mechanism of BMS formation, we found that selenite was reduced non-specifically by reduced glutathione (GSH) or protein sulfhydryl groups in the tissue soluble fraction and then reacted with methylmercury, forming BMS [7]. It seems probable that if a complex of selenium and methylmercury could be formed *in vivo*, its formation could account for the modification of methylmercury toxicity by selenite *in vivo*.

The present study confirms that BMS is actually formed in the blood and tissues of methymercury-treated rats after injection of selenite. The possible role of BMS in selenite-induced redistribution of methylmercury is discussed.

MATERIALS AND METHODS

Chemicals. [^{203}Hg]-Methylmercuric chloride (MMC, 1.70 mCi/mg Hg) was purchased from the New England Nuclear Corp., Boston, MA, U.S.A. Unlabeled MMC and sodium selenite were from Wako Chemicals, Osaka, Japan and Nakarai Chemicals, Kyoto, Japan, respectively. Silica gel plates were from Merck, Darmstadt, F.R.G. BMS was synthesized by the method of Breitinger and Morell [8]. Other reagents used were of analytical grade.

Animal treatment and assay of mercury. Male Wistar rats weighing 150–200 g were used. Twenty-eight rats were injected intraperitoneally with [^{203}Hg]-MMC at a dose of 20 μmoles (30 μCi)/kg. One hour after the injection, four rats were killed and the remaining twenty-four were divided into two groups of twelve rats each. One group was injected subcutaneously with sodium selenite (20 μmoles/kg) and the other was treated with saline as a control. At various times after selenite administration, four rats from each group were decapitated. Blood was collected in a heparinized tube. The liver, kidney and brian were homogenized in 5 vol. of 0.05 M Tris–HCl buffer (pH 7.4) in an ice bath. Volumes of 1 ml of blood or homogenate were transferred to two stoppered tubes. One sample was used for measuring total mercury, and the other was mixed with 2 ml of benzene, shaken vigorously, and centrifuged at 3000 rpm for 5 min. The γ-ray emission of [^{203}Hg] in the benzene was measured in a well-type NaI scintillation counter (NDW-351E, Aloka, Japan).

In another experiment, sodium selenite was injected into rats 1 hr before, at the same time as, or 1 hr after administration of [^{203}Hg]-MMC. The rats were killed 1.5 hr after administration of [^{203}Hg]-MMC, and total, and benzene-extractable, mercury in the blood and tissues were measured.

Whole body autoradiography. Sodium selenite (20 μmoles/kg) was injected subcutaneously into rats 1 hr after [^{203}Hg]-MMC [20 μmoles (500 μCi)/kg] and 2 hr later, the rats were anesthetized by inhalation of chloroform and were immersed into a mixture of dry-ice and acetone. Freeze-dried sections of the whole body of 30 μm thickness were prepared by the method of Ullberg [9] and exposed to X-ray film

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(LKB ultrofilm ^{3}H, LKB Produkter, Sweden) for 2 weeks.

Thin-layer chromatography. Blood and tissues were homogenized with an equal volume of benzene in an ice bath and centrifuged at 3000 rpm for 5 min. The benzene extract (0.5 ml) was applied to a silica gel plate with a concentrated zone, together with MMC and BMS as reference standards. The plate was developed to a distance of 10 cm from the origin with petroleum benzin–diethyl ether (7:2 v/v), and mercury was located by spraying the plate with 0.1% dithizone–chloroform solution. Then a length of 1 cm of silica gel was scraped off the plate and used for measurement of radioactivity.

RESULTS

Whole body autoradiograms of rats after injection of [^{203}Hg]-MMC alone or in combination with selenite are shown in Fig. 1. The radioactivities in the liver, kidney, lung and peritoneal cavity were markedly lower in selenite-treated rats than in saline-treated control animals. In contrast, the brain, testes and skeletal muscle, which had low radioactivities in the control, had considerably higher activities in the selenite-treated rat. The labeled mercury was more uniformly distributed in the animal body by selenite.

As shown in Fig. 2, administration of selenite resulted in significant changes in the total mercury levels in the liver, kidney and brain, but not in the blood; the total mercury level decreased in the liver and kidney, but increased in the brain. This change of mercury retention in these tissues was mainly

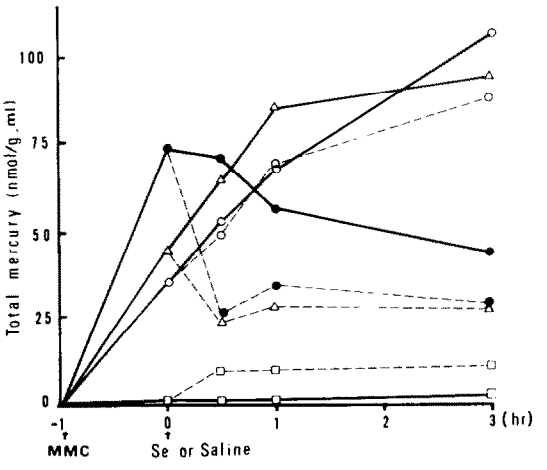

Fig. 2. Effects of selenite treatment on the total mercury levels in the blood and tissues of rats injected with [^{203}Hg]-MMC. Points are mean values for four rats. Rats were treated with [^{203}Hg]-MMC (20 μmoles/kg, i.p.) and 1 hr later with either saline (solid lines) or sodium selenite (20 μmoles/kg, s.c., broken lines). Key: blood (○), liver (●), kidney (△), and brain (□).

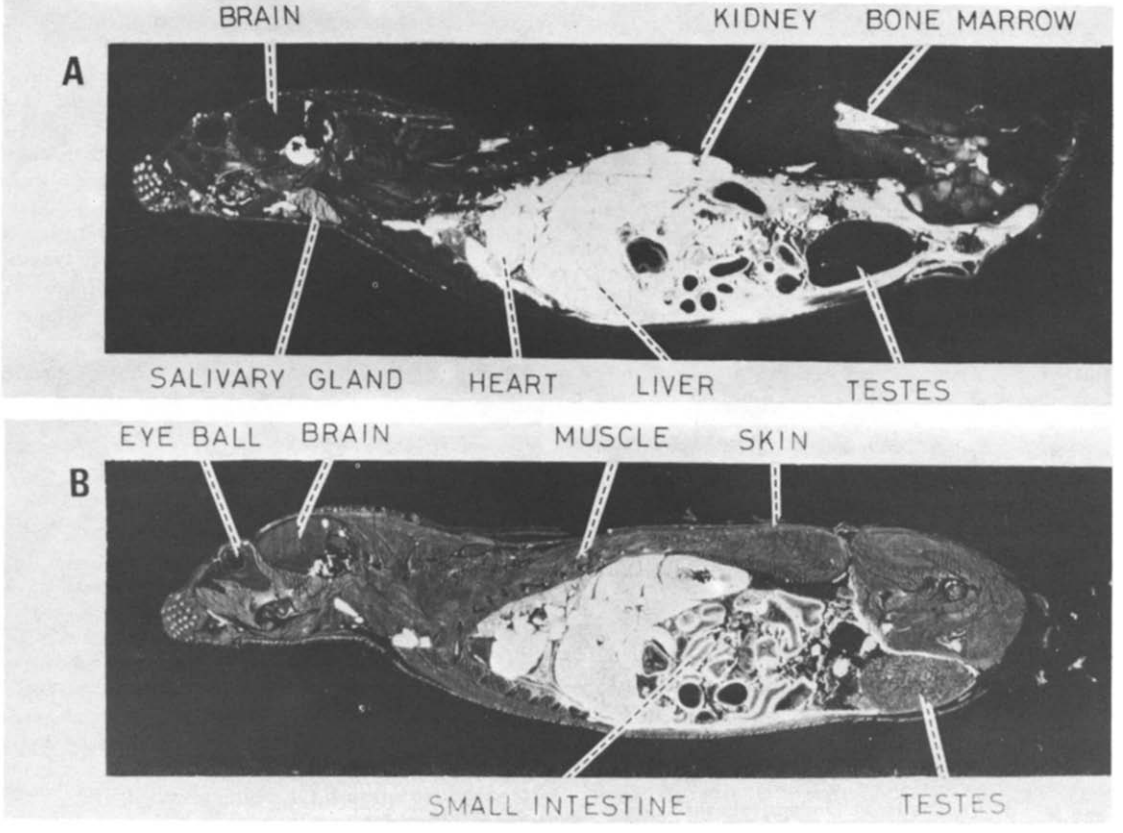

Fig. 1. Whole body autoradiograms showing the effect of selenite treatment on the distribution of [^{203}Hg]-MMC in rats. White areas correspond to radioactivity. The rats were injected with either saline (A) or sodium selenite (B) 1 hr after treatment with [^{203}Hg]-MMC and were killed 2 hr later.

induced within the first 30 min after selenite administration. The total mercury concentrations in the liver and kidney of selenite-treated rats were about half those of control animals, whereas the concentration in brain was five times the control value of 30 min after selenite injection.

The time courses of change in mercury extracted with benzene in the blood and tissues are shown in Fig. 3. Benzene-extractable mercury in the blood and tissues of the control animals was less than 2 percent of the total mercury at all times examined, but it was markedly more in selenite-treated rats.

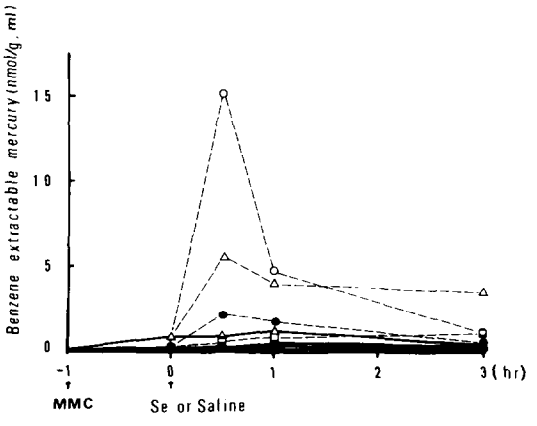

Fig. 3. Effects of selenite treatment on the concentration of benzene-extractable mercury in the blood and tissues of rats injected with [^{203}Hg]-MMC. Points are means for four rats. Rats were treated with [^{203}Hg]-MMC (20 μmoles/kg, i.p.) and 1 hr later with either saline (solid lines) or sodium selenite (20 μmoles/kg, s.c., broken lines). Key: blood (○), liver (●), kidney (△), and brain (□).

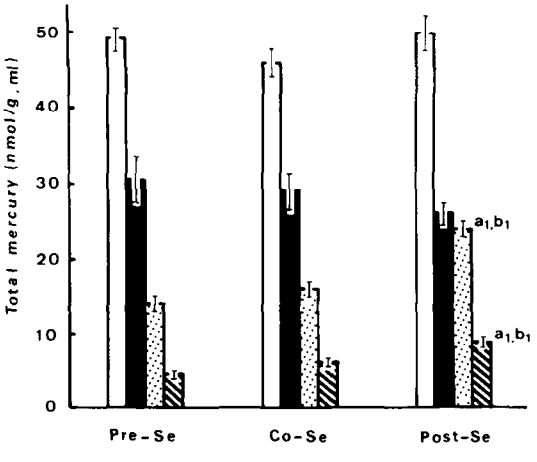

Fig. 4. Influence of time of injection of selenite on the total mercury levels in the blood and tissues of rats 1.5 hr after administration of [^{203}Hg]-MMC. Columns and vertical bars are means and S.E. for four rats. Sodium selenite (20 μmoles/kg, s.c.) was injected 1 hr before (Pre-Se), simultaneously with (Co-Se), or 1 hr after [^{203}Hg]-MMC (20 μmoles/kg, i.p.) (Post-Se). Key: blood (□), liver (■), kidney (▩), and brain (▧); a_1b_1: significant difference between Post-Se and Co-Se or Pre-Se ($P < 0.01$).

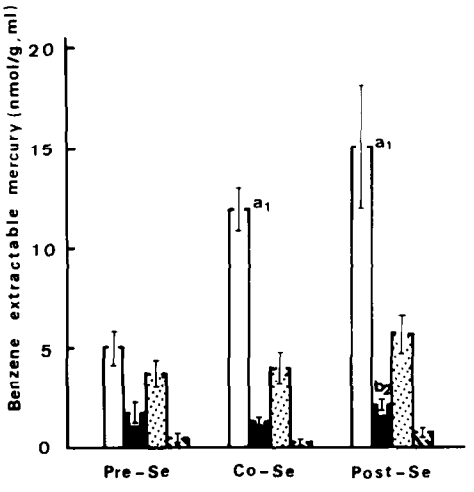

Fig. 5. Influence of selenite injection time on the concentration of benzene-extractable mercury in the blood and tissues of rats 1.5 hr after administration of [^{203}Hg]-MMC. Columns and vertical bars are means and S.E. for four rats. Sodium selenite (20 μmoles/kg, s.c.) was injected into rats 1 hr before (Pre-Se), simultaneously with (Co-Se), or 1 hr after [^{203}Hg]-MMC (20 μmoles/kg, i.p.) (Post-Se). Key: blood (□), liver (■), kidney (▩), and brain (▧); a_1: significant difference between Pre-Se and Co-Se or Post-Se ($P < 0.01$); b_2: significant difference between Co-Se and Post-Se ($P < 0.05$).

Large amounts of benzene-extractable mercury in the blood, kidney and liver were found 30 min after selenite injection and amounted to 30, 23 and 8 percent, respectively, of the total mercury. However, the level in the brain increased gradually for up to 3 hr after selenite injection to 9 percent of the total mercury.

The concentrations of total and benzene-extractable mercury were compared in groups of rats injected with selenite 1 hr before (Pre-Se), simultaneously with (Co-Se), and 1 hr after [^{203}Hg]-MMC (Post-Se) (Figs. 4 and 5). Although total blood mercury was not affected by the time of selenite treatment, the benzene-soluble mercury levels were 2.4 and 3.0 times higher in the Co-Se and Post-Se groups, respectively, than in the Pre-Se group, indicating that benzene-soluble mercury increased with delay in the time of selenite administration. In the kidney and brain, the total mercury levels were significantly higher in the Post-Se group than in the other groups, but the benzene-soluble mercury was similar in the three groups.

A thin-layer chromatogram of the benzene-soluble mercury is shown in Fig. 6. The radioactivity in the benzene extract of the blood of selenite-treated rats migrated mainly at an R_f value of 0.73, unlike authentic MMC (R_f value: 0.35). BMS, used as a reference, had the same R_f value as benzene-extractable mercury. The radioactivities in the benzene extracts of liver, kidney and brain were located mainly in the position of BMS. Since the benzene-soluble mercury had the same properties as those of BMS determined in our previous study [7], it was assumed to be BMS.

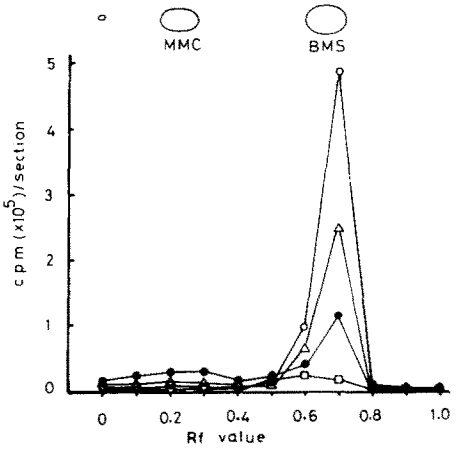

Fig. 6. Thin-layer chromatography of benzene-soluble mercury. Benzene-soluble mercury was obtained from the blood and tissues of [^{203}Hg]-MMC-treated rats 30 min after selenite injection. Key: blood (○), liver (●), kidney (△), and brain (□).

DISCUSSION

Selenite is known to cause considerable change in the distribution of methylmercury. In this work, whole body autoradiograms showed the effect of selenite on redistribution of methylmercury, confirming previous reports of others [10, 11].

Since injected MMC is nearly all bound to plasma and tissue proteins, its distribution between blood and other tissues should depend on diffusible chemical species of methylmercury, such as a methylmercury–cysteine complex [12]. The mechanism of selenite-induced redistribution of methylmercury is unknown. Selenite may convert methylmercury to a diffusible form suitable for transfer between intravascular, interstitial and intracellular fluids. Since injected MMC is nearly all bound to tissue proteins, it could not be extracted with benzene at neutral pH. However, in the present study, selenite significantly increased the benzene-extractable mercury in the blood and tissues of rats after injection of [^{203}Hg]-MMC. The increased accumulation of mercury in the brain and the decrease in its concentrations in the liver and kidney occurred at the time when benzene-soluble mercury was maintained at a maximum level in the blood and tissues. This suggests that benzene-soluble mercury can diffuse between the blood and tissues.

Naganuma and Imura [6] reported that MMC reacts with selenite to form BMS in the presence of rabbit blood or GSH. They suggested that the cycle of formation and decomposition of BMS may occur repeatedly, since BMS was rapidly decomposed *in vitro* and was hardly detectable in mice injected with MMC and selenite [13]. However, as shown in Fig. 6, our results indicated that considerable formation of BMS occurs *in vivo*. In particular, the level of BMS in the blood increased remarkably, and this increase was found to be greatest 30 min after selenite injection, when the selenium concentration in the blood is thought to be high (Fig. 5). Thus, from the present study, blood seems to be an important site of formation of BMS.

However, our previous studies showed that BMS was formed by the reaction of MMC and selenite in the presence of a soluble fraction of liver, kidney or brain in place of the blood [7]. In fact, BMS was found to increase in spite of a decrease in the total mercury in the liver and kidney, suggesting that BMS synthesized in the liver and kidney may be expelled into the blood, resulting in a decrease in the total mercury level. The increased accumulation of total mercury in the brain may reflect enhanced uptake of BMS from the blood rather than synthesis of BMS in the brain. These phenomena may be explained by supposing that BMS readily penetrates through cell membranes, including the blood–brain barrier, since it is a non-ionic, lipid-soluble substance, unlike methylmercury itself.

Since BMS was detected relatively soon after selenite injection, it may not be directly responsible for the protective action of selenite against methylmercury toxicity. Further investigations are needed on the subsequent fate and biological properties of BMS in the body, including its toxicity. However, from our results we suggest that selenium may have a protective effect because it alters the distribution of methylmercury in the tissues, preventing methylmercury from binding to possible critical sites. The present findings that BMS may function as a diffusible form in the process of selenite-induced redistribution of methylmercury seem to indicate one way in which selenium modifies methylmercury toxicity.

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