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Sex Differential of Methylmercury Toxicity in Spontaneously Hypertensive Rats (SHR)

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Sex differences in methylmercury (MeHg) toxicity have been reported in man and several laboratory animal species (Magos et al. 1976, 1981; Fowler 1972a,b; Tagashira et al. 1980a,b). For example, the records of 51 randomly selected autopsy samples from the Iraqi MeHg epidemic revealed a 3:1 ratio of females to males (Magos et al. 1976), a finding consistent with that of Greenwood (1975; cited in Magos et al. 1981). Studies on rats reveal females are more susceptible to MeHg than males (Fowler 1972a,b). In ICR-strain mice severe methylmercury chloride (MMC) poisoning appears earlier in females than in males (Tagashira et al. 1980a,b). The findings of the above cited studies suggest females rather than males are more sensitive to the toxic effects of MeHg.

During a study of the effect of MeHg on blood pressure in spontaneously hypertensive rats (SHR), we observed extensive differences between males and females in mercury toxicity. The SHR model, which was developed for studying spontaneous hypertension in animals and essential hypertension in man (Okamoto and Aoki 1963), is used widely today for this purpose. Since the sex differences were so profound and since apparently knowledge of such differences in MeHg intoxication have never been reported in SHR, it was thought the findings worthy of publication. Herein, the findings on sex differences in morbidity, mortality and blood pressure of SHR treated orally with MMC (2 mg/kg/day) for 26 consecutive days are presented.

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MATERIALS AND METHODS

The study was conducted between January 24 and March 11, 1985, at the animal research facility at the National Institute for Minamata Disease. Male and female SHR/NCrj, 7 weeks old, were studied. Three to four rats were caged together with food and water available ad libitum throughout the experiment. The animals were housed under light from 0600 to 1800 alternating with 12 hours of darkness; room temperature was kept at 22 ± 1°C. MMC (2 mg/kg/day) was administered orally once daily between the clock hours of 0900 and 1000 for 26 consecutive days.

Blood pressure was measured by an indirect method using a programmed Electrosphygmomanometer (model PE-300, Nacro Biosystems, Houston, Texas) in conjunction with an occulted tail cuff and pulse transducer. Blood pressure measurements were carried out while the animal was placed in a special restrainer. During measurement body temperature was maintained through warming by a hot plate (40°C). All rats were initially accustomed to the procedure for measuring blood pressure by exposure to the experimental conditions during four different pre-studies. During the experiment, blood pressure was monitored once weekly. All measurements were made during the span of diurnal rest for the animals, mostly between 1000 and 1700. There were ten rats per treatment group. Tail blood pressure of the male SHR before MMC administration varied from 170.0 to 195.0 mmHg with a mean of 180.5 ± 2.6 (standard error) mmHg; blood pressure of the female SHR varied from 160.0 to 185.0 mmHg with an average of 170.5 ± 2.7 mmHg.

The body weight of each animal and the intake of food and water of all animals in each cage were monitored daily. The mean body weight at the start of the experiment (day 0) was 253.2 ± 7.6g for males and 163.1 ± 9.9g for females in the experimental group and 256.6 ± 14.2g (males) and 163.5 ± 6.0g (females) in the control group. Crossing of the hind limbs, disturbed righting reflex and abnormal gait (common neurological signs of MMC intoxication in rodents) as well as mortality were monitored daily also. Neurologic disturbances of the treated rats were evaluated as described elsewhere (Inoue et al. 1985).

Differences between the means of the body weight and blood pressure measurements were compared by student t-test (Snedecor and Cochran 1967). The Kolmogorov-Smirnov two-sample test (Siegel 1956) was applied to the cumulative distributions of neurological manifestations and mortality.

RESULTS AND DISCUSSION

Body weight differed significantly between male and female SHRs at the beginning of the experiment. Males weighed on the average 90 grams more than females on day 0; however, the difference in body weight between experimental and control groups within the same sex was not statistically significant. The weight

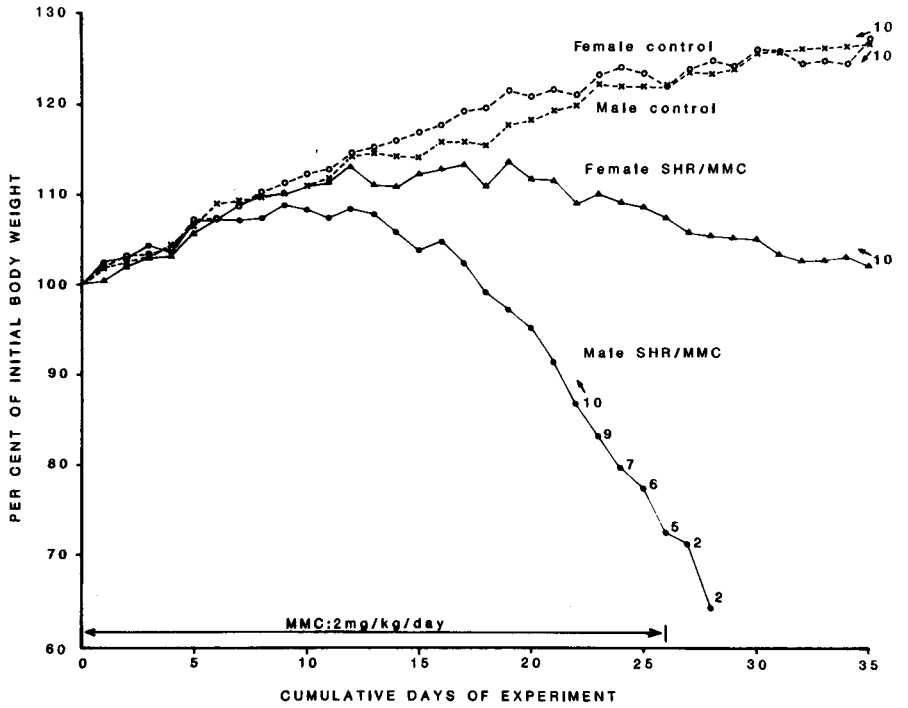

Figure 1. Changes of body weight of SHRs during and after methylmercury chloride administration. (Numbers indicate how many animals upon which the daily body weight means are based.)

gains of untreated male and female SHRs were the same during the observation period in terms of percent of the initial body weight (on day 0). The body weight of male SHRs treated with MMC began to decline on the 13th treatment day, reaching a level lower than the initial body weight after 18 days of MCC treatment (Figure 1). Body weight continued to decrease even at a faster rate thereafter, the maximum decline being approximately 65% below the initial body weight.

In comparison to males, the body weight of female MMC-treated rats did not begin decreasing until the 20th treatment day; too, the rate of body weight loss thereafter was much slower than that of male SHRs. The body weight of females never fell below the initial day 0 level during MMC treatment, although a continuous decline was observed. Overall, loss of body weight (or decline in weight gain), an early sign of MeHg intoxication, was more marked in male than female SHRs.

Deaths began to occur on day 23 among male SHRs, with all succumbing by the 29th post-treatment day (Figure 2). There was no mortality in female SHRs during the entire treatment and

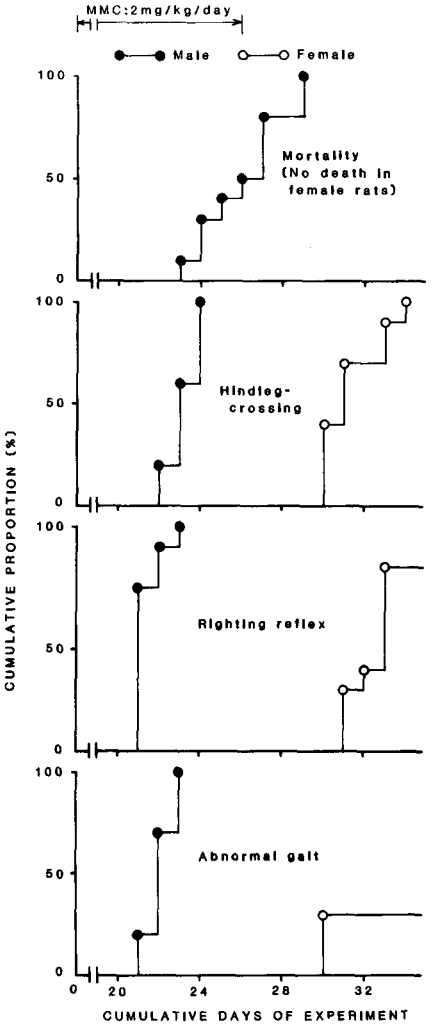

Figure 2. Cumulative proportion of mortality, hindleg-crossing, abnormal righting reflex and abnormal gait in methylmercury chloride-treated SHRs.

observation period. Neurologic disturbances, such as hindleg-crossing, disturbed righting movement and abnormal gait, established neurologic signs of MeHg poisoning in rats (Inoue et al. 1985), appeared 9 days earlier among male (on day 21 or 22) than among female SHRs (on day 30 or 31). Neurological signs always preceded death. It is of interest that abnormal gait was exhibited only by 3 of 10 female rats although all showed crossing of the hindlimbs. All male rats exhibited both abnormal gait and hindleg crossing almost simultaneously. In general, the neurologic manifestation of hindleg-crossing occurs in most laboratory

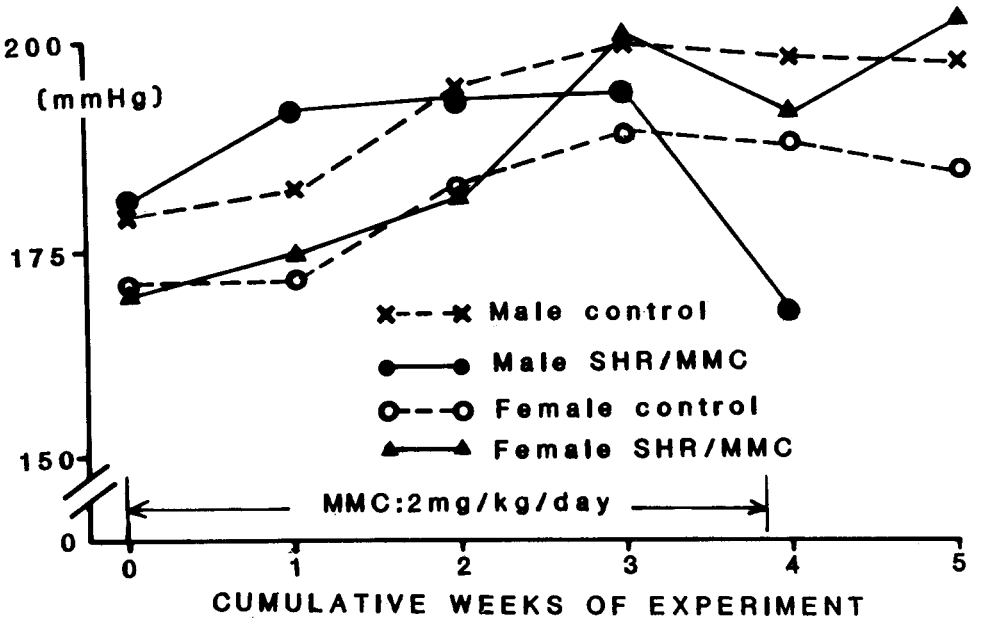

Figure 3. Changes of blood pressure during and after administration of methylmercury chloride in SHRs.

animal species around the same time as or before abnormal gait. Typically, the incidence of abnormal gait is usually higher than that of hindleg-crossing in male SHRs and Wistar Kyoto rats, the progeny of SHR.

During the observation period, the blood pressure of control animals increased to a plateau around the 3rd week when it ranged from 180.5 ± 2.6 to 195.8 ± 5.5 mmHg among male SHRs and from 170.5 ± 2.7 to 184.0 ± 2.9 mmHg among females (Figure 3). Blood pressure was always higher in control male than female SHRs, the difference (about 10 mmHg) between the sexes being almost constant throughout the observation period.

On the other hand, the blood pressure among the experimental groups, both male and female, varied considerably during observation, particularly at the end of the period. Relative to the appropriate sex control group, the blood pressure of the MMC-treated female SHRs was higher during the 3rd and 5th weeks of the experiment. Too, the blood pressure of the MMC-treated rats was higher in female than male SHRs during weeks 3-4. Relative to the male controls, surviving MMC-treated males exhibited reduced blood pressure during the 4th week. All male MMC-treated SHRs succumbed to death during the 5th week of observation.

Wide individual, sex, species and/or genetic differences in the organ distribution, biological half-time and toxic dose of MeHg in man and laboratory animals have been reported. Recognition of this variability is important in terms of preventing future outbreaks of MeHg poisoning (Doi and Kobayashi 1982). We have stressed elsewhere that it is necessary to take into consideration the heterogeneity of the exposed population due to the possibility of varying susceptibilities and multiple environmental factors introduced to the population cumulatively and simultaneously (Tamashiro et al. 1985b).

The present study indicates a large sex difference in the susceptibility of SHRs to the toxic effects of MeHg. No mortality occurred in female rats treated with MMC, while all the male rats so treated died. Neurological manifestations were significantly lower in occurrence or later in onset in female as compared to male rats. Contrary to previous reports of higher toxicity of MeHg in females (Fowler 1972a,b; Tagashira et al. 1980a,b), the findings of this investigation on SHRs revealed males much more susceptible to neurotoxic effects than females. These results are consistent with those of Takahashi et al. (1978), although less impressive. Takahashi et al. (1978) showed MeHg exerted not only greater toxicity but also induced higher mortality in male DD and hairless mice.

Significant differences in MMC effects on body weight and blood pressure between sexes were noted throughout the experiment; however, it is not clear whether such account for the sex-related patterns of MMC-induced morbidity and mortality in the SHRs. It has been suggested loss of body weight may accelerate development of neurotoxicity through enhancing MeHg uptake by the nervous system (Magos et al. 1981). Tagashira et al. (1980a,b) and Magos et al. (1981), who found female rodents more susceptible to MMC, hypothesized the early onset of neurotoxicity in female mice attributable chiefly to sex-related differences in the amount of mercury deposited in the brain and other organs. Indeed, the former group found mercury levels of all organs, except the kidneys, of female mice higher. Distribution of mercury in organs was not measured in the present experiment; however, it is known SHRs have a characteristic organ distribution of MeHg which differs from that of Wistar Kyoto rats (Tamashiro et al. 1985a). Thomas et al. (1982) found significant differences in whole body mercury retention between male and female Long Evans rats after taking into consideration faster growth rate and larger body weight of male versus female rats. During our study the growth rate as monitored by weight gain of untreated rats did not differ between males and females. A study is now in progress to clarify sex-related differences, if any, in the organ distribution, retention, excretion and biological half-time of MeHg in SHR for comparison to findings in the Wistar Kyoto rat.

The blood mercury concentration in mice after MeHg administration also has been demonstrated to be in part genetically determined

(Doi et al. 1983). Strain difference in blood mercury concentration is due mainly to the structure of the Hb-β chain; the cysteinyl residue located outside of the $\alpha_1\beta_2$ contact junction appears to play an important role in the binding of hemoglobin to MeHg.

It seems unlikely differences in blood pressure levels affected the differences in morbidity/mortality of male and female SHRs since the blood pressure of the females reached the same level as the males in the later period of observation. However, the early differential in blood pressure cannot be ruled out. It is pertinent, nonetheless, that the longest surviving male SHRs exhibited lower blood pressure than those males succumbing earlier in the experiment.

The role that sex hormones play, if any, in MeHg toxicity requires study, bearing in mind the existence of sex differences of SHRs (presumably associated with levels of androgens and estrogens) in the incidence of stroke, life span, cerebrovascular lesions and renal vascular damage (Yamori et al., 1976). It is of interest that female SHRs treated with androgen after gonadectomy are more vulnerable to hypoxia than nontreated intact females (Sasagawa et al. 1976). Since MeHg-treated monkeys experimentally subjected to hypoxia (Ikuta et al. 1982) exhibit pathological changes commonly seen in Minamata disease patients, a mechanism underlying sex differences in MeHg intoxication may involve physiologic alterations which are reproductive-hormone dependent.

Other sex-related traits may be important as well. For example, both male and female SHRs are more locomotor-active than Wistar Kyoto rats (Tamashiro et al. unpublished data). The behavior of SHR females also differs from that of males. The extent to which sex-dependent behaviors are associated with differential MeHg toxicity awaits clarification.

Hypertension is one of the most significant health problems among inhabitants of MeHg-polluted areas in Japan. The prevalence of hypertension, including borderline hypertension, in 1984 in this country was reported to be 55.5 percent in those aged 40 years or more in these areas (Futatsuka et al. 1985). However, increased mortality from hypertensive and cerebrocardiovascular diseases has been observed neither in Minamata-disease patients nor in the general population of Japan exposed to MeHg (Tamashiro et al. 1983, 1984). The findings described herein underline the requirement that both environmental potentiators as well as individual differences in resistance-susceptibility to MMC be recognized when setting environmental standards for mercury and in studying dose-response or dose-effect relationships for human beings and other animal species (Tamashiro et al. 1985a).

In summary, treatment of SHRs with MMC (2 mg/kg/day) for 26 consecutive days resulted in differences by sex in the rate of onset and magnitude of toxic effect. In comparison to untreated male and female SHRs, which gained weight throughout the experiment, MCC-treated male SHRs underwent weight loss sooner and

more extensively than MCC-treated females. Too, the manifestation of neurological symptoms and mortality was significantly greater in male than in female MCC-treated SHRs. In SHRs the blood pressure difference between MCC-treated males and females does not seem to account for the sex differential of methylmercury toxicity. The finding of MCC-induced toxicity being greater in male than female SHRs is contrary to previous reports, suggesting strain differences in male-female toxicity of MCC.

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