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Methylmercury Toxicity in Spontaneously Hypertensive Rats (SHR)

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The toxic effects of methylmercury (MeHg) in experimental animals have been reported to be influenced by various factors such as environmental temperature (Nomiyama et al. 1980; Yamaguchi et al. 1984), selenium in food (Oda et al. 1984), and ethanol intake (Takahashi et al. 1978; Turner et al. 1981; Tamashiro et al. 1985). Results of these studies suggest modifying factors are important with regard to evaluating currently proposed standards for maximum safe levels of MeHg as well as dose-response and dose-effect relationships in man and laboratory animals. Nonetheless, information is scant on both environmental and individual factors as potentiators of MeHg toxicity in human beings and other animal species.

Hypertension is quite common among the inhabitants of MeHg-polluted areas. The prevalence of hypertension, including borderline hypertension, in 1984 was reported to be 55.5 percent at age 40 years or more in MeHg-exposed persons (Futatsuka et al. 1985). Thus, hypertension is one of the most significant health problems among the population exposed to MeHg. It is of special interest to learn what is the health consequence among the hypertensives who have been exposed to MeHg for a prolonged period of time.

This study was designed to delineate the toxicity of MeHg in animals having high blood pressure using the laboratory model of spontaneously hypertensive rats (SHR). This rodent model was developed for the study of spontaneous hypertension in animals and of essential hypertension in man. This established animal model has been used widely for this purpose (Okamoto and Aoki 1963). This paper presents the mortality as well as distribution of mercury in the tissues of SHR and control rats treated orally with methylmercury chloride (MMC: 5 mg/kg/day) for 10 consecutive days.

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MATERIALS AND METHODS

This study was conducted between December 10, 1984, and January 10, 1985, at the animal research facility at the National Institute for Minamata Disease. Male SHR/NCrj and Wistar Kyoto rats (WKY/NCrj), 10 weeks old, were studied. Three or four rats were housed per cage. Food and water were available ad libitum throughout the experiment. The animals were exposed to light from 0700 to 1900 alternating with 12 hours of darkness. The animal room was kept at 22° ± 1° C. MMC (5 mg/kg/day) was administered orally once daily between the clock hours of 0900 and 1000 for 10 consecutive days.

Indirect blood pressures were measured using a programmed Electrosphygmomanometer (mode PE-300, Narco Biosystems, Houston, TX) in conjunction with an occluded tail cuff and pulse transducer. The body temperature of the rats was maintained by warming using a hot plate (40°C) for 15-20 minutes before the blood pressure measurements; the latter were obtained while the animals were placed in a special restrainer. All rats were initially accustomed to the described procedure for measuring blood pressure by conducting four different pre-study sessions. During the experiment, blood pressure was monitored once weekly using nine to ten rats per treatment group. Measurements were made during the span of diurnal rest in the nocturnally active rats, between 1000 and 1700. Tail blood pressure of the SHR before MMC administration varied from 190 to 231 mmHg with a mean of 211 mmHg. In comparison, the average pressure of the WKY rats was 142 mmHg, with the range from 130 to 165 mmHg.

The body weight of each animal and the intake of food and water of animals in each cage were measured daily. Hindleg-crossing, abnormal gait (both common neurological symptoms of MMC intoxication) and mortality were observed daily.

A separate experiment was carried out to measure the total mercury contents in the brain, liver, kidneys, plasma, and blood cells of rats housed and treated according to the conditions described above. One and five days after the final administration of MMC, 5 rats of each group were sacrificed under pentobarbital anesthetization and autopsied. Tissue samples were obtained after thorough perfusion with saline solution. Total mercury levels of tissue homogenates were determined by the oxygen combustion-gold-amalgam method using the Sugiyama-Gen Mercury Analyzer (Tokyo, Japan) following procedures described by Nishi et al. (1974).

Differences between means of the body weight and the MMC concentration of the various tissue samples were teted by a student t-test (Snedecor and Cochran, 1967). Kolnogorov-Smirnov two-sample test (Siegel 1956) was applied to

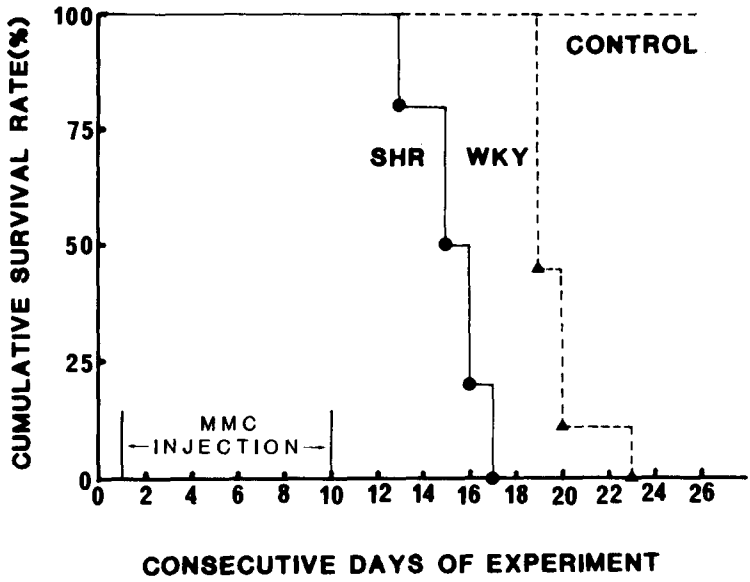

Figure 1. Cumulative survival rate (%) of the spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats treated with methylmercury chloride (MMC).

the data on the patterns of hindleg-crossing, abnormal gait, and mortality to evaluate statistical significance.

RESULTS AND DISCUSSION

Death occurred significantly ($p < 0.01$) earlier in the MMC-treated SHR than in the MMC-treated WKY rats, but the final mortality rates were not significantly different between the two groups (Figure 1). Although not graphically shown, the neurological manifestations of hindleg-crossing and abnormal gait appeared earlier in MMC-treated SHR; similarly, the decrease in body weight of SHR was more marked in comparison to that of WKY rats during and after MMC treatment.

Figure 2 compares the concentration of total mercury in the tissue samples of SHR and WKY rats 1 and 5 days after the final MMC treatment for 10 days. On day 1, the content of total mercury was highest in the blood cells (680.3 μg/g for SHR, 661.0 μg/g for WKY rats) followed by the kidneys (149.5, 116.6 μg/g), liver (80.8, 67.3 μg/g), brain (19.3, 16.9 μg/g) and plasma (2.0, 1.7 μg/g). The concentration of mercury by organ on day 5 exhibited the same pattern as found on day 1. These findings are consistent with those described by Imura (1980). We also have reported elsewhere (Tamashiro et al.

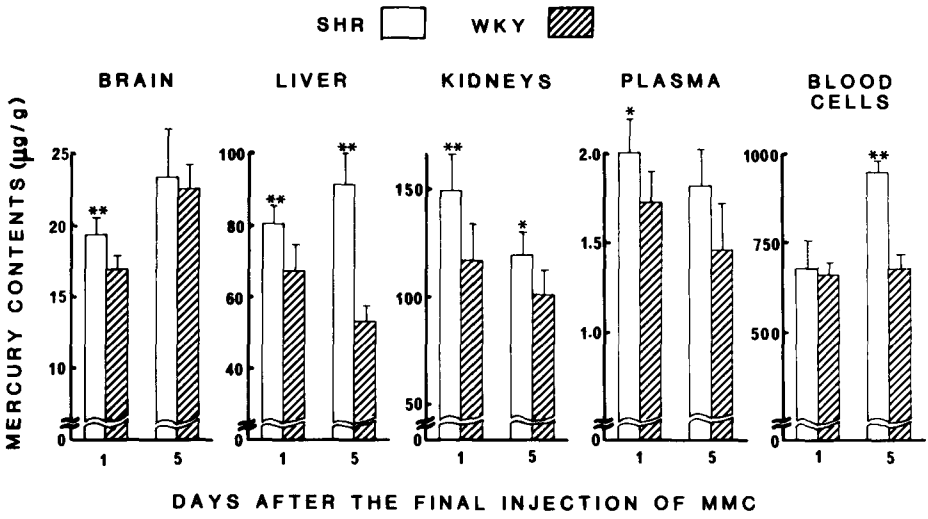

Figure 2. Mercury content (μg/g) by organ for SHR and WKY rats.

1985) that the proportion of mercury as MeHg is more than 86 percent in the tissues of WKY rats treated with MMC.

Compared to the day 1 post-MMC treatment data, the mercury level in the brain and blood cells of both SHR and WKY rats increased by day 5, while that in the liver, kidneys and plasma of both groups of rats was higher on day 1 than on day 5, with the exception of the liver of SHR.

It has been shown that the brain reaches its maximum concentration several days later than the other organs during or after MMC treatment, probably because the blood-brain barrier delays distribution. Too, the blood mercury level has been shown to be highest immediately after MMC administration and decrease faster than the other organs (Imura 1980). The liver mercury concentration also has been shown to decrease rapidly in comparison with the kidneys. However, the present results are not comparable with the previous findings, particularly with regard to the blood cells and liver of SHR whose concentrations did not peak until day 5. A study is in progress to clarify the discrepancy of retention and excretion of MeHg in SHR in comparison to other strains of rats.

There were significant differences between SHR and WKY rats in the concentration of mercury in the brain, liver, kidneys, and plasma on day 1 and again in the liver, kidneys, and blood cells on day 5 (Figure 2). The concentration of mercury was always higher in SHR than in WKY rats for all organs and both days examined. This, perhaps, contributes to the higher

mortality among SHR as compared to WKY MMC-treated rats. Overall, the findings of this study indicate that MeHg toxicity can be potentiated in the hypertensive animals and, by implication, in human hypertensives.

It has been suggested mercury may be associated with cardiovascular disease (Shaper 1979). Perry et al. (1974) have shown that rats fed diets with mercury (5 and 10 ppm) for a year, but not for a shorter duration of 6 months, had significant increase in systolic blood pressure. There has been extensive public health concern about the effects of MeHg pollution as a risk factor in the development of cerebrocardiovascular diseases, besides neurological disorders, particularly since pathological changes in cerebral blood vessels have been found in human and animal cases of organic mercurial intoxication (Shiraki 1969; Shiraki and Nagashima 1977; Shaw et al. 1979). The significance of this with regard to the subsequent development of cerebral blood vessel atherosclerosis associated with aging has been the subject of increasing concern in recent years (Shaw et al. 1979). Yet, increased mortality from hypertensive and cerebrocardiovascular diseases has not been observed in Minamata-disease patients (Tamashiro et al. 1983, 1984).

The data of this study, obtained from investigations using two strains of rats, indicate the necessity of taking into consideration individual variability in susceptibility to Minamata disease and MeHg intoxication. The findings of the experiment described herein underline the requirement that both environmental potentiators of MMC as well as individual differences in resistance-susceptibility to MMC be recognized when setting environmental standards for mercury and for the study of dose-response or dose-effect relationships for human beings as well as other animal species.

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