Protective Effect of Melatonin on Methylmercury-Induced Mortality in Mice

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KIM, C.-Y., NAKAI, K., KAMEO, S., KUROKAWA, N., LIU, Z.-M. and SATOH, H. Protective Effect of Melatonin on Methylmercury-Induced Mortality in Mice. Tohoku J. Exp. Med., 2000, 191 (4), 241-246 — Effect of melatonin on the mortality in methylmercury chloride (MMC)-intoxicated mice was evaluated. Mice were given MMC in the diet (40 mgHg/g) with or without melatonin in drinking water (20 mg/ml) for 5 weeks. In the control group, given MMC alone, 4 of 10 mice began to show neurological signs (e.g., abnormal righting reflex, staggering gait and posture on its side) concomitant with loss of body weight 4-7 days before death. This group also showed 60% of survival rate on the 35th day. However, the treated group, concomitantly given melatonin, showed a 100% of survival rate on the 35th day, although 1 of 10 mice began to show the neurological signs on the 33rd day. The level of thiobarbituric acid reactive substance in the brain, as an indication of oxidative damage, showed a significant decrease in the treated group compared with the control group. Thus, the 100% survival rate in the treated group may be partly due to antioxidative effect of melatonin on the MMC induced neurotoxicity. —— methylmercury; melatonin; intoxication; survival rate; neurotoxicity © 2000 Tohoku University Medical Press

Methylmercury is highly neurotoxic as evidenced by the tragic epidemics in Japan and Iraq (Watanabe and Satoh 1996). The most prominent toxic features of methylmercury in human are neurological disturbances including ataxia, sensory loss and constriction of the visual fields (WHO 1990). In experimental mice intoxicated with methylmercury, they also showed neurological signs such as abnormal righting reflex, staggering gait and fallen posture on its side (Suzuki and Miyama 1971).

An antioxidant such as vitamin E was reported to have a protective effect against the neurotoxicity of methylmercury in experimental animals (Chang et al. 1978; Welsh 1979). More recently, it has been reported that the neurotoxicity of
methylmercury may be involved with oxidative damage-inducing free radicals (Sarafian and Verity 1991; Ali et al. 1992; Park et al. 1996). Thus, it is possible that the neurotoxicity of methylmercury might be decreased by enhancing antioxidative capacity in the brain.

Melatonin, a pineal hormone, plays an important role in the circadian regulation of sleep, and is often utilized as a sleep-inducing agent (Klein et al. 1971; Wetterberg 1999). It is also known to easily penetrate the blood-brain barrier (Reiter et al. 1997) and possess a potent antioxidant activity in the brain (Reiter 1998); it is an effective free radical scavenger and also stimulates the mRNA level of glutathione-peroxidase as well as the activity of the enzyme (Barlow-Walden et al. 1995; Kotler et al. 1998; Reiter 1998). It has been shown to reduce lipid peroxidation in both in vivo and in vitro experiments (Reiter et al. 1995).

In the present study, we investigated the possible protective effect of melatonin against the neurotoxicity of methylmercury in mice by observing the survival rate. We also determined thiobarbituric acid reactive substance (TBARS) as an indication of oxidative damage in the brain.

**Materials and Methods**

*Animals and procedures*

Male ICR mice, 6 week old and approximately weighing 30–32 g, were purchased from Nihon SLC (Hamamatsu). They were kept in a temperature-controlled room (23 ± 2°C) with a 12 hour light-dark cycle (light period; 0800-2000). Three or four mice were placed in a plastic cage with free access to food and water.

The mice were allotted to two groups administered with MMC in diet (40 mgHg/g; Nihon CLEA, Osaka) with or without melatonin (20 mg/ml) in drinking water containing 0.02% ethanol. Each group consists of 10 mice and designated either the treated group or the control group, respectively.

After four weeks of treatment, both groups were evaluated by neurological signs (e.g., abnormal righting reflex, staggering gait, and fallen posture on its side) according to Suzuki and Miyama (1971). They were sacrificed on the 35th day of the treatment and tissues were collected for mercury and TBARS analyses.

*TBARS assay*

TBARS was determined by a method based on the original method of Ohkawa et al. (1979) but after slight modification. Briefly, to 200 ml of 2.5% homogenate, 200 ml of 0.8% sodium dodecyl sulfate, 1.5 ml of 20% acetate solution (pH 3.5) and 1.5 ml of 0.5% TBA were added, and the mixture was heated at 95°C for 60 minutes. The value was expressed as nmol of TBARS (malondialdehyde equivalent) per gram of the brain. Malondialdehyde standard was prepared from 1, 1, 3, 3-tetramethoxypropane.
Total mercury determination

The tissues were weighed and wet-ashed in a Pyrex tube with a mixture of nitrate/sulfate/perchloric acids (1:4:1 v/v) at 160°C for 30 minutes. The concentration of mercury in the tissues was determined by cold-vapor atomic absorption spectrometry, using stannous chloride as the reducing agent (Kim et al. 1995). To assure the accuracy of the determination, bovine liver (BCR No. 185; Bureau of Reference, Commission of the European Communities) was used as a reference material. Determined values fell within the range of the certified value.

Statistical analysis

Statistical analysis were performed by Student’s t-test where appropriate. Survival rates are analyzed by Log-Rank test (JMP version 3, SAS Institute Inc.).

RESULTS AND DISCUSSION

Until the third week of the treatment period, both the treated and control groups showed a small but gradual increase in the body weight (Fig. 1). On the forth week, the control group showed small decrease. But, there is no significant difference between the body weights of the two groups during this period.

The control group also showed a marked decrease in survival rate over the period of 30th to 35th days compared with 100% of survival rate of the treated group (Fig. 2: p = 0.029 by Log-Rank test). In the control group, 4 mice began to show neurological signs such as abnormal righting reflex, staggering gait and fallen posture on its side concomitantly with loss of body weight 4-7 days before death. However, in the treated group only 1 mice began to show those signs on the 33rd day and was alive at sacrifice on the 35th day.

![Body weight changes during experiment](image)

Means and SDs are shown (n = 10). There was no statistical difference between the Control (○) and Treated (●) groups.
Fig. 2. Survival rate.

The Treated (●) group showed statistically significant improvement of survival rate compared with that of the Control (○) group (p = 0.029 by Log-Rank test).

These findings indicate that the melatonin treatment reduced manifestation of neurological signs concomitantly with loss of body weight and it ameliorated the survival rate of the fifth week. These effects are due to, presumably, antioxidative capacity of melatonin. As shown in Fig. 3, the treated group showed decreased TBARS in the brain compared with that of the control group.

Mercury concentration in the brain of the treated group is higher than that of

![Graph showing survival rate over days.]

Fig. 3. TBARS in the brain.

Mean ± S.D. (n = 5 for Control group and 8 for Treated group). On the 35th day with MMC treatment with (□) or without (■) melatonin, mice were sacrificed and the brain was collected for TBARS assay.

***p < 0.001 by Student's t-test.
the control group, though there is no statistical significance because of relatively large SDs (Table 1). The mice of which brain was analyzed for mercury were sacrificed on the 35th day. Therefore, it is possible that survival mice with relatively lower Hg concentration in the brain among the control group were sacrificed and analyzed for mercury. This may enhance the difference in mercury concentration between the two groups. Food consumption on the basis of each cage was always greater in the treated group than in the control group (about 1.1–1.5 times greater; data not shown). Since melatonin has hyperphagia effect (Shaji and Kulkarni 1998), this might be associated with this effect. In spite of greater intake and slightly higher concentration of mercury in the brain, it is concluded that the treated group showed less neurological disturbance and mortality.

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References


