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Comparison of Monothiols and Vitamin Therapy Administered Alone or in Combinations during Methylmercury Poisoning

C. Bapu, K. Vijayalakshmi, P. P. Sood

Laboratory of Neurobiology, Department of Biosciences, Saurashtra University, Rajkot-360005, India

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Methylmercury chloride (MMC) is an important environmental pollutant. It is considered as a cumulative poison that unleashes several pathological symptoms in animals and man. During the past few years, scientists have tried several chemical agents as antidotes to decrease the mercury burden from the body and claimed positive results in non-nervous tissues (Mulder and Kostyniak, 1985), while in the nervous system mercury burden is further increased (Thomas and Smith, 1984). This condition is seen with the most of the mono- and dithiols used so far in methylmercury detoxication (See Sood et al., 1993 for review).

Since suitable antagonists are not available till date for the effective removal of mercury, especially from central nervous system (Kojima et al., 1989), we have tried glutathione (GSH) and its precursor like N-acetyl-DL-homocysteine thiolactone (NAHT). Along with this, we also used various vitamins viz., B Complex, C, B12 and E. The monothiols and vitamins are studied either alone or in various combinations. These chemical agents are used as they are body's own constituents, hence not injurious when used in proper doses. Further, the monothiols accelerate the mercury elimination (Aaseth, 1975; Watanabe et al., 1988), while the vitamins have cell repairing capacity as well as enhance the cellular metabolic properties (see McDowell, 1989; Dreyfus, 1985 for review). In order to compare the relative therapeutic abilities of various antagonists, both nervous and non-nervous tissues are included in this investigation. Since biochemical lesions are considered to be the most primary effect, and glycosidases are highly sensitive to MMC (Vinay et al., 1990), their fluctuation is considered as an indicator of toxicity in the study.

Correspondence to: P. P. Sood

MATERIALS AND METHODS

Eighty, three months old male albino mice (inbred strain obtained from National Institute of Occupational Health, Ahmedabad) weighing 30 ± 5 gms were used in this investigation. The animals were kept in highly hygienic conditions using polypropylene cages maintained at 28 ± 5°C with lighting conditions of 12h of light and 12h of darkness. They were fed with balanced food prepared with the following mixture; cracked wheat (70%), cracked bengal gram (20%), fish meal (5%), yeast powder (4%), shark liver oil (0.5%) and ground nut oil (0.5%) and water ad libitum. The animals were divided into 20 groups (each cage containing 4 animals).

Four groups of animals were used as control: (1) treated with the buffer (10 mM Na 2 CO 3 - NaHCO 3, pH 9.2) for 7 days and sacrificed; (2) treated with the buffer for 7 days and sacrificed on the 15th day (withdrawal group); (3) treated with the buffer for 7 days and then treated with saline for 7 days prior to sacrifice; and (4) treated with the buffer for 7 days and then treated with olive oil for 7 days. The volume, the dose and the interval were same in all the groups. Sixteen groups of animals were injected with methylmercury chloride (MMC) at a daily dose of 1 mg/kg body weight for 7 days. Out of these, two groups were sacrificed on the 8th day. Two groups of 7 days MMC pretreated animals were kept without toxicant for another 7 days and sacrificed on the 15th day. These were considered as normal withdrawal groups. Two more 7 day MMC pretreated groups were given separately the antagonists namely NAHT (40 mg/kg) and GSH (50 mg/kg) from 8th to 14th day and sacrificed on the 15th day. Likewise, four vitamins namely B Complex, C, B12 and E were administered subcutaneously at different regions of back to four separate groups of 7 day MMC pretreated animals from 8th to 14th day at a daily dose of 20 mg/kg, 5 mg/kg, 2 mg/kg and 60 mg/kg respectively. These animals were sacrificed on the 15th day. Another four groups of 7 day MMC pretreated animals were given NAHT (40 mg/kg body weight) and after half an hour vitamin B Complex (one group), vitamin C (one group), vitamin B 12 (one group) and Vitamin E (one group). The dose of vitamins and the durations of treatment were similar to the earlier groups. The last four groups of 7 day MMC pretoxicated animals were treated with GSH (50mg/kg body weight) and the vitamins similar to that of NAHT.

The antagonists and vitamins were diluted or dissolved in physiological saline except vitamin E which was diluted in olive oil. Antagonists and vitamins were always injected after a gap of half an hour. All injections were subcutaneous and given in between 9.00 to 10.00 A.M.

The animals were sacrificed by decapitation on the scheduled days between 6.00 and 7.00 A.M. without using anaesthesia. The brain, spinal cord, liver, kidney and testes were quickly dissected out, washed in chilled saline (4°C), blotted and weighed. Tissues were minced with sharp scissors and homogenized in a glass mortar with a glass pestle in sodium citrate (1mg/ml). Complete homogenization was obtained by adding non-acidic sand to the medium. This was followed by centrifugation in Remi make refrigerated centrifuge (-10°C) at 3000 rpm (500g) for 10 minutes in case of brain and spinal cord and at 5000 rpm (1400g) for 10 minutes in case of liver, kidney, and testis. The supernatants were treated with chilled acetone and resubjected to centrifugation at 10,000 rpm (5600g) for 30 minutes. The supernatants were discarded and the residue was dissolved in sodium citrate and used for the estimations of alpha-glucosidase and beta-glucosidase (alpha-gluco and beta-gluco) according to the technique of Tettamanti and Masserini (1984). Specific activities of the enzyme were calculated as µmol p-nitrophenol liberated/min/mgm protein wet weight tissue. Protein was estimated according to Lowry et al. (1951) method. All analyses were carried out in triplicate and statistical significance of the data was calculated by one way ANOVA according to Sokal and Rohlf (1969).

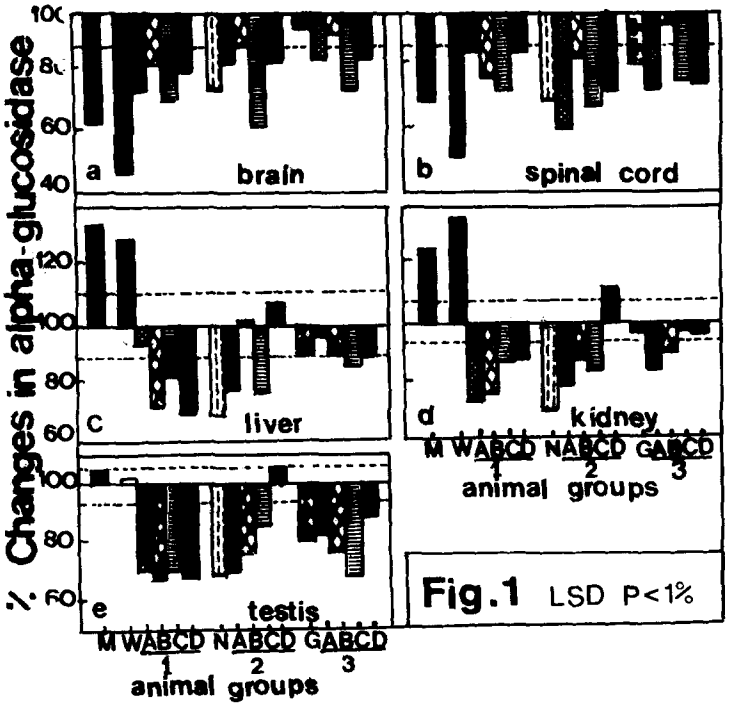
RESULTS AND DISCUSSION

Figures 1 and 2 represent the levels of alpha-gluco (Fig.1) and beta-gluco (Fig.2) in brain (a), spinal cord (b), liver (c), kidney (d) and testis (e) during MMC toxication and chelation therapy. The figures themselves are self explanatory, therefore, only the highlights of the study are mentioned in the text.

A daily dose of 1 mg/kg of MMC for 7 days showed an acute inhibition of both the glucosidases in the brain and spinal cord (M; Figs. 1,2 a,b). In sharp contrast to the CNS, the liver, kidney and testis demonstrated an increased activity of both the enzymes, which is statistically significant in all the tissues (Figs. 1,2 c,d,e).

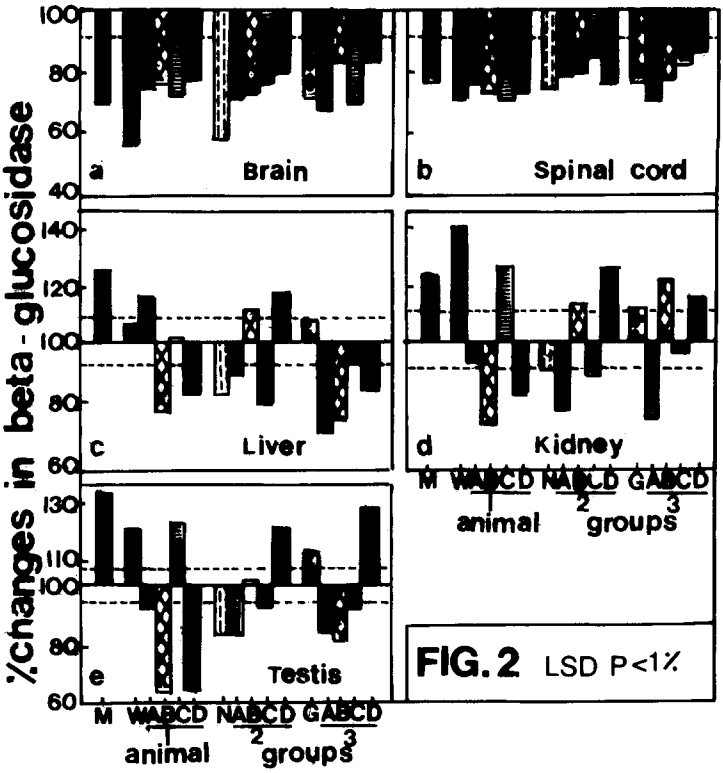
The seven days MMC treated animals when kept without toxicant for another 7 days showed a further inhibition of both enzymes in brain (W; Figs. 1-3 a) and spinal cord (W; Figs. 1,2 b) as compared to 7 days MMC treated mice. In kidney, the enzyme levels are further increased (W; Figs. 1,2 d), but in liver and testis they are recovered W; Figs. 1,2 c,e).

Post treatment with vitamins for 7 days caused recoveries of the enzymes in the brain and spinal cord



Figures 1a-e represents the percentage changes in alpha-glucosidase activity in different tissues during methylmercury (M) application as well as during normal (W), N-acetyl-DL-homocysteine thiolactone (N), glutathione (G) and vitamins (B Complex, C, B12, and E) induced withdrawals (A, B, C). Abbreviations used in the text and figures: M- 7 days MMC; W- 7 days MMC and sacrificed on 15th day; A1- 7 days MMC and 8th-14th day vitamin B Complex; B1- 7 days MMC and 8th-14th day vitamin C; C1- 7 days MMC and 8th-14th day vitamin B12; D1- 7 days MMC and 8th-14th day vitamin E; N- 7 days MMC and 8th-14th day NAHT; A2- 7 days MMC and 8th-14th day NAHT + vitamin B Complex; B2- 7 days MMC and 8th-14th day NAHT + vitamin C; C2- 7 days MMC and 8th-14th day NAHT + vitamin B12; D2- 7 days MMC and 8th-14th day NAHT + vitamin E; G- 7 days MMC and 8th-14th day GSH: A3- 7 days MMC and 8th-14th day GSH + vitamin B complex; B3- 7 days MMC and 8th-14th day GSH + vitamin C; C3- 7 days MMC and 8th-14th day GSH + vitamin B12; D3- 7 days MMC and 8th-14th day GSH + vitamin E.

(A1,B1,C1,D1; Figs. 1,2 a,b). Amongst the various vitamins, the maximum recovery of alpha-gluco in brain is shown by vitamin C (B1; Fig.1a) and in spinal cord with vitamin B complex and E (A1,D1;Fig.1b). In case of beta-gluco vitamin E showed maximum recovery in brain (D1; Fig.2a) and vitamin B Complex in spinal cord (A1; Fig.2b), though in the latter case it is insignificant. The vitamin showed different results in liver, kidney



Figures 2a-e represent the percentage changes in Beta-glucosidase activity in different tissues during methylmercury (M) application as well as during normal (W), N-acetyl-DL-homocysteine thiolactone (N), glutathione (G) and vitamins (B Complex, C, B12 and E) induced withdrawals (A, B, C, D). For details see text.

and testis. In most of the cases the enzymes reached to control level and then further inhibited upto a great extent (For details see Figs. 1,2 c,d,e).

Post treatment with NAHT and GSH showed recovery of the enzymes in the nervous tissue however, the control level is achieved only in the case of alpha-gluco (G; Figs.1a). In non-nervous tissues beta-gluco is significantly recovered (G; Fig.2 c,d,e) while alpha-gluco is recovered 100% and then further inhibited (G; Fig. 1 c,d,e).

Only some combinations of vitamins and thiols are found to be effective in CNS (Figs. 1,2 a,b). As for example, the combination of GSH and vitamin C showed a maximum recovery of alpha-gluco (B3; Fig.1 a,b), while in the case of beta-gluco, the combination of GSH and vitamin E showed the maximum recovery both in brain and spinal cord (D3; Figs. 2 a,b). The antagonists and vitamins combinations showed different results in non-nervous tissues (for details see Figs. 1,2 c,d,e).

The overall data clearly reveals the differential effect of MMC on nervous and non-nervous tissues. In 7 days MMC treated animals the glucosidases are significantly inhibited in CNS, while in the non-nervous tissues they are enhanced. Seven days MMC abstinence showed a still higher levels of enzymatic inhibition in nervous tissue but in non-nervous tissue fluctuation is less significant. Neverthless, the effect of MMC on both the enzymes is almost similar.

The GSH and NAHT are the cysteine derivatives and play an important role in xenobiotic metabolism (Dolphin et al., 1989; Meister and Anderson, 1983) and maintains -SH groups in the reduced state required for various biological activities. In the present experiment, these chelators are applied for a short duration as post therapeutic agents. Since in non-nervous tissues the enzymes, significantly enhanced with MMC, are not only 100% recovered but further inhibited with these chelators, it gives an indication that either we should have applied for a shorter duration in order to get theoretically the normal level of the enzymes. However, when we compare this data with nervous tissue, it gives a reverse impression. Moreover, the mercury analysis data still reveal sufficient metal in the tissues under similar experimental conditions (Sood et al., 1993) indicating that higher doses of chelators and longer duration of treatment is required. Thus, the situation is quite paradoxical, if we increase the dose and duration of chelators, there is a severe inhibition of enzymes in the non-nervous tissues but simultaneously it is necessary to mobilize the mercury content from various organs. Under such conditions, the application of thiols alone is not much helpful. Almost identical results are obtained with other metal chelators (Aaseth, 1975; Kostyniak and Soiefer, 1984; Raghu et al., 1990).

The effect of MMC on vitamin metabolism is completely unknown except a few isolated reports indicating that toxication decreases vitamins C and E levels in different tissues (Blackstone et al., 1974; Sakamoto, 1985). In a recent study from this laboratory, we also found decreased level of vitamin B1 and E both in nervous and non-nervous tissues during toxication (unpublished data). Since vitamins are essential for normal body functions, their deficiency can cause histopathological, ultrastructural, biochemical and behavioural abnormalities (see McDowell, 1989 for review). Therefore, the application of vitamins compensate their deficiencies leading to normal body function, recover all foresaid abnormalities and simultaneously help in decreasing the mercury burden.

The glycosidases are the group of lysosomal enzymes involved in various physiological functions and their deficiency causes severe metabolic disorders including myelin degeneration (Charlton, 1974; Vinay et al.,1990) which is also confirmed by us in mice (Bapu et al., 1992). Thus the myelin is quite sensitive to MMC, therefore, the inhibition of the enzymes related to myelin metabolism during toxication clearly indicate the metabolic disturbances in myelin sheath leading to its degeneration. The MMC elimination (Sood et al., 1993), repairing of myelin sheath (Bapu et al., 1992) and the recoveries of various glycosidases during vitamins and thiol therapy definitely show the beneficial effect of foresaid chelators in MMC detoxication.

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