Bull. Environ. Contam. Toxicol. (1992) 49:71–77

Environmental Contamination and Toxicology

Differential Effects of Methylmercury, Thiols, and Vitamins on Galactosidases of Nervous and Non-Nervous Tissues

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A rational pharmacological attack on heavy metal poisoning has only been possible with the advent of non-toxic binding of chelating agents. In the recent past, a number of chelators have been used to detoxicate the mercury content from the body (see Winship, 1985 for a review). When all the well known chelators were subjected for their therapeutic capacities in the central nervous system, most of the findings were discouraging (Unnikumar and Sood, 1985; Raghu et al., 1990; Vinay et al., 1990). In a recent study we have demonstrated the superiority of vitamins over thiol compounds in methylmercury mobilization (Bapu et al., 1990), which otherwise has been considered difficult and often an impossible task for clinicians as well as toxicologists.

Biochemical lesions are considered to be the most primary effects of methylmercury toxication (Unnikumar and Sood, 1985), and lysosomes are the critical cellular organelles which are easily ruptured and release enzymes (Lauwerys and Buchet, 1972; Sood et al., 1988; Vachhrajani et al., 1986). In the present study, the biochemical analyses of two lysosomal enzymes (alpha and beta-galactosidases) in various nervous and non-nervous tissues of mice during methylmercury toxication as well as detoxication with vitamins and thiols have been studied in the light of previous investigation related to methylmercury mobilization with these agents.

MATERIALS AND METHODS

Ninety six 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 12 hrs of light and 12 hrs of darkness. They were fed with balanced food and water ad libitum. The animals were divided into 24 groups. Each cage contained 4 animals.

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Eight groups of animals (controls) were subcutaneously injected with vehicle (10 mM Na_2CO_3-$NaHCO_3$, pH, 9.2). The volume, the dose and the interval were same in all 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 day MMC pretreated animals were kept without drug 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 N-acetyl-DL-homocysteine thiolactone (NAHT - 40 mg/kg) and glutathione (GSH - 50 mg/kg) from 8th to 14th day and sacrificed on the 15th day. Likewise, four vitamins, vitamin B complex, vitamin C, vitamin B_{12} and vitamin 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 doses of vitamins and the duration of treatment were the same as the earlier groups. The last four groups of 7 day MMC pretreated animals were treated with GSH (50 mg/kg body weight) and the vitamins separately similar to that of NAHT.

The antagonists and vitamins were 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 the injections were subcutaneous and given in between 9-10 AM. The controls were always injected along with the treated groups.

The animals were sacrificed by decapitation on the scheduled days between 6 and 7 AM without using any anaesthesia. The brain, spinal cord, liver, kidney and testis were quickly dissected out, washed in 4°C saline, blotted and weighed. Tissues were minced with sharp scissors and homogenized in a glass mortar using glass pestle in sodium citrate (1 mg/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 (500 g) for 10 minutes in case of brain and spinal cord and at 5000 rpm (1400 g) for 10 minutes in case of liver, kidney and testis. The supernatants were treated with chilled acetone and resubjected to centrifugation at 10000 rpm (5600 g) for 30 minutes. The supernatants were discarded and the residues were dissolved in sodium citrate and used for the estimations of alpha-galactosidase (A-GAL) and beta-galactosidase (B-GAL) according to the technique of Tettamanti and Masserini (1984). Specific activity of the enzyme was calculated as μ mol/mg protein/gm wet weight tissue. Protein was estimated according to the Lowry et al. (1951) method. All the analyses were carried out in triplicate and the statistical significance of the data was calculated by one way ANOVA according to Sokal and Rohlf (1969).

RESULTS AND DISCUSSION

A daily dose (1 mg/kg) of MMC for 7 days showed different pattern of enzymatic alterations in various tissues. In brain (M; Figs. 1a, 2a), spinal cord (M; Figs. 1b, 2b) and testis (M; Figs. 1e, 2e) both the galactosidases were inhibited; in liver (M; Figs. 1c, 2c) the activity was increased, while in kidney A-GAL was inhibited (M; Fig. 1d) and B-GAL was enhanced (M; Fig. 2d). All the results were statistically significant except B-GAL in testis (M; Fig. 2e).

The withdrawal group of animals (treated for 7 days with MMC and kept for another 7 days without drug and sacrificed on 15th day) showed different pattern of enzymatic alterations in nervous and non-nervous tissues. Both the enzymes were further inhibited in brain and spinal cord (W; Figs. 1, 2a, b). In liver, the activity was recovered (W; Figs. 1, 2c). In kidney A-GAL was significantly recovered (W; Fig. 1d), while B-GAL was further increased (W; Fig. 2d). Nevertheless, both the enzymes showed a significant increased activity in kidney of withdrawal animals. Likewise, in testis A-GAL was recovered (W; Fig. 1e) and B-GAL was further inhibited (W; Fig. 2e).

When 7 day MMC pretreated animals were given vitamins for another 7 days, they showed significant recoveries of both the enzymes in brain (A1, B1, C1, D1; Figs. 1a, 2a) and spinal cord (A1, B1, C1, D1; Figs. 1b, 2b). Amongst the four vitamins applied, vitamin E revealed maximum recovery in brain (D1; Figs. 1a, 2a), while in spinal cord vitamin B complex and vitamin B_{12} showed maximum recoveries of A-GAL (A1; Fig. 1b) and B-GAL (C1; Fig. 2b) respectively.

In kidney and testis, all vitamins significantly recovered B-GAL (A1, B1, C1; Fig. 2d, e), except vitamin E which showed significant inhibition (D1; Fig. 2d, 2e). A-GAL was also reverted in kidney with vitamins (A1, B1, C1; Fig. 1d) but in liver and testis the vitamins were not of much help, except vitamin B_{12} (C1; Fig. 1c, e). For details see figure 1,2.

The antagonists, NAHT and GSH, when applied from day 8 to 14th to 7 day MMC pretreated animals, revealed significant recoveries of both the enzymes in brain and spinal cord (N,G; Figs. 1a, b; 2a, b). Glutathione also recovered both the enzymes in non-nervous tissues though the percentage of recovery was quite variable. A-GAL in liver and testis and B-GAL in kidney and testis were completely recovered by GSH (G; Fig. 2d, e). The other antagonist, NAHT, recovered B-GAL only in liver (N; Fig. 2c), while in other tissues the alterations were either insignificant (N; Figs. 1c, 2c) or there was an acute inhibition (N; Figs. 1d, 2d). These results were quite different from brain and spinal cord.

When 7 day MMC pretreated groups of animals were administered with vitamins and thiols for another 7 days, they showed mixed results both in nervous and non-nervous tissues. Only a few combinations

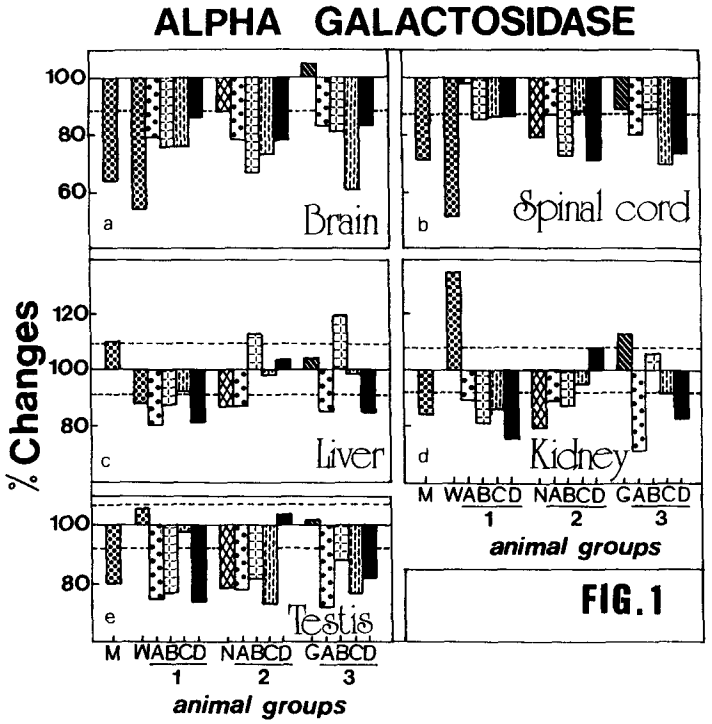
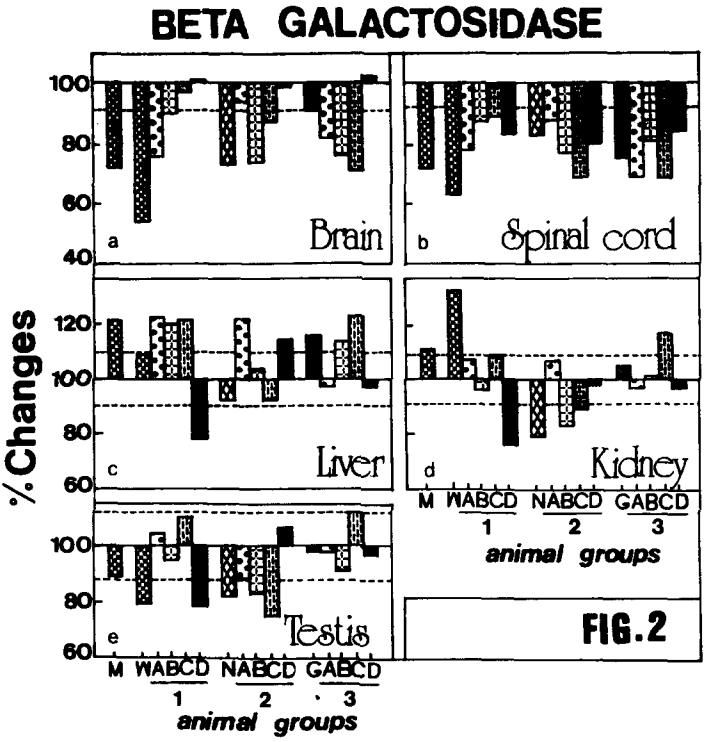


Figure 1a-e represent the percentage changes in alpha-galactosidase activity in different tissue during methylmercury (M) application as well as during normal (W), N-acetyl-DL-homocysteine thiolactone (N), glutathione (G) and vitamins (B complex, C, B_{12} and E) induced withdrawals (A,B,C,D). For details see text.

Abbreviations used in text and figures : M - 7 days MMC; W - 7 days MMC and sacrificed on 15th day; A1 - 7 days MMC and 8-14th day vitamin B complex; B1 - 7 days MMC and 8-14th day vitamin C; C1 - 7 days MMC and 8-14th day vitamin B_{12}; D1 - 7 days MMC and 8-14th day vitamin E; N - 7 days MMC and 8-14th day NAHT; A2 - 7 days MMC and 8-14th day NAHT + vitamin B complex; B2 - 7 days MMC and 8-14th day NAHT + vitamin C; C2 - 7 days MMC and 8-14th day NAHT + vitamin B_{12}; D2 - 7 days MMC and 8-14th day NAHT + vitamin E; G - 7 days MMC and 8-14th day GSH; A3 - 7 days MMC and 8-14th day GSH + vitamin B complex; B3 - 7 days MMC and 8-14th day GSH + vitamin C; C3 - 7 days MMC and 8-14th day GSH + vitamin B_{12}; D3 - 7 days MMC and 8-14th day GSH + vitamin E.

showed desired results. For example, B-GAL was completely recovered in brain with NAHT and vitamin B complex and E combinations. GSH combinations with vitamin E in brain, with vitamin B complex and E in liver and vitamin E with both the thiols in kidney were found to be quite meaningful. The rest of the combinations were discouraging. Likewise, in A-GAL only a few combinations have worked (for details see figure 1).

In our earlier study we have demonstrated differential effect of



Figures 2a-e represent the percentage changes in beta-galactosidase 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, B_{12} and E) induced withdrawals (A, B, C, D). For details see text.

NAHT, GSH and various vitamins in the mobilization of methylmercury content in the nervous and non-nervous tissues (Bapu et al., 1990). It was found that during drug abstinence period (normal withdrawal), the mercury content was increased in brain and spinal cord and decreased in liver, kidney and testis (Bapu et al., 1990). The present biochemical analysis also showed an inhibition of both the galactosidases in the nervous tissue. However, these enzymes in non-nervous tissues showed mixed results. For example, though mercury was mobilized in withdrawal groups, enzymes either recovered or were further inhibited or further increased, demonstrating no correlation with methylmercury mobilization. Almost similar results have been obtained with alpha and beta-glucosidases and alpha-mannosidase (unpublished data).

The thiol compounds, NAHT and GSH, mobilized mercury from all the tissues (except spinal cord) and simultaneously recovered the enzymes. Interestingly enough, though methylmercury content increased in the spinal cord, both the thiols were able to recover the enzymes to some extent. The same results have been found with

other glycosidases (unpublished data). In non-nervous tissues both the enzymes were recovered in some groups, while in others they further inhibited, indicating that the dose or duration of antidote application should be reduced. But this view does not appear to be correct as non-nervous tissue still revealed about 30 to 70 % mercury content with the antidotes.

The application of vitamins showed better results in B-GAL recovery in nervous tissue. The vitamins and thiols combinations usually do not give desired results in brain and spinal cord. In non-nervous tissues, the vitamins or either of the thiols showed better results only in a few groups and not in others. Nevertheless, the analytical data revealed much more elimination of mercury with vitamins when they were applied alone as compared to thiols or their combinations with vitamins (Bapu et al., 1990). Surprisingly enough, though a large amount of mercury is present in the non-nervous tissues in thiols and vitamins withdrawal groups, still the galactosidases levels were not only 100% recovered but also further inhibited. Such a situation was not seen in the CNS. Therefore, probably this stage may be temporary and the enzymes may normalize with time. Since methylmercury is a potent neurotoxicant and most of the metal chelators have failed to mobilize mercury as well as to recover the biochemical lesions, the extra application of vitamins appear to be much more advantageous in mercury mobilization as demonstrated in our earlier therapeutic study (Bapu et al., 1990) and the recovery of various enzymes as revealed in the present investigation.

Acknowledgments. This work was supported by UGC, New Delhi and GUJCOST, Gandhinagar. Authors are thankful for the same.

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Received May 30, 1991; accepted January 12, 1992.