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Differential Therapeutic Responses of Thiol Compounds in the Reversal of Methylmercury Inhibited Acid Phosphatase and Cathepsin E in the Central Nervous System of Rat

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Though considerable headway has been made in elucidating the effect of methylmercury on the biochemical machinery of nervous system, the studies on the alterations in the levels of acid hydrolases received less attention (Sood et al 1988). Being a lysosomal marker, acid phosphatase is one of the most extensively studied enzymes amongst the acid hydrolases. Its significance in various key physiological as well as pathological processes is well preserved in literature (see Nadler, 1973). Cathepsin E, an aspartic proteinase, has been demonstrated in a number of cells and tissues within the human body, rat, E.coli where its role is implicated in a number of important metabolic processes (see Yonezawa et al 1988). In the present paper, we report the results of the differential levels of inhibition of these enzymes with methylmercury as well as their differential recoveries with two thiols (N-acetyl-DL-homocysteine thiolactone and glutathione) in various neuroanatomical areas (olfactory bulbs, cerebral hemispheres, cerebellum, medulla oblongata and spinal cord) of rat.

MATERIALS AND METHODS

Seventy eight young healthy, male Wistar albino rats (275±10 g) were used in this investigation. The animals were kept in highly hygienic conditions, using polypropylene cages, maintained at 27±2°C with lighting conditions of 12 hrs of light and 12 hrs of darkness. They were fed with balanced food pellets and water ad libitum. The animals were divided into 26 groups and each group contained 3 animals. Two groups of animals, meant for control studies, were injected intramuscularly with the vehicle (10 mM Na_2CO_3-$NaHCO_3$, pH 9.2). The volume, the mode, the duration and the interval was the same in all the groups. Eight groups of animals were used for neurotoxicological studies. Methylmercury chloride (MMC ; Wako Pure Chemicals Ltd., Japan ; 85.0 % Pure) was dissolved in the above stated vehicle and was injected intramuscularly at a daily dose of 1 and 10 mg/kg body weights in two separate sets of experiments.

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Sixteen groups of animals were utilized for therapeutic studies. Two thiol compounds, N-acetyl-DL-homocysteine thiolactone (NAHT) and glutathione (GSH) were dissolved in physiological saline and injected to MMC pretreated groups as a daily intramuscular dose of 40 and 80 mg/kg (NAHT) and 100 and 150 mg/kg (GSH) body weights.

The controls, MMC and thiols treated animals were sacrificed, in the morning hours in order to avoid diurnal fluctuations in the enzymes level, on 3rd, 8th, 15th and 16th day post-treatment. The quickly dissected out brains and spinal cords were placed in 4°C normal saline to remove excess of blood and adhering meninges. Various neuroanatomical areas (olfactory bulbs, cerebral hemispheres, cerebellum, medulla oblongata and spinal cord) were weighed, minced with sharp scissors and homogenized in a glass mortar using glass pestle.

The homogenates were then subjected to centrifugation (at -10°C) for 5 min. at 430 x g. The supernatants thus obtained were treated with chilled acetone and resubjected to centrifugation for 10 min. at 1070 x g. The supernatants were again treated with chilled acetone and were subjected to final centrifugation for 30 min. at 5375 x g. The supernatants were discarded and the residue was washed with 0.25 M sucrose before dissolving in the same medium. This partially purified extract was used for the estimation of acid phosphatase and cathepsin E according to the techniques provided by Shinowara et al (1942), Fiske and Subbarow (1925) and Lapresle and Webb (1962) respectively. The specific activity of the enzymes was expressed in terms of u mol/hr/37°C/mg protein. Protein was determined according to Lowry et al (1951). All the analyses were done in triplicate and the statistical analysis of the data was obtained by employing Analysis of Variance (ANOVA) as per the procedure described by Sokal and Rohlf (1969).

RESULTS AND DISCUSSION

Various CNS areas of control animals displayed a great degree of variation in the activities of acid phosphatase and cathepsin E (Fig. 1). Two days of MMC treatment did not show any statistically significant variations in any of the neuroanatomical areas with either of the metal compound doses (M2 - Figs. 2-5). Zimmer and Carter (1979) have also demonstrated that methylmercury entry into brain requires 2-7 days. However, when the duration of MMC application was extended beyond two days, both the enzymes showed significant enzymes inhibition in all the neuroanatomical areas with both the doses except for a few isolated instances like olfactory bulbs (M7 - Fig. 2a), cerebral hemispheres (M7 - Fig. 2b) and medulla oblongata (M7 - Fig. 2d). On the otherhand, a maximum inhibition of both the enzymes in all the neuroanatomical areas was exhibited in the animals treated for 15 consecutive days (M15 - Figs. 2-5). However, the magnitude of cathepsin E inhibition with both the doses is higher as compared to acid phosphatase (compare figures 4,5 with 2,3). This trend is similar in all the CNS areas.

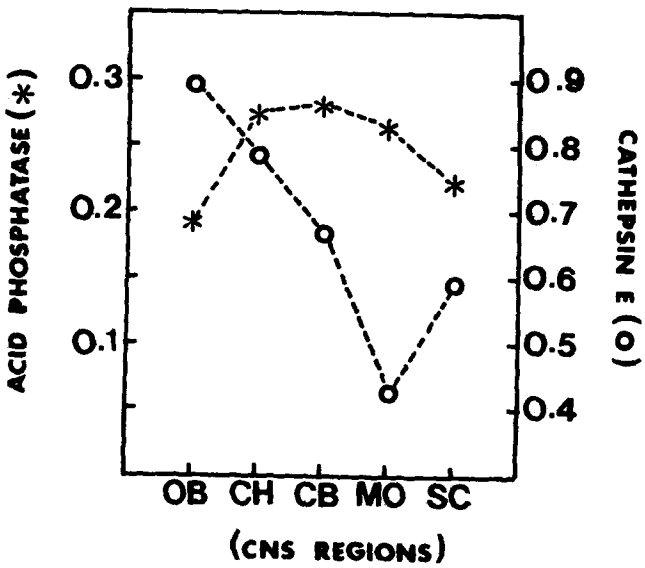

Figure 1. represents the specific activity (u mol/hr/37°C/ mg protein) of acid phosphatase and cathepsin E in olfactory bulbs (OB), cerebral hemisphere (CH), cerebellum (CB), medulla oblongata (MO) and spinal cord (SC) of control animals.

The application of thiol compounds to the MMC treated groups showed significant recovery of acid phosphatase in all the groups as well as in all the neuroanatomical areas (B and C - Fig. 2a-e). However, the extent of significant enzyme recovery is less pronounced with the high doses (B and C - Fig. 3a-e). In contrast to acid phosphatase, cathepsin E recovery is quite negligible with NAHT and GSH. However, in all the cases, GSH always exhibited better results as compared to NAHT (C - Figs. 4 and 5a-e). It is also clearly evident that in no case control level of the enzymes was achieved in any of the groups with either NAHT or GSH (B,C - Fig. 2-5). Further, there was a negligible variation in the respective enzymatic recoveries by both the thiol compounds especially with cathepsin E (B,C - Figs. 4, 5). Similar results with other lysosomal enzymes were also noted in our earlier work (Vinay and Sood 1991).

The fascinating aspect of the present study was the differential inhibitions as well as recovery levels of both the enzymes with MMC and thiols treatments. It was also clearly evident that the degree of acid phosphatase inhibition, in all the neuroanatomical areas, was comparatively less with both the MMC doses as compared to cathepsin E under similar experimental conditions. Such a variability in the degree of inhibition of other lysosomal enzymes like arylsulfatases A and B (Vinay and Sood 1991) and glycosidases (Vinay et al 1990) is also reported. It is known that methylmercury deposits in the lysosomes (Thorlacius-Ussing and

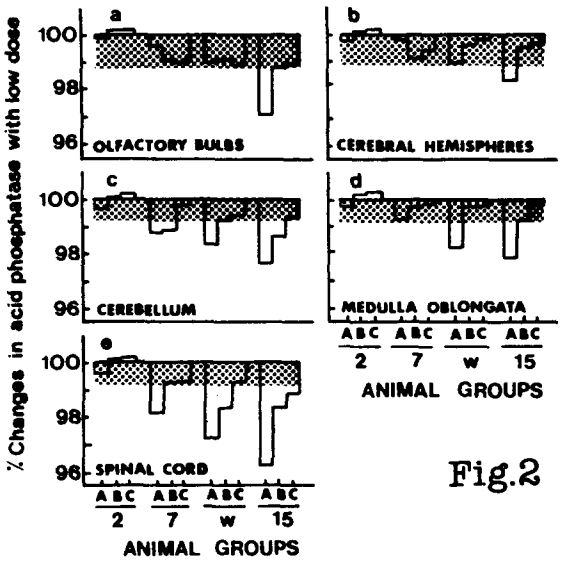

Fig.2

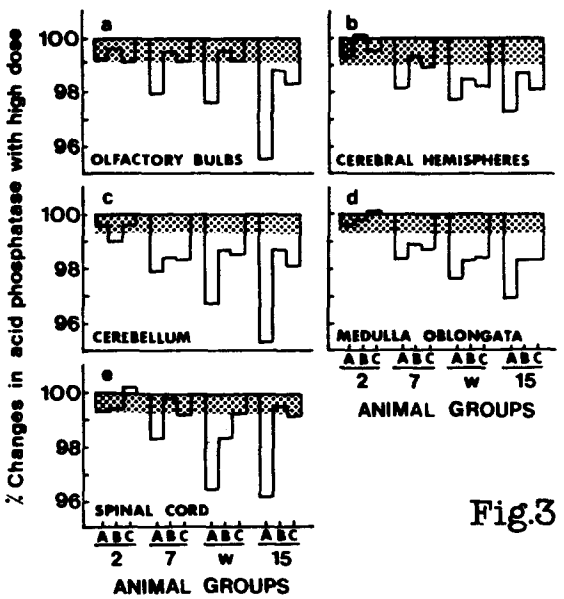

Fig.3

Figures 2 and 3. represent the percentage changes of acid phosphatase (u mol inorganic phosphate/hr/mg protein/37°C) in different CNS areas with low and high doses of MMC (A2,A7,Aw,A15), NAHT (B2,B7,Bw,B15) and GSH (C2,C7,Cw,C15). Control is regarded as 100% and any deviations from it are represented in histogrammes. Shaded area denotes the statistical significant limit at a particular P level ($P < 0.001$).

Graabek 1986) and ruptures the lysosomal membrane due to overburdening and liberates the enzymes (Lauwerys and Buchet, 1972; Sood et al 1988), yet the inhibitory action of methylmercury

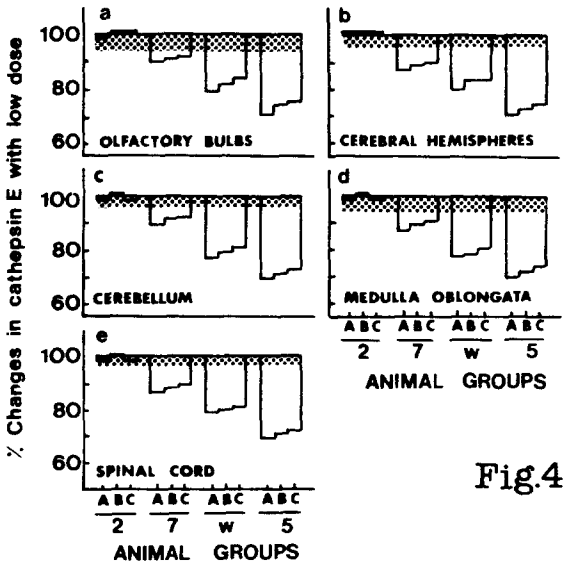

Fig.4

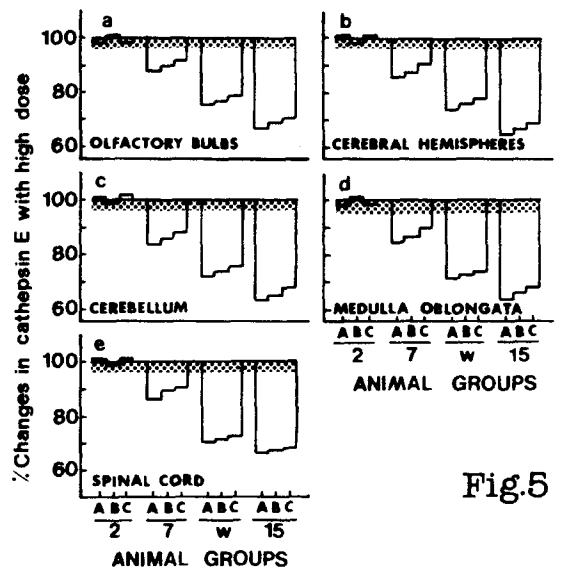

Fig.5

Figures 4 and 5. represent the percentage changes of cathepsin E (u moles peptides/hr/mg protein/37°C) in different CNS areas with low and high doses of MMC and thiol compounds. For abbreviations and details see figures 2 and 3.

varies for different acid hydrolases.

It is known that different neuroanatomical areas have differential capacities to store, metabolize, retain and excrete mercury both under methylmercury (Hargreaves et al 1985) as well as antagonists

treatment (Vinay et al 1990). However, the relationship of mercury accumulation and the inhibition of both the enzymes is concerned, the trend is just reverse, as in all the cases there is a dose and duration dependent increase of mercury deposition and simultaneously the inhibition of the enzymes. The tissue mercury analysis data, under identical experimental conditions, showed the highest mercury accumulation in cerebellum and lowest in olfactory bulbs with both the drug doses (Vinay et al 1990), but it does not correspond well with the degree of enzymatic inhibition as demonstrated in the present study. Such a view is also put forth by Omata et al (1982). It may be noted that the catabolism of proteins within the cells require ATP, and acid phosphatase and cathepsin E depend on ATP for their respective activities (Hinton and Koeing, 1975 ; Thomas et al 1989). Since methylmercury is known to deplete ATP levels (Cheung and Verity, 1981), diminish cellular energetics (Ally et al 1984) and interfere in Krebs' cycle (Yoshino et al 1966), it is possible that these ATP dependent enzymes are affected indirectly. However, no direct casual relationship is available on the effect of methylmercury on these enzymes to facilitate the molecular interpretation of such an inhibitory action.

In the light of the present investigation, where the enzymes are greatly effected and the efficacy of the thiol compounds is kept at the bearest minimum, it appears to us that the application of any thiol compound alone will never be able to recover the altered biochemical machinery of the nerve cells, though they have been proved beneficial in non-nervous tissues (Aaseth, 1975 ; Mulder and Kostyniak, 1985).

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REFERENCES

Aaseth J (1975) The effect of N-acetyl-DL-homocysteine and its thiolactone on the distribution and excretion of mercury in methylmercury chloride injected mice. Acta Pharmacol Toxicol 36 : 193-202.

Ally A, Phipps J, Miller DR (1984) Interaction of methylmercury chloride with cellular energetics and related processes. Toxicol Appl Pharmacol 76 : 207-218.

Cheung M, Verity MA (1981) Methylmercury inhibition of synaptosome protein synthesis. The role of mitochondrial dysfunction. Environ. Res 24 : 286-298.

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66 : 375-400.

Hargreaves RJ, Foster JR, Pelling D, Moorhouse SR, Gangolli SD, Rowland IR (1985) Changes in the distribution of histochemically localized mercury in the CNS and in tissue levels of organic and inorganic mercury during the development of intoxication of methylmercury treated rats. Neuropathol Appl Neurobiol 11 : 383-401.

Hinton DE, Koeing JC (1975) Acid phosphatase activity in

subcellular fractions of fish liver exposed methylmercuric chloride. Comp Biochem Physiol 50 : 621-625.
Lapresle C, Webb T (1962) Biochem J 84: 455-462 In: Dingle JT (ed) (1972) Lysosomes - A laboratory hand book, North-Holland Publishing Co., Amsterdam, London, p 45.
Lauwerys R, Buchet JD (1972) Study on the mechanism of lysosome labilization by inorganic mercury In vitro. Eur J Biochem 26: 535-542.
Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin-phenol reagent. J Biol Chem 193: 265-275.
Mulder KM, Kostyniak PJ (1985) Involvement of glutathione in the enhanced renal excretion of methylmercury in CFW Swiss mice. Toxicol Appl Pharmacol 78 : 451-457.
Nadler HL (1973) Acid phosphatase deficiency. In: Hers HG, Van Hoof F (eds) Lysosomes and storage diseases. Acad Press, New York, p 474.
Omata S, Momose Y, Ueki H, Sugano H (1982) In vivo effect of methylmercury on protein synthesis in peripheral nervous tissues of rat. Arch Toxicol 49 : 203-214.
Shinowara GY, Jones JM, Reinhart HL (1942) The estimation of serum inorganic phosphate and acid and alkaline phosphatase activity. J Biol Chem 142 : 912-921.
Sokal RR, Rohlf FJ (1969) Biometry. Freeman & Co., San Fransisco, p 227.
Sood PP, Unnikumar KR, Vinay SD, Raghu KG, Wegmann R (1988) Duration dependent effect of methylmercury chloride and antagonists on the enzymes of the central nervous system of rat. II Acid phosphatase study on the brain. Cell Mol Biol 34: 271-277.
Thomas DJ, Richards AD, Kay J (1989) Inhibition of aspartic proteinase by alpha$_2$-macroglobulin. Biochem J 259: 905-907.
Thorlacius-Ussing O, Graabek PM (1986) Simultaneous ultrastructural demonstration of heavy metals (silver, mercury) and acid phosphatase. Histochem J 18 : 639-646.
Vinay SD, Raghu KG, Sood PP (1990) Dose and duration related methylmercury deposition, glycosidases inhibition, myelin degeneration and chelation therapy. Cell Mol Biol 36: 609-623.
Vinay SD, Sood PP (1991) Inability of thiol compounds to restore CNS arylsulfatases inhibited by methylmercury. Pharmacol Toxicol 69 : 71-74.
Yonezawa S, Fujii K, Maejima Y, Tamoto K, Mori Y, Muto N (1988) Further studies on rat cathepsin E : Subcellular localization and existence of the active subunit form. Arch Biochem Biophys 267 : 176-183.
Yoshino Y, Mozai T, Nakao K (1966) Biochemical changes in the brain in rats poisoned with an alkylmercury compound with special reference to the inhibition of protein synthesis in brain cortex slices. J Neurochem 13 : 1223-1230.
Zimmer L, Carter DE (1979) Effect of complexing treatment administered with the onset of methylmercury neurotoxic signs. Toxicol Appl Pharmacol 51 : 29-37.

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