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Protective Role of Thiol Chelators Against Dimethylmercury Induced Toxicity in Male Rats

Deepmala Joshi · Deepak Kumar Mittal · Sadhana Shrivastava · Sangeeta Shukla

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Abstract The present study was undertaken to establish mode of action, comparative therapeutic efficacy and safety evaluation of *N*-acetyl cysteine and dithiothreitol against acute dimethylmercury poisoning in rats. Male *Sprague–Dawley* albino rats (150 ± 10 g) were randomly divided into six groups. Group 1 served as control. Group 2–4 were administered dimethylmercury (10 mg/kg, *p.o.*) once only and group 2 served as experimental control. Animals of group 3 and 4 were received *N*-acetyl cysteine and dithiothreitol. Compared to the control, significant increase ($p \leq 0.05$) was observed in the activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lipid peroxidation level and mercury ion concentration, however reduced glutathione, catalase, adenosine triphosphatase, acetyl cholinesterase (in brain only) were also decreased. It was concluded that *N*-acetyl cysteine provided maximum protection when compared with dithiothreitol group.

Keywords Dimethylmercury · *N*-acetyl cysteine · Dithiothreitol · Lipid peroxidation

Mercury is a toxic non-radioactive, volatile metal. The basis of mercury toxicity is undoubtedly multifactorial. Approximately, 400 billion metric tons of mercury is released into the atmosphere every year (US EPA 2000). The World Health Organization estimates that approximately 10,000 tonnes of mercury is released worldwide from both natural and manmade sources each year (Agrawal and Wankade 2003).These have a vast array of remarkably adverse effects, including those of carcinogenicity, neurotoxicity and immunotoxicity. It is well known that mercury produces oxidative stress by generating reactive oxygen species (H_2O_2) and free radicals resulting in lipid peroxidation, DNA damage, cytotoxicity and alters physiological and biochemical characteristics of biological system. It is a transition metal, promotes the formation of reactive oxygen species such as hydrogen peroxides. These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell (Sharma et al. 2007).

The chelation is one of the effective methods to treat metal induced toxicity in the modern era. Chelating agent is generally non-specific with regard to the affinity of the metal. The most commonly chelating agents that have been the forerunners against mercury toxicity were investigated by various authors as calcium disodium versenate, diethylenetriaminepentaacetic acid, ethylenediaminetetraacetic acid, 2,3–dimercaptopropane–1–sulfonic acid and meso–2,3–dimercaptosuccinic acid. Calcium disodium versenate showed good absorption but is very painful at injection site and have major toxic effects on the renal system. meso–2,3–dimercaptosuccinic acid is effective chelator of soft tissues but it is unable to chelate mercury from bones. 2,3–dimercaptopropane–1–sulfonic acid is effective only in extra cellular spaces. *N*-acetyl cysteine is an excellent source of sulfhydryl (–SH) groups and some essential nutrients, its therapy have been found to be beneficial in increasing mercury mobilization and assisting the recovery of altered biochemical parameters (Aremu et al. 2008). Dithiothreitol is another chelator which protects free sulfhydryl groups from oxidation or reduce disulfide linkages to free sulfhydryl groups in protein and enzymes (Hultberg

D. Joshi (✉) · D. K. Mittal · S. Shrivastava · S. Shukla
Reproductive Biology and Toxicology Laboratory, UNESCO Setellite center of Trace Element Research & School of Studies in Zoology, Jiwaji University, Gwalior, Madhya Pradesh, India
e-mail: deepmalajoshi953@gmail.com

et al. 2001). Thus, the present study has been undertaken to assess the effectiveness of *N*-acetyl cysteine and dithiothreitol so as to develop a thiol antidote as an intervention strategy and thus a practical solution to mercury intoxication.

Materials and Methods

Adult male Sprague–Dawley rats (150 ± 10 g body wt) were obtained from the departmental animal facility where they were housed under standard husbandry conditions with standard rat feed (Pranav Agro Industries, India) and water ad libitum. Experiments were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and experimental protocols were approved by the institutional animal ethics committee (CPCSEA/501/01/A). dimethylmercury, *N*-acetyl cysteine and dithiothreitol were procured from Sigma–Aldrich.

Dimethylmercury (DMM) (10 mg/kg) (RTECS 1986) was suspended in olive oil and was administered orally. The selection of doses of *N*-acetyl cysteine (NAC) (2 mM/kg, *i.p.*) and dithiothreitol (DTT) (15.4 mg/kg *i.p.*) were based on earlier works (Giradi and Elias 1993) and (Barnes et al. 1980) as well as our previous studies (Singh et al. 2007). Olive oil was given as vehicle to control animals. Animals were divided into four groups consisting of six animals each and groups 2–4 received DMM once only and all the treated groups received therapy intraperitoneally for 3 days after 72 h of toxicant administration.

- Group 1: control, received vehicle only.
- Group 2: experimental control, received DMM for once only
- Group 3: received DMM (once only) followed by NAC
- Group 4: received DMM (once only) followed by DTT

Animals of all the groups were euthanized after 48 h of last administration; blood was collected, serum was isolated to assess various biochemical variables. Liver kidney and brain were immediately processed for the biochemical analysis.

After keeping the blood for 1 h at room temperature, serum was isolated by centrifugation at 1,000×*g* for 15 min and stored at −20°C until analyzed. Brain was homogenized in 0.25 M sucrose solution for the estimation of acetyl cholinesterase activity. Homogenates of liver, kidney and brain were prepared in chilled hypotonic solution (10%, w/v) for the estimation of total proteins, adenosine triphosphatase (ATPase) and catalase activities. Tissues were digested (Jacobs et al. 1960) for analysis of mercury concentration. Serum was used for the estimation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel 1957) and alkaline phosphatase (SALP) (Hawk et al. 1954). Fresh tissues of liver, kidney and brain were immediately processed for the estimation of lipid peroxidation (Sharma and Krishna Murti 1968), reduced glutathione (Brehe and Burch 1976) and enzymatic activities, adenosine triphosphatase (ATPase) (Seth and Tangri 1966) and catalase (CAT) (Sinha 1972). Acetyl cholinesterase (AChE) assay was measured as described by Ellman et al. (1961).

Statistical analysis was carried out using one-way analysis of variance (ANOVA) taking significant at $p \leq 0.05$ followed by student's *t*-test taking significant at $p \leq 0.05$ (Snedecor and Cochran 1994). Percent protection was also used for comparison for different treatment groups.

Results and Discussion

Table 1 assesses the effect of therapeutic potential of thiol protectors on blood biochemical variables. The activities of transaminases, alkaline phosphatase and protein in serum

Table 1 Effect of *N*-acetyl cysteine and dithiothreitol against dimethylmercury induced toxicity on blood biochemistry

Treatments	AST (IU/L)	ALT (IU/L)	SALP (mg Pi/100 mL/h)
Control	65.0 ± 3.59	47.0 ± 2.63	205 ± 11.3
DMM *per se*	151 ± 8.35$^{\#}$	130 ± 7.18$^{\#}$	1,450 ± 80.1$^{\#}$
DMM + NAC	98.0 ± 5.41*	78.0 ± 4.31*$^{,\Omega}$	994 ± 54.9
% Protection	*61.6%	63.10%	36.60%
DMM + DTT	113 ± 6.27*	99.0 ± 3.44*	1,131 ± 62.5
% Protection	*43.5%	37.70%	25.60%
F value	44.4$^{@}$	66.7$^{@}$	163$^{@}$

Data are mean ± SE, $N = 6$; $^{\#}$ DMM *per se* vs. control at $p \leq 0.05$; * Treatment vs. DMM *per se* at $p \leq 0.05$

$^{\Omega}$ NAC vs. DTT for Tukey's HSD *post hoc* test ($p \leq 0.05$); $^{@}$ Significant at $p \leq 0.05$ for ANOVA

Abbreviations: *DMM* dimethylmercury, *NAC* *N*-acetyl cysteine, *DTT* dithiothreitol

were raised significantly after 72 h of dimethylmercury administration ($p \leq 0.05$). The therapy with *N*-acetyl cysteine and dithiothreitol prevented the enhanced release of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and protein contents in serum towards control ($p \leq 0.05$). Percent protection showed that the therapy of *N*-acetyl cysteine prevented the release of aspartate aminotransferase and alanine aminotransferase more than 60% and alkaline phosphatase 36.6% serum protein 70%, respectively. Tukey's HSD *post hoc* test showed significant difference between *N*-acetyl cysteine and dithiothreitol treatments in alanine aminotransferase activity, thus confirming profound efficacy of *N*-acetyl cysteine than dithiothreitol at 5% level. Control group did not register any significant change during the entire regimen.

Table 2 indicates significant elevation in lipid peroxidation level with acute dimethylmercury exposure ($p \leq 0.05$). *N*-acetyl cysteine showed significant protective effect ($p \leq 0.05$) in liver, kidney and brain lipid peroxidation as seen by statistical analysis ($p \leq 0.05$). Acute mercury administration declined reduced glutathione contents (Table 2). Tukey's HSD *post hoc* test showed significant increase in the reduced glutathione content of liver, kidney and brain with *N*-acetyl cysteine treatment ($p \leq 0.05$) and thus confirming better antioxidant properties of *N*-acetyl cysteine over dithiothreitol. Table 3 reported the short term toxic exposure of mercury in the acetyl cholinesterase activity. Three days curative treatment with *N*-acetyl cysteine restored the enzymatic activity significantly, where as dithiothreitol was found to be less effective. Therapy with *N*-acetyl cysteine showed its better protective effect on brain function when compared with dithiothreitol treatment at 5% level by Tukey's HSD *post hoc* test. On the other hand, Table 3 also depicted the significantly increased activity of catalase in liver, kidney and brain after acute dimethylmercury exposure ($p \leq 0.05$). Both *N*-acetyl cysteine and dithiothreitol treated group significantly arrested the enzymatic activity but *N*-acetyl cysteine exhibited maximum protection equivalent to normal when compared to dithiothreitol *per se* group. Table 4 represents the effect of –SH compounds on the metal ion concentration and distribution of dimethylmercury in the various tissues. Three days post treatment with *N*-acetyl cysteine was significantly effective in removing mercury from liver, kidney and brain, where as dithiothreitol was significantly effective in kidney only ($p \leq 0.05$). Tukey's HSD *post hoc* test showed significant difference in metal chelating properties with *N*-acetyl cysteine and dithiothreitol therapies ($p \leq 0.05$).

A single dose of toxicant caused significant increase in the activity of serum enzymes as compared to normal control group. Raised activity of serum transaminases in intoxicated rats as found in present study can be attributed to the severe damaged structural integrity of the liver (Singh et al. 2007). The stabilization of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase level by thiol chelators, *N*-acetyl cysteine and dithiothreitol clearly indicated improvement in functional status of the liver cells, however *N*-acetyl cysteine showed better recovery. Significant rise in the lipid peroxidation is one of the primary effects induced by oxidative stress. It may also be correlated with the reduction in the antioxidative defense enzyme systems. Mercury has high affinity for glutathione (Ramanathan et al. 2003) so, metal-glutathione conjugation formed depletes the glutathione from the cell and thus decreasing antioxidant potential. One enzyme that is inhibited is choline acetyl transferase and esterase, which is involved in the final step of acetylcholine production and lead to acetylcholine deficiency. The loss of acetylcholine may be attributed to the signs and symptoms of motor dysfunctions. Catalase plays an important role in the protection against the deleterious effects of membrane peroxidation, *N*-acetyl cysteine detoxifies several toxic

Table 2 Potential of *N*-acetyl cysteine and dithiothreitol on lipid peroxidation and reduced glutathione against dimethylmercury intoxication

Treatment	Lipid peroxidation (n moles MDA/mg protein)			Reduced glutathione (μ mole/g)		
	Liver	Kidney	Brain	Liver	Kidney	Brain
Control	.27 ± 0.02	0.32 ± 0.02	0.30 ± 0.02	7.95 ± 0.43	7.12 ± 0.39	7.58 ± 0.41
DMM *per se*	1.31 ± 0.07$^{\#}$	2.90 ± 0.17$^{\#}$	1.96 ± 0.11$^{\#}$	2.79 ± 0.15$^{\#}$	2.45 ± 0.13$^{\#}$	2.76 ± 0.15$^{\#}$
DMM + NAC	1.14 ± 0.06	1.90 ± 0.10*	1.74 ± 0.09	5.32 ± 0.29*$^{,\Omega}$	5.28 ± 0.29*$^{,\Omega}$	5.39 ± 0.29*$^{,\Omega}$
% Protection	16.4%	41.9%	13.1%	49.0%	60.6%	54.5%
DMM + DTT	1.24 ± 0.07	2.00 ± 0.11*	1.81 ± 0.11	3.32 ± 0.18	3.80 ± 0.21*	3.08 ± 0.17
% Protection	6.55%	37.1%	8.90%	10.2%	29.0%	6.63%
F value	138$^{@}$	184$^{@}$	160$^{@}$	58.2$^{@}$	46.7$^{@}$	55.7$^{@}$
F value	138$^{@}$	184$^{@}$	160$^{@}$	58.2$^{@}$	46.7$^{@}$	55.7$^{@}$

Data are mean ± SE, $N = 6$; $^{\#}$ DMM *per se* vs. control at $p \leq 0.05$; * Treatment vs. DMM *per se* at $p \leq 0.05$

$^{\Omega}$ NAC vs. DTT for Tukey's HSD *post hoc* test ($p \leq 0.05$); $^{@}$ Significant at $p \leq 0.05$ for ANOVA

Abbreviations: *DMM* dimethylmercury, *NAC* *N*-acetyl cysteine, *DTT* dithiothreitol

Table 3 Potential of *N*-acetyl cysteine and dithiothreitol on acetyl cholinesterase and catalase activities against dimethylmercury intoxication

Treatments	Acetyl cholinesterase (μ mole/min/mg protein)			Catalase (μ mole/min/mg protein)		
	Fore brain	Mid brain	Hind brain	Liver	Kidney	Brain
Control	39.3 ± 2.17	20.1 ± 1.11	44.6 ± 2.46	54.5 ± 3.01	56.9 ± 3.14	57.4 ± 3.17
DMM *per se*	9.93 ± 0.55$^{\#}$	7.37 ± 0.41$^{\#}$	12.0 ± 0.66$^{\#}$	86.0 ± 4.75$^{\#}$	40.8 ± 2.26$^{\#}$	85.0 ± 4.69$^{\#}$
DMM + NAC	16.8 ± 0.93*$^{,\Omega}$	12.9 ± 0.71*$^{,\Omega}$	15.6 ± 0.86*$^{,\Omega}$	62.0 ± 3.42*	50.2 ± 2.77*	64.8 ± 3.58*
% Protection	23.6%	43.5%	10.8%	76.2%	57.9%	73.1%
DMM + DTT	14.0 ± 0.77*	9.25 ± 0.51*	13.0 ± 0.72	68.0 ± 3.76*	47.0 ± 2.59	76.0 ± 4.20
% Protection	13.9%	14.7%	2.98%	57.1%	38.5%	32.6%
F value	87.9$^{@}$	50.0$^{@}$	159$^{@}$	16.0$^{@}$	6.15$^{@}$	12.0$^{@}$

Data are mean ± SE, $N = 6$; $^{\#}$ DMM *per se* vs. control at $p \leq 0.05$; * Treatment vs. DMM *per se* at $p \leq 0.05$

$^{\Omega}$ NAC vs. DTT for Tukey's HSD *post hoc* test ($p \leq 0.05$); $^{@}$ Significant at $p \leq 0.05$ for ANOVA

Abbreviations: *DMM* dimethylmercury, *NAC N*-acetyl cysteine, *DTT* dithiothreitol

Table 4 Potential of *N*-acetyl cysteine and dithiothreitol on mercury concentration against dimethylmercury intoxication

Treatments	Mercury concentration(μg/g)		
	Liver	Kidney	Brain
Control	0.04 ± 0.002	0.02 ± 0.001	0.01 ± 0.001
DMM *per se*	41.0 ± 2.26$^{\#}$	41.5 ± 2.29$^{\#}$	29.0 ± 1.60$^{\#}$
DMM + NAC	21.5 ± 1.18*$^{,\Omega}$	26.0 ± 1.43*$^{,\Omega}$	20.0 ± 1.10*
% Protection	47.6%	37.3%	31.0%
DMM + DTT	35.0 ± 1.93	31.0 ± 1.71*	27.0 ± 1.49
% Protection	14.6%	25.3%	6.89%
F value	154$^{@}$	145$^{@}$	139$^{@}$

Data are mean ± SE, $N = 6$; $^{\#}$ DMM *per se* vs. control at $p \leq 0.05$; * Treatment vs. DMM *per se* at $p \leq 0.05$

$^{\Omega}$ NAC vs. DTT for Tukey's HSD *post hoc* test ($p \leq 0.05$); $^{@}$ Significant at $p \leq 0.05$ for ANOVA

Abbreviations: *DMM* dimethylmercury, *NAC N*-acetyl cysteine, *DTT* dithiothreitol

agents including the heavy metals such as mercury, lead and cadmium (Ketterer et al. 1983). It helps produce optimal amounts of glutathione, which also conjugates with most "foreign" compounds and excess oxidizers that enter cells (Pal and Chatterjee 2004). Organic mercury is distributed throughout the body accumulating in organs and blood. The present study showed the effect of chelators, *N*-acetyl cysteine/dithiothreitol on the metal distribution and mobilization of dimethylmercury in the tissues. Treatment with *N*-acetyl cysteine as compared to dithiothreitol was significantly effective in removing mercury from liver and kidney both.

In conclusion, mercury is one of the best-studied environmental toxicants, but there are still some gaps in understanding a number of global issues. Based on the above role and significance, in the present study it can be concluded that present research has identified the role of chelating agents in the mitigation of mercury toxicity. The *N*-acetyl cysteine has certain advantages over dithiothreitol. *N*-acetyl cysteine is a thiol containing compound. It is acetylated variant of the amino acid L-cysteine and is an excellent source of sulfhydryl (−SH) groups. It is a more stable form because it has an acetyl group but is more water soluble and bioavailable than L-cysteine. The significant recovery of *N*-acetyl cysteine have been ascribed to its ability to serve as an analogue of cysteine as well as a precursor of reduced glutathione, to enhance the activities of glutathione *S*-transferases, glutathione peroxidase, glutathione reductase, NADH and NADPH-quinone reductase, and probably, to promote DNA repair by protecting ADP ribosyltransferase activity. The key to *N*-acetyl cysteine's protection may be the sulfur and sulfhydryl groups contained in *N*-acetyl cysteine and its derivative, Glutathione. Both cysteine and methionine are good precursors of glutathione, but *N*-acetyl cysteine is better. L-cysteine loses approximately 85% of its sulfur group (which becomes the active part of glutathione) in the digestion process, while *N*-acetyl cysteine, a more stable compound, loses only 15%. This means that *N*-acetyl cysteine has almost six times more effective sulfur groups left after digestion. The

complex is more stable as it forms folded structure due to its two functional groups.

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