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# Melatonin Protects Against Mercury(II)-Induced Oxidative Tissue Damage in Rats

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*Abstract:* Mercury exerts a variety of toxic effects in the body. Lipid peroxidation, DNA damage and depletion of reduced glutathione by Hg(II) suggest an oxidative stress-like mechanism for Hg(II) toxicity. Melatonin, the main secretory product of the pineal gland, was recently found to be a potent free radical scavenger and antioxidant. N-Acetylcysteine, a precursor of reduced glutathione and an antioxidant, is used in the therapy of acute heavy metal poisoning. In this study the protective effects of melatonin in comparison to that of N-acetylcysteine against Hg-induced oxidative damage in the kidney, liver, lung and brain tissues were investigated. Wistar albino rats of either sex (200–250 g) were divided into six groups, each consisting of 8 animals. Rats were intraperitoneally injected with 1) 0.9% NaCl, control (C) group; 2) a single dose of 5 mg/kg mercuric chloride ($HgCl_2$), Hg group; 3) melatonin in a dose of 10 mg/kg, 1 hr after $HgCl_2$ injection, Hg-melatonin group; 4) melatonin in a dose of 10 mg/kg one day before and 1 hr after $HgCl_2$ injection, melatonin-Hg-melatonin group; 5) N-acetylcysteine in a dose of 150 mg/kg, 1 hr after $HgCl_2$ injection, Hg-N-acetylcysteine group, and 6) N-acetylcysteine in a dose of 150 mg/kg one day before and 1 hr after $HgCl_2$ injection, N-acetylcysteine-Hg-N-acetylcysteine group. Animals were killed by decapitation 24 hr after the injection of $HgCl_2$. Tissue samples were taken for determination of malondialdehyde, an end-product of lipid peroxidation; glutathione (GSH), a key antioxidant, and myeloperoxidase activity, an index of neutrophil infiltration. The results revealed that $HgCl_2$ induced oxidative tissue damage, as evidenced by increases in malondialdehyde levels. Myeloperoxidase activity was also increased, and GSH levels were decreased in the liver, kidney and the lungs. All of these effects were reversed by melatonin or N-acetylcysteine treatment. Since melatonin or N-acetylcysteine administration reversed these responses, it seems likely that melatonin or N-acetylcysteine can protect all these tissues against $HgCl_2$-induced oxidative damage.

Mercury is a widespread environmental and industrial pollutant, which induces severe alterations in the tissues of both animals and men (Stacey & Kappas 1982; Lund *et al.* 1993; Mahboob *et al* 2001). Various mechanisms, including lipid peroxidation have been proposed for the biological toxicity of mercuric chloride ($HgCl_2$), and it has been demonstrated that lipid peroxidation occurs in the kidney, liver and other tissues of the rats and mice following parenteral administration of $HgCl_2$ (Huang *et al.* 1996; Mahboob *et al.* 2001).

Stacey & Kappas (1982) reported induction of lipid peroxidation associated with Hg(II) treatment of isolated rat hepatocytes, and suggested a causative role of oxidative stress in mercury cytotoxicity. Woods et al. (1990) investigated the aetiology of mercury-induced porphyrinuria under *in vitro* conditions; their findings support the view that Hg(II) ions both compromise the antioxidant potential of GSH and promote formation of reactive species via thiol complexation. Acute oxidative stress response was also observed in cultured astrocytes treated with $HgCl_2$ (Brawer *et al.* 1998) or rat brain cell cultures treated with methyl mercury (Sorg *et al.* 1998). The cellular mechanisms by which mercury compounds exert their neurotoxic action were obtained from *in vitro* studies for which high concentrations were used in acute conditions. It has been proposed by Sorg *et al.* (1998) that the mechanism of toxicity of mercury could be via binding to thiol groups.

An imbalance in the antioxidant protective mechanisms leading to oxidative stress in the cells is being identified as a common factor in $HgCl_2$ exposure. A drastic decrease in the antioxidant enzymes catalase and glutathione peroxidase accompanies a drastic increase in reactive oxygen species production. This could be due either to a loss of the cells expressing these enzymes, to a direct effect of reactive oxygen species on the enzymes, or due to a direct inhibition from Hg(II) causing impairment of the antioxidant function and hence, increased reactive oxygen species production (Sorg *et al.* 1998). It has been reported that Hg(II) can inactivate a number of enzymes by blocking the functional sites through binding to sulfhydryl groups, which are part of catalytic or binding domains (Sanders *et al.* 1996). Thus, it was suggested that in addition to depletion of intracellular thiol pools, the oxidant pathway may be a primary mechanism of induction of the response for Hg to induce oxygene free radicals or promote formation of lipid peroxides. Hg(II) can also alter protein conformation by covalently binding to sulfhydryl groups, or creating protein adducts through modification of side chains leading to changes in protein shape and activity; such changes are known to be the result of the generation of free radicals by metals (Sanders *et al.* 1996). In fact, Sorg *et al.* (1998) reported

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that acute treatment with mercury induced the dramatic increase in reactive oxygen species accumulating in rat brain cell cultures, leading to lipid peroxidation, protein degradation, and finally to cell death. Hg(II) applied chronically at low concentrations also seems to induce reactive oxygen species release as well as inhibiting the enzymes that neutralise reactive oxygen species, but during chronic exposure reactive oxygen species seems to be neutralized by antioxidant defence mechanisms of the cell.

Melatonin, the main secretory product of the pineal gland, was recently found to be a potent free radical scavenger and antioxidant (Tan *et al.* 1993; Reiter *et al.* 1995). It scavenges hydrochlorous acid at a rate sufficient to protect catalase against inactivation by this molecule (Reiter *et al.* 2000a). It also detoxifies highly toxic hydroxyl radical and peroxyl radical *in vitro* (Gilad *et al.* 1997; Reiter *et al.* 2000c & 2001).

Melatonin has been demonstrated to protect against copper-mediated free radical damage in liver homogenates by binding copper ions as well as by protecting against free radical damage by its antioxidant properties (Parmar *et al.* 2002). It has also been demonstrated to have binding affinities for aluminium, cadmium, iron (III), lead and zinc (Limson *et al.* 1998). It was reported that melatonin protects against methyl mercury-induced death in mice (Kim *et al.* 2000) and to attenuate the acute renal failure and oxidative stress due to $HgCl_2$ in rats (Nava *et al.* 2000). In the latter study levels of malondialdehyde and glutathione (GSH), and activities of catalase and glutathione peroxidase (GSH peroxidase)were determined, and it was observer that the increment in renal content of MDA and decrease in GSH resulting from $HgCl_2$ toxicity were prevented, and the increment in creatinin clearance as well as renal tubular necrosis were decreased by melatonin pretreatment. Metabolism of GSH by GSH peroxidase requires $H_2O_2$ or other hydroperoxides, and as a result of this reaction, GSH is converted to oxidized glutathione. Within cells, the GSH:oxidized GSH ratio is normally greatly in favour of GSH, and to maintain this ratio, oxidized GSH is rapidly metabolized back to GSH by GSH peroxidase. It has been demonstrated that melatonin also increases the activity of the endogenous antioxidant enzyme, GSH peroxidase, which may be due to the effect of the hormone to remove hydrogen peroxide (Reiter *et al.* 2000b & 2001).

N-Acetylcysteine is a precursor of reduced glutathione and is being used and studied in conditions characterized by decreased GSH or oxidative stress. As a source of thiol groups, N-acetylcysteine can stimulate GSH synthesis, enhance glutathione S-transferase activity, promote detoxification, and act directly on reactive oxidant radicals (Kelly 1998). Evidence indicates that N-acetylcysteine is useful as a chelating agent in the therapy of acute heavy metal poisoning. *In vitro,* N-acetylcysteine effectively chelates gold, silver and mercury. It has been suggested that the ability of mercury to accumulate in the liver and kidneys might be inversely related to the supply of non-protein thiol groups, and N-acetylcysteine as a source of these groups, significantly reduces mercury content in and is protective against mercury-induced damage to these organs (Kelly 1998). Girardi & Elias (1993) demonstrated that the ability of Hg(II) to accumulate in the kidneys might be inversely related to the supply of non-protein sulfhydryl levels (mainly GSH) in this tissue, and that in N-acetylcysteine treated rats, there is less mercury accumulation in the kidneys and a higher clearance of $HgCl_2$ compared to controls. Thus, in the present study we wanted to compare the protective effects of melatonin to that of N-acetylcysteine against acute $HgCl_2$ toxicity in rats. For the purpose, malondialdehyde, an end product of lipid peroxidation; glutathione (GSH), a key antioxidant, and myeloperoxidase activity, an index of neutrophil infiltration were determined in various tissues of the rats. Since it was reported previously (Huang *et al.* 1996) that following subcutaneous injection of $HgCl_2$, MDA concentrations in the tissues (liver and kidney) peak at 24 hr, in the present study, animals were sacrificed at 24 hr. It was also reported that a near maximal burden of inorganic mercury is present in the total renal mass at this time after exposure (Zalups *et al.* 1999). Doses of melatonin and N-acetylcysteine are the doses used in other investigations (Şener *et al.*, 2001, 2002 & 2003).

## Materials and Methods

*Animals.* Rats were housed in a room at a constant temperature of $22\pm2\,^{\circ}$ with 12 hr light/dark cycles and fed standard pellet chow and water *ad libitum*. The study was approved by the Marmara University, School of Medicine, Animal Care and Use Committee.

Experimental design. Wistar albino rats of either sex (200–250 g) were divided into six groups, each consisting of 8 animals. Rats were intraperitoneally injected with 1) Control (C) group; 0.9% NaCl, 2) Hg group, a single dose of 5 mg/kg mercuric chloride ($HgCl_2$);

(This dosage has been determined from previous studies (Lund *et al.* 1993) as sufficient to elicit mild or moderate oxidative stress in kidney cells.) 3) Hg-melatonin group, melatonin in a dose of 10 mg/kg, 1 hr after $HgCl_2$ injection, 4) melatonin-Hg-melatonin group, melatonin in a dose of 10 mg/kg one day before and 1 hr after $HgCl_2$ injection, 5) Hg-N-acetylcysteine group, N-acetylcysteine in a dose of 150 mg/kg, 1 hr after $HgCl_2$ injection, and 6) N-acetylcysteine-Hg-N-acetylcysteine group, N-acetylcysteine in a dose of 150 mg/kg one day before and 1 hr after $HgCl_2$ injection. Animals were killed by decapitation 24 hr after the injection of $HgCl_2$. Since it was reported previously (Huang *et al.* 1996) that after subcutaneous injection of $HgCl_2$, malondialdehyde concentrations in the tissues (liver and kidney) peak at 24 hr, in the present study, animals were sacrificed at 24 hr. In groups 3 and 5, melatonin or N-acetylcysteine were administered 1 hr after the injection of $HgCl_2$ to determine if they can reverse the toxicity. On the other hand, in groups 4 and 6, melatonin and N-acetylcysteine were administered twice, both before and after $HgCl_2$ to determine if they have a preventive effect against toxicity when given before $HgCl_2$, and if their efficacy changes when administered twice.

*Biochemical analysis.* After decapitation, the kidney, liver, lung and brain tissues were immediately removed and stored at $-70\,^{\circ}$. Afterwards, malondialdehyde and GSH levels, as well as tissue-associated myeloperoxidase activity were measured in all wet tissue samples.

*Malondialdehyde and glutathione(GSH) assays.* Tissue samples were homogenized with ice-cold 150 mM KCl for determination of

malondialdehyde and GSH levels. The malondialdehyde levels were assayed for products of lipid peroxidation (Buege & Aust 1978). Briefly, 1 ml of thiobarbituric acid solution (0.375% w/v+15% trichloroacetic acid+0.25 N HCl) was added to 0.5 ml homogenate (10% w/v). It was boiled in a water bath for 15 min. at 100 °. After cooling, the tubes were centrifuged at 3000 rpm for 15 min. at room temperature. The absorbance of supernatant was read at 532 nm in spectrophotometry. As the standard, 1, 1, 3, 3, tetraethoxypropane was used. Results were expressed as ηmol malondialdehyde/g tissue.

Glutathione was determined by the spectrophotometric method, which was based on the use of Ellman's reagent (Beutler 1975). Briefly, 0.2 ml trichloroacetic acid was added to 0.4 ml homogenate (10% w/v). After centrifuging at 3000 rpm for 15 min., 0.2 ml supernatant was mixed 1 ml of 0.3 N $Na_2HPO_4$ and 50 µl of 5,5-dithiobis-(2-nitrobenzoate). The mixture was set to react for 5 min., and absorbance was read at 412 nm. Reduced glutathione was used as the standard. Results were expressed as µmol GSH/g tissue.

*Myeloperoxidase activity.* Myeloperoxidase activity was measured in tissues in a procedure similar to that documented by Hillegass *et al.* (1990). Tissue samples were homogenized in 50 mM potassium phosphate buffer (pH 6.0), and centrifuged at 41,400 ×*g* (10 min.); pellets were suspended in 50 mM phosphate buffer containing 0.5 % hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41,400 ×*g* for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM phosphate buffer, o-dianisidine, and 20 mM $H_2O_2$ solution. One unit of enzyme activity was defined as the amount of the myeloperoxidase present that caused a change in the absorbance measured at 460 nm for 3 min. myeloperoxidase activity was expressed as U/g tissue.

*Statistics.* Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). All data were expressed as means±S.E.M. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $P<0.05$ were regarded as significant.

## Results

After parenteral administration of mercuric chloride ($HgCl_2$), enhanced lipid peroxidation in the liver, kidney, lungs and the brain was observed.

All treatments protected the tissues against the increase in malondialdehyde levels significantly, and brought the levels back to the control values (fig. 1). However, protective effects of melatonin and N-acetylcysteine seemed to be similar and administration of melatonin or N-acetylcysteine twice, i.e. both before and after $HgCl_2$ treatment did not increase the protective effect significantly.

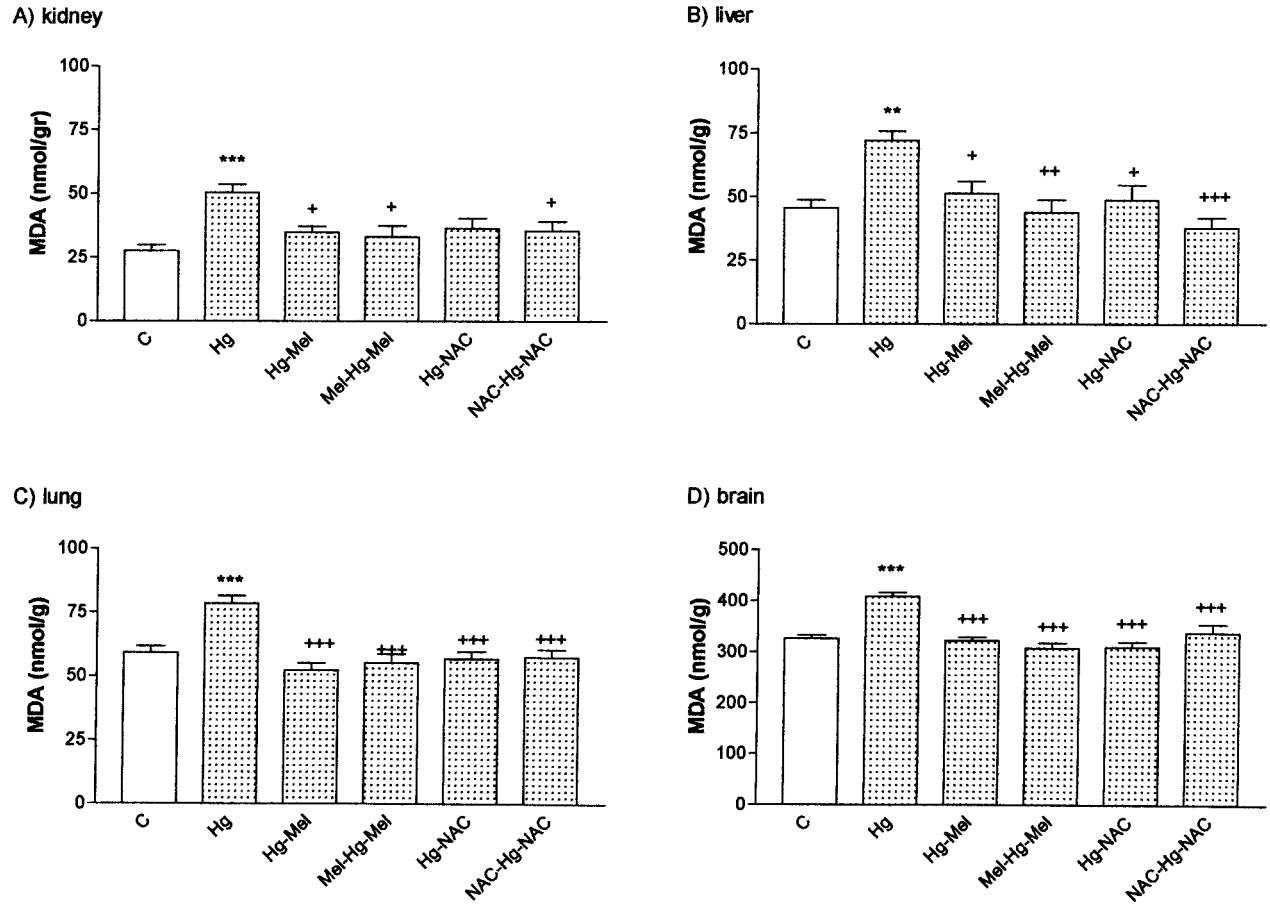


Fig. 1. Malondialdehyde (MDA) levels in A) kidney, B) liver, C) lung and D) brain tissues. C: Control, Hg: Mercuric chloride, Hg-Mel: Mercuric chloride+Melatonin, Mel-Hg-Mel: Melatonin+Mercuric chloride+Melatonin, Hg-NAC: Mercuric chloride+N-acetylcysteine and NAC-Hg-NAC: N-acetylcysteine + Mercuric chloride+N-acetylcysteine groups. ***: $P<0.001$ compared with C group. $^{+}$: $P<0.05$, $^{++}$: $P<0.01$, $^{+++}$: $P<0.001$ compared with Hg group.

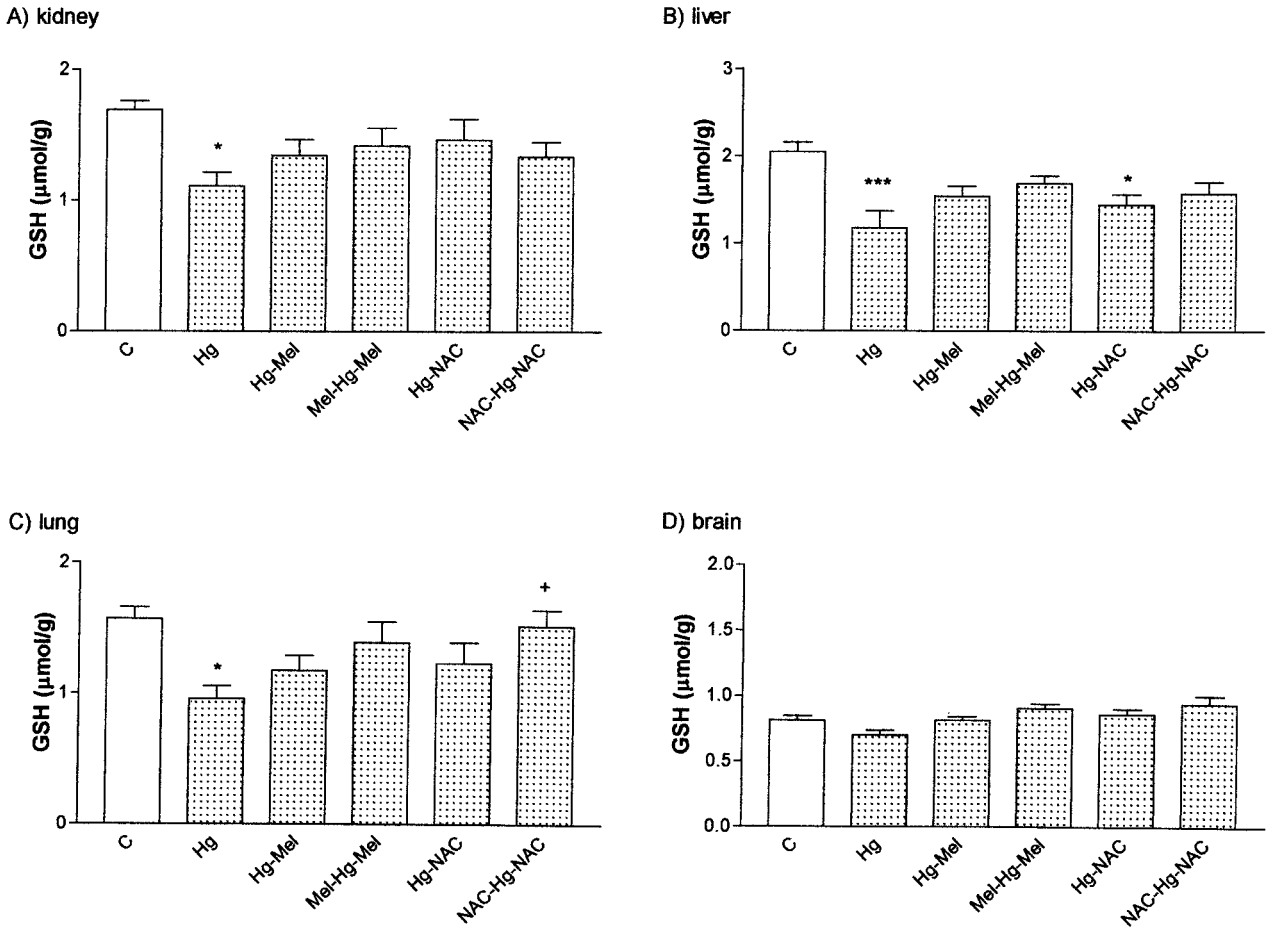


Fig. 2. Glutathione (GSH) levels in A) kidney, B) liver, C) lung and D) brain tissues. C: Control, Hg: Mercuric chloride, Hg-Mel: Mercuric chloride+Melatonin, Mel-Hg-Mel: Melatonin+Mercuric chloride+Melatonin, Hg-NAC: Mercuric chloride+N-acetylcysteine and NAC-Hg-NAC: N-acetylcysteine+Mercuric chloride+N-acetylcysteine groups. *: $P<0.05$, ***: $P<0.001$ compared with C group. $^{+}$: $P<0.05$ compared with Hg group.

$HgCl_2$ treatment decreased the GSH levels significantly in the liver, kidneys and lungs, and all types of treatments did increase the GSH levels in these tissues, however, the levels were not significantly different from either the control or the Hg(II) treated groups (fig. 2). Only in the liver after treatment with N-acetylcysteine (Hg-N-acetylcysteine group) were GSH levels still significantly lower than the control. In the lungs after N-acetylcysteine-Hg-N-acetylcysteine treatment, GSH levels were very close to the control and significantly higher than the Hg(II) group.

Myeloperoxidase levels were increased significantly after $HgCl_2$ treatment, demonstrating the neutrophil infiltration induced by $HgCl_2$ (fig. 3). All treatments decreased the myeloperoxidase levels significantly. In the kidneys, and the liver, only when N-acetylcysteine or melatonin were given both before and after $HgCl_2$, the myeloperoxidase levels went back to the control levels. In the lungs and the brain, all treatments seemed to offer the same degree of protection against neutrophil infiltration induced by $HgCl_2$, and the myeloperoxidase levels were almost back to the control levels.

## Discussion

In the present study, acute administration of $HgCl_2$ to rats was observed to cause toxic effects in the kidney, liver, and the lungs, and this damage was observed to be associated with increases in lipid peroxides, indicating oxidative tissue damage, as well as an increase in myeloperoxidase activity, and a significant reduction in glutathione levels. In fact, treatment of the animals with the antioxidants melatonin or N-acetylcysteine seemed to afford protection against this noxious stimulus.

Interpretation of the findings in the brain tissue was difficult; brain malondialdehyde and myeloperoxidase levels were increased after treatment with $HgCl_2$ while GSH levels were not changed significantly, although GSH levels are known to decrease in tissues under an oxidative damage. GSH levels of a tissue are an expressin of the compound in that tissue, whereas myeloperoxidase is an enzyme that is released from the neutrophils and myeloperoxidase is a product of oxidative reactions, and these compounds can be carried to other parts of the body by circulation. Since

$HgCl_2$ can not penetrate the blood-brain barrier, it may be assumed that the increases in myeloperoxidase and malondialdehyde levels in the brain do not necessarily demonstrate the oxidative damage in this tissue, but probably are an expression of changes in blood levels of these compounds during oxidative damage.

Mercury compounds have strong toxicity, and because of the high affinity of mercury to thiol groups, they are known to affect living organisms by damaging proteins and thiol enzymes. Divalent ions of transition metals, especially iron, mercury and copper can promote lipid peroxidation *in vitro* and much attention is currently focused on lipid peroxidation in the pathogenesis of metal toxicity. Lipid peroxidation is an autocatalytic free-radical process whereby polyunsaturated fatty acids in cell membranes undergo degradation by a chain reaction to yield lipid hydroperoxides which subsequently decompose to form a variety of products, including malondialdehyde.

Lipid peroxidation by Hg(II) has been demonstrated in the rat tissues, as well as in isolated rat hepatocytes, and it has been suggested that the cell membrane permeability may be affected by this process (Nava *et al.* 2000; Mahboob *et al.* 2001).

It has been demonstrated that the principal toxic effects of Hg(II) arise from alterations in the structural integrity of mitochondrial inner membrane, resulting in loss of the normal cation selectivity which permits it to participate effectively in oxidative metabolism (Lund *et al.* 1993). The mitochondrial electron transport chain is the principal site of cellular production of reactive oxidants, superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$). Action of Hg(II) to perturb mitochondrial inner membrane function results in depletion of mitochondrial reduced glutathione content and increased formation of $H_2O_2$ by the mitochondrial electron transport chain *in vitro* (Lund *et al.* 1991). It was suggested that such reactions occur *in vivo* after treatment with $HgCl_2$, and moreover, that increased $H_2O_2$ formation may be accompanied by increased peroxidation of mitochondrial lipids, consistent with an oxidative stress-like condition (Lund *et al.* 1993; Woods & Ellis 1995).

It has been stated that, because of the toxicokinetics of Hg(II), kidneys may be predisposed to the toxic effects of

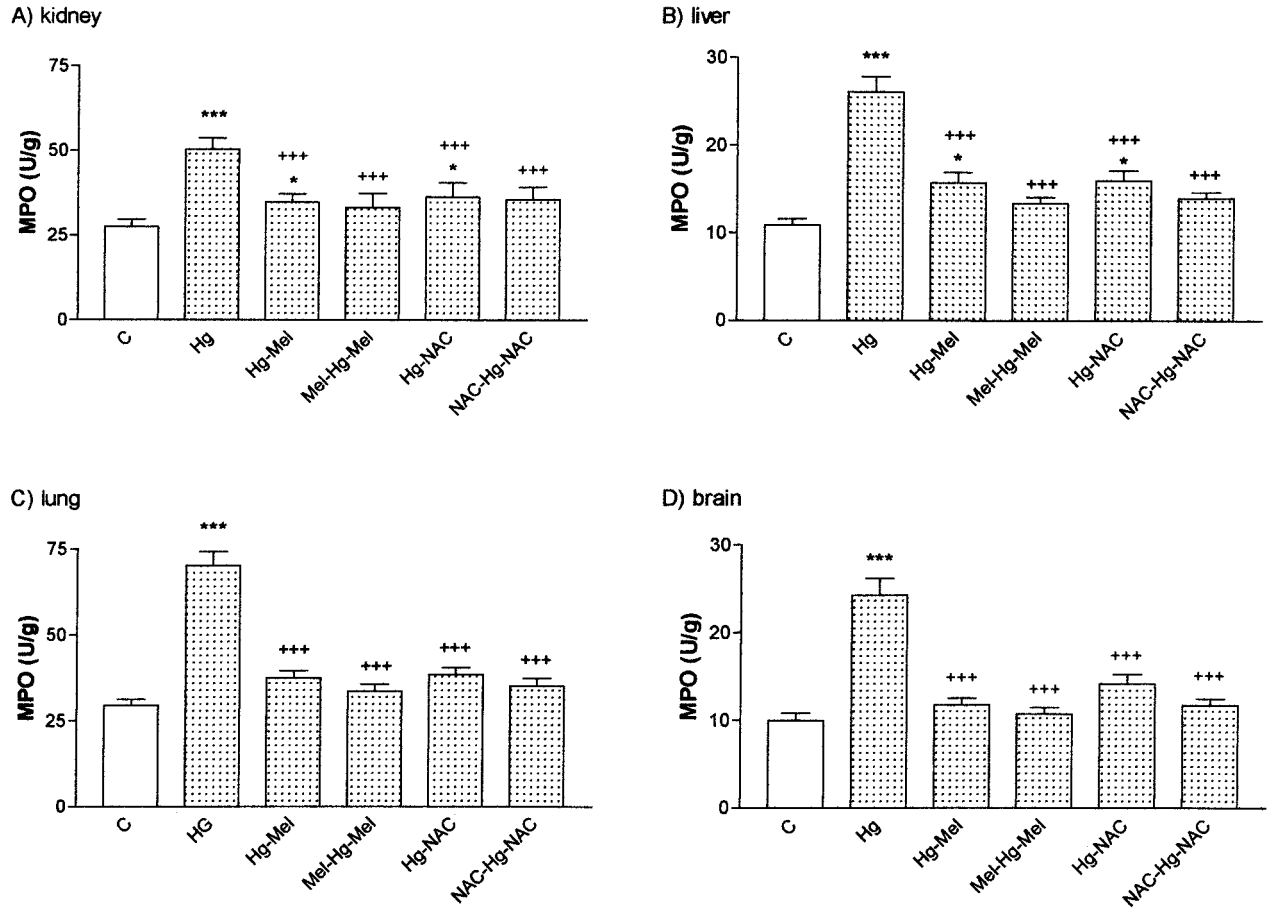


Fig. 3. Myeloperoxidase (MPO) activity in A) kidney, B) liver, C) lung and D) brain tissues. C: Control, Hg: Mercuric chloride, Hg-Mel: Mercuric chloride+Melatonin, Mel-Hg-Mel: Melatonin+Mercuric chloride+Melatonin, Hg-NAC: Mercuric chloride+N-acetylcysteine and NAC-Hg-NAC: N-acetylcysteine+Mercuric chloride+N-acetylcysteine groups. *: $P<0.05$, ***: $P<0.001$ compared with C group. $^{+++}$: $P<0.001$ compared with Hg group.

this metal more than the other tissues (Lund *et al.* 1993; Tandon *et al.* 2001). However, *in vitro* studies have also documented mercury-induced oxidative damage to mitochondria from hepatocytes, heart and other tissues, suggesting that mitochondria, regardless of cellular origin, respond similarly to Hg(II) (Lund *et al.* 1993), and findings of the present study support this view.

Lund *et al.* (1993) have suggested that Hg(II) administration selectively depletes mitochondrial GSH, and produces local toxicity by altering membrane permeability and decreasing the efficiency of oxidative phosphorylation. This makes mitochondria more susceptible to oxidative damage, especially during increased free radical production. Under conditions where GSH is experimentally depleted, concomitant restoration of mitochondrial respiration, oxidative phosphorylation, membrane permeability and GSH levels by melatonin and/or N-acetylcysteine was consistent with the importance of the mitochondrial GSH pool for the protection of the mitochondrial membrane against oxidative damage (Reiter *et al.* 1995, 2000a, 2000b; Kelly, 1998).

Findings of Sanders *et al.* (1996) provide further support for the hypothesis that Hg(II) toxicity is mediated by lipid peroxidation and protein oxidation (rather than covalent binding phenomenon). The administration of N-acetylcysteine was found to markedly protect the kidneys against Hg(II) toxicity, lipid and protein oxidation and the decline in tissue GSH concentration (Girardi & Elias 1991). Since N-acetylcysteine has been reported to prompt GSH synthesis in the mouse following acetaminophen challenge (Corcoran & Wong 1986), it would be reasonable to speculate that N-acetylcysteine in the present experiment protects against $HgCl_2$ by a similar mechanism.

Myeloperoxidase activity is used as an indirect evidence of neutrophil infiltration in oxidant–induced tissue injury. The present study demonstrates increased myeloperoxidase activity following Hg(II) treatment. Furthermore, melatonin and N-acetylcysteine reversed this effect. Reactive oxygen species can generate hydrochlorous acid in the presence of neutrophil-derived myeloperoxidase, and initiate the deactivation of antiproteases and activation of latent proteases, which lead to tissue damage (Kettle & Winterbourn 1997). In many diseases and acute inflammatory disorders, important components of the pathologic process are linked to the neutrophils' ability to release a complex assortment of agents that can destroy normal cells and dissolve connective tissue. Increasing evidence suggests that mesengial cells and neutrophils release chemotactic substances, which further promote neutrophil migration to tissues, activating neutrophils and increasing injury (Kettle & Winterbourn 1997; Reiter *et al.* 2000a). Thus, observations of the present study demonstrate that $HgCl_2$ induces tissue injury, both directly by promoting oxidative damage and by binding to tissue proteins, as well as indirectly by stimulating neutrophil infiltration.

Antioxidants have a protective effect against some tissue injuries in the pathogenesis of which lipid peroxidation may be involved. Melatonin has been shown to be protective against neurodegenerative disorders as well as the copper induced-lipid peroxidation in liver homogenates by binding cupric chloride (Cu(II)). Nava *et al.* (2000) have evaluated the effect of melatonin in the course of $HgCl_2$-induced acute renal failure, and observed that melatonin pretreatment decreased the renal toxicity of $HgCl_2$ and prevented induction of acute renal failure. They proposed that the beneficial effects of pharmacological doses of melatonin were due to its antioxidant properties.

Melatonin has been reported to exert a protective effect under certain pathological conditions such as damage caused by ionizing radiation or drug toxicity where a common feature is the existence of mitochondrial damage (Reiter *et al.* 1995; Karbownik & Reiter 2000). During ionizing radiation or drug toxicity, there is an increased production of superoxide ions during the synthesis of ATP via the mitochondrial respiratory chain, and the oxidative damage cannot be prevented by intra-mitochondrial antioxidant system. The mechanism of action of melatonin in mitochondria explains some of its protective effects on cell survival (Acuna-Castroviejo *et al.* 2001).

N-Acetylcysteine is a sulfhydryl group donor serving as a precursor of GSH synthesis and inhibiting formation of extracellular reactive oxygen intermediates (Kelly 1998; Dobrzynska *et al.* 2000). Moreover it may directly react with electrophilic compounds such as free radicals. Although GSH depletion may be insufficient to cause lipid peroxidation, it is agreed that it may favour the peroxidation produced by other factors. Since N-acetylcysteine metabolism is related to reduced GSH, at least part of the beneficial effects of N-acetylcysteine may be ascribed to the inhibition of lipoperoxidative processes. These therapeutic effects of N-acetylcysteine may be linked to its antioxidant effect and the free radical scavenging actions. The administration of N-acetylcysteine was found to markedly protect the rat kidneys against Hg(II) toxicity, lipid and protein oxidation and the decline in tissue GSH concentration (Girardi & Elias 1991) and it increased the renal clearence of $HgCl_2$ (Girardi & Elias 1993). Since N-acetylcysteine has been reported to prompt GSH synthesis in the mouse following acetaminophen challange (Corcoran & Wong 1986) it would be reasonable to speculate that N-acetylcysteine in the present experiment protects against $HgCl_2$ by a similar mechanism, as well as by increasing its elimination, and thus decreasing the renal accumulation of Hg(II).

However although GSH levels were increased by melatonin or N-acetylcysteine treatment, this increase was not significant in most cases (except N-acetylcysteine-Hg-N-acetylcysteine treatment in lungs). This can be ascribed to the utilisation of GSH by the oxidative reactions taking place in the tissue or it may have been bound by Hg(II).

In conclusion, the present study demonstrates that acute $HgCl_2$-induced toxicity involves oxidative damage in various tissues. In this study it was also demonstrated that melatonin and N-acetylcysteine, in the doses used, may afford the same degree of protection against acute $HgCl_2$ toxicity by reduction of free radical accumulation and preventing neutrophil infiltration, as well as by serving as a precursor

of GSH synthesis. It is most probable that N-acetylcysteine also decreased the binding of Hg(II) in the cells. GSH levels were increased by melatonin or N-acetylcysteine treatment, but this increase was not significant in most cases. This may be ascribed to the utilisation of GSH by the oxidative reactions taking place in tissues. Administration of the antioxidant (melatonin or N-acetylcysteine) both before and after $HgCl_2$ did not seem to afford additional protection in comparison to a single administration after $HgCl_2$ treatment.

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