

EFFECT OF METHIMAZOLE AND FISH OIL TREATMENT ON GENTAMICIN NEPHROTOXICITY IN RATS

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SUMMARY: Gentamicin, a nephrotoxic aminoglycoside antibiotic, was injected into adult male albino rats, alone or together with methimazole and fish oil. The effects on renal and liver functions and renal thiol status were studied. Gentamicin was administered as two i.p. injections (40 mg/kg body weight) for 3, 7 and 10 consecutive days. The animals were sacrificed 12 hours after the last injection. In gentamicin-treated rats, for 7 and 10 days, blood urea nitrogen (BUN) and serum creatinine concentrations and urinary N-acetyl-beta-D-glucosaminidase (NAG) activity were significantly increased compared with saline treated controls. Administration of methimazole (20 mg/kg) and fish oil (5 ml/kg) together with gentamicin partially protected against the nephrotoxicity induced by gentamicin by returning the urea and creatinine concentrations and urinary NAG activity to normal levels, despite having higher kidney gentamicin concentrations especially with methimazole. Rats given gentamicin alone for 3 days exhibited no elevation of BUN, serum creatinine and urinary NAG values, However, these rats exhibited an increase in nonprotein disulfide concentrations and a decrease in renal protein thiol and protein disulfide concentrations, as opposed to rats given gentamicin and methimazole and rats given gentamicin and fish oil. These results show that methimazole and fish oil were effective antagonists of gentamicin-induced nephrotoxicity. Methimazole did not inhibit gentamicin renal uptake but may protect against gentamicin-induced nephrotoxicity by acting as an antioxidant within the kidneys. On the other hand, fish oil may protect against gentamicin-induced nephrotoxicity by counteracting the biochemical alterations induced by the drug in the renal cortex.

We conclude the methimazole and fish oil may be compounds for reducing gentamicin-toxic side effects, including nephrotoxicity, without compromising its antibiotic activity.

Key Words: Methimazole, fish oil, gentamicin, nephrotoxicity.

INTRODUCTION

Various drugs and other useful chemicals are known to have the side effect of causing renal damage (5,18,20,40,44). In the case of drugs, this places unde-

sirable restrictions on the amount and frequency with which the drug can be used, and limits use of the drug to patients who can tolerate its side effects. In the case of nondrug chemicals (e.g. workplace chemicals), nephrotoxic effects may in an analogous manner, require that exposure to these chemicals be limited.

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Depending on the chemical, the mechanism of nephrotoxicity may involve direct interference with tubular or mitochondrial transport processes (44), covalent modification of critical cellular constituents or generation of free radicals (5,18). For the latter situations, cytotoxicity is usually observed only after cellular defenses particularly glutathione (GSH), the major non-protein thiol (NPT), are significantly depleted (37). Indeed, a large body of *in vivo* and *in vitro* evidence supports the concept that oxidative stress may play an important role in the pathophysiology of chemically induced acute renal failure. For example, cephaloridine, a nephrotoxic cephalosporin antibiotic (44), was shown to change the thiol status in the renal cortex before the development of significant morphological changes.

Gentamicin is an aminoglycoside antibiotic, which is commonly used in life-threatening gram negative bacterial infections. Nearly 10-25% of human patients treated with gentamicin exhibit increased blood urea nitrogen (BUN) concentrations subsequent to a reduction of glomerular filtration rate, which is closely associated with acute tubular necrosis (15,24). A number of biochemical alterations, including renal lysosomal phospholipidosis, alteration of mitochondrial function, alteration of glutathione disulfide (GSSG) concentrations, and inhibition of active transport of organic cations, have been observed before the changes in renal morphology or the increase in BUN concentrations (20,36,46), but it remains unclear how these events lead to cell necrosis.

Methimazole, a sulfur-containing drug commonly used to treat hyperthyroidism (34), was found to reduce free radical metabolites of prostaglandin H synthase (33), to inhibit hepatic and renal cysteine conjugates S-oxidase activities (39) and to protect against kidney damage induced in rats, mice, and dogs by cisplatin, an antitumor drug (6,40,45). Furthermore, methimazole protected rats against nephrotoxicity elicited by cephaloridine, S-(1.2- dichlorovinyl)-L-cysteine, 2-bromohydroquinone, bromobenzene and gentamicin (20,40). Because these chemicals are known to require

different mechanisms of activation, the methimazole mechanism of protection was suggested to be independent of the activation mechanisms of these chemicals (19). Further evidence for this hypothesis was provided by the findings that 1 h after cephaloridine treatment, rats given cephaloridine and methimazole had serum and kidney cephaloridine concentrations similar to those of rats given cephaloridine only, but the methimazole-pretreated rats were protected against cephaloridine-induced oxidation of renal nonprotein thiols (NPTS), an early event in cephaloridine induced toxicity (40). Because the rat kidney was shown to maintain the second highest percentage of the *in vivo* administered methimazole dose per gram of wet tissue and methimazole was also shown to be taken up by rat kidney cortical slices *in vitro* in a concentration-and-time-dependent manner (40,41), these results suggest that methimazole may protect against chemically induced renal damage by acting as an antioxidant within the kidneys.

Fish oil has been shown to protect against cyclosporine nephrotoxicity in rats (21) and in renal transplant recipients (22), and against proteinuria in passive Heymann nephritis (47). In addition, fish oil also has been reported to protect against acetaminophen (paracetamol)- induced hepatotoxicity (42), ethanol-induced gastric mucosal injury in rats (26) and in a number of inflammatory diseases (12).

Although several agents have been shown to modify gentamicin nephrotoxicity (23) there is still a need to search for safe, practical and effective agents for reducing or preventing gentamicin nephrotoxicity. Thus, in the present experiment, the effects of methimazole and fish oil treatment on gentamicin-induced nephrotoxicity, renal gentamicin concentration, and renal thiol status were discussed to determine whether methimazole and fish oil administration would reduce gentamicin-induced nephrotoxicity, and to possibly provide new insights into the mechanism of gentamicin-induced nephrotoxicity. The effects of methimazole and fish oil liver function was also studied by determining SGPT.

MATERIALS AND METHODS

Animals

Male adult albino rats (*Ratus norvegicus*) weighing 150-170 g were obtained from the experimental breeding station at Helwan, Egypt, and were maintained with free access to standard laboratory chow and tap water. They were housed singly in metabolic cages to permit collection of urine, at a room temperature of $23 \pm 2^\circ\text{C}$ and relative humidity of 55-65%. Rats were accustomed to metabolic cages 7 days before experimentation.

Chemicals

Gentamicin sulfate, glutathione (GSH), glutathione disulfide (GSSG), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), bovine serum albumin, Triton X-100 were purchased from Sigma Chemical Company (St. Louis, Mo.). Methimazole and NaBH_4 were purchased from Aldrich Chemical Company (Milwaukee, Wisc.). Fish oil was obtained from Sigma; Lot 93 HO 538 from Menhaden, containing 25-30% or ω -3 octadecate-traenoic, eicosapentaenoic and docosahexaenoic fatty acids as triglycerides.

Most other chemicals used were of the highest grade commercially available.

Treatment of Animals

In these experiments, gentamicin was administered twice daily for 3 or 7 or 10 days rather than by a single treatment to more closely mimic its use in the clinical setting. The gentamicin dose (40 mg/kg) for coadministration with methimazole or fish oil was chosen on the basis of nephrotoxicity results obtained with a wide range of gentamicin doses (20-180 mg/kg) (20). The methimazole dose (20 mg/kg) and fish oil dose (5 ml/kg) were selected on the basis of previous findings that these doses were effective in reducing cisplatin-, cephaloridine- and gentamicin-induced nephrotoxicity in rats (2,20,40). Rats were cotreated with gentamicin (40 mg/kg) and methimazole (20 mg/kg) dissolved in saline or given gentamicin, methimazole (20 mg/kg), or saline only, *i.p.*, twice daily at 07:00-08:00 and 19:00-20:00 for 3 or 7 or 10 days. In some experiments, animals were cotreated with gentamicin (40 mg/kg, *i.p.*) and fish oil (5 ml/kg, orally) or given fish oil only, twice daily for 3 or 7 or 10 days. All animals were killed 12 h after the last treatment. For all experiments, immediately after decapitation, blood samples were collected in centrifuge tubes and centrifuged at 3000 r.p.m for 15 min. The obtained serum and urine samples were frozen at -80°C pending analysis. Immediately after sacrifice, kidneys of rats also were excised and frozen until needed for analyses. BUN and serum

creatinine concentrations and serum glutamate-pyruvate transaminase activity, indicators of glomerular filtration rate and liver function respectively. To assess the renal tubular damage, urinary NAG levels were determined in rats.

Methods

BUN, serum creatinine and urinary NAG were measured because it was previously shown that it correlates with the extent of gentamicin-induced proximal tubular necrosis (20). Serum glutamate - pyruvate transaminase activity was determined on samples obtained from rats given methimazole or fish oil only to ascertain whether the methimazole or fish oil treatments are associated with hepatotoxicity since four cases liver damage in humans after chronic methimazole administration have been reported (29).

BUN, serum creatinine concentrations and serum glutamate-pyruvate transaminase activity were analyzed using reagent kits from Bio-Analytics Company (P.O. Box 388, Palm City, FL, U.S.A.). Total protein content was determined by the method of Lowry *et al.* (27). Urinary NAG concentrations were measured by MCP method (32).

Determination of Kidney Gentamicin Concentrations

A portion of the kidney was homogenized in 3 ml of buffer (0.1 M KH_2PO_4 , 0.1 M KCl, 5 mM EDTA, pH 7.4) per gram of tissue. Mixtures of 0.5 ml absolute ethanol and 0.5 ml kidney homogenate were stirred using a vortex mixer, and the protein was precipitated by centrifugation for 10 min in an Eppendorf centrifuge. Gentamicin concentrations in the supernatants were determined using the gentamicin fluorescence polarization immunoassay (Abbott Laboratories, North Chicago, 111).

Determination of Renal Nonprotein (NPT), Nonprotein Disulfides (NPT disulfide), Protein Thiols, and Protein Disulfide Concentrations

Renal tissue (1g) was homogenized in a 10-ml solution (pH 4.3) of KCl (0.15 mM) and EDTA (30 mM). To 2 ml homogenate, 3 ml of a solution containing NaCl (0.3 g/ml), metaphosphoric acid (0.017 g/ml), and EDTA (0.002 g/ml) was added, and the solution was centrifuged for 20 min at 3000 rpm. The supernatants were used for NPT and NPT disulfide determinations as previously described (40); in the determination of NPT disulfide, a modification involving the inclusion of 20 μl n-octanol/ml supernatant was used to decrease foaming after the addition of the NaBH_4 solution (10). Protein thiols were determined as glutathione (GSH) equivalents by a modified assay based on previously published methods (11,17). Briefly, the pellets obtained after the above-described centrifu-

Table 1: Effect of administration on methimazole and fish oil on glutamatepyruvate transaminase activity after 7 and 10 days in adult male albino rats.

Treatment	Enzyme (GPT) activity (U/ml)		
	Days of Sacrifice	7 days	10 days
Saline		10.50 ± 1.20	10.82 ± 1.15
Methimazole (20 mg/kg)		10.99 ± 1.38	11.21 ± 1.40
Fish oil (5ml/kg)		10.70 ± 1.22	10.65 ± 1.30

Values represent the mean S.D. for 5 rats.

gation step were washed twice by resuspension in 5 ml IM HClO_4 and centrifugation for 10 min at 3000 rpm. The supernatant was discarded, and the resulting pellet was resuspended in 9.9 ml Tris-HCl (0.5M) containing 5 M urea (pH 8.8 unadjusted) and 0.1 ml 10% Triton X-100. The resuspended pellet solution (0.5 ml) was added to tubes containing 2 ml Na_2HPO_4 , followed by the addition of 0.5 ml of 0.04% DTNB in 10% sodium citrate. The solution was stirred using a Vortex mixer, and the absorbance at 412 nm was determined immediately. Data were expressed as nanomoles protein, calculated on the basis of a GSH standard curve. For total protein thiol (thiols and disulfides) determinations, similar assays were performed with protein samples that were treated with sodium borohydride to reduce disulfide linkages (11,40).

Statistics

Data are given as means \pm SD. All appropriate data were analyzed by ANOVA, and when significant F values were obtained, the two groups were subsequently analyzed with student's t test. $P < 0.05$ was used as the criterion for significance.

RESULTS

Figure 1 shows BUN and serum creatinine concentrations and urinary NAG activity following injection of gentamicin (40 mg/kg), methimazole (20 mg/kg) and methimazole and gentamicin twice daily for 3, 7 and 10 days. BUN, serum creatinine and urinary NAG levels in rats treated with gentamicin or methimazole or gentamicin and methimazole twice daily for 3 consecutive days showed no significant changes when compared to the control saline animals. On the other hand, BUN and serum creatinine concentrations and urinary NAG

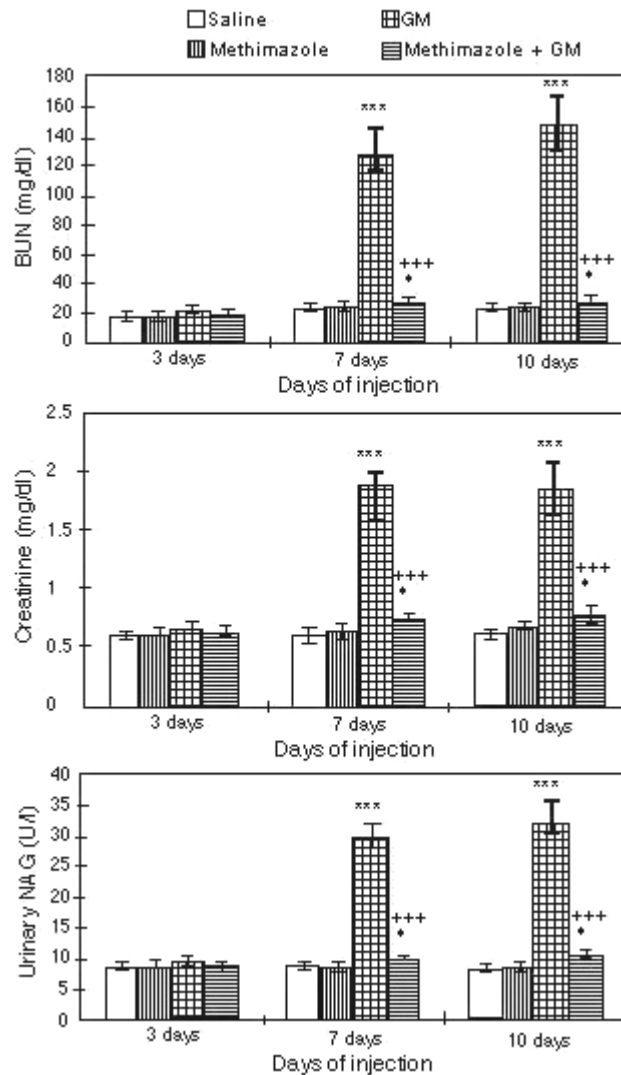
activity were significantly ($P < 0.001$) lower following administration of methimazole with gentamicin in rats for 7 and 10 days when compared with rats given gentamicin only.

As shown in Figure 2, the data of BUN, serum creatinine and urinary NAG concentrations in rats treated with gentamicin (40 mg/kg) or fish oil (5 ml/kg) or gentamicin and fish oil twice daily for 3 consecutive days reflect the occurrence of a non-significant ($P > 0.05$) changes as compared to the saline group. On the other hand, BUN, serum creatinine and urinary NAG levels on days 7 and 10 following injection of gentamicin or fish oil with gentamicin are shown also Figure 2. These parameters in rats given fish oil with gentamicin significantly ($P < 0.001$) decreased on days 7 and 10 compared with those in animals treated with gentamicin only.

To determine whether methimazole or fish oil treatments for 7 and 10 days causes toxicity, effects of methimazole or fish oil treatment on kidney and liver functions were evaluated. Rats given methimazole alone or fish oil alone did not exhibit alterations of BUN, serum creatinine and urinary NAG levels (Figures 1 and 2) or serum glutamate-pyruvate transaminase activities (Table 1).

Effects of methimazole or fish oil administration on renal gentamicin concentrations after gentamicin was administered at 40 mg/kg twice daily for 7 and 10 days were also investigated in an attempt to determine whether the methimazole or fish oil mechanism of pro-

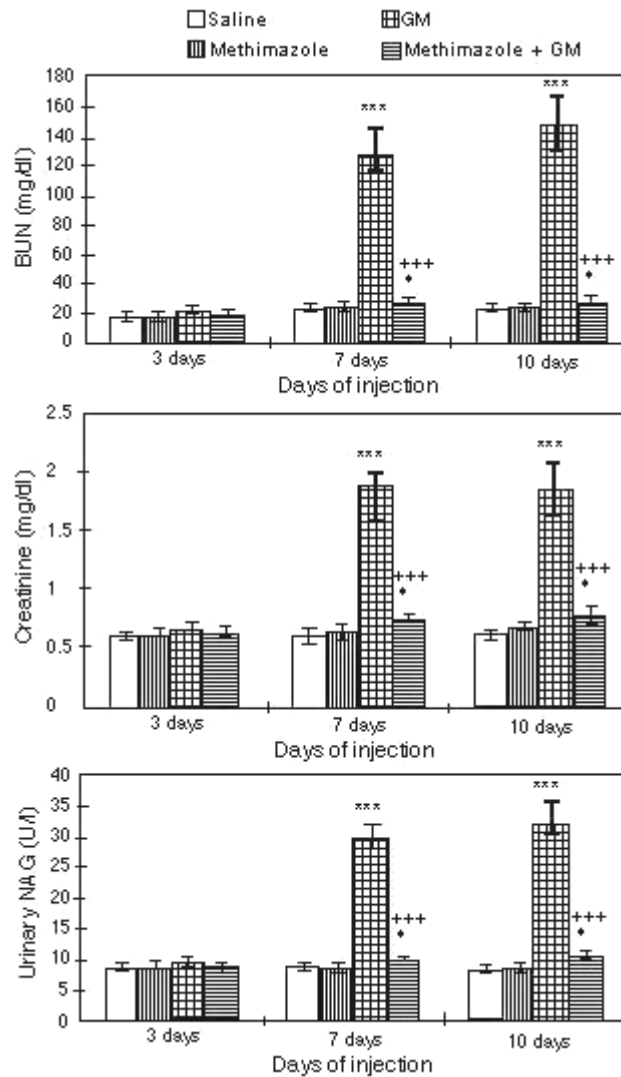
Figure 1: Effect of methimazole administration on GM-induced changes in BUN, creatinine and urinary NAG concentrations after 3, 7 and 10 days in adult male albino rats. Each bar represent mean \pm SD of 5 rats. * $p < 0.05$, *** $p < 0.001$ vs the saline-treated controls. +++ $p < 0.001$ vs the GM-treated rats.



tection may involve inhibition of gentamicin renal uptake by methimazole or fish oil. The data recorded in Table 2 shows that rats given methimazole, paradoxically exhibited higher gentamicin concentrations than rats given gentamicin only. On the other hand, a small but significant ($P < 0.05$) decrease were recorded in renal gentamicin concentrations in animals cotreated with gentamicin and fish oil compared with rats given gentamicin only.

The effects of the various treatments on renal thiol status after 3 days were conducted in an attempt to investigate its role in the mechanism of gentamicin-induced nephrotoxicity. The 3-day dosage schedule was selected because it was not associated with nephrotoxicity as assessed by determinations of BUN, serum creatinine and urinary NAG levels. Rats given gentamicin, gentamicin and methimazole, gentamicin and fish oil or methimazole or fish oil or saline only

Figure 2: Effect of fish oil administration on GM-induced changes in BUN, creatinine and urinary NAG concentrations after 3, 7 and 10 days in adult male albino rats. Each bar represents mean \pm SD of 5 rats. * $p < 0.05$, *** $p < 0.001$ vs the saline - treated controls +++ $p < 0.001$ vs the GM-treated rats.



twice daily for 3 consecutive days exhibited non-significant ($P > 0.05$) changes in BUN, serum creatinine and urinary NAG values (Figures 1 and 2). In this 3-day study, rats given gentamicin only, as opposed to rats given gentamicin and methimazole, gentamicin and fish oil or methimazole or fish oil only, exhibited significant elevations in renal NPT disulfide concentrations and reductions in both renal protein thiol and protein disulfide concentrations, compared with rats given saline only (Figure 3).

DISCUSSION

The effects of methimazole and fish oil treatment on gentamicin induced nephrotoxicity were studied using previously established protocols to induce nephrotoxicity (2,15,20). The nephrotoxic potential of gentamicin limits its clinical use, especially in patients with pre-existing renal dysfunction (15). The present data show that treatment of rats with gentamicin, twice daily for 7 or 10 consecutive days, produced the typical pattern of nephrotoxicity as shown by increases in BUN, serum

creatinine concentrations and urinary NAG activity. Conversely, rats given gentamicin for 3 days exhibited similar BUN, serum creatinine and urinary NAG values as compared to the saline control animals.

BUN and serum creatinine are very common parameters for the evaluation of renal function. However, increases in BUN or creatinine levels are considered to be indirect findings of renal dysfunction, because BUN and creatinine are waste products which are cleared from the blood into the urine, and therefore, a time lag is observed between the onset of renal impairment and the elevation of BUN or serum creatinine. Histological examination demonstrated that the kidneys of all rats given gentamicin exhibited severe and virtually complete necrosis of the proximal tubules throughout of the cortex with some extension into the outer stripe of the medulla (2,20).

Also urinary NAG is a well known parameter for early detection of renal toxicity induced by gentamicin. Previous studies indicated that the increase in this uri-

nary enzyme activity and in BUN and serum creatinine concentrations appeared following gentamicin administration in rats (2,20).

In contrast, the results of the present study indicate that cotreatment of rats with methimazole protected against the *in vivo* nephrotoxicity of gentamicin at 7 and 10 days, despite a paradoxical increase in renal accumulation of gentamicin. Rats given gentamicin and methimazole exhibited higher gentamicin concentrations in their kidneys than rats given gentamicin only, possibly because they were protected against gentamicin-induced renal damage, which may affect gentamicin uptake and (or) retention by the kidney. Similar protection effects of methimazole against gentamicin, cisplatin, cephaloridine, 2-bromohydroquinone- and S-(1,2-dichlorovinyl) -L-cysteine-induced nephrotoxicity has been observed in rats (20,40). The present observations which provide clear evidence that the methimazole mechanisms of protection does not involve inhibition of gentamicin uptake by the kidney, is similar

Table 2: Effect of methimazole and fish oil administration on renal gentamicin (GM) concentration after 7 and 10 days.

Treatment		GM (40 mg/kg)	GM + methimazole (200 mg/kg)	GM + fish oil (5 ml/kg)
No. of days	Gentamicin Concentration			
7 days	GM concentration ($\mu\text{g/g}$ tissue)	240.35 \pm 25.18	635.19 \pm 69.15***	200.91 \pm 20.69*
	GM concentration ($\mu\text{g/mg}$ protein)	2.75 \pm 0.25	8.17 \pm 1.02***	2.30 \pm 0.21*
10 days	GM concentration ($\mu\text{g/g}$ tissue)	272.18 \pm 31.22	720.21 \pm 80.12***	225.18 \pm 21.15*
	GM concentration ($\mu\text{g/mg}$ protein)	3.14 \pm 0.48	9.20 \pm 1.20***	2.58 \pm 0.30*

Values represent the mean \pm SD for 5 rats. * $p < 0.05$, *** $p < 0.001$ vs the GM-treated rats.

to this reported previously by Elfarra *et al.* (20) and are similar to the reported effects of poly-L-aspartic acid on gentamicin-induced nephrotoxicity and on gentamicin renal concentrations (7,35).

Our results indicate that fish oil at high doses has exerted protection against gentamicin nephrotoxicity. The mechanism(s) of this protective effect is not certain. However, fish oil may have antagonized the biochemical action of gentamicin on phospholipids in the proximal tubules. The antibiotic is known to cause a number of morphological, metabolic and functional alterations (23). Abdel Gayoum *et al.* (1) have reported that gentamicin significantly increased plasma triglycerides and decreased plasma phospholipid concentration. Fish oil, on the other hand, is known to be a major source of ω -3 fatty acids and alters membrane phospholipid fatty acid composition and eicosanoid production (4). It has been hypothesized that gentamicin may cause nephrotoxicity by altering the metabolism of the eicosanoid thromboxane A (38). Dietary fish oil alters the fatty acid composition in various organs including the kidney (3). Therefore it is possible that fish oil in gentamicin-treated rats for 7 or 10 days has counteracted the biochemical alterations induced by the drug in the renal cortex. Most of the protectant drugs decrease gentamicin concentration in renal cortex (23). Those results are in agreement with the present data, which indicate that rats given gentamicin and fish oil exhibited lower gentamicin concentrations in their kidneys than rats given gentamicin only (Table 2). Other mechanisms may also be involved in this protective effect of fish oil. The potential usefulness of fish oil in ameliorating gentamicin nephrotoxicity may be limited by the relatively high doses needed (2), which would probably allow acute treatments only.

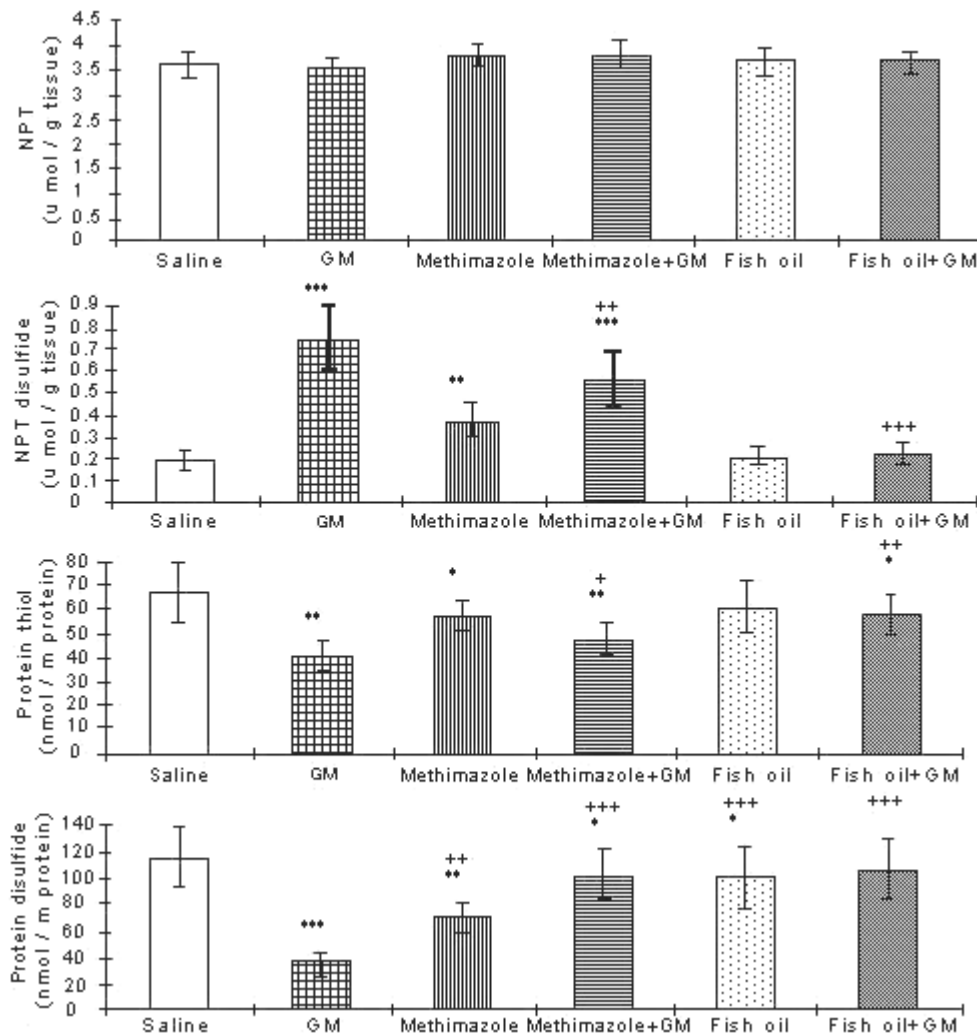
The methimazole and fish oil treatment did not cause significant changes in liver or kidney functions and morphology (2,20), whereas rats given poly-L-aspartic acid were reported to have a large number of apical vacuoles in their proximal tubules (7). Although the toxicological significance of these vacuoles is unknown, methimazole, a drug commonly used to treat

hyperthyroidism, and fish oil which is known to be useful clinically in preventing or ameliorating the tissue damage in a number of important diseases and conditions, may provide an alternative approach for the reduction of gentamicin-induced nephrotoxicity. Moreover, the present observations revealed that methimazole and fish oil doses needed to protect against nephrotoxicity did not exhibit liver or kidney damage (Figures 1 and 2, Table 1). When methimazole is used as an antithyroid drug in patients for 1-2 years, it is usually tolerated well and subsequent hypothyroidism after this long-term use is not observed (28). It has also been shown that rats do not exhibit hypothyroidism until at least 2-3 weeks after they have been on 0.025% methimazole in drinking water (8). Thus, the relatively long time needed for methimazole to cause antithyroid effects may allow the use of this drug to selectively block chemically induced nephrotoxicity with minimal or no inhibition of the thyroid function.

The methimazole mechanism of protection against gentamicin-induced nephrotoxicity is unlikely to be related to inhibition of thyroid hormone biosynthesis, since exogenous thyroxine treatment for 10 days prior to gentamicin administration protected against gentamicin-induced nephrotoxicity (14). Because hypothyroidism is commonly associated with a decrease in glomerular filtration rate, which can lead to an increase in BUN concentrations (9), the finding that rats given methimazole only did not exhibit changes in BUN, serum creatinine concentrations and urinary NAG activity (Figure 1) provides further support for the hypothesis that the methimazole mechanism of protection is not related to its inhibition of thyroid function.

Our results about the reductions in both protein thiols and protein disulfide concentrations in rats given gentamicin only, twice daily for 3 days, are similar to those of Elfarra *et al.* (20). Loss of protein thiols, a consequence of oxidative stress by many chemicals, was suggested as one of the critical factors leading to cell death after acetaminophen exposure (30,43). Our findings that rats given gentamicin only exhibited reductions in both protein thiol and protein disulfide

Figure 3: Effect of methimazole and fish oil administration on GM-induced changes in renal thiol status after 3 days in adult male albino rats. Each bar represent mean \pm SD of 5 rats. * p <0.05, ** p <0.01, *** p <0.001 vs the saline-treated controls. + p <0.05, ++ p <0.01, +++ p <0.001 vs the GM-treated rats.



concentrations before the development of cellular necrosis (20) suggests a role for irreversible protein thiol oxidation in the mechanism of gentamicin - induced nephrotoxicity. The data that methimazole protected rats against gentamicin-induced irreversible loss of renal protein thiols provides further evidence for this hypothesis.

It is therefore reasonable to suggest that gentamicin-induced irreversible loss of protein thiols may lead to renal tubular necrosis (20) and renal dysfunction as shown by increases in BUN and serum creatinine con-

centrations, and urinary NAG activity after 7 and 10 days of gentamicin injection in the present work. Irreversible peroxide-dependent oxidation of protein thiols with formation of products other than disulfides similar to that proposed here for gentamicin, has been reported (13).

Rats given methimazole or fish oil only have significant changes in the renal thiol status (Figure 3) (2,20,40). However, the small decline in protein thiol and protein disulfide concentrations observed in rats given methimazole only may have been a conse-

quence of a small portion of the methimazole dose being oxidized in the presence of NADPH in microsomes to yield reactive metabolites that modify protein thiols (16).

Further evidence for the formation of reactive oxygen species by gentamicin was obtained by the finding that renal nonprotein thiol (NPT) disulfide concentrations were elevated in rats given gentamicin for 3 days compared to saline-treated controls (Figure 3). Similarly, Wu *et al.* (48) and Elfarra *et al.* (20) reported an increase in the NPT status in the kidneys of rats given gentamicin in doses of 75 mg/kg and 40 mg/kg, respectively, twice daily for 3 days. Additionally, Ram-sammy *et al.* (36) established that 24 h after rats were given GM, 100 mg/kg per day for 4 days, exhibited an increase in renal glutathione disulfide (GSSG). In support of the hypothesis that alteration of the renal protein thiol status plays an important role in the mechanism of gentamicin-induced nephrotoxicity, *in vitro* gentamicin-dependent enhancement of H₂O₂ generation by renal cortical mitochondria was reported previously by Walker and Shah (46). In addition, Nakajima *et al.* (31) established that rats treated with hydroxyl radical scavengers or iron chelators were significantly protected against gentamicin-induced nephrotoxicity. Moreover, Kays *et al.*, (25) reported that high dietary iron potentiated gentamicin-induced nephrotoxicity.

The proposed role of irreversible oxidation of protein thiols in gentamicin-induced nephrotoxicity is also compatible with the previous findings that inhibition of GHS biosynthesis did not enhance gentamicin nephrotoxicity and that treatment of rats with diphenyl phenylenediamine, an antioxidant that blocked gentamicin-induced elevation in GSSG concentrations, exhibited not protective effects against gentamicin-induced nephrotoxicity (36,48). Collectively, the data suggest that GSSG formation is not a critical event for the development of gentamicin nephrotoxicity, but rather it may indicate the generation of reactive oxygen species that predominantly interact with renal protein thiols leading to irreversible oxidation of renal protein thiols rather than GSSG formation.

In summary, the present results provide evidence that methimazole and fish oil treatments protected against gentamicin-induced nephrotoxicity. The methimazole mechanism of protection is not due to inhibition of gentamicin uptake by the kidneys, since the presence of higher gentamicin concentrations in the kidneys of rats given gentamicin and methimazole compared with rats given gentamicin only were recorded, but rather may involve the antioxidant properties of methimazole and the maintenance of the NPT status within the kidneys (20,40). On the other hand, as fish oil is composed, it is possible that its protective actions may be due to one or more of these compounds. Also its possible that fish oil in gentamicin-treated rats has counteracted the biochemical alterations induced by the drug in the renal cortex.

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