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ORIGINAL ARTICLE

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Vitamin C may be beneficial in the prevention of paracetamol-induced renal damage

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Abstract
Background. There is no specific treatment for paracetamol-induced renal damage. Vitamin C is an outstanding chain-breaking antioxidant and a free radical scavenger. The present study was undertaken to determine whether large doses of vitamin C are useful in the treatment of paracetamol-induced renal damage.
Methods. Renal injury was induced in rats by the administration of 1 g/kg body weight paracetamol intraperitoneally. Some rats received intraperitoneal injections of vitamin C (250, 500, or 1000 mg/kg body wt) at 1.5 h, 6 h, 9 h, or 16 h after the administration of paracetamol, and the rats were killed 24 h after the administration of paracetamol.
Results. Renal injury was accompanied by a decrease in nonprotein thiol and protein thiol in the kidneys of paracetamol-treated rats. The administration of vitamin C to the paracetamol-treated rats prevented renal damage either completely or partially. Lower doses of vitamin C were beneficial in the prevention of paracetamol-induced renal injury when administered early and higher doses were beneficial when administered later. In the paracetamol-treated rats that responded to vitamin C, renal nonprotein thiol level and protein thiol were restored almost completely. Interestingly, a highly significant inverse correlation was obtained between renal nonprotein thiol level and plasma creatinine.
Conclusions. Megadoses of vitamin C may be beneficial in the treatment of paracetamol-induced renal damage. The mechanism of protection by vitamin C appears to be the regeneration of nonprotein thiol.

Key words Paracetamol toxicity · Renal damage · Oxidative stress · Vitamin C

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Introduction

Paracetamol (acetaminophen, APAP) is a widely used over-the–counter analgesic and antipyretic drug that is safe at therapeutic dosages. Even though paracetamol is considered a safe drug, in overdose situations (such as accidental or suicidal ingestion) it produces hepatic necrosis and renal failure in both humans[1–3] and experimental animals.[4,5] Renal tubular damage and acute renal failure can occur even in the absence of liver injury.[3,6,7] The characteristic features of paracetamol-induced renal damage are acute tubular necrosis, increase in plasma creatinine levels, and decrease in glomerular filtration rate. Oxidative stress is reported to play a role in the pathogenesis of paracetamol-induced renal damage, as evidenced by increase in lipid peroxidation[8,9] and depletion of nonprotein thiol, about 95% of which is glutathione.[10,11] *N*-Acetyl–cysteine, a glutathione precursor, protects against APAP hepatotoxicity in humans,[12,13] but it is unable to protect against APAP nephrotoxicity.[14,15] Because APAP can induce life-threatening renal lesions, the search for antidotes or treatments for APAP nephrotoxicity is of clear toxicological importance.

Our defenses against oxidative stress are the antioxidants synthesized in the body (catalase, glutathione peroxidase) and antioxidant vitamins (vitamin C, vitamin E, and carotenoids). The levels of antioxidants synthesized in the body cannot be manipulated by simple means. On the other hand, the levels of antioxidant vitamins can be increased easily by dietary means or supplementation.

Vitamin C is known to act as an antioxidant both in vitro and in vivo.[16–20] Vitamin C functions as a chain-breaking antioxidant,[21] a free radical scavenger,[22] and is involved in the recycling of vitamin E and glutathione.[23,24] Thus far, there are no studies on the effect of vitamin C in the prevention of paracetamol-induced renal damage, to the best of my knowledge. The aim of the present study was to determine the effect of different doses of vitamin C (administered at different time intervals after the administration of paracetamol) in the prevention of paracetamol-induced renal damage.

The dose of 1 g per kilogram body weight of paracetamol was chosen based on the study by Trumpher et al.[25] The effect of administration of different doses of vitamin C (250, 500, or 1000 mg/kg body weight) at different time intervals (1.5, 6, 9, or 16 h) after the administration of paracetamol on the prevention of renal injury was studied in rats.

Materials and methods

Chemicals

Paracetamol was obtained from Vambi Laboratories, Maharashtra, India. Bis-(3-Carboxy-4-nitrophenyl) disulfide, HEPES buffer, 1,1,3,3-tetramethoxy propane, and glutathione were obtained from Sigma Chemical, St. Louis, MO, USA. All other chemicals were of analytical grade.

Animals

Adult male Wistar rats weighing 200–250 g were used for the study. The rats were housed in galvanized iron cages and maintained on standard laboratory chow (Lipton India Limited) with free access to water in a thermostatically controlled room under 12-h dark/light cycle.

Animal treatment

Some rats received paracetamol (1 g/kg body wt) intraperitoneally dissolved in normal saline; some received paracetamol and vitamin C at different doses (250, 500, or 1000 mg/kg body wt orally) at various time intervals (1.5, 6, 9, or 16 h) after the administration of paracetamol. Saline-treated animals served as normal controls. To the vitamin C controls was administered the corresponding dose of vitamin C.

The rats were killed 24 h after the administration of paracetamol/vehicle, after withdrawal of blood under light ether anaesthesia. The kidneys were removed and used for biochemical assays as well as for histopathological examination. Plasma was separated and used for biochemical assays.

Biochemical studies

Assay of lipid peroxide in the kidney

Approximately 0.5 g of kidney was homogenized in 4.5 ml ice-cold 1.15% KCl. Lipid peroxide was measured in the whole homogenate by thiobarbituric reaction.[26] To 0.1 ml kidney homogenate were added 0.2 ml 8.1% sodium dodecyl sulfate, 1.5 ml 20% acetic acid adjusted to pH 3.5, and 1.5 ml 0.8% aqueous solution of thiobarbituric acid. The mixture was made up to 4 ml with distilled water and heated at 95°C for 60 min using a glass ball as condenser. After cooling with tap water, 1 ml distilled water and 5 ml *n*-butanol and pyridine mixture (15:1) were added and the solution was shaken vigorously. After centrifugation at 2000 *g* for 10 minutes the absorbency of the organic layer was measured at 532 nm.

Assay of protein thiol in the kidney supernatants

About 0.5 g kidney was minced and homogenized using a Potter-Elvehjem homogenizer in 4.5 ml ice-cold 10 mmol/l HEPES buffer, pH 7.4. The homogenate was centrifuged at 16000 *g* at 4°C. The supernatant was used for the assay of protein thiol by the reaction of protein thiol with bis-(3-carboxy-4-nitrophenyl) disulfide.[27] Protein content of supernatant was determined by the method described by Lowry et al.[28]

Nonprotein thiol determination in the kidney

Kidney was homogenized in 4.5 volumes of ice-cold 0.1 M phosphate buffer, pH 7.4. Nonprotein thiol was determined according to the method of Ellman.[27] The protein in an aliquot of the kidney homogenate was precipitated by adding an equal volume of 4% sulfosalicylic acid. After centrifugation, 0.5 ml supernatant was added to 4.5 ml bis-(3-carboxy-4-nitrophenyl) disulfide in 0.1 M phosphate buffer, pH 8.0). Nonprotein thiol was proportional to the absorbancy at 412 nm.

Plasma nonprotein thiol

Plasma nonprotein thiol was determined by the method described by Sedlak and Lindsay.[29] Briefly, proteins were removed by the addition of 21 µl 50% trichloroacetic acid (TCA) to 400 µl plasma. The samples were centrifuged at 12000 rpm for 10 min. Then, 50 µl obtained TCA extract and 100 µl 6 mmol/l dithionitrobenzene (DTNB) (Ellman's reagent) were added successively to 850 µl 0.2 mmol/l phosphate buffer, pH 8.2, and after 1 h the absorbance was measured at 412 nm. The results were read from a standard curve prepared from 1 mmol/l solution of reduced glutathione.

Estimation of plasma creatinine and urea

Creatinine and urea in plasma were estimated by the spectrophotometric method.[30,31]

Histological studies

Slices of kidney tissue were fixed in buffered formalin, processed, and stained with hematoxylin and eosin stain for histological assessment.

Statistical analysis

The data represent mean value ± SD. Means of five groups were compared by analysis of variance.[32] Student's *t* test with Bonferroni correction was used to compare individual means in the case of a significant *F* in analysis of variance. The level of significance was set at $P < 0.05$. For correlation studies, Pearson rank correlation was used.

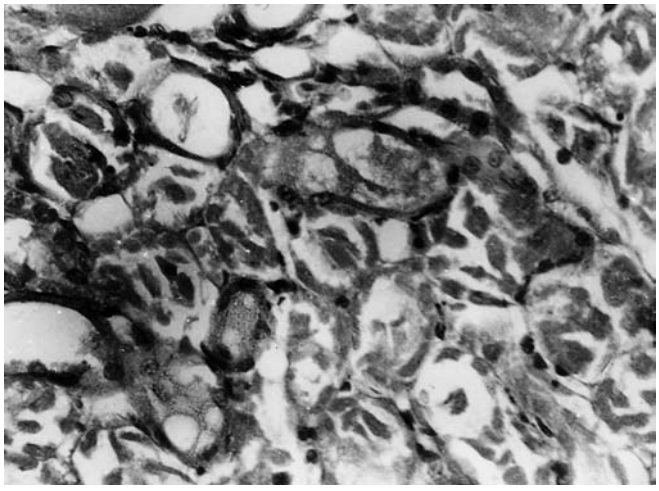

Fig. 1. Kidney of a paracetamol-treated rat showing extensive tubular necrosis. H & E. ×40

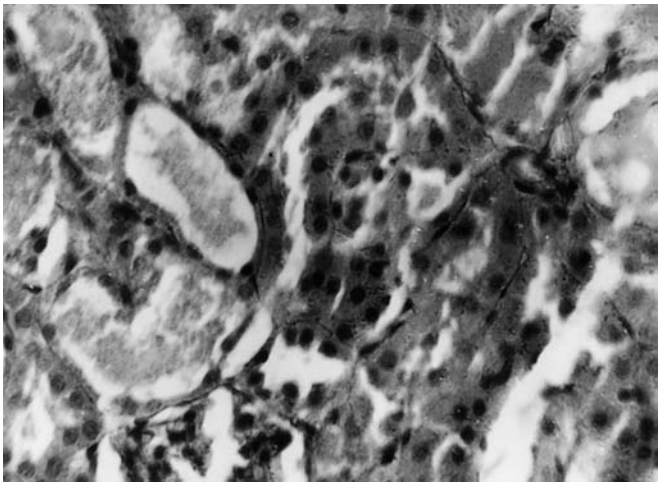

Fig. 3. Kidney of a paracetamol + vitamin C (250 mg/kg body weight)-treated rat showing mild tubular necrosis. H & E. ×40

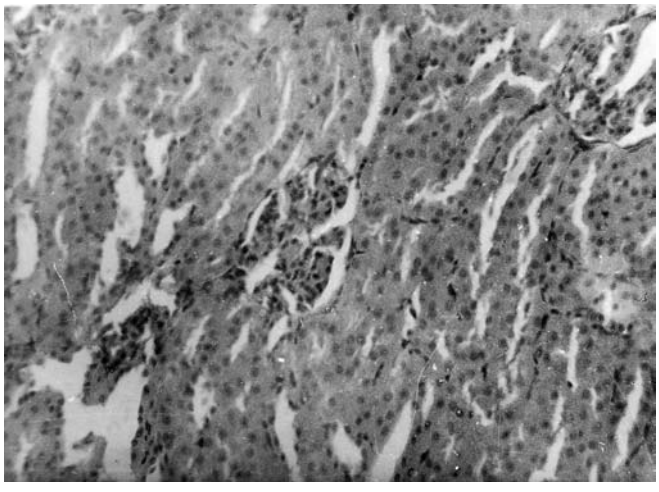

Fig. 2. Kidney of a paracetamol + vitamin C (250 mg/kg body weight)-treated rat showing normal architecture. H & E. ×20

Results

Histopathology

All the paracetamol-treated rats showed evidence of renal damage, as indicated by the presence of interstitial congestion and massive proximal tubular necrosis (Fig. 1). The administration of 250 mg vitamin C 1.5 h after the administration of paracetamol completely prevented renal damage in 60% of the rats (Fig. 2), and in the other 40% the renal damage was reduced (mild tubular necrosis) as compared with the paracetamol-treated rats (Fig. 3). However, the administration of 250 mg vitamin C 6 h after the administration of paracetamol prevented renal damage in only 20% of the rats.

Because 250 mg/kg vitamin C prevented renal damage in the rats at 1.5 h, it was assumed that 500 mg/kg would prevent renal damage at this time period; therefore, the later time period was chosen for studies using 500 and 1000 mg/kg. The administration of 500 mg/kg vitamin C, 6 h after the administration of APAP completely prevented renal damage in 50% of the rats; in the other 50%, the renal damage was reduced (mild tubular necrosis) as compared with the APAP-treated rats. However, the administration of 500 mg/kg vitamin C 16 h after the administration of APAP did not prevent renal damage in any of the APAP-treated rats.

The administration of 1000 mg/kg vitamin C 9 h after the administration of APAP completely prevented renal damage in 25% of the rats; in the other 75%, the renal damage was reduced (mild tubular necrosis) as compared with the APAP-treated rats. However, the administration of 1000 mg vitamin C 16 h after the administration of paracetamol did not prevent the renal damage induced by paracetamol.

The kidneys of all the vitamin C-treated rats and the saline control rats showed normal architecture (Figs. 4 and 5, respectively).

Effect of paracetamol on mortality and renal injury

Only 60% of the paracetamol-treated rats survived the treatment period. On the other hand, all the paracetamol/vitamin C-treated rats survived. Plasma creatinine and urea levels were elevated in all the paracetamol-treated rats.

Effect of 250 mg/kg body wt of vitamin C on various biochemical parameters in paracetamol-treated rats

In 60% of rats treated with 250 mg/kg vitamin C 1.5 h after paracetamol administration, the plasma creatinine values were normal, and in the other 40%, the values were less as compared with the paracetamol-treated rats (Table 1). The administration of 250 mg/kg body wt vitamin C 6 h after the

Table 1. Effect of 250 mg vitamin C on plasma creatinine, nonprotein thiol, and urea and nonprotein thiol, protein thiol, and lipid peroxide level in kidney of paracetamol-treated rats and control rats

Parameters	Saline control	Vitamin C control	APAP	APAP + vitamin C (1.5 h)	APAP + vitamin C (6 h)
Plasma creatinine (mg %)	0.61 ± 0.22	0.65 ± 0.02	1.69 ± 0.47[a]	1.07 ± 0.47[b]	1.34 ± 0.64
Plasma urea (mg %)	44.6 ± 9.20	37.8 ± 8.40	154.3 ± 34.5[a]	–	105.9 ± 70.40
Renal nonprotein thiol (μmol/g)	1.03 ± 0.11	0.97 ± 0.16	0.57 ± 0.08[a]	0.99 ± 0.06[b]	0.68 ± 0.10
Renal protein thiol (nmol/mg protein)	107.5 ± 5.60	102.6 ± 7.20	62.9 ± 6.20[a]	99.8 ± 8.90[b]	57.9 ± 4.30
Renal lipid peroxide level (nmol/g)	143.04 ± 16.90	122.20 ± 7.50	209.37 ± 38.80[a]	195.40 ± 35.60	136.4 ± 23.00[b]
Plasma nonprotein thiol (μmol/100 ml)	3.85 ± 0.30	3.10 ± 0.21	2.35 ± 0.37[a]	3.40 ± 1.21[b]	4.02 ± 0.64[b]

Data represent mean ± SD of 5–7 rats
APAP, paracetamol (acetaminophen)
[a] $P < 0.05$ compared with control; [b] $P < 0.05$ compared with APAP

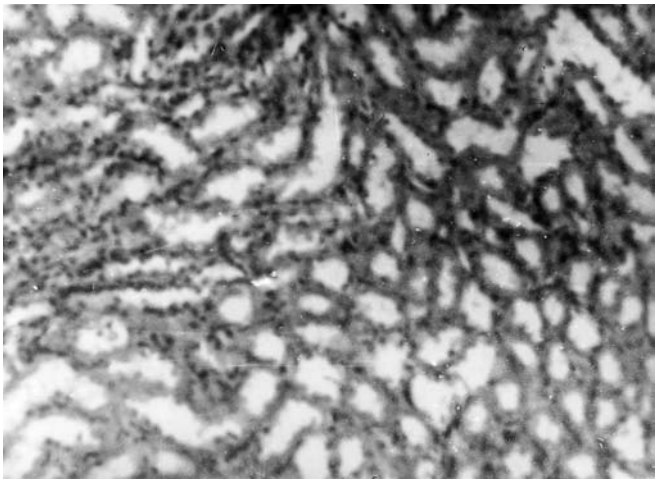

Fig. 4. Kidney of vitamin C-treated rat showing normal architecture. H & E. ×20

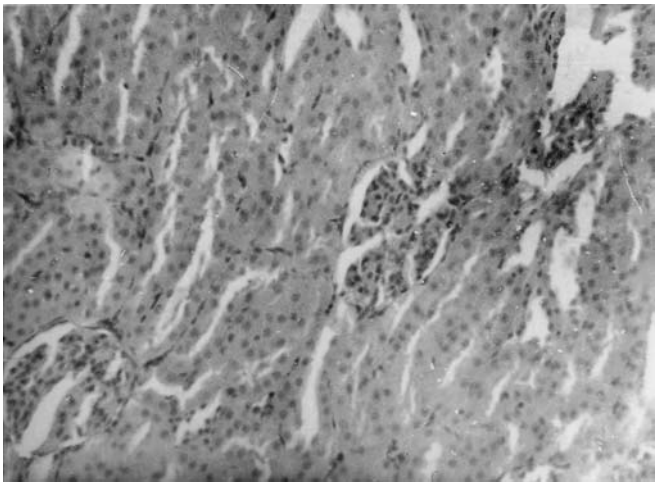

Fig. 5. Kidney of saline control rat showing normal architecture. H & E. ×20

administration of paracetamol prevented a rise in plasma creatinine values in 20% of the rats; however, in the other 80%, the plasma creatinine values were similar to the paracetamol-treated rats. Similar results were obtained for plasma urea. Renal nonprotein thiol was reduced by 43% in the paracetamol-treated rats, and the administration of 250 mg/kg body wt of vitamin C 1.5 h after the administration of paracetamol completely prevented the loss of nonprotein thiol. However, the administration of this dose of vitamin C 6 h after the administration of paracetamol did not prevent the loss of nonprotein thiol induced by paracetamol significantly. Administration of 250 mg vitamin C 1.5 h after administration of paracetamol did not prevent the increase in lipid peroxidation induced by paracetamol; however, administration of the vitamin 6 h later reduced lipid peroxidation in the kidney significantly. The administration of this dose of vitamin C at 1.5 h almost completely prevented the decrease in protein thiol induced by paracetamol; however, the administration of vitamin C at a later time (6 h) did not prevent the decrease in protein thiol induced by paracetamol. Administration of vitamin C at 1.5 or 6 h almost completely prevented the decrease in plasma nonprotein thiol induced by paracetamol.

Effect of 500 mg/kg body wt of vitamin C on various biochemical parameters in paracetamol-treated rats

In 50% of rats treated with 500 mg/kg vitamin C 6 h after APAP administration, the plasma creatinine values were normal, and in the other 50%, the values were less as compared with the APAP-treated rats (Table 2). The administration of 500 mg/kg body wt vitamin C 16 h after the administration of APAP did not prevent the rise in plasma creatinine values in any of the rats. Similar results were obtained for plasma urea. Administration of 500 mg/kg body wt of vitamin C 6 h after the administration of APAP almost completely prevented the loss of renal nonprotein thiol induced by APAP. However, administration of this dose of vitamin C 16 h after administration of APAP did not prevent the loss of nonprotein thiol induced by APAP.

Table 2. Effect of 500 mg vitamin C on plasma creatinine, nonprotein thiol, and urea and nonprotein thiol, protein thiol, and lipid peroxide level in kidney of paracetamol-treated rats and control rats

Parameters	Saline control	Vitamin C control	APAP	APAP + vitamin C (6 h)	APAP + vitamin C (16 h)
Plasma creatinine (mg %)	0.55 ± 0.25	0.53 ± 0.15	1.42 ± 0.52[a]	0.80 ± 0.24[b]	1.20 ± 0.19
Plasma urea (mg %)	44.2 ± 9.00	38.7 ± 9.20	174.3 ± 40.2[a]	104.2 ± 54.20	201.9 ± 60.50
Renal nonprotein thiol (μmol/gm)	0.99 ± 0.08	0.91 ± 0.14	0.57 ± 0.10[a]	0.89 ± 0.21[b]	0.55 ± 0.11
Renal protein thiol (nmol/mg protein)	96.7 ± 4.70	92.5 ± 8.10	53.3 ± 4.30[a]	73.6 ± 4.10[b]	59.6 ± 8.60
Renal lipid peroxide level (nmol/g)	143.40 ± 8.60	142.20 ± 12.20	210.80 ± 23.40[a]	146.30 ± 32.80[b]	149.5 ± 12.24[b]
Plasma nonprotein thiol (μmol/100 ml)	3.17 ± 0.37	3.20 ± 0.24	2.05 ± 0.15[a]	2.06 ± 1.20	1.90 ± 0.17

Data represent mean ± SD of 5–7 rats
[a] $P < 0.05$ compared with control; [b] $P < 0.05$ compared with APAP

Table 3. Effect of 1000 mg vitamin C on plasma creatinine, nonprotein thiol, and urea and nonprotein thiol, protein thiol, and lipid peroxide level in kidney of paracetamol-treated rats and control rats

Parameters	Saline control	Vitamin C control	APAP	APAP + vitamin C (9 h)	APAP + vitamin C (16 h)
Plasma creatinine (mg %)	0.56 ± 0.24	0.54 ± 0.16	1.62 ± 0.42[a]	0.74 ± 0.43[b]	1.84 ± 0.43
Plasma urea (mg %)	41.2 ± 6.20	35.9 ± 4.30	199.0 ± 40.1[a]	140.4 ± 30.4[b]	222.7 ± 20.70
Renal nonprotein thiol (μmol/gm)	1.02 ± 0.09	0.91 ± 0.14	0.60 ± 0.09[a]	0.62 ± 0.16	0.50 ± 0.15
Renal protein thiol (nmol/mg protein)	107.5 ± 5.60	104.5 ± 7.50	60.2 ± 7.10[a]	70.3 ± 6.00	68.10 ± 5.80
Renal lipid peroxide level (nmol/g)	141.60 ± 15.20	142.30 ± 12.10	236.70 ± 74.50[a]	185.40 ± 17.80	155.60 ± 10.20[b]
Plasma nonprotein thiol (μmol/100 ml)	3.33 ± 0.44	3.22 ± 0.25	1.99 ± 0.13[a]	1.55 ± 1.20	1.83 ± 0.34

Data represent mean ± SD of 5–7 rats
[a] $P < 0.05$ compared with control; [b] $P < 0.05$ compared with APAP

Administration of 500 mg vitamin C 6 or 16 h after the administration of paracetamol almost completely prevented the increase in lipid peroxidation induced by paracetamol. Administration of this dose of vitamin C at 6 h partially (but significantly) prevented the decrease in renal protein thiol induced by paracetamol; however, administration of vitamin C at a later time (16 h) did not prevent the decrease in protein thiol induced by paracetamol. Administration of vitamin C at 6 or 16 h did not prevent the decrease in plasma nonprotein thiol induced by paracetamol.

Effect of 1000 mg/kg body wt of vitamin C on various biochemical parameters in paracetamol-treated rats

In 75% of rats treated with 1000 mg/kg vitamin C 9 h after APAP administration, the plasma creatinine values were elevated, but were less than in the APAP-treated rats, and in the other 25%, the values were comparable with the control (Table 3). Administration of 1000 mg/kg body wt vitamin C 16 h after the administration of APAP did not prevent the rise in plasma creatinine values in any of the rats. In fact, the plasma creatinine values were slightly higher than in the APAP-treated rats. Similar results were obtained for plasma urea. The administration of 1000 mg/kg body wt vitamin C 9 or 16 h after the administration of paracetamol did not prevent the loss of renal nonprotein thiol induced by paracetamol. Administration of 1000 mg/kg vitamin C 6 h after administration of paracetamol partially prevented the increase in lipid peroxidation induced by paracetamol; however, at 16 h lipid peroxidation was significantly reduced as compared with the paracetamol-treated rats. Administration of this dose of vitamin C at 9 or 16 h did not significantly prevent the decrease in renal protein thiol induced by paracetamol. Also, administration of vitamin C at 9 or 16 h did not prevent the decrease in plasma nonprotein thiol induced by paracetamol.

Correlation between renal nonprotein thiol and plasma creatinine

A highly significant inverse correlation (Fig. 6) was obtained between renal nonprotein thiol and plasma creatinine ($r = -0.890$, $P < 0.001$).

Correlation between renal protein thiol and plasma creatinine

Although vitamin C was able to prevent the decrease in protein thiol induced by APAP, no significant correlation

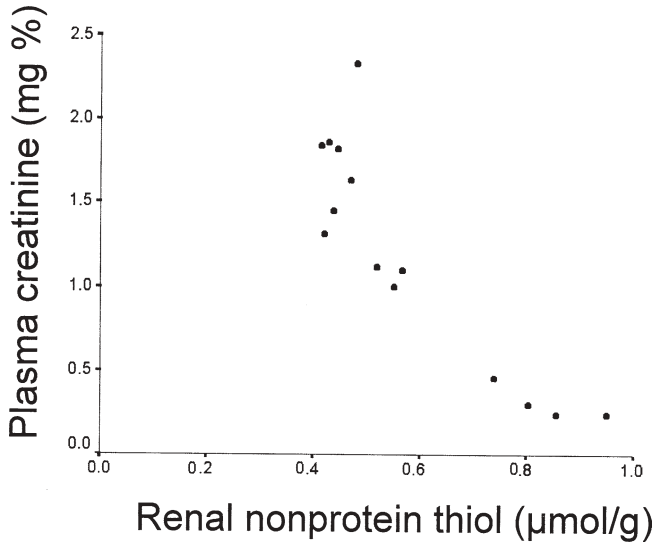

Fig. 6. Correlation between renal nonprotein thiol and plasma creatinine

was obtained between renal protein thiol and plasma creatinine.

Discussion

In the present study, paracetamol-induced renal damage was accompanied by significant depletion of nonprotein thiol, about 95% of which is glutathione and protein thiol in the kidney, as well as in enhanced lipid peroxidation. These results are in agreement with those reported earlier by other workers.[8–11] Paracetamol-induced renal injury may be due to the metabolic activation of paracetamol to the reactive metabolite, *N*-acetyl-*p*-benzoquinone imine.[33] This metabolite can directly react with glutathione, and at overdose of paracetamol, depletion of cellular glutathione may occur.[33] Depletion of glutathione may have two adverse consequences. First, it reduces the inactivation of the reactive metabolite and tends to increase its covalent binding to macromolecules.[34,35] Second, it may have several deleterious effects on cell homeostasis,[36,37] and may therefore aggravate the toxic effects of the reactive metabolite.

A novel finding in the present study is that a lower dose of vitamin C (250 mg/kg) when administered soon after paracetamol intoxication (1.5 h) helps in the prevention of renal damage (either completely or partially) induced by paracetamol, and a higher dose (500 or 1000 mg/kg) when administered later (6 or 9 h) helps in the prevention (either completely or partially) of renal damage induced by paracetamol. The protective effect of vitamin C appears to be due to its regeneration of nonprotein thiol, about 95% of which is glutathione, because a highly significant inverse correlation was obtained between renal nonprotein thiol and plasma creatinine in the rats treated with paracetamol/vitamin C. As mentioned earlier, vitamin C has been shown to recycle glutathione[24] and is also reported to have a sparing effect on glutathione.[38] The ability of vitamin C to prevent renal damage may also be due to its regeneration of vitamin E and maintenance of the chain-breaking activity of vitamin E.[23] In addition, the ability of vitamin C to prevent APAP-induced renal damage may be attributed, at least in part, to its prevention of depletion of protein thiol. Protein thiols are physiological free radical scavengers and may serve as antioxidants by several mechanisms.[39] The decrease in protein thiol in APAP intoxication may be due to the binding of the reactive metabolite generated from APAP to protein thiols in the kidney.[40] However, the mechanism by which vitamin C restores protein thiol is not clear.

Although paracetamol-induced renal damage was accompanied by an increase in lipid peroxidation, vitamin C was able to prevent renal damage without inhibition of lipid peroxidation; i.e., in paracetamol/vitamin C-treated rats that had no evidence of renal damage, lipid peroxide levels were elevated and, in some paracetamol/vitamin C-treated rats which had evidence of renal damage, the lipid peroxide levels were comparable with the normal controls. These findings suggest that lipid peroxidation is not mainly responsible for paracetamol-induced kidney damage. A few earlier studies have also suggested that lipid peroxidation may not play a causative role in the pathogenesis of paracetamol-induced renal injury.[41,42]

Depletion of glutathione may not be the only mechanism of paracetamol-induced renal injury. Paracetamol-induced renal injury could also be due to hepatic-derived paracetamol metabolites, particularly glutathione conjugates,[43] and inhibition of renal organic anion transporter.[44] A recent study has shown that nitric oxide may play a role in the pathogenesis of paracetamol-induced renal damage. The nitric oxide donor V-PYRRO/NO has been shown to protect against paracetamol-induced renal damage in mice.[9] It is interesting to note that vitamin C can increase the bioavailability of nitric oxide.[45] Hence, the protective effect of vitamin in paracetamol-induced renal damage may also be due to its ability to increase the availability of nitric oxide.

The reason why the glutathione prodrug *N*-acetyl cysteine, which increases cellular glutathione, protects against paracetamol-induced hepatotoxicity but is unable to protect against paracetamol-induced nephrotoxicity could be explained on the basis of the fact that the liver is capable of synthesizing glutathione from the precursors and, in fact, liver is the main producer and exporter of glutathione.[46,47] On the other hand, kidney cannot synthesize glutathione from its precursors and is dependent on plasma for its requirement.[47]

In summary, this study demonstrated that vitamin C is effective in protecting against paracetamol-induced renal injury in the rat. The protective effect appears to be due to its regeneration of glutathione. More animal experiments are to be conducted to confirm the present results. Clinical trials need to be carried out to determine the usefulness and dose of vitamin C in the prevention of paracetamol-induced renal damage in humans. Higher doses of vitamin C may be required to prevent paracetamol-induced renal damage in humans compared to rats because humans cannot synthesize vitamin C.

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