Protection by Taurine Against Adriamycin-induced Proteinuria and Hyperlipidemia in Rats (44122)

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Abstract. **Taurine was used in the present study to evaluate its beneficial effects against proteinuria and hyperlipidemia associated with nephrotic syndrome. Rats made nephrotic with adriamycin had a high excretion of protein, albumin, and N-acetyl-β-D-glucosaminidase compared with nonnephrotic rats. Nephrotic rats manifested hyperlipidemia with significant elevation in all major lipoprotein fractions. Treatment with taurine significantly suppressed adriamycin-induced proteinuria, albuminuria, and urinary excretion of N-acetyl-β-D-glucosaminidase. Treatment of rats with taurine for 7 days before adriamycin, and daily thereafter, significantly lowered plasma cholesterol, triglycerides, phospholipids, lipid peroxides, and malondialdehyde associated with lipoprotein fractions. Similarly, total lipids, cholesterol, triglycerides, lipid peroxides, hydroperoxides, and hydroxyl radicals in the liver and kidneys of taurine-treated adriamycin rats were decreased significantly compared with adriamycin alone. Lecithin cholesterol acyl transferase activity and free fatty acid levels in plasma, lipoprotein lipase activity, glutathione, total thiol, and ascorbic acid in the liver and kidneys of taurine-treated adriamycin groups were significantly elevated compared with adriamycin alone. These results suggest that taurine might be applicable as a protective agent for proteinuria and hyperlipidemia associated with nephrotic syndrome.** [P.S.E.B.M. 1997, Vol 215]

The anthracycline antibiotic adriamycin (ADR) is one of the most important antitumor agents, having a broad spectrum of therapeutic potency against a variety of human tumors, including soft-tissue sarcoma, breast cancer, small-cell carcinoma of the lung, and acute leukemias (1). However, the clinical efficacy of this antitumor drug is greatly limited because of severe cytotoxic side effects, the most serious being cardiotoxicity (2, 3). In addition to its well-known cardiotoxicity, renal injury has been reported to occur as part of the toxic syndrome induced by adriamycin (4–6). Intravenous administration of adriamycin in rats induces nephrotic syndrome, which is characterized by heavy proteinuria, hypoalbuminemia, albuminuria, and hyperlipidemia (7–10).

Hyperlipidemia associated with the nephrotic syndrome is a complex disorder involving abnormalities of both synthesis and degradation of lipoproteins most likely induced by the glomerular barrier defect and the secondary reduction in serum oncotic pressure which occurs as hypoalbuminemia ensues (11). It has been reported that hyperlipidemia secondary to nephrosis can aggravate the primary renal disease (12, 13). Previous reports have documented that hyperlipidemia has an adverse effect on glomerular function in normal and experimental animals (14–16). Although various *in vivo* models of nephrotoxicity induced by adriamycin have been described, there have been few animal studies carried out assessing compounds that may prevent ADR-induced nephrosis, particularly proteinuria and hyperlipidemia. Interestingly, the pathogenesis of ADR-induced nephrosis resembles, in many ways, the older model of nephrotic syndrome induced by administration of puromycin aminonucleoside (PAN) (17).

We have recently demonstrated that administration of taurine ameliorated PAN-induced proteinuria (18) and hy-

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perlipidemia (19). In view of the attenuating effect produced by taurine, the present study led us to hypothesize that treatment of proteinuria in ADR rats with taurine may ameliorate hyperlipidemia associated with nephrotic syndrome. The results of these experiments form the basis of this report. We confirm here that taurine plays a protective role against ADR-induced nephrosis.

Materials and Methods

Chemicals. Adriamycin, taurine, bovine serum albumin, *p*-nitrophenyl N-acetyl-β-D-glucosaminide (NAG) and thiobarbituric acid were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of analytical grade.

Experimental Design. Healthy male Wistar rats weighing 100 g were kept in groups of three in polyvinyl cages. A 12:12-hr light:dark cycle was maintained. Food and water were available *ad libitum.* Rats were divided into four experimental groups: (i) saline (SA); (ii) taurine + saline (T + SA); (iii) adriamycin (ADR); and (iv) taurine + adriamycin (T + ADR). Rats were treated with taurine (1%) in drinking water to appropriate groups, 7 days prior to intravenous administration of saline or adriamycin (6 mg/kg body wt) and thereafter throughout this study. Thirty days after the administration of ADR, all control and experimental rats were housed for 24-hr urine collection in individual metabolic cages with access to water only.

Biochemical Analyses. Rats were sacrificed under pentobarbital anaesthesia (75 mg/kg), blood was collected, and plasma was separated by low-speed centrifugation (5000*g*). High-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL) were separated by the dual precipitation method as described by Wilson and Spiger (20), and they were analyzed for cholesterol (21), phospholipids (22), triglycerides (23), and lipid peroxides (24). Plasma was also analyzed for free fatty acids (25) and lecithin cholesterol acyl transferase (LCAT) activity (26). A portion of the collected blood was used for the separation of serum. Serum was analyzed for protein (27) and albumin (28). Excised kidneys and liver rinsed in ice-cold physiological saline to remove blood and adherent tissues were used for lipid extraction (29), lipid peroxide measurement (30), lipoprotein lipase activity (31), hydroperoxides (32), hydroxyl radicals (33, 34), reduced glutathione (35), total thiol (36), and ascorbic acid (37). Urine was analyzed for protein (27), creatinine (38), albumin (28), cholesterol (21), and N-acetyl-β-D-glucosaminidase activity (39).

Statistical Analysis. All values are reported as the mean ± SD of six observations, and the data were analyzed by one-way analysis of variance (ANOVA).

Results

Body Weight. Nephrotic rats lost weight ($P < 0.01$), whereas control rats gained weight during 30 days of experiment. Treatment with taurine over the same period of time significantly diminished ADR-induced decreases in body weight, although the values were lower than those of SA– or T + SA–treated groups (Table I).

Urinary Excretion of Protein, Albumin and N-acetyl-β-D-glucosaminidase. Treatment of rats with ADR produced the characteristic symptoms of nephrotic syndrome including edema of kidneys and liver, and fluid accumulation in the peritoneal cavity. Saline– and saline plus taurine–treated rats showed no statistical difference between them in kidney weight (0.49 ± 0.05 versus 0.51 ± 0.09 g wet tissue, respectively). Kidney weight in nephrotic rats was significantly higher (0.88 ± 0.04 g wet tissue; $P < 0.05$), however, taurine treatment in nephrotic rats produced no change in kidney wet weight (0.86 ± 0.09 g). Compared with that of saline and saline plus taurine rats, the liver weight of nephrotic rats showed a significant difference (1.81 ± 0.26 and 1.78 ± 0.30 versus 2.61 ± 0.12 g wet tissue for saline and saline plus taurine versus nephrotic rats, respectively; $P < 0.05$). Taurine administration to nephrotic rats, however, produced no change in liver wet weight

Table I. Effect of Taurine on Body Weight, Serum Protein and Albumin, Urinary Protein and Albumin, N-acetyl-glucosaminidase, and Cholesterol Excretion in Control and Nephrotic Rats

Variable	Saline (SA)	Taurine + saline (T + SA)	Adriamycin (ADR)	Taurine + adriamycin (T + ADR)
Body weight (g)	145 ± 10	150 ± 7	71 ± 5[d]	104 ± 6[b]
Serum protein (g/dl)	6.3 ± 1.0	5.8 ± 0.8	3.1 ± 0.4[d]	4.9 ± 0.7[b]
Serum albumin (g/dl)	3.0 ± 0.9	3.2 ± 1.0	1.4 ± 0.3[d]	2.4 ± 0.8[b]
Urine protein (mg/mg creatinine)	1.1 ± 0.1	0.9 ± 0.1	50.3 ± 4.5[a]	20.5 ± 40[c]
Urine albumin (mg/mg creatinine)	0.4 ± 0.05	0.36 ± 0.04	15.8 ± 3.6[a]	6.5 ± 1.9[c]
N-acetyl-glucosaminidase (nmol/mg creatinine)	265 ± 25.6	270 ± 20.7	515 ± 36.5[a]	360 ± 25.7[c]
Urinary cholesterol (mg/24 hr)	0.30 ± 0.05	0.35 ± 0.04	2.25 ± 0.08[a]	0.95 ± 0.05[c]

Note. Rats were treated with 1% taurine (in drinking water) 7 days prior to adriamycin (6 mg/kg body wt, iv) administration, and 30 days after the iv adriamycin injection biochemical analyses were carried out as described in Materials and Methods. Values are expressed as mean ± SD of six observations.

[a] Significantly higher ($P < 0.01$) than all other groups.
[b] Significantly higher ($P < 0.05$) than the ADR group.
[c] Significantly lower ($P < 0.05$) than the ADR group.
[d] Significantly lower ($P < 0.01$) than all other groups.

Table II. Effect of Taurine on Plasma Lipoprotein Profile and Malondialdehyde (MDA) Content Associated with the Lipoprotein of Control and Nephrotic Rats

Lipoproteins	Saline (SA)	Taurine + saline (T + SA)	Adriamycin (ADR)	Taurine + adriamycin (T + ADR)
VLDL				
Cholesterol (mg/dl)	12.65 ± 2.49	13.01 ± 1.85	28.13 ± 3.58[a]	15.36 ± 2.06[b]
Triglycerides (mg/dl)	33.51 ± 4.65	34.07 ± 4.01	47.87 ± 6.07[a]	38.54 ± 5.12[b]
Phospholipids (mg/dl)	19.14 ± 3.06	18.49 ± 2.89	31.07 ± 3.86[a]	22.06 ± 2.93[b]
MDA (nmol/ml)	0.61 ± 0.11	0.59 ± 0.14	2.87 ± 0.86[a]	1.35 ± 0.54[b]
LDL				
Cholesterol (mg/dl)	30.34 ± 6.59	29.65 ± 6.04	60.10 ± 8.98[a]	38.35 ± 6.56[b]
Triglycerides (mg/dl)	17.56 ± 4.03	18.35 ± 3.87	31.78 ± 6.16[a]	20.49 ± 3.85[b]
Phospholipids (mg/dl)	41.05 ± 7.89	40.04 ± 6.59	54.32 ± 8.16[a]	43.54 ± 6.85[b]
MDA (nmol/ml)	1.12 ± 0.61	1.25 ± 0.70	4.67 ± 1.30[a]	2.55 ± 0.91[b]
HDL				
Cholesterol (mg/dl)	26.13 ± 4.58	27.08 ± 4.69	10.50 ± 3.06[c]	22.30 ± 4.56[d]
Triglycerides (mg/dl)	7.10 ± 1.25	8.01 ± 2.06	16.13 ± 3.18[a]	10.36 ± 2.25[b]
Phospholipids (mg/dl)	24.78 ± 5.07	25.54 ± 4.49	40.45 ± 6.70[a]	30.30 ± 5.15[b]
MDA (nmol/ml)	3.06 ± 0.86	3.00 ± 0.93	7.51 ± 1.85[a]	4.15 ± 1.01[b]

Note. Rats were treated with 1% taurine (in drinking water) 7 days prior to adriamycin (6 mg/kg body wt, iv) administration, and 30 days after the iv adriamycin injection biochemical analyses were carried out as described in Materials and Methods. Values are expressed as mean ± SD of six experiments.
[a] Significantly higher ($P < 0.01$) than all groups.
[b] Significantly lower ($P < 0.01$) than the ADR group.
[c] Significantly lower ($P < 0.01$) than all groups.
[d] Significantly higher ($P < 0.05$) than the ADR group.

(2.49 ± 0.26 g wet tissue). No attempt was made to find the dry weight of these tissues. Rats with nephrosis had a high excretion of protein, albumin, and N-acetyl-β-D-glucosaminidase compared with non-nephrotic rats. However, 30 days after taurine treatment urinary protein, albumin, and NAG excretion were significantly reduced (Table I).

Serum Protein and Albumin. As shown in Table I, rats with nephrosis manifested a decline in serum total protein and albumin. Treatment with taurine significantly reduced the ADR-induced decreases in serum total protein and albumin.

Plasma Lipoprotein, Lipids, Lipid Peroxide Content, and Lecithin Cholesterol Acyl Transferase. The effect of the administered dose of taurine on the elevation of plasma lipoprotein, lipids, and lipid peroxides at the 30th day after ADR treatment was examined (Tables II and III). Nephrotic rats were severely hyperlipidemic, characterized by increased levels of cholesterol, triglycerides, and phospholipids. Significant elevation in plasma LDL and VLDL cholesterol was observed in nephrotic rats. Interestingly, HDL cholesterol was reduced in parallel with the increased urinary cholesterol (Table I) excretion in nephrotic rats. Treatment with taurine significantly attenuated the ADR-induced decreases in HDL cholesterol levels. Phospholipid and triglyceride content in the plasma after ADR treatment was significantly elevated in all lipoprotein fraction. In a similar fashion, ADR increased malondialdehyde content in all the three lipoprotein fraction. As shown in Tables II and III, taurine treatment prevented the ADR-

Table III. Effect of Taurine on Plasma Lipids, Lipid Peroxide, and Lecithin Cholesterol Acyl Transferase (LCAT) Activity in Control and Nephrotic Rats

Parameters assayed	Saline (SA)	Taurine + saline (T + SA)	Adriamycin (ADR)	Taurine + adriamycin (T + ADR)
Total cholesterol (mg/dl)	71.85 ± 9.68	70.15 ± 8.56	103.0 ± 16.56[a]	78.05 ± 9.98[b]
Total triglycerides (mg/dl)	59.95 ± 5.85	61.25 ± 6.03	98.30 ± 9.16[a]	68.65 ± 7.00[b]
Total phospholipids (mg/dl)	85.60 ± 3.08	86.95 ± 3.14	129.35 ± 5.85[a]	93.89 ± 4.44[b]
Free fatty acids (mg/dl)	18.35 ± 3.64	19.30 ± 3.85	9.45 ± 1.86[c]	16.35 ± 2.56[d]
Lipid peroxide (nmol MDA/ml)	2.56 ± 0.58	2.30 ± 0.55	7.65 ± 1.85[a]	3.16 ± 0.89[b]
LCAT (nmol cholesterol esterified/hr/ml)	71.26 ± 8.56	73.65 ± 8.06	32.56 ± 5.65[c]	66.45 ± 7.67[d]

Note. Rats were treated with 1% taurine (in drinking water) 7 days prior to adriamycin (6 mg/kg body wt, iv) administration, and 30 days after the iv adriamycin injection biochemical analyses were carried out as described in Materials and Methods. Values are expressed as mean ± SD of six observations.
[a] Significantly higher ($P < 0.01$) than all groups.
[b] Significantly lower ($P < 0.01$) than the ADR group.
[c] Significantly lower ($P < 0.01$) than all groups.
[d] Significantly higher ($P < 0.01$) than the ADR group.

induced increases of the above biochemical parameters, although these values remained higher than their respective control values. Nephrotic rats had decreased levels of plasma lecithin cholesterol acyl transferase, an effect attenuated by taurine treatment (Table III).

Lipoprotein Lipase. Kidney and liver lipoprotein lipase activity was lower in nephrotic rats than in saline– or taurine plus saline–treated rats. The lowered lipoprotein lipase activity in the kidney and liver of nephrotic rats showed significant elevation with taurine treatment (Table IV).

Oxidant and Antioxidant Status. ADR administration produced a significant increase in lipid peroxide, hydroperoxides, and hydroxyl radical content in kidney and liver (Tables V and VI). However, nephrotic rats treated with taurine did achieve significantly lower levels of these biochemical parameters than those of nephrotic rats treated without taurine in their drinking water. On the contrary, a significant reduction in kidney and liver glutathione, total thiol, and ascorbic acid content occurred after 30 days in rats given ADR. Taurine administration to ADR rats significantly elevated the antioxidant levels (Tables V and VI).

Discussion

Intravenous administration of ADR has been used to evaluate the pathological features of nephrotic syndrome caused by this anticancer drug in animal models (7–10). We recently reported that taurine was effective in blocking PAN-induced proteinuria and hyperlipidemia associated with nephrotic syndrome (18, 19). On the basis of our previous studies, we hypothesized that taurine might play an important role as an antiproteinuric agent in ADR model of nephrosis. Treatment with taurine produced a significant reduction in ADR-induced proteinuria and hyperlipidemia.

In the present study, renal toxicity was a prominent feature of adriamycin administration. The administration of ADR to rats produces heavy proteinuria accompanied by widespread fusion of the glomerular foot processes and detachment from the capillary basement membrane (5, 7, 9). However, the specific cellular targets and molecular mechanisms responsible for the pathogenesis of ADR-induced renal injury are not clearly defined. The potential biochemical mechanism of this nephrotoxicity has not yet been established, although many hypotheses have been put forward. One of the most possible mechanisms for the toxicity of ADR may be the consequence of oxidative stress (40)—that is, oxidation and cross-linking of cellular thiols and membrane lipid peroxidation. Earlier reports have shown that ADR enhances the generation of superoxide and hydrogen peroxide by renal cortical microsomes (41) and that oxygen radicals generated by ADR are important mediators in renal injury (42). Considerable evidence suggests that the involvement of ADR semiquinone radicals gives rise to reactive oxygen species (ROS) (40). The prime target of the oxygen radicals is the unsaturated bonds in membrane lipids, which can form lipid peroxides followed by the initiation of a chain reaction of lipid peroxidation. Malondialdehyde is one of the end products of lipid peroxidation, and a variety of cross-linking reactions including linking of lipids and proteins and linking between lipids and proteins can be mediated by malondialdehyde (43). This mechanism is supported by the report that ADR-enhanced kidney microsomal lipid peroxidation was diminished by the inclusion of oxyradical scavenger, superoxide dismutase, and 1,3-dimethylurea, and by the chelating agents ethylenediaminetetraacetic acid (EDTA) and diethylenetriamine-pentaacetic acid (DETPAC).

Bertolatus *et al.* (44) have found no evidence that oxi-

Table IV. Effect of Taurine on Liver and Kidney Total Lipids, Cholesterol, Triglycerides, and Lipoprotein Lipase Levels in Control and Nephrotic Rats

Parameters assayed	Saline (SA)	Taurine + saline (T + SA)	Adriamycin (ADR)	Taurine + adriamycin (T + ADR)
Liver				
Total lipids[a]	60.23 ± 8.75	62.11 ± 7.86	101 ± 10.34[b]	71.5 ± 8.13[c]
Cholesterol[a]	7.13 ± 1.56	6.96 ± 2.13	13.5 ± 3.89[b]	8.53 ± 2.30[c]
Triglycerides[a]	8.21 ± 1.69	7.95 ± 1.80	15.1 ± 2.45[b]	10.35 ± 1.56[c]
Lipoprotein lipase[d]	5.18 ± 1.01	5.50 ± 1.51	1.25 ± 0.68[e]	4.06 ± 0.96[f]
Kidney				
Total lipids[a]	31.34 ± 4.36	33.56 ± 4.65	55.16 ± 6.65[b]	39.85 ± 3.65[c]
Cholesterol[a]	4.85 ± 0.76	4.40 ± 0.65	8.31 ± 1.61[b]	5.75 ± 0.81[c]
Triglycerides[a]	5.12 ± 1.01	5.36 ± 1.81	9.75 ± 2.95[b]	6.25 ± 1.75[c]
Lipoprotein lipase[d]	3.25 ± 0.96	3.15 ± 0.78	0.95 ± 0.06[e]	2.46 ± 0.35[f]

Note. Rats were treated with 1% taurine (in drinking water) 7 days prior to adriamycin (6 mg/kg body wt, i.v.) administration, and 30 days after the iv adriamycin injection biochemical analyses were carried out as described in Materials and Methods. Values are expressed as mean ± SD of six observations.

[a] mg/g wet tissue.

[b] Significantly higher ($P < 0.01$) than all groups.

[c] Significantly lower ($P < 0.05$) than the ADR group.

[d] μmol of glycerol liberated/hr/mg protein.

[e] Significantly lower ($P < 0.01$) than all groups.

[f] Significantly higher ($P < 0.01$) than the ADR group.

Table V. Effect of Taurine on Lipid Peroxide, Hydroperoxides, Hydroxyl radicals, and Antioxidant in the Liver of Control and Nephrotic Rats

Parameters assayed	Saline (SA)	Taurine + saline (T + SA)	Adriamycin (ADR)	Taurine + adriamycin (T + ADR)
Lipid peroxides (nmol/mg protein)	1.97 ± 0.53	1.70 ± 0.49	7.51 ± 2.05[a]	3.06 ± 0.79[b]
Hydroperoxides[c]	2.57 ± 0.78	2.31 ± 0.50	4.95 ± 0.94[a]	3.21 ± 1.01[b]
Hydroxyl radicals[d]	3.05 ± 0.91	2.90 ± 0.75	6.34 ± 1.85[a]	3.69 ± 0.96[b]
Glutathione (nmol/g wet tissue)	3.45 ± 0.87	3.63 ± 0.78	1.01 ± 0.13[e]	2.89 ± 0.65[f]
Total thiol (μg SH/mg protein)	22.87 ± 3.16	24.15 ± 2.89	8.35 ± 1.06[e]	19.85 ± 2.90[f]
Ascorbic acid (μg/mg protein)	1.89 ± 0.81	1.70 ± 0.70	0.30 ± 0.12[e]	1.25 ± 0.69[f]

Note. Rats were treated with 1% taurine (in drinking water) 7 days prior to adriamycin (6 mg/kg body wt, iv) administration, and 30 days after the iv adriamycin injection biochemical analyses were carried out as described in Materials and Methods. Values are expressed as mean ± SD of six observations.
[a] Significantly higher ($P < 0.01$) than all groups.
[b] Significantly lower ($P < 0.05$) than the ADR group.
[c] μg of *t*-butyl hydroperoxide/mg protein.
[d] nmol of formaldehyde formed/min/mg protein.
[e] Significantly lower ($P < 0.01$) than all groups.
[f] Significantly higher ($P < 0.01$) than the ADR group.

dative injury plays a major role in ADR nephrosis. Significantly, the findings of Ghiggeri *et al.* (45) appear to conflict with that of Bertolatus and co-workers. The results presented by these authors reiterate and strengthen previous findings that ADR in the presence of glomeruli and glomerular epithelial cells in culture induces O_2 generation, which is partially responsible for its cytotoxicity. Our data support the hypothesis that ROS are involved in ADR-induced renal toxicity based upon the elevation of lipid peroxides, hydroperoxides, and hydroxyl radicals in the liver and kidneys of ADR rats with concomitant reduction in nonenzymic antioxidant levels. The protective role of taurine is consistent with this proposal. Involvement of ROS in nephrotoxicity is indirectly suggested by the decrease in antioxidant enzymes (data not shown) that we observed after ADR administration. A recent study also suggests a role of ROS in the induction and progression of ADR nephrosis (46). Nevertheless, conclusive evidence of whether oxygen free radicals are formed and play a pathogenetic role in ADR-induced renal toxicity remains to be clarified.

It has been demonstrated that 30 days after ADR treatment there is an increase in urinary protein and NAG excretion. Nephrotic rats had hypoalbuminemia, hyperlipidemia, and significant elevation in lipid and lipoprotein profiles. The abnormal lipid and lipoprotein profiles in ADR nephrotic rats could be the result of complex abnormalities of both synthesis and catabolism as observed in PAN nephrosis (47–49). Hyperlipidemia is an intrinsic component of the nephrotic syndrome (11), and the role of hyperlipidemia in the evolution of renal disease has been reviewed (11). The causative factor for hyperlipidemia in nephrotic syndrome has not been established; however, abnormal glomerular permeability to plasma proteins and reduced serum oncotic pressure may contribute (11). Pharmacological or dietary treatment of hyperlipidemia in animals with proteinuric disease has been efficacious in reducing proteinuria and glomerulosclerosis (50). The results of this study indicate that the ability of taurine to inhibit proteinuria could have additional beneficial effects in ameliorating hyperlipidemia. Our results are in agreement with those of Kasiske and

Table VI. Effect of Taurine on Lipid Peroxide, Hydroperoxides, Hydroxyl Radicals, and Antioxidants in the Kidney of Control and Nephrotic Rats

Parameters assayed	Saline (SA)	Taurine + saline (T + SA)	Adriamycin (ADR)	Taurine + adriamycin (T + ADR)
Lipid peroxides (nmol/mg protein)	2.35 ± 0.79	2.01 ± 0.54	8.45 ± 1.49[a]	3.15 ± 0.48[b]
Hydroperoxides[c]	1.62 ± 0.45	1.48 ± 0.40	4.24 ± 0.85[a]	1.67 ± 0.51[b]
Hydroxyl radicals[d]	3.19 ± 0.76	3.01 ± 0.58	6.55 ± 1.47[a]	4.10 ± 0.67[b]
Glutathione (nmol/g wet tissue)	2.76 ± 0.39	2.95 ± 0.43	0.89 ± 0.34[e]	2.05 ± 0.38[f]
Total thiol (μg SH/mg protein)	15.16 ± 2.95	15.60 ± 3.04	5.89 ± 1.01[e]	12.81 ± 2.85[f]
Ascorbic acid (μg/mg protein)	1.01 ± 0.43	0.95 ± 0.36	0.34 ± 0.08[e]	0.89 ± 0.18[f]

Note. Rats were treated with 1% taurine (in drinking water) 7 days prior to adriamycin (6 mg/kg body wt, iv) administration, and 30 days after the iv adriamycin injection biochemical analyses were carried out as described in Materials and Methods. Values are expressed as mean ± SD of six observations.
[a] Significantly higher ($P < 0.01$) than all groups.
[b] Significantly lower ($P < 0.05$) than the ADR group.
[c] μg of *t*-butyl hydroperoxide/mg protein.
[d] nmol of formaldehyde formed/min/mg protein.
[e] Significantly lower ($P < 0.01$) than all groups.
[f] Significantly higher ($P < 0.01$) than the ADR group.

co-workers (51), who demonstrated that the hyperlipidemic agents mevinolin and clofibric acid reduced proteinuria in obese Zucker rats. Kasiske *et al.* (16) have demonstrated that hyperlipidemia induced by cholesterol-rich diet increased glomerular capillary pressure and therefore damaged the kidney through hemodynamic mechanisms in the rat. A similar mechanism could be operative in ADR rats. Glomerular filtration is considered to be the early renal hemodynamic alteration in ADR-administered rats, and disorder of the lipid metabolism and renal barrier function are related to the abnormal leakiness of glomeruli in ADR rats (52). The reduction in proteinuria in taurine-treated nephrotic rats could be associated with a direct effect on glomerular filtration, or, alternatively, could be secondary to its hypolipidemic property.

It is believed that the initial injury caused by ADR involves ROS, which presumably cause glomerular and tubular injury (8). It has also been demonstrated that tubular and interstitial changes are commonly found in ADR and are even thought to predispose to subsequent glomerulosclerosis (53), although influx of interstitial inflammatory cells is a relatively late occurrence in this model (8). The inflammatory cells secrete a variety of potent degradative lysosomal enzymes, which may cause the destruction of structural components of the kidney (54). The increase in urinary protein and NAG excretion in ADR group may reflect cellular injury with increased vascular permeability, and this could account for increased level of renal injury. These changes were attenuated by taurine treatment. The beneficial effects of taurine in counteracting renal damage may be attributed to its antioxidative and anti-inflammatory properties.

On the basis of these findings, we suggest that administration of taurine attenuates ADR-induced nephrosis in the rat. In conjunction with the evidence that taurine is also effective against puromycin aminonucleoside nephrotic syndrome, our results further suggest that taurine could be applicable as a therapeutic agent in treating chemically induced nephrosis in which proteinuria and hyperlipidemia are customary hall marks of the nephrotic process. However, more detailed studies are required to determine whether oxygen free radicals do indeed play a pathogenetic role in adriamycin-induced renal injury. Additionally, ultrastructural studies are called for to understand the sequence of physiologic and histologic changes in taurine-treated ADR-model nephrosis and whether taurine is protecting directly against ADR-induced renal toxicity or simply blunting secondary biochemical derangements.

1. Blum RH, Carter SK. Adriamycin: A new anticancer drug with significant clinical activity. Ann Intern Med **80:**249–259, 1974.
2. Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. Clinicopathologic analysis of adriamycin cardiotoxicity. Cancer **32:**302–314, 1973.
3. Cortes EP, Lutman G, Wanta J, Pickren J, Wallace J, Holland J. Adriamycin (NSC 123127) cardiotoxicity: A clinicopathologic correlation. Cancer Chemother Rep **6:**215–225, 1975.
4. Burke JF Jr., Laucius JF, Brodovsky HS, Soriano RZ. Doxorubicin hydrochloride-associated renal failure. Arch Intern Med **137:**385–389, 1977.
5. O'Donnell MP, Michels L, Kasiske BL, Raij L, Keane WF. Adriamycin-indced chronic proteinuria: A structural and functional study. J Lab Clin Med **106:**62–67, 1985.
6. Borch RF. The nephrotoxicity of antineoplastic drugs. In: Hook J, Goldstein R, Eds. Toxicology of the Kidney. New York: Raven Press, 1993.
7. Bertani T, Poggi A, Pozzoni R, Delaini F, Sacchi G, Thoua Y, Mecca G, Remuzzi G, Donati MB. Adriamycin induced nephrotic syndrome in rats: Sequence of pathologic events. Lab Invest **46:**16–23, 1982.
8. Milner LS, Wei SH, Houser MT. Amelioration of glomerular injury in doxorubicin hydrochloride nephrosis by dimethylthiourea. J Lab Clin Med **118:**427–434, 1991.
9. Venkatesan N, Ramesh CV, Jayakumar R, Chandrakasan G. Angiotensin I converting enzyme activity in adriamycin induced nephrosis in rats. Toxicology **85:**137–148, 1993.
10. Geetha A, Catherine J, Sankar R, Devi CSS. Lipids and lipoprotein profile in doxorubicin treated rats: Influence of α-tocopherol administration. Indian J Exp Biol **28:**1071–1074, 1990.
11. Hutchison FN. Proteinuria, hyperlipidemia and the kidney. Miner Electrolyte Metab **19:**127–136, 1993.
12. Moorhead JF, Chan MK, El-Nahas M, Varghese Z. Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. Lancet **ii:**1309–1311, 1982.
13. Watanabe Y, Ozaki I, Yoshida F. A case of nephrotic syndrome with glomerular lipoprotein deposition with capillary ballooning and mesangiolysis. Nephron **51:**265–270, 1989.
14. French JW, Yamanaka BS, Ostwald R. Dietary induced glomerulosclerosis in the guinea pig. Arch Pathol **83:**204–220, 1967.
15. Peric-Golia L, Peric-Golia M. Aortic and renal lesions in hypercholesterolemic adult male virgin Sprague Dawley rats. Atherosclerosis **46:**57–65, 1983.
16. Kasiske BL, O'Donnell MP, Schmitz PG, Kim Y, Keane WF. Renal injury of diet-induced hypercholesterolemia in rats. Kidney Int **37:**880–891, 1990.
17. Kokui K, Yoshikawa N, Nakamura H, Itoh H. Cyclosporin reduces proteinuria in rats with aminonucleoside nephrosis. J Pathol **166:**297–301, 1992.
18. Venkatesan N, Rao PV, Arumugam V. In vivo protective effect of taurine on puromycin aminonucleoside induced nephrosis in rats. J Clin Biochem Nutr **16:**91–100, 1994.
19. Venkatesan N, Rao PV, Arumugam V. Inhibitory effect of taurine on puromycin aminonucleoside induced hyperlipidemia in rats. J Clin Biochem Nutr **15:**203–210, 1993.
20. Wilson DE, Spiger MJ. A dual precipitation method for quantitative plasma lipoprotein measurement without centrifugation. J Lab Clin Med **82:**473–482, 1973.
21. Parekh AC, Jung DH. Cholesterol determination with ferric acetate-uranium acetate and sulfuric acid-ferrous sulfate reagents. Anal Chem **42:**1423–1427, 1970.
22. Zilversmit DB, Diluzio NR. Synthesis of phospholipids in diabetic dogs. J Biol Chem **194:**673–683, 1952.
23. Foster LB, Dunn RT. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. Clin Chem **19:**338–339, 1973.
24. Cynamon HA, Isenberg NJ, Nguyen Co H. Erythrocyte malondialdehyde release in vitro: A functional measure of vitamin E status. Clin Chim Acta **151:**169–176, 1985.
25. Hron WT, Menahan LA. A sensitive method for the determination of free fatty acids in plasma. J Lipid Res **23:**377–381, 1981.
26. Hitz J. Steinmetz J, Siest G. Plasma lecithin: Cholesterol acyl transferase reference values and effects of xenobiotics. Clin Chim Acta **133:**85–96, 1983.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193:**265–275, 1951.

28. Varley H. Practical Clinical Biochemistry. Delhi: CBS Publishers and Distributors, 1988.
29. Folsch J, Lees M, Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem **226:**497–509, 1957.
30. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem **95:**351–358, 1979.
31. Lambert M, Neish AC. Rapid method for estimation of glycerol in fermentation solutions. Can J Res **28:**83–89, 1950.
32. Beuge JA, Aust SB. Microsomal lipid peroxidation. In: Fleisher S, Packer L, Eds. Methods in Enzymology. New York: Academic Press, Vol **52:**pp302–310, 1978.
33. Puntarulo S, Cederbaum AI. Effect of oxygen concentration on microsomal oxidation of ethanol and generation of oxygen radicals. Biochem J **251:**787–794, 1988.
34. Burton RM. The determination of glycerol and dihydroxyacetone. In: Colowick SP, Kaplan NO, Eds. Methods in Enzymology. New York: Academic Press, Vol **3:**pp246–249, 1957.
35. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta **582:**67–78, 1979.
36. Sedlak J, Lindsay RH. Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem **25:**192–205, 1968.
37. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCormick DB, Wright LD, Eds. Methods in Enzymology. New York: Academic Press, Vol **62:**pp7–8, 1979.
38. Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by the Jaffe reaction. J Biol Chem **158:**581–589, 1945.
39. Moore JC, Morris JW. A simple automated colorimetric method for determination of N-acetyl-β-D-glucosaminidase. Ann Clin Biochem **19:**157–159, 1982.
40. Mimnaugh EG, Trush MA, Gram TE. A possible role for membrane lipid peroxidation in anthracycline nephrotoxicity. Biochem Pharmacol **35:**4327–4335.
41. Shah SV, Price L, Baricos W. Adriamycin-induced stimulation of superoxide anion (O_2) production in renal cortical microsomes. Clin Res **30:**864A, 1982.
42. Wu SH, Yang YC, Wang ZM. Role of oxygen radicals in adriamycin-induced nephrosis. Chin Med J Engl **103:**283–289, 1990.
43. Freeman BA, Crapo JD. Free radicals and tissue injury. Lab Invest **47:**412–426, 1982.
44. Bertolatus JA, Klinzman D, Bronsema DA, Ridnour L, Oberley LW. Evaluation of the role of reactive oxygen species in doxorubicin hydrochloride nephrosis. J Lab Clin Med **118:**435–445, 1991.
45. Ghiggeri GM, Bertelli R, Ginervi F, Oleggini R, Altieri P, Trivelli A, Gusmano R. Multiple mechanisms for doxorubicin cytotoxicity on glomerular epithelial cells "in vitro." Eur J Pharmacol **228:**77–83, 1992.
46. Futenma A, Miyai H, Iida T, Hara T, Watanabe T, Shiono S, Fukatsu A, Kato K. The protective effect of probucol on adriamycin nephrosis in the rat. Nippon Jinzo Gakkai Shi **37:**616–621, 1995.
47. Marsh JB, Sparks CE. Hepatic secretion of lipoproteins in the rat and the effect of experimental nephrosis. J Clin Invest **64:**1229–1237, 1979.
48. Yamada M, Matsuda I. Lipoprotein lipase in clinical and experimental nephrosis. Clin Chim Acta **30:**787–794, 1970.
49. Kashyap ML, Srivastava LS, Hynd BA, Brady D, Perisutti G, Glueck CJ, Gartside PS. Apolipoprotein C II and lipoprotein lipase in human nephrotic syndrome. Atherosclerosis **35:**29–40, 1980.
50. Hirano T, Mamo JCL, Nagona S, Sugisaki T. The lowering effect of probucol on plasma lipoprotein and proteinuria in puromycin aminonucleoside-nephrotic rats. Nephron **58:**95–100, 1991.
51. Kasiske BL, O'Donnell MP, Cleary MP, Keane WF. Treatment of hyperlipidemia reduces glomerular injury in obese Zucker rats. Kidney Int **33:**667–672, 1988.
52. Soose M, Haberstroh U, Rovira-Halbach G, Brunkhorst R, Stolte H. Heterogeneity of glomerular barrier function in early adriamycin nephrosis of MWF rats. Clin Physiol Biochem **6:**310–315, 1988.
53. Bertani T, Rocchi G, Sacchi G. Adriamycin-induced glomerulosclerosis in the rats. Am J Kidney Dis **12:**12–19, 1986.
54. Weiss SJ. Tissue destruction of neutrophils. N Engl J Med **320:**365–376, 1989.