

Efficacy of deferoxamine, N-acetylcysteine and selenium treatments in rats with Adriamycin-induced nephrotic syndrome

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ABSTRACT

Background: Various experimental models related to Adriamycin (ADR)-induced nephropathy have been reported. The purpose of the present study was to evaluate the efficacy of N-acetylcysteine (NAC), deferoxamine (DFO) and selenium in protection against renal injury in ADR nephropathy.

Methods: The study included 53 Sprague Dawley male rats. Nephrotic syndrome was induced by injection of ADR 5 mg/kg intravenously (n=46). Control rats (n=7) were injected with an equal volume of isotonic saline. After ADR administration, they were divided into a group given only ADR (n=17) and 3 antioxidant treatment groups: (i) NAC (n=10), (ii) DFO (n=10) and (iii) selenium (n=9). In both renal tissue and erythrocytes, oxidative system parameters and trace elements were determined.

Results: Nephrotic syndrome was proven in ADR-injected rats 4 weeks after injections, with proteinuria, higher blood lipids and hypoalbuminemia. All of the antioxidant agents used in the present study to prevent the development of nephrotic syndrome provided benefits for the nephrotic state. Of them, selenium seemed to offer relatively lower and statistically insignificant efficacy for preventing proteinuria compared with the others.

Conclusions: Our results showed that concomitant administration of some antioxidants with ADR injections seems to have beneficial effects on clinical parameters even if antioxidants were given in a single dose. NAC and DFO are more effective than selenium to prevent renal injury.

Key words: N-Acetylcysteine, Adriamycin, Deferoxamine, Nephrotic syndrome, Selenium

INTRODUCTION

Increased oxidant stress plays an important role in the pathophysiological processes of a wide variety of disorders (1) including nephrotic syndrome (2). In the kidneys, oxidant injury is initiated by free radicals and reactive oxygen intermediates (ROIs) that can be generated either in the circulation or locally by glomerular cells (3).

Adriamycin (ADR) is an anticancer drug, and intravenous administration in rats induces nephrotic syndrome (4). ADR generates semiquinone radicals, which in turn react with molecular oxygen and provide other ROIs at an early stage after administration (4). Semiquinone radicals react with molecular oxygen under aerobic conditions to form superoxide and hydrogen peroxide and, consequently, hydroxyl radicals in the presence of transition metals (iron and copper [Cu]).

N-Acetylcysteine (NAC), a potent antioxidant by itself, may serve as a precursor for glutathione synthesis. Park et al reported that NAC showed good effectiveness in prevention of ADR-induced nephrotoxicity (5). Conversely, it has been demonstrated that rats pretreated with NAC had no significant reduction in proteinuria at 3 weeks after ADR administration when compared with rats receiving ADR in the absence of scavengers (6).

On the other hand, ADR free radicals come from a nonenzymatic mechanism that involves reactions with iron. This iron-ADR complex can reduce oxygen to hydrogen peroxide and other active oxygen species. In this context, the water-soluble iron chelator deferoxamine (DFO) is mainly used in non-iron overload conditions to produce antioxidant and antiproliferative effects.

In an experimental study, selenium showed good effectiveness in prevention of ADR-induced nephrotoxicity (5). We reported previously that both low plasma selenium levels and reduced glutathione peroxidase (GPX) activities were encountered in patients with nephrotic syndrome (2). Therefore, supplementation of selenium may have ameliorating effects on nephrotic syndrome. The purpose of the present study was to evaluate the efficacy of NAC, DFO and selenium in protection against renal injury in ADR nephropathy.

SUBJECTS AND METHODS

Animals and treatments

The study included 53 Sprague Dawley male rats (mean weight 250 ± 50 g). All rats were housed in pathogen-free conditions, with a 12-hour light/dark cycle, using standard animal cages with free access to food and water. Nephrotic syndrome was induced in 46 rats by injection of ADR 5 mg/kg intravenously via the tail vein under anaesthesia. Control rats ($n=7$) were injected with an equal volume of isotonic saline. After ADR administration, the study rats were divided into a group given only ADR ($n=17$) and 3 antioxidant treatment groups: (i) NAC ($n=10$), (ii) DFO ($n=10$) and (iii) selenium ($n=9$). Both NAC and DFO were administered intravenously in a single dose of 20 mg/kg. Selenium was given intraperitoneally at a dose of 15 mg/kg. All of these agents were given immediately after ADR injections. The animal ethics committee of Gülhane Military Medical Academy approved all animal procedures. The spontaneously voided urine specimens from each animal were collected by using the metabolic cage, for determination of protein and creatinine on the night before sacrifice. Blood samples were obtained by substernal cardiac puncture under anaesthetic and then rat were sacrificed by pneumocardectomy. Blood samples were drawn into tubes coated with sodium-ethylenediaminetetraacetic acid (EDTA).

Measurements

Blood chemistry tests such as serum creatinine, total cholesterol, triglycerides and albumin were analyzed using an autoanalyzer (Olympus AU 2700; Olympus Diagnostics, Hamburg, Germany), and commercially available kits were used according to the manufacturer's instructions. Urinary protein was measured by a colorimetric method

with the Olympus AU 2700 autoanalyzer using reagents from Olympus Diagnostics (Hamburg, Germany). Urinary creatinine was measured by modified Jaffe method with the Olympus AU 2700 autoanalyzer using reagents from Olympus Diagnostics (Hamburg, Germany). Urinary protein to urinary creatinine ratio (PCR) was also calculated. Plasma and erythrocyte fractions were separated from whole blood by centrifugation at 4,000g for 10 minutes. The erythrocyte fractions were washed 3 times with saline. Then erythrocytes were lysed with cold distilled water (1:4), stored at 4°C for 15 minutes, and membranes were removed by centrifugation at 4°C for 30 minutes at 20,000g. Plasma samples and erythrocyte lysate were stored at -70°C until assay.

Rats were sacrificed on day 28 after ADR administration. One kidney was removed before sacrifice and stored at -80°C until the assay was performed for determination of renal oxidative stress parameters in tissue homogenates. Tissues were homogenized with ice-cold KCl (1.15%) using a glass homogenizer. The homogenates were then centrifuged at 4,400g for 10 minutes at 4°C to remove the cell debris, and the supernatant obtained was used for the determination of antioxidant markers.

Erythrocyte and renal tissue oxidative system parameters and trace elements

GPX activity in erythrocyte lysate was measured by the method of Pleban et al (7). The reaction mixture was 50 mmol/L Tris buffer, pH 7.6, containing 1 mmol/L Na_2 EDTA, 2 mmol/L reduced glutathione (GSH), 0.2 mmol/L nicotinamide adenine dinucleotide phosphate, reduced form, (NADPH), 4 mmol/L sodium azide and 1,000 U glutathione reductase (GR). Twenty microliters of lysate and 980 μL of reaction mixture were incubated for 5 minutes at 37°C. Then, the reaction was initiated with 8.8 mmol/L H_2O_2 , and the decrease in NADPH absorbance at 340 nm was recorded for 3 minutes. Erythrocyte CuZn-superoxide dismutase (SOD) activity was measured as described by Fitzgerald et al (8). Erythrocyte lysate samples were diluted with 10 nm phosphate buffer (pH 7.0) about 400 fold and mixed with 850 μL substrate solution containing 0.05 mmol/L xanthine and 0.0025 mmol/L 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (INT) in a buffer solution containing 50 mmol/L 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS) and 0.94 mmol/L EDTA (pH 10.2). Then, 125 μL xanthine oxidase (80 U/L) was added to the mixture, and absorbance increase was followed at 505 nm for 3 minutes against air blank. Tissue SOD and

GPX activities were measured on a UV-VIS Recording Spectrophotometer (UV-2100S; Shimadzu Co, Kyoto, Japan) as previously described by Aydin et al (9). Tissue SOD and GPX activities are expressed in U/g tissue.

Erythrocyte and renal tissue catalase (CAT) activities were measured in hemolysates and supernatant of kidney homogenates, respectively, at 25°C by the method of Aebi (10). The decomposition rate of the substrate H₂O₂ was monitored spectrophotometrically at 240 nm for 30 seconds. The activity is expressed in MU/L for erythrocytes and mU/g tissue for tissue homogenates; 1 U is equal to 1 μmol of H₂O₂ decomposed/minute.

Erythrocyte and tissue malondialdehyde (MDA) as an estimation of lipid peroxidation were measured as thiobarbituric acid reactive substances in both erythrocyte lysates and supernatant of kidney homogenates in accordance with the methods previously described (2, 9). After the reaction of MDA with thiobarbituric acid, the reaction product was followed spectrophotometrically at 532 nm, using tetramethoxypropane as a standard. The results are expressed as nmol/mL for erythrocytes and nmol/g tissue for renal tissue.

Erythrocyte and tissue zinc (Zn) levels were measured by flame atomic absorption spectrophotometry using a

Varian Atomic Absorption Spectrophotometer (30/40 model; Varian Techtron Pty Ltd, Victoria, Australia). The results were expressed as μg/mL for erythrocytes and μg/g tissue for renal tissue. Plasma and tissue Cu levels were measured by atomic absorption spectrophotometry. The results were expressed as μg/dL for plasma samples and μg/g tissue for renal tissue. Tissue iron concentrations were determined by the atomic absorption spectrophotometer. The results were expressed as μg/g tissue for renal tissue. Selenium levels in erythrocytes were measured using an atomic absorption spectrometer and electrothermal atomization technique. Measured selenium levels were expressed as nanograms per 10⁶ cells.

Statistical analyses

Data were analyzed with SPSS software (SPSS Inc, Chicago, IL, USA). Data are shown as means ± SD. Normality of the parameters was tested with the Kolmogorov-Smirnov test. Parameter differences among the groups were investigated with 1-way ANOVA and least significant difference (LSD) as the post hoc test. The Spearman coefficients of correlation were calculated. A p value of ≤0.05 was considered as significant.

TABLE I

COMPARISON OF BLOOD AND URINARY PARAMETERS OF CONTROL RATS, ADRIAMYCIN-ONLY AND TREATMENT GROUPS (1-WAY ANOVA AND LEAST SIGNIFICANT DIFFERENCE)

Parameters	Control rats (n=7)	Adriamycin- only rats (n=17)	N-Acetylcysteine- treated rats (n=10)	Deferoxamine- treated rats (n=10)	Selenium- treated rats (n=9)	F	p Value
Urinary protein to creatinine ratio*	15.14 ± 10.86 ^a	92.48 ± 57.45 ^b	12.33 ± 8.67 ^c	34.43 ± 27.55 ^d	56.28 ± 57.34 ^e	5.896	0.001
Serum albumin (g/L)	29.3 ± 1.1 ^f	21.0 ± 3.6 ^g	27.8 ± 1.8 ^h	25.3 ± 2.9 ⁱ	25.4 ± 4.7 ^j	11.044	<0.001
Serum total cholesterol (mmol/L)	1.77 ± 0.23 ^k	6.87 ± 4.76 ^l	1.78 ± 0.18 ^m	2.40 ± 0.69 ⁿ	3.33 ± 2.10 ^o	6.919	<0.001
Serum triglycerides (mmol/L)	0.78 ± 0.09 ^p	5.34 ± 3.68 ^q	1.37 ± 0.38 ^r	1.47 ± 0.47 ^s	2.65 ± 1.93 ^t	7.935	<0.001
Plasma copper (μmol/L)	0.19 ± 0.02 ^u	0.12 ± 0.05 ^v	0.19 ± 0.01 ^w	0.26 ± 0.06 ^x	0.18 ± 0.04 ^y	18.062	<0.001

Data are means ± SD, except for statistical values.

*The unit used for urinary protein and creatinine concentrations in the calculation of ratio was mg/dL.

^ap<0.001, vs. ^b, ^b vs. ^c, ^f vs. ^g, ^g vs. ^h, ^k vs. ^l, ^l vs. ^m, ^l vs. ⁿ, ^p vs. ^q, ^q vs. ^r, ^q vs. ^s, ^u vs. ^v, ^v vs. ^w, ^v vs. ^x, ^x vs. ^y; p=0.001, for ^v vs. ^y; p=0.002, for ^g vs. ⁱ, ^g vs. ^j, ^w vs. ^x; p=0.003, for ^b vs. ^d; p=0.005, for ^u vs. ^x; p=0.006, for ^l vs. ^o; p=0.008, for ^q vs. ^t; p=0.016, for ^f vs. ⁱ; p=0.023, for ^f vs. ^j; p=0.029, for ^c vs. ^e, p=0.049, for ^a vs. ^e.

RESULTS

Nephrotic syndrome was proven in ADR-injected rats 4 weeks after injections, with higher PCR ($p < 0.001$), higher serum total cholesterol ($p < 0.001$), higher serum triglycerides ($p < 0.001$) and lower serum albumin ($p < 0.001$) (Tab. I). At that time, proteinuria decreased significantly after NAC ($p < 0.001$) and DFO ($p = 0.003$) treatment, whereas selenium did not. When compared with the ADR-only group, NAC, DFO, and selenium treatments raised the serum albumin levels significantly and decreased the serum total cholesterol and triglyceride levels (Tab. I). Plasma Cu decreased significantly after ADR administration ($p < 0.001$). NAC ($p < 0.001$), DFO ($p < 0.001$) and selenium ($p = 0.001$) treatments increased the plasma Cu levels significantly. Serum creatinine levels did not differ between groups (data not shown).

Tissue MDA did not significantly change after ADR administration. NAC ($p = 0.002$) and selenium ($p < 0.001$) increased the tissue MDA significantly, whereas DFO reduced the tissue MDA ($p < 0.001$) (Tab. II). Tissue SOD ($p = 0.002$) and CAT ($p < 0.001$) activities decreased significantly after ADR administration, whereas GPX activity did not change. Selenium increased tissue SOD activity significantly ($p < 0.001$), whereas NAC or DFO did not. NAC ($p = 0.042$),

DFO ($p = 0.004$) and selenium ($p < 0.001$) increased tissue CAT activity significantly. DFO ($p = 0.003$) and selenium ($p = 0.036$) decreased the tissue GPX activity significantly, whereas NAC did not. Tissue Zn increased after ADR administration ($p = 0.012$), whereas tissue Cu did not. NAC decreased tissue Zn significantly ($p = 0.001$), whereas DFO and selenium did not. DFO decreased tissue Cu significantly ($p < 0.001$), whereas NAC and selenium did not (Tab. II). Tissue iron levels did not differ between groups (data not shown).

Erythrocyte MDA did not significantly differ after ADR administration. DFO decreased the erythrocyte MDA significantly ($p < 0.001$), whereas NAC and selenium did not (Tab. III). Erythrocyte SOD activity increased significantly after ADR administration ($p = 0.011$). NAC increased the erythrocyte SOD activity ($p < 0.001$), whereas DFO ($p = 0.024$) and selenium ($p < 0.001$) reduced it significantly. Erythrocyte CAT and GPX activity did not change after ADR administration. NAC only increased erythrocyte GPX activity significantly ($p = 0.024$), while DFO only increased erythrocyte CAT activity ($p = 0.003$). Erythrocyte Zn decreased significantly after ADR administration ($p < 0.001$). NAC ($p = 0.007$), DFO ($p < 0.001$) and selenium ($p = 0.003$) increased the erythrocyte Zn. Erythrocyte selenium increased significantly after ADR administration

TABLE II

COMPARISON OF OXIDATIVE SYSTEM PARAMETERS AND TRACE ELEMENTS IN THE KIDNEY TISSUE OF CONTROL RATS, ADRIAMYCIN-ONLY AND TREATMENT GROUPS (1-WAY ANOVA AND LEAST SIGNIFICANT DIFFERENCE)

Parameters	Control rats (n=7)	Adriamycin- only rats (n=17)	N-Acetylcysteine- treated rats (n=10)	Deferoxamine- treated rats (n=10)	Selenium- treated rats (n=9)	F	p Value
Malondialdehyde (nmol/g tissue)	6.63 ± 2.16 ^a	5.25 ± 1.45 ^b	7.80 ± 3.11 ^c	2.93 ± 0.51 ^d	10.05 ± 2.27 ^e	17.604	<0.001
Superoxide dismutase (U/g tissue)	201.00 ± 40.79 ^f	112.12 ± 48.47 ^g	131.90 ± 53.21 ^h	158.10 ± 66.10 ⁱ	247.89 ± 85.70 ^j	8.987	<0.001
Catalase (mU/g tissue)	19.43 ± 3.74 ^k	11.29 ± 4.25 ^l	14.40 ± 2.17 ^m	16.00 ± 4.92 ⁿ	25.56 ± 3.21 ^o	21.833	<0.001
Glutathione peroxidase (U/g tissue)	20.81 ± 7.05	24.33 ± 5.48 ^p	24.31 ± 6.28 ^q	17.78 ± 1.15 ^r	19.70 ± 4.36 ^s	3.516	0.013
Zinc (µg/g tissue)	8.18 ± 1.40 ^t	9.98 ± 1.40 ^u	7.80 ± 1.16 ^v	8.98 ± 1.74	9.19 ± 2.02	3.758	0.010
Copper (µg/g tissue)	3.13 ± 1.26 ^w	2.97 ± 1.29 ^x	3.28 ± 0.94 ^y	0.74 ± 0.74 ^z	2.15 ± 0.32 ^{aa}	10.720	<0.001

Data are means ± SD, except for statistical values.

^a $p < 0.001$, vs. ^d, ^b vs. ^e, ^c vs. ^d, ^d vs. ^e, ^g vs. ⁱ, ^h vs. ^j, ^k vs. ^l, ^l vs. ^o, ^m vs. ^o, ⁿ vs. ^o, ^w vs. ^z, ^x vs. ^z, ^y vs. ^z; $p = 0.001$, for ^a vs. ^e, ^u vs. ^v; $p = 0.002$, for ^b vs. ^c, ^f vs. ^g, ⁱ vs. ^j; $p = 0.003$, for ^p vs. ^r, ^k vs. ^o; $p = 0.004$, for ^l vs. ⁿ, ^z vs. ^{aa}; $p = 0.006$, for ^b vs. ^d; $p = 0.007$, for ^q vs. ^r; $p = 0.011$, for ^k vs. ^m; $p = 0.012$, for ^t vs. ^u; $p = 0.018$, for ^c vs. ^e; $p = 0.020$, for ^y vs. ^{aa}; $p = 0.023$, for ^f vs. ^h; $p = 0.036$, for ^p vs. ^s; $p = 0.042$, for ^l vs. ^m.

($p=0.005$). NAC ($p=0.005$), DFO ($p<0.001$) and selenium ($p=0.007$) decreased the erythrocyte selenium (Tab. III). In the DFO-only group, there was a significant negative correlation between tissue MDA and tissue CAT activity ($r=-0.652$; $p=0.005$) and a positive correlation was observed between tissue SOD activity and tissue Zn ($r=0.543$; $p=0.024$). In the NAC group, tissue CAT activity negatively correlated with tissue MDA ($r=-0.735$; $p=0.016$).

DISCUSSION

In reviewing the literature, there are quite a few studies published on the effects of these drugs in this model (11-13). However, the results from these studies are in conflict. Therefore, our study was considered to have a contribution to make to this debate. In the present study, ADR administration induced a severe nephrotic syndrome with proteinuria, hypoalbuminemia and hyperlipidemia. These observed characteristic features of ADR-induced nephropathy are similar to those previously reported by other investigators (4). Tissue and erythrocyte MDA did not significantly change after ADR administration. Similar to our results, Bertolatus et al demonstrated no evidence

of increased lipid peroxidation after administration of ADR (6). However, in a previous report, lipid peroxidation was found to be significantly increased in the blood of rats with ADR nephropathy (14). Saad et al found increased MDA levels 48 hours after Adriamycin injections (15). Bertani et al showed that podocyte damage was observed in the first day after Adriamycin injection and some focal fusion of foot processes could be observed by electron microscopy in the early phase after injection (28 hours) (16). Moreover, colloidal iron staining of kidney biopsy samples revealed loss of glomerular polyanions as early as 3 hours and very marked loss at 28 hours after Adriamycin administration (16). Therefore, we designed the present study to prevent these early effects of ADR on renal tissue by using different antioxidant agents. On the other hand, Bertolatus et al also reported there were no changes in the renal cortical levels of either enzyme activity or immunoreactive protein for the endogenous antioxidant enzymes such as SOD or CAT after administration of ADR (6). Likewise, we observed decreased or unchanged antioxidant enzyme activities of renal tissue after ADR administration. Normally, decreased activities of glomerular antioxidative enzymes might be, at least partly, responsible for increased ROI levels.

TABLE III
COMPARISON OF OXIDATIVE SYSTEM PARAMETERS AND TRACE ELEMENTS IN ERYTHROCYTES OF CONTROL RATS, ADRIAMYCIN-ONLY AND TREATMENT GROUPS (1-WAY ANOVA AND LEAST SIGNIFICANT DIFFERENCE)

Parameters	Control rats (n=7)	Adriamycin- only rats (n=17)	N-Acetylcysteine- treated rats (n=10)	Deferoxamine- treated rats (n=10)	Selenium- treated rats (n=9)	F	p Value
Malondialdehyde (nmol/mL)	3.16 ± 0.89 ^a	3.01 ± 0.88 ^b	2.43 ± 0.85 ^c	1.33 ± 0.19 ^d	2.94 ± 0.58 ^e	10.063	<0.001
Superoxide dismutase (U/g Hb)	369.86 ± 54.12 ^f	507.41 ± 174.18 ^g	679.50 ± 65.25 ^h	400.70 ± 53.39 ⁱ	327.78 ± 92.91 ^j	14.394	<0.001
Catalase (mU/L)	33.92 ± 6.39 ^k	36.46 ± 12.30 ^l	38.69 ± 5.08 ^m	47.12 ± 7.45 ⁿ	40.10 ± 1.31	3.379	0.016
Glutathione peroxidase (U/g Hb)	0.52 ± 0.04	0.52 ± 0.10 ^o	0.59 ± 0.07 ^p	0.50 ± 0.06 ^q	0.53 ± 0.07	2.055	NS
Zinc (µg/mL)	12.96 ± 1.41 ^r	10.42 ± 1.54 ^s	11.81 ± 0.77 ^t	13.20 ± 1.10 ^u	12.04 ± 0.97 ^v	10.149	<0.001
Selenium (ng per 10 ⁶ cells)	463.00 ± 192.27 ^w	737.24 ± 320.73 ^x	496.20 ± 67.37 ^y	318.60 ± 122.05 ^z	497.33 ± 22.01 ^{aa}	7.199	<0.001

Data are means ± SD, except for statistical values.

^a $p<0.001$, vs. ^d, ^b vs. ^d, ^d vs. ^e, ^f vs. ^h, ^g vs. ^h, ^g vs. ⁱ, ^h vs. ⁱ, ^r vs. ^s, ^s vs. ^u, ^x vs. ^z; $p=0.002$, for ^c vs. ^d; $p=0.003$, for ^k vs. ⁿ, ^l vs. ⁿ, ^s vs. ^v; $p=0.005$, for ^w vs. ^x, ^x vs. ^y; $p=0.007$, for ^s vs. ^t, ^x vs. ^{aa}; $p=0.011$, for ^f vs. ^g, ^p vs. ^q; $p=0.015$, for ^t vs. ^u; $p=0.024$, for ^g vs. ⁱ, ^o vs. ^p; $p=0.030$, for ^m vs. ⁿ.

NAC treatment, in contrast to the results in some previous report (6, 12), improved the parameters concerning nephrotic syndrome without any diminishing effect on MDA levels. Although a study suggested that NAC is ineffective for protection in ADR nephropathy (12), that study differed from our study with respect to treatment beginning time, duration, dose and route of administration. In the study of Bertolatus et al (6), NAC showed no significant reduction in proteinuria at 3 weeks after ADR administration. When compared with the present study, NAC dosage used in their study was 10 times higher than that used by us, and their NAC-treated group was smaller (n=4). Since NAC may also have prooxidant effects (17), the possible reason behind these different results seems related to dose-dependent effects of NAC. Moreover, NAC increased antioxidant enzymes such as tissue CAT, erythrocyte SOD and GPX. In the NAC group, tissue CAT activity negatively correlated with tissue MDA. However, these antioxidant enzyme activities seemed unable to cope with increased tissue MDA levels in NAC treatment. Consequently, this could indicate that NAC improves the nephrotic state independently from the oxidant system. Interestingly, although NAC is an oxygen-radical scavenger, it increased the tissue MDA significantly. However, erythrocyte MDA levels showed no increase in the NAC-treated group. It has been reported that the use of NAC alone may have prooxidant effects, due to the facility with which it interacts with iron (17). On the one hand, the oxidative metabolism of NAC can generate thiol-free radicals that have been increasingly considered as intermediates in processes that may be involved in the development of biological damage resulting from oxidative stress (17). In vitro, NAC was reported to increase hydroxyl radical generation in a system with Fe(III)-citrate and H₂O₂ by reducing ferric iron to its catalytic, active Fe⁺² form (17). NAC treatment seemed to increase total nonheme iron content, and this could be associated with the effects of NAC on iron regulatory protein and ferritin synthesis (18). On the other hand, because both increases and decreases of cellular Zn elicit oxidative stress (19), decreased tissue Zn levels after NAC treatment may be another factor in enhanced lipid peroxidation. We also found a positive correlation between tissue SOD activity and tissue Zn. In this way, it has been shown that NAC has also a heavy-metal chelating effect at high intravenous doses, which may induce excessive Zn urinary excretion (20). This effect can still be enhanced by the action of NAC metabolites, among which cysteine is the most powerful Zn-sequestering agent (21).

Because of the central role of iron in the redox cycling of anthracycline-iron complexes (22), DFO has been studied for possible protection against ADR-induced nephrotic syndrome. In DFO-treated rats, like those treated with NAC, we observed a significant decrease in both proteinuria and blood lipid levels, coupled with an increase in serum albumin levels. In contrast to our finding, it has been reported that rats pretreated with DFO had no significant reduction in proteinuria after ADR administration when compared with rats receiving only ADR (6). Moreover, same authors reported that no evidence was seen of increased lipid peroxidation and no changes were seen in the renal cortical enzyme activities of either SOD or CAT after ADR (6). However, in our study, treatment with DFO also reduced both tissue and erythrocyte MDA, whereas it increased both tissue and erythrocyte CAT activity significantly, suggesting that DFO reduced systemic and renal oxidative stress by activating CAT enzyme. Nevertheless, lack of any correlation between MDA and antioxidant enzyme activities in the DFO treatment group may indicate direct scavenging effect of DFO in ADR-induced renal injury. Another protective effect of DFO on oxidative stress may be decreased tissue Cu levels. In vitro studies have suggested that similar to iron, Cu acts as a catalyst in the formation of ROIs that can lead to oxidative stress and destructive lipid peroxidative damage (23). Cu-accelerated lipid peroxidation in human erythrocytes has also been reported (24). In the current study, there was no beneficial role of selenium supplementation in proteinuria, although other parameters related with nephrotic syndrome improved. Interestingly, like NAC, selenium increased MDA levels in kidney tissue. Concerning ADR toxicity, several animal models, with different results, have been assayed. Hermansen and Wassermann did not observe any protection against ADR from selenium in mice (25). However, in another group of studies, beneficial effects of selenium supplementation have been described (26). The different results observed with NAC, DFO and selenium in the present study may be explained by usage of different types of antioxidants. The present study was designed to explore the most effective among well-known antioxidants. On the other hand, it is also known that scavenger enzymes exhibit different activity levels in different tissues (27). This may also be a factor influencing our results. Human studies of improvements in nephrotic state by administering the various antioxidants, based upon preliminary data from studies in animals, are also needed, and it will be interesting to see if these human studies will agree with animal studies.

Several limitations in this study need to be mentioned. First, although nephrotic syndrome is a glomerular disease, we had to conduct the study in whole kidney homogenates. Second, as redox cycling reactions of ADR have been shown to happen within minutes or hours, it would be more informative to determine the effect of ADR and antioxidants at earlier times after ADR injections, as well. Third, direct measurement of ROIs instead of MDA would be better. Fourth, histological analysis of renal tissue with respect to oxidative stress would render this study more meaningful. Fifth, it would also be more helpful to give the antioxidant agents in different doses and durations to confirm their preventive and therapeutic effects and to compare the results obtained with single doses versus repeated doses, with respect to improving clinical effects on nephrotic state, such as reducing proteinuria. In conclusion, our results suggest that concomitant administration of some antioxidants with ADR injections seemed to have beneficial effects on clinical parameters even if antioxidants were given in a single dose. Moreover, all of the antioxidant agents used in the present study to prevent the development of nephrotic syndrome provided benefits for the nephrotic state in varying degrees. Of them, selenium in particular seemed to have a relatively lower and statistically insignificant efficacy for preventing proteinuria, compared with the others. Furthermore, it also seems that NAC and DFO improve markedly the parameters related to nephrotic state. Although they affect some of the antioxidant system parameters differently both in tissue and erythrocytes, their functions in relieving the

nephrotic state seem to be a result of their direct scavenging properties, as indicated by the lack of any significant correlation between lipid peroxidation and scavenging enzymes in the DFO- and NAC-treated groups. To investigate the development of nephrotic syndrome and the treatment options, serial determinations of the parameters in the course of the disease and prolonged treatment with NAC, DFO and selenium should be undertaken. In any case, NAC and DFO show promise for the treatment of human nephrotic syndrome.

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