Detrimental Effects of N-Acetylcysteine Plus Desferoxamine Combination in an Experimental Nephrotic Syndrome Model


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The aim of this study was to evaluate the effects of N-acetylcysteine (NAC) and desferoxamine (DFO) administered alone or in combination together in rats with doxorubicin (DOX)-induced nephrotic syndrome, by monitoring oxidative stress parameters and trace elements in renal tissue and erythrocytes. Fifty-four male Sprague-Dawley rats were included the study. Equal volume of isotonic saline was injected to control rats. After DOX administration, the animals were divided into four experimental groups: (a) rats given only DOX; (b) rats treated with NAC; (c) rats treated with DFO; (d) rats treated with NAC plus DFO. The combination of N-acetylcysteine and DFO has no beneficial effect on reducing proteinuria in experimentally nephrotic rats, although both of these agents ameliorate the condition when administered separately. It seems likely that detrimental effects of NAC plus DFO could be secondary to its effects on erythrocyte selenium levels demonstrated here. Consequently, the results may propose caution to the use of antioxidant therapeutic strategies such as NAC plus DFO against nephropathy.

Keywords Desferoxamine, Doxorubicin, N-Acetylcysteine, Nephrotic Syndrome

Oxidative damage by free radicals has been implicated in many disease conditions (Ozata et al. 2002), including pathogenesis of nephrotic syndrome (Bulucu et al. 2000). Reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide are capable of causing tissue damage and inflammation. Doxorubicin (DOX) has been widely used for the treatment of a variety of carcinomas and sarcomas. However, DOX was reported to induce changes in glomerular capillary permeability, tubulointerstitial damage with heavy proteinuria, hypoalbuminemia, and hyperlipidemia known as nephrotic syndrome (Hall, Wilke, and Fettman 1986; Bertani et al. 1982; Okasora et al. 1992). It has been reported that DOX leads to direct oxidative injury to DNA (Feinstein, Canaani, and Weiner 1993) and generates lipid peroxidation (Huertas et al. 1992). DOX treatment also induced peroxidative alterations in various tissues, which were evident by significant elevation in malondialdehyde (MDA) production and depletion of glutathione contents in the rat kidney (Saad, Najjar, and Al-Rikabi 2001). DOX-induced nephrotic syndrome in rats is a suitable model for rapidly observing the oxygen free radicals that are possible mediators of renal injury and protective effects of investigated antioxidant interventions on this experimental damage (Okasora et al. 1992).

Pharmacologic methods of interrupting the cycle of ROS generation have involved numerous antioxidants, such as N-acetylcysteine (NAC), desferoxamine (DFO), and metal...
catalysts (selenium, manganese, copper [Cu], zinc [Zn]) of anti-
oxidative enzymes. In this way, NAC is widely used antioxidant
agent in clinical and preclinical setting. NAC is the acetylated
precursor of both the amino acid l-cysteine and reduced glu-
thione. NAC supplementation was found to reduce oxidative
stress by improving the thiol redox status, to inhibit neutrophil
and monocyte chemotaxis and oxidative metabolism, and to
scavenge superoxide, hydrogen peroxide, and hydroxyl radicals
(Araujo et al. 1989; Bernard et al. 1984). However, the use of
NAC alone may have limitations and present pro-oxidant effects,
due to the facility with which it interacts with iron (Ritter et al.
2004a). Therefore, it seems reasonable to use an iron chelator in
addition to NAC to improve its therapeutic effects. DFO is an
indiscriminate and very powerful iron chelator. DFO acts as a
direct scavenger of superoxide anion, an activated oxygen species
that participates in hydroxyl radical formation (Meraszhe et al.
1987). DFO eliminates the harmful effects of iron overloading
and resulted in a partial inhibition of DOX toxicity in both nor-
mal and iron-loaded cultured heart cells (Link et al. 1996). It
seems likely that the combination of drugs is more efficient than
the use of either alone. Recent reports demonstrated that the
combination of NAC plus DFO, but not their isolated use, is
an effective treatment of severe sepsis (Ritter et al. 2004a) and
acute hepatic failure rats (Ritter et al. 2004b). It is possible that
the addition of DFO to the NAC regimen prevents its oxidation,
and the occurrence of the Fenton chemistry maintained by the
iron recycling mediated by NAC.

There are no reports in the literature that describe the ef-
ects of NAC plus DFO in animal or humans with nephrotic
syndrome. Therefore, the main aim of the present study was to
verify whether NAC administered alone or in combination with
DFO significantly reduced the oxidative damage in the kidneys
of rats with DOX-induced nephrotic syndrome.

MATERIALS AND METHODS

Experimental Design
Fifty-four male Sprague-Dawley rats (mean weight
250 ± 50 g) supplied by the Research Center of Gülhane Mili-
tary Medical Academy were used in the study. They were kept
on a 12-h light/dark cycle, in a room with a temperature of
24°C ± 1°C and humidity 50% ± 10%. All animals had ad li-
bition access to food and water throughout the course of the ex-
periment. They were fed with a standard laboratory diet. Nephrotic
syndrome was induced by single intravenous (i.v.) administra-
tion of 5 mg/kg DOX intravenously via the tail vein under anes-
thesia (n = 47). An equal volume of isotonic saline was injected
to control rats (control group) (n = 7). After DOX administra-
tion, the animals were divided into four experimental groups:
(a) rats given only DOX (DOX group) (n = 17); (b) rats treated
with NAC (NAC group) (n = 10); (c) rats treated with DFO
(DFO group) (n = 10); (d) rats treated with NAC plus DFO
(NAC plus DFO group) (n = 10). Both NAC and DFO were
administered in a single dose of 20 mg/kg intravenously. Exper-
iments were conducted according to the guidelines of Animal
Ethical Committee of Gülhane Military Medical Academy.

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 sam-
pies were obtained by substernal cardiac puncture under anes-
thetic and then sacrificed by pneumocardectomy. Blood samples
for oxidative stress parameter analysis were drawn into tubes
coated with Na-EDTA.

Biochemical Procedure
Blood chemistry tests (serum creatinine, total cholesterol,
triglyceride, and albumin) were analyzed on autoanalyzer
(Olympus AU 2700; Olympus Diagnostics, Hamburg, Germany)
and commercially available kits were used by using the methods
according to the manufacturer’s instructions.

Urinary protein was measured by a colorimetric method, ur-
inary creatinine was measured by modified Jaffe method, both
with an Olympus AU 2700 autoanalyzer using reagents from
Olympus Diagnostics. Urinary protein to urinary creatinine ra-
tio was also calculated.

Plasma Cu levels were measured by atomic absorption spec-
trophotometry.

Plasma and erythrocyte fractions were separated from whole
blood by centrifugation at 4000 × g for 10 min. The erythrocyte
fractions were washed three times with saline. Then, erythro-
cytes were lysed with cold distilled water (1:4), stored at 4°C
for 15 min and membranes were removed by centrifugating at
4°C for 30 min with 20000 × g. Plasma samples and erythrocyte
lysate were stored at −70°C until assay.

Oxidative System Parameters and Trace Elements in
Erythrocytes
Erythrocyte thiobarbituric acid reactive substances (TBARS)
as the estimation of lipid peroxidation were determined in ery-
throcyte lysates by the method previously described by Aydin
et al. (2001). 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.

Glutathione peroxidase (GSH-Px) activity in erythrocyte
lysate was measured with the method of Pleban, Munyani,
and Beachum (1982). The reaction mixture was 50 mmol/L Tris
buffer, pH 7.6 containing 1 mmol/L NADH, 2 mmol/L re-
duced glutathione, 0.2 mmol/L NADPH, 4 mmol/L sodium
azide, and 1000 U glutathione reductase. Twenty microliters of
lysat and 980 μl of reaction mixture were incubated for 5 min
at 37°C. Then, the reaction was initiated with 8.8 mmol/L H2O2
and the decrease in NADPH absorbance at 340 nm was recorded
for 3 min. The results are expressed as u/g/h.
Erythrocyte copper zinc-superoxide dismutase (CuZn-SOD) activity was measured by the method described by Fitzgerald, Campbell, and Lamont (1992). Erythrocyte lysate samples were diluted with 10 mm 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 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 min against air blank. The results are expressed as μg/g.

Erythrocyte catalase (CAT) activity was measured in hemolysates at 25°C by the method of Aebi (1984). The activity is expressed as mU/L. One unit is equal to 1 μmol of H₂O₂ decomposed/min.

Erythrocyte Zn levels were measured by flame atomic absorption spectrophotometry using Varian Atomic Absorption Spectrophotometer (model 3040; Varian Techtron, Victoria, Australia). The results are expressed as μg/ml.

Selenium levels in erythrocytes were measured using an atomic absorption spectrometer and electrothermal atomization technique. Measured selenium levels were expressed as ng/ml.

Statistical Analysis
Data were analyzed with SPSS (SPSS, Chicago, IL, USA) software. Descriptives were shown as the mean ± SD notation. Normality of the parameters were tested with Kolmogorov-Smirnov test. Parameter differences among the groups were investigated with one-way analysis of variance (ANOVA) and least significance difference (LSD) as the post hoc test. Spearman coefficients of correlation were calculated. p value was set at ≤0.05.

RESULTS
Full-blown nephrotic syndrome developed 4 weeks after DOX administration. In DOX and NAC plus DFO groups, proteinuria, hypoalbuminemia, hypercholesterolemia, and hypertriglyceridemia were significantly different compared to those of controls (Table 1). Single dose of both NAC and DFO administered simultaneously with DOX ameliorated proteinuria, hypoalbuminemia, hypercholesterolemia, and hypertriglyceridemia (p < .001, for all parameters). Plasma Cu reduced significantly after DOX administration as compared to controls (p < .001). DFO was more effective to cause an elevation in plasma Cu levels than other treatments (Table 1). Serum creatinine levels did not differ among groups (data not shown).

Activity of both CAT and CuZn-SOD in kidney tissue was significantly decreased in DOX group when compared to Control group (Table 2). Despite these decreases in above-mentioned enzyme activities, GSH-Px activity remained unchanged and, surprisingly, TBARS levels of controls and DOX-treated groups were not different significantly from each other (Table 2). Tissue Zn levels showed a significant increase in DOX group when compared to controls (Table 2). Erythrocyte selenium levels increased in statistically significant fashion in the DOX-treated group when compared to controls, whereas erythrocyte Zn levels decreased significantly (Table 3).

When animals treated simultaneously with DOX and NAC were compared to only DOX-treated ones, tissue CAT activities had a statistically increase in NAC group, whereas scavenger enzyme activities showed no improvement (Table 2). Tissue TBARS levels in the NAC-treated group showed a significant increase in comparison with only DOX group (Table 2). Tissue Cu content increased in NAC-treated group when compared to DOX-alone group (Table 2). NAC administration caused a significant increase both in erythrocyte CuZn-SOD activity and Zn levels, whereas it reduced erythrocyte selenium levels significantly (Table 3).

DFO administration along with DOX increased tissue CAT activity and decreased tissue GSH-Px activity, TBARS, Zn, and Cu levels (Table 2). When DFO was given with DOX, it increased erythrocyte CAT activity and Zn levels, whereas erythrocyte TBARS and selenium levels showed statistically significant decreases in DFO added group (Table 3).
TABLE 1
Comparison of the blood and urinary parameters of the rats with Control, DOX, NAC, DFO, and NAC plus DFO groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>DOX group</th>
<th>NAC group</th>
<th>DFO group</th>
<th>NAC plus DFO group</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 17)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>29.3 ± 1.1</td>
<td>21.0 ± 3.6</td>
<td>27.8 ± 1.8</td>
<td>25.3 ± 2.9</td>
<td>20.2 ± 2.5</td>
<td>20.005</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/L)</td>
<td>1.77 ± 0.23</td>
<td>6.87 ± 4.76</td>
<td>1.78 ± 0.18</td>
<td>2.40 ± 0.69</td>
<td>7.01 ± 3.18</td>
<td>8.008</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serum Triglyceride (mmol/L)</td>
<td>0.78 ± 0.09</td>
<td>5.34 ± 3.68</td>
<td>1.37 ± 0.38</td>
<td>1.47 ± 0.47</td>
<td>3.58 ± 1.43</td>
<td>8.664</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Plasma copper (nmol/L)</td>
<td>0.19 ± 0.02</td>
<td>0.12 ± 0.05</td>
<td>0.19 ± 0.01</td>
<td>0.26 ± 0.06</td>
<td>0.16 ± 0.06</td>
<td>15.492</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Urinary protein/creatinine ratio</td>
<td>15.14 ± 10.86</td>
<td>92.48 ± 57.45</td>
<td>12.33 ± 8.67</td>
<td>34.43 ± 27.55</td>
<td>67.49 ± 18.50</td>
<td>10.565</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Note: DOX, doxorubicin; NAC, N-acetylcysteine; DFO, desferoxamine.

p < .001 for a versus b, a versus e, b versus c, b versus d, c versus e, d versus e, g versus h, l versus m, p versus q, q versus r, q versus s, s versus t, u versus v, v versus w, w versus x; p = .001 for f versus g, f versus j, g versus i, h versus j, k versus l, k versus o, l versus n, w versus y; p = .002 for i versus j, u versus y; p = .003 for r versus s; p = .006 for a versus d; p = .009 for p versus s; p = .022 for q versus t; p = .026 for x versus y; p = 0.041 for n versus o; p = .044 for m versus o.

Tissue TBARS in NAC plus DFO group was statistically significant higher than those of only DOX-treated rats (Table 2). Both tissue Zn and Cu levels had statistically significant declines in animals treated with NAC plus DFO compared to those in the DOX-only group (Table 2). NAC plus DFO administration along with DOX caused a statistically significant decrease in CuZn-SOD activity and TBARS levels in erythrocytes when compared to the DOX-alone group (Table 3). On the other hand, both

TABLE 2
Comparison of the oxidative system parameters and trace elements in the kidney tissue of the rats with Control, DOX, NAC, DFO, and NAC plus DFO groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>DOX group</th>
<th>NAC group</th>
<th>DFO group</th>
<th>NAC plus DFO group</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 17)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase (mU/g tissue)</td>
<td>19.43 ± 3.74</td>
<td>11.29 ± 4.25</td>
<td>14.40 ± 2.17</td>
<td>16.00 ± 4.92</td>
<td>9.70 ± 2.41</td>
<td>9.714</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CuZn-superoxide dismutase (U/g tissue)</td>
<td>201.00 ± 40.79</td>
<td>112.12 ± 48.47</td>
<td>131.90 ± 53.21</td>
<td>158.10 ± 66.10</td>
<td>84.70 ± 39.68</td>
<td>6.693</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glutathione peroxidase (U/g tissue)</td>
<td>20.81 ± 7.05</td>
<td>24.33 ± 5.48</td>
<td>24.31 ± 6.28</td>
<td>17.78 ± 1.15</td>
<td>25.62 ± 7.04</td>
<td>3.245</td>
<td>.019</td>
</tr>
<tr>
<td>TBARS (nmol/g tissue)</td>
<td>6.63 ± 2.16</td>
<td>5.25 ± 1.45</td>
<td>7.80 ± 3.11</td>
<td>2.93 ± 0.51</td>
<td>7.39 ± 2.47</td>
<td>9.368</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Zinc (μg/g tissue)</td>
<td>8.18 ± 1.40</td>
<td>9.98 ± 1.40</td>
<td>7.80 ± 1.16</td>
<td>8.98 ± 1.74</td>
<td>6.37 ± 0.91</td>
<td>12.384</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Copper (μg/g tissue)</td>
<td>3.13 ± 1.26</td>
<td>2.97 ± 1.29</td>
<td>3.28 ± 0.94</td>
<td>0.74 ± 0.74</td>
<td>0.84 ± 0.24</td>
<td>16.605</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Note: TBARS, thiobarbituric acid reactive substances; DOX, doxorubicin; NAC, N-acetylcysteine; DFO, desferoxamine.

p < .001 for a versus b, a versus e, d versus e, f versus g, f versus j, g versus i, h versus j, k versus l, k versus o, l versus n, w versus y; p = .002 for i versus j, u versus y; p = .003 for r versus s; p = .006 for a versus d; p = .009 for p versus s; p = .022 for q versus t; p = .026 for x versus y; p = 0.041 for n versus o; p = .044 for m versus o.
NAC PLUS DESFEROXAMINE IN EXPERIMENTAL NEPHROTIC SYNDROME

TABLE 3
Comparison of the oxidative system parameters and trace elements in erythrocytes of the rats with Control, DOX, NAC, DFO, and NAC plus DFO groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (n = 7)</th>
<th>DOX group (n = 17)</th>
<th>NAC group (n = 10)</th>
<th>DFO group (n = 10)</th>
<th>NAC plus DFO group (n = 10)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (mU/L)</td>
<td>33.92 ± 6.39a</td>
<td>36.46 ± 12.30b</td>
<td>38.69 ± 5.08c</td>
<td>47.12 ± 7.45d</td>
<td>41.60 ± 8.02</td>
<td>3.101</td>
<td>.024</td>
</tr>
<tr>
<td>CuZn-superoxide dismutase</td>
<td>369.86 ± 54.12e</td>
<td>507.41 ± 174.18f</td>
<td>679.50 ± 65.25g</td>
<td>400.70 ± 53.39h</td>
<td>312.10 ± 68.53i</td>
<td>16.673</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Glutathione peroxidase (U/gHb)</td>
<td>0.52 ± 0.04</td>
<td>0.52 ± 0.10d</td>
<td>0.59 ± 0.07e</td>
<td>0.50 ± 0.06f</td>
<td>0.57 ± 0.05g</td>
<td>2.923</td>
<td>.030</td>
</tr>
<tr>
<td>TBARS (nmol/ml)</td>
<td>3.16 ± 0.89a</td>
<td>3.01 ± 0.88h</td>
<td>2.43 ± 0.85e</td>
<td>1.33 ± 0.19w</td>
<td>1.38 ± 0.24v</td>
<td>15.791</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Selenium (ng/ml)</td>
<td>463.00 ± 192.27k</td>
<td>737.24 ± 320.73l</td>
<td>496.20 ± 67.37m</td>
<td>318.60 ± 122.05n</td>
<td>1339.60 ± 97.07o</td>
<td>36.699</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Zinc (µg/ml)</td>
<td>12.96 ± 1.41l</td>
<td>10.42 ± 1.54o</td>
<td>11.81 ± 0.77c</td>
<td>13.20 ± 1.10r</td>
<td>12.36 ± 1.59s</td>
<td>8.876</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Note. TBARS, thiobarbituric acid reactive substances; DOX, doxorubicin; NAC, N-acetylcysteine; DFO, desferoxamine.

*p < 0.001 for e versus g, f versus g, f versus i, h versus i, n versus q, o versus r, p versus q, p versus s, versus w, t versus w, u versus w, v versus w, x versus y, z versus w; p = .001 for y versus z; p = .003 for z versus w, p = .005 for a versus b, c versus d, s versus t, t versus u; p = .008 for e versus f, k versus l; p = .012 for f versus h, j versus k, y versus z, p = .020 for f versus h, j versus k, y versus z, p = .026 for l versus m, z versus w; p = .042 for c versus d, n versus p, o versus p.

erythrocyte selenium and Zn contents significantly increased in rates treated with NAC plus DFO after DOX administration (Table 3).

In the DOX-only group, there was a negative significant correlation between tissue TBARS and tissue CAT activity (r = -.652; p = .005), and a positive correlation was observed between tissue CuZn-SOD activity and tissue Zn (r = .543; p = .024). In NAC group, tissue CAT activity negatively correlated with tissue TBARS (r = -.735; p = .016).

DISCUSSION

In the present study, single-dose DOX administration induced nephrotic syndrome with proteinuria, hypoalbuminemia, and hyperlipidemia in rats. The lipid peroxidation product, tissue and erythrocyte TBARS, after DOX administration showed no significant change in the present study. This is in contrast to reports of other workers, where there has been an increase in the concentrations of TBARS in nephrotic syndrome (Fdyryk et al. 1998; Kinra, Rath, and Kahi 2000; Rajbala et al. 1997; Turi et al. 1997). However, similar to our results, it has been suggested that there is no evidence of increased lipid peroxidation or changes in the activity of antioxidant enzymes such as CuZn-SOD or CAT after administration of DOX (Bertolatus et al. 1991). We also observed decreased antioxidant enzyme activities, in spite of unchanged TBARS levels, of renal tissue after DOX administration. Classically, peroxidation of lipid membranes raises the concentration of the by-product TBARS, which results in a lowering of the concentration of antioxidants as a result of consumption. Therefore, our findings suggest that lipid peroxidation is not the main cause of DOX-induced nephrotoxicity.

Our results demonstrated that NAC lowered proteinuria and blood lipid levels and enhanced serum albumin levels without any declining effect on lipid peroxidation. Activities of antioxidant enzymes such as tissue CAT, erythrocyte CuZn-SOD, and GSH-Px increased significantly in NAC group. Moreover, tissue TBARS negatively correlated with tissue CAT activity. Classically, glutathione (GSH), the most abundant low-molecular-weight thiol in animal cells, plays a central role in the antioxidant defense against ROS. For tissue GSH synthesis, the availability of cysteine is generally the limiting factor, and one of the effective precursors of cysteine is its synthetic derivative, NAC. NAC may also provide SH groups for GSH synthesis and scavenge ROS itself by enhancing glutathione S-transferase, an antioxidant enzyme, activity. In the present study, our observations concerning an increase in some of antioxidant enzyme activities (catalase in kidney tissue, CuZn-SOD and GSH-Px in erythrocytes) may be attributed to direct superoxide scavenging effect of NAC related to its thiol group (Aruoma et al. 1989). This direct antioxidant effect may ameliorate both antioxidant enzyme activities and nephrotic state after NAC administration.
It seems likely that these increased antioxidant enzyme activities are unable to cope with increased tissue TBARS levels due to NAC treatment. Thus, this allowed us to speculate that NAC ameliorate nephrotic state independently from oxidative stress in DOX-induced nephrotoxicity.

In our study, TBARS levels in kidney tissue increased in NAC group. NAC is a well-known oxygen-radical scavenger, but the use of NAC alone may have limitations and present pro-oxidant effects, due to the facility with which it interacts with iron (Ritter et al. 2004a). The oxidative metabolism of NAC can generate thyl free radicals, which are increasingly considered as intermediates in processes that may be involved in the development of biological damage resulting from oxidative stress (Sagrista et al. 2002). In vitro, NAC increased hydroxyl radical generation in a system with Fe(III) citrate and H2O2 by reducing ferric iron to its catalytic, active Fe(II) form (Sagrista et al. 2002; Sprong et al. 1998). 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 (Smith et al. 2003; Corna et al. 2004). On the other hand, decreased tissue Zn levels after NAC treatment may be another factor for enhanced lipid peroxidation. We also observed a positive correlation between tissue CuZn-SOD activity and tissue Zn. In spite of the fact that Zn ions are redox inert in biology, they have profound effects on redox metabolism (Martel et al. 2006). Both Zn deficiency and Zn overload elicit oxidative stress. In this context, NAC is known to exhibit a Zn-chelating probability (Martell and Smith 1992), and cysteine, a NAC metabolite, is the most powerful Zn-sequestering agent (Huertas et al. 1992).

DFO treatment, like NAC, improved the parameters concerning nephrotic syndrome with significant diminishing effect on both tissue and erythrocyte TBARS levels. In addition, tissue and erythrocyte CAT activity increased significantly after DFO treatment. These may suggest that DFO reduced systemic and renal oxidative stress. Conversely, Bertolatus et al. (1991) reported that rats pretreated with DFO had no significant reduction in proteinuria after DOX administration when compared with rats receiving only DOX. Moreover, same authors reported that no evidence was observed of increased lipid peroxidation or no changes were observed in the activity of CuZn-SOD or CAT after DOX (Bertolatus et al. 1991). Their roles in causing various diseases and the catalytic effects of iron in this process have generated much interest in the possible benefits of iron chelators in preventing oxidative tissue damage in experimental nephrotic syndrome. It has been reported that evidence for the Fenton-like ability of ferrioxamine reduced by pararquat cation radicals to consume H2O2 and produce hydroxyl radicals in the process (Borg and Schaich 1986). Similar reactions may explain previously reported potentiation of the oxidizing toxicity of pararquat in rats by high doses of DFO, as well as several other examples of prooxidant actions of DFO in vivo. The authors (Borg and Schaich 1986) hypothesized that biphasic antioxidant/prooxidant behavior of DFO as a function of dose may be common with iron-catalyzed oxidizing reactions when mobile, strong reducing agents are present. Hence, the real possibility of amplifying oxidizing damage must be considered when planning treatment with DFO, and failure of DFO to inhibit a particular response to oxidizing stress or its enhancement by DFO cannot, by itself, be considered sufficient evidence to rule out an iron-dependent process.

Another preventive effect of DFO on oxidative stress may be decreased tissue Cu levels. DFO is more specific for iron than some of the other iron chelators. Metal binding constant of DFO is 30.6 for ferric iron, 14.0 for Cu, 11.0 for cobalt, and 11.1 for Zn (Dayam et al. 2004). Cu-accelerated lipid peroxidation in human erythrocytes has also been reported (Hochstein, Kumar, and Forman 1980). Cu-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical by a mechanism analogous to the iron-catalyzed Harber-Weiss reaction (Hanna and Mason 1992). Consequently, lack of any correlation between TBARS and antioxidant enzyme activities in DFO treatment group may indicate direct scavenging effect of DFO on DOX-induced renal injury.

In an attempt to maximize renal protection after DOX administration, we tested NAC and DFO in combination, without expecting the detrimental effects of this combination on nephrotic state parameters. Our data provide the first experimental demonstration that NAC plus DFO worsened proteinuria, hypoalbuminemia, hypercholesterolemia, and hypertriglyceridemia, depressed antioxidant enzymes, and decreased tissue Zn levels. In addition, the demonstrated effect of NAC plus DFO on erythrocyte selenium levels seems to be of importance. Selenium is a well-known modulator of cell growth and the biological effects are highly concentration dependent (Spallholz 1997; Wilson et al. 1992). In lower concentrations, selenium stimulates cell growth and induces synthesis of selenoproteins. In higher concentrations, as seen in the present study, selenium compounds such as selenite, selenium dioxide, and diselenides turn from antioxidants to pro-oxidants with potent inhibitory effects on cell growth (Spallholz 1997; Wilson et al. 1992). Certain selenium compounds are strongly redox active and act as potent pro-oxidants, oxidizing intracellular thiols nonstoichiometrically, thereby leading to excessive oxidative stress (Spallholz 1997; Spallholz 1994), oxygen consumption, and apoptosis (Harrison et al. 1997; Kim et al. 2001). Finally, because at higher concentrations selenium is extremely toxic and may cause necrosis and tissue destruction, this may be responsible factor for detrimental effect of NAC plus DFO treatment.

Several limitations need to be mentioned. First, although nephrotic syndrome is a glomerular disease, we had to conduct the study in whole kidney homogenates. Second, direct measurements of ROS instead of TBARS would be better. Third, accumulation of intracellular selenium in erythrocytes after NAC plus
DFO treatment needs to be elucidate and one cannot conclude a cause and effect relationship from our observation. Additionally, histological analysis of renal tissue with respect to oxidative stress would render this study more meaningful.

In conclusion, important findings of the present study are (1) lipid peroxidation is not the main cause of DOX-induced nephrotoxicity and (2) DFO has direct scavenging effect on DOX-induced renal injury. Moreover, we, for the first time, demonstrated that the administration of NAC plus DFO has undesirable effect on nephrotic state in rats, although both NAC and DFO have beneficial effect separately. It seems likely that detrimental effects of NAC plus DFO could be secondary to its effects on erythrocyte selenium levels demonstrated here. Consequently, our results may propose caution to the use of antioxidant therapeutic strategies such as NAC plus DFO against nephropathy.

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