Deneysel Nefrotik Sendromda Plazma ve Böbrek Dokusunda Oksidatif Stres Durumu

Oxidative Stress Status in Renal Tissue and Plasma of Experimental Nephrotic Syndrome

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ÖZET

Bu çalışmada, adriamisin ile oluşturulan deneysel sıçan nefrotik sendromunda plazma ve böbrek dokusunda oksidatif sistemin durumu araştırılmıştır. Plazma glutatiyon peroksidaz (GSH-Px) aktivitesi ve malondialdehit (MDA) düzeyleri ile böbrek homojenatlarında MDA düzeyleri, GSH-Px ve süperoksit dizmutaz (SOD) aktiviteleri incelenmiştir. Plazma MDA düzeyleri ve doku SOD aktiviteleri kontroller ile karşılaştırıldığında nefrotik sıçanlarda belirgin olarak daha yüksektir. Doku ve plazma MDA düzeyleri arasında belirgin bir ilişki saptanamazken, nefrotik sıçanlarda üriner protein atılımı ve serum total kolesterol düzeyleri ile plazma MDA düzeyleri belirgin bir pozitif korelasyon içindedir. Nefrotik sıçanlarda serum albümin düzeyleri plazma MDA düzeyleri ile belirgin negatif korelasyon göstermiştir. Bu bulgular, nefrotik sendromlu sıçanlarda saptanan yüksek MDA düzeylerinin esas sorumlusunun artmış renal doku oksidatif stresi olmadığını göstermektedir. Nefrotik durum yüksek plazma MDA düzeylerine belirgin olarak etki ederken, nefrotik sendromun ağırlığı ile plazma MDA düzeylerinin yüksekliği ilşkilidir.

Anahtar sözcükler: adriamisin, nefrotik sendrom, oksidatif stres, plazma, renal doku

ABSTRACT

In the present study, oxidative system status both in plasma and renal tissue of rats with adriamycin induced experimental rat nephrotic syndrome was investigated. Glutathion peroxidase (GSH-Px) activities and malondialdehyde (MDA) levels in plasma and MDA levels, GSH-Px and superoxide dismutase (SOD) activities in kidney homogenates were determined. Plasma MDA levels and tissue SOD activities were significantly higher in nephrotic rats when compared to those of the controls. No correlation was found between plasma and tissue MDA levels while plasma MDA levels of nephrotic rats had positively significant correlations with urinary protein excretion and serum total cholesterol levels. Serum albumin levels showed negatively significant correlations with plasma MDA levels in nephrotic rats. The data suggest that increased oxidative stress in renal tissue seems not to be responsible principally for high plasma MDA levels observed in rats with nephrotic syndrome. Nephrotic state has significant contributions to elevated plasma MDA levels and severity of nephrotic syndrome is related to the high levels of plasma MDA.

Keywords: adriamycin, nephrotic syndrome, oxidative stress, plasma, renal tissue

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Introduction

Oxidative system takes part in numerous physiological processes in organism and it also has a role in ethiopathogenesis and course of various diseases (1,2,3,4,5,6). In recent years, oxidative system and related therapeutic modalities in kidney diseases have been investigated and its importance has been emphasized (7,8,9,10,11,12,13,14,15,16). Reactive oxygen species have been considered to have a role in pathogenesis of glomerular diseases such as minimal change disease and membranous nephropathy, postischemic or toxic acute renal failure and pyelonephritis (8). Renal cells, neutrophils or other cells in circulation may be the sources of reactive

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oxygen species. Oxidant radicals produced in kidney have been associated with tissue injury and they are cleared by antioxidative defence system of the kidney (17,18). An abnormality in oxidative system in patients with nephrotic syndrome has been reported (8,9,10,12,14,15,16). However, it is to be explained whether the antioxidative system changes observed in nephrotic syndrome are due to the similar changes in the glomerulus or they are consequences of nephrotic state of the patients. In the present study, oxidative system in plasma and kidney homogenates of rats with adriamycin induced experimental nephrotic syndrome was investigated. Oxidative system parameters were measured in plasma and it was sought whether the changes in plasma parameters were results of oxidative stress status in glomeruli or they are due to nephrotic state.

Material and Methods

Twenty male Sprague-Dawley rats (surviving ones of 35) weighing between 200 and 300 g were included in the study. They were fed with standard laboratory chow and tap water with free access. Adriamycin (Adriablastina, Deva Ilaç, Turkey) at a dose of 5 mg/kg was administered intravenously via their penile veins under anaesthesia. The rats became proteinuric during the 2nd and 4th weeks following injections. Proteinuria was detected in urine samples collected using metabolic cage. 24-hour urinary protein was measured by trichloroacetic acid precipitation, with a spectrophotometer (ERMA AE-300, Japan). Blood samples were obtained by cardiac puncture and immediately thereafter they were scarified and one kidney was removed to prepare homogenate. Glutathion peroxidase (GSH-Px) activities and malondialdehyde (MDA) levels were determined in both plasma and tissue. Copper-zinc superoxide dismutase (Cu-Zn SOD) activities were measured only in tissue. Additionally, serum total protein, albumin, total cholesterol and creatinin levels were also determined. The control group was consisted of 10 male rats from the same species with same weight range. Isotonic saline was injected to the control rats, also via penile vein. Same procedures were applied to the control rats to obtain blood samples, renal tissue homogenate.

Removed kidneys were stored at -80°C until assay. Kidney tissue was homogenized with KCL (1.5%) solution in a glass tube settled on ice. Subsequently, these homogenates were centrifuged and supernatant was used for the measurements of oxidative stress parameters. Blood samples in tubes with EDTA were centrifuged for 10 minutes with 4000 rpm at 4°C to obtain for plasma. Plasma samples were stored at -70°C. Chemicals used in antioxidant enzyme activity and MDA level measurements were provided from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstad, Germany). Measurements for oxidative stress parameters were performed with UV/Vis spectrophotometer (Shimadzu, Japan). GSH- Px activities in plasma and tissue homogenate were measured by the method of Aydın et al. 2001 (19). Reaction mixture was 50 mmol/L tris buffer, pH 7.6 containing 1 mmol/L of Na_2 EDTA, 2 mmol/L of reduced glutathione (GSH), 0.2 mmol/L of NADPH, 4 mmol/L of sodium azide and 1000 U of glutathione reductase (GR). Fifty microlitre of lysate and 950 µL of reaction mixture were mixed and 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 followed for 3 minutes. Enzyme activities were reported as U/µL and U/g wet tissue for plasma and homogenates, respectively. CuZn-SOD activity in kidney homogenate was also measured by the method described by Aydin et al., 2001 (19). Homogenate samples were diluted with 10 mM phospate buffer pH 7.0 about 400 fold. Twenty-five microlitre diluted homogenate samples were 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 minutes against air. Twenty-five microlitre phosphate buffer or 25 µL various standard concentrations in place of sample were used as blank and standard determinations. CuZn-SOD activity was expressed as U/g wet tissue. Plasma and tissue MDA levels were determined by the method described by Richard et al. in 1991 (20). After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol and was measured spectrofluometrically (excitation: 532 nm, emission:553). Tetrametoxy propane solution was used as standard. MDA levels were expressed

as nmol/ml and nmol/g wet tissue for plasma and homogenates, respectively.

Blood samples taken for serum studies were centrifuged following clotting and sera were stored at -80°C. Biochemical parameters were measured in DAX-48 autoanalyzer (Technicon, USA).

Statistical analyses were done by SPSS (Statistical Package for the Social Sciences Program) statistical program. Mann-Whitney U test was used for the comparison of the patient and control groups. Correlations were investigated with Pearson's correlation test. The results were expressed as mean ± standard deviation and the p values less than 0.05 were accepted as significant.

Results

The comparison of the nephrotic rats and controls with respect to their urinary protein excretion and some serum parameters is shown in Table I. Adriamycin administered rats had significantly higher urinary protein excretion ($p<0.001$) and serum total cholesterol levels ($p<0.001$) than those of the plasebo injected ones. Serum total protein ($p<0.001$) and albumin ($p<0.001$) levels were also significantly reduced in adriamycin injected group when compared to the controls while serum creatinine levels ($p=0.051$) remained unchanged.

There were statistically significant increases in plasma MDA levels ($p<0.001$) and tissue Cu-Zn SOD activities ($p=0.005$) in nephrotic rats when compared with controls (Table II). Plasma GSH-Px ($p=0.839$), tissue GSH-Px ($p=0.307$) activities and tissue MDA levels ($p=0.155$) of the nephrotic rats were not significantly different from those of the control rats.

High plasma MDA levels of nephrotic rats seemed to be correlated negatively with tissue GSH-Px activities ($r= - 0.745$, $p<0.001$) and serum albumin levels ($r= - 0.606$, $p=0.013$) in a statistically significant manner. Urinary protein excretion ($r= 0.681$, $p=0.002$) and serum total cholesterol levels ($r= 0.781$, $p<0.001$) showed statistically significant positive correlations with plasma MDA levels in nephrotic rats (Table III).

Discussion

The presence of an abnormality in antioxidative system in patients with nephrotic system has been documented with clinical studies (9,10,11,12,14,15, 16,18,21,22,23,24,25,26,27,28,29,30). Experimental studies in which antioxidative system was investigated have also been conducted and the data from these studies indicate an abnormality in oxidative system status in nephrotic syndrome as well (13,29, 31,32,33,34,35,36,37,38,39,40,41,42). Furthermore,

Tablo I. Comparison of the nephrotic rats and controls with respect to their urinary protein excretion and some serum parameters

Parameters	Controls (n=10)	Study group (n=20)	p value
Urinary protein (g/dl)	0.28 ± 0.14	4.53 ± 3.87	<0.001
Serum total protein (g/dl)	7.44 ± 0.27	5.42 ± 0.76	<0.001
Serum albumin (g/dl)	3.38 ± 0.13	1.90 ± 0.69	<0.001
Serum total cholesterol (mg/dl)	89.87 ± 7.80	289.11 ± 157.64	<0.001

Tablo II. Comparison of the controls and study groups in regard to studied oxidative stress parameters

Parameters	Controls (n=10)	Study group (n=20)	p value
Plasma MDA (nmol/ml)	0.54 ± 0.44	2.40 ± 1.23	<0.001
Tissue MDA (nmol/g wet tissue)	2.15 ± 0.63	1.86 ± 0.64	0.155
Plasma GSH- Px (U/ml)	3.86 ± 0.76	3.97 ± 1.27	0.839
Tissue GSH- Px (U/g wet tissue)	151.50 ± 30.94	161.85 ± 49.22	0.307
Tissue Cu-Zn SOD (U/g wet tissue)	0.46 ± 0.01	0.051 ± 0.07	0.005

Tablo III. Correlations between plasma MDA levels and other studied parameters

	Plasma MDA levels	
	r	p value
Plasma GSH-Px	-0.040	0.871
Tissue GSH-Px	-0.745	<0.001*
Tissue Cu-Zn SOD	-0.175	0.473
Tissue MDA	-0.226	0.352
Urinary protein	0.681	0.002*
Serum total protein	-0.256	0.321
Serum albumin	-0.606	0.013*
Serum total cholesterol	0.781	<0.001*
Serum creatinine	-0.856	0.161

some of the therapeutical modalities used in treatment of nephrotic syndrome and drugs affecting the oxidative system have been reported to have beneficial effects on oxidative system parameters in nephrotic syndrome and an improvement in the course of the nephrotic syndrome has also been suggested (32,34,37,38,39,42). Therefore, antioxidant system can be mentioned as having an important role in nephrotic syndrome.

In experimental nephrotic syndrome model presented here, a statistically significant increase in plasma MDA levels was observed in nephrotic rats. Rajbala et al., Fydryk et al., Mocan et al. and Kinra et al. also reported significantly increased serum and plasma MDA levels, respectively, in nephrotic syndrome as we did in a previous report of us (9,12,14,15,16). Although some studies, Bertolatus et al. and Podracka et al., report that there is no evidence for a change in antioxidative system, an impaired antioxidant protection in nephrotic syndrome both in plasma and erytrocytes oxidative stress in experimental nephrotic syndrome has been reported (9,10,12,15,21,25,28,29,43). However, the data from the present study did not yield a change in plasma GSH-Px activies. Since there is a controversy about the role of scavenger enzyme abnormalities on plasma MDA levels, an elucidation of high plasma levels of MDA in nephrotic syndrome is needed. Therefore, it is required to explain whether the high plasma MDA levels in nephrotic syndrome were due to similar changes in renal tissue or they were consequences of nephrotic state of the patients. There are conflicting reports about MDA levels in renal tissue. Ohtake et al. reported increased lipid peroxide levels in renal cortical tissue in mice (36). Total MDA levels in kidney homogenates from the nephrotic rats were not different from those of the controls in the study of Zima et al. (25). Our data about MDA levels in kidney homogenates from the rats with adriamycin induced nephrotic syndrome supports the data of the latter study. The results of the two previous studies (Zima et al. and Ohtake et al.) and those of the present study are consistent with respect to unchanged GSH-Px activities in renal tissue of experimentally nephrotic rats (23,36). Surprisingly, although decreased (Pedraza-Chaverri et al.) and unchanged (Zima et al. and Ohtake et al.) renal tissue SOD activities in nephrotic rat models have been reported, statistically significant high renal tissue SOD activities were observed in our experimental nephrotic syndrome model (23,34,36). This may be a contributing factor that unchanged MDA levels in renal tissue.

Increased plasma MDA levels are almost consistent finding in nephrotic syndrome both in human and animal studies and it leaves little or no doubt about the presence of an oxidative stress in this disorder. Oxidative stress parameters except tissue GSH-Px activities did not show any statistically significant correlation with plasma MDA levels in the present study. This relationship between two parameters, plasma MDA levels and tissue GSH-Px activities, is hard to be elucidated and it may be resulted incidentally. On the other hand, urinary protein excretion, serum albumin levels and serum total

cholesterol levels had statistically significant correlations with plasma MDA levels and these correlations indicate that nephrotic state itself has an important contribution in increased lipid peroxidation seen in nephrotic syndrome. Similarly, the study of Dogra et al. demonstrated that in nephrotic syndrome there was decreased free-radical trapping capacity of plasma that was inversely correlated with hypoalbuminemia (29). Abnormalities in antioxidative system of nephrotic patients have also been associated with lipid abnormalities (12,28).

In conclusion, our data from experimental nephrotic syndrome suggest that plasma abnormalities in antioxidative system in nephrotic syndrome are related with nephrotic state rather than being associated with antioxidative system changes occurred in renal tissue. Elevated plasma MDA levels in nephrotic syndrome are strongly associated with the severity of nephrotic syndrome and renal injury did not seem to have a direct contribution to increased lipid peroxidation in this disorder.

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