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Antioxidant status of children with steroid-sensitive nephrotic syndrome

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Abstract. Eighteen children with steroid-sensitive nephrotic syndrome (SSNS) were studied. The control group comprised 20 healthy children. The following indirect parameters of reactive oxygen species activity were determined in nephrotic patients during four stages of the disease (full relapse before prednisone administration, disappearance of proteinuria, prednisone cessation, unmaintained remission): plasma malondialdehyde (MDA) levels, copper/zinc superoxide dismutase (CuZn SOD) activity and glutathione peroxidase (GPX) activity in erythrocytes, reduced glutathione (GSH) and vitamin C levels in whole blood, and vitamin E level in serum. Increased MDA levels, reduced vitamin C levels, and enhanced CuZn SOD activity were found in relapse. GSH concentration was high during all four stages. Vitamin E level was also increased, parallel to the pattern of serum lipids. GPX activity remained low during the proteinuria stage and in remission. We conclude that the majority of abnormal findings can be attributed to the hyperlipidemia of NS. Low GPX activity may be a factor limiting the antioxidant capacity in NS. The present study is inconclusive regarding the role of free radicals in the proteinuria of NS.

Key words: Nephrotic syndrome – Antioxidants – Reactive oxygen species

Introduction

A change of glomerular filtration barrier integrity resulting in proteinuria may affect the structure of the glomerular capillary wall, its function, or both [1]. Several pieces of experimental evidence suggest alteration within proteoglycans and glycosaminoglycans of the glomerular filtration barrier concurrent with proteinuria in the absence of major glomerular pathology [2–4]. Many factors induce proteinuria under experimental conditions [1]. Reactive oxygen species (ROS), which are strong oxidants, can also produce proteinuria by inducing injury to glomerular epithelial cells and subsequent alterations in glomerular filtration barrier components synthesized by these cells, by reducing of the electronegative charge of the glomerular filtration barrier or by other unknown mechanisms [5–11]. ROS that affect barrier to macromolecular filtration can be generated in the circulation [12–15] or locally by glomerular cells [16–18]. The triggering mechanism leading to free radical production remains speculative. It may be related to infection, allergy, or some other immune stimulus. In clinical practice both infection and exposure to allergens frequently precede relapse of the nephrotic syndrome.

In the clinical setting direct investigation of the role of ROS in inducing proteinuria is not feasible. However, the natural antioxidants present in the blood may provide indirect information about the extent of free radical reactions in the body. The object of the present study was to examine indirect evidence of ROS activity in various stages of steroid-sensitive nephrotic syndrome (SSNS).

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Patients and methods

Patients. Eighteen children aged 4–13 years with SSNS were investigated during four stages of SSNS: full relapse before prednisone administration (I), disappearance of proteinuria (II), prednisone cessation (III), and remission at least 4 weeks after prednisone withdrawal (IV). All patients were infrequent relapsers [19], had normal blood pressure and serum creatinine levels, and ingested a diet appropriate for age without additional vitamin supplementation. Relapse, defined as daily urinary protein excretion ≥40 mg/h per m^2 [20], was treated with daily prednisone (60 mg/m^2 per day in 3 divided doses) until the urine was protein free for 3 days, with subsequent alternate-day prednisone (40 mg/m^2 per day) in a single morning dose for 4 weeks. Kidney biopsy was not performed. Clinical data of nephrotic patients are shown in Table 1. The control group comprised 20 healthy children of comparable age, on a normal diet without vitamin supplementation.

Table 1. Clinical data of patients

Patient no.	Age (years)	No. of relapse	Proteinuria (mg/h per m^2) Serum albumin (g/dl)			
			I	II	III	IV
1	4	1	46.0	–	–	–
			3.4	3.5	4.0	ND
2	7.5	3	134.0	–	–	–
			2.3	2.7	3.9	ND
3	4	1	172.0	–	–	–
			1.5	3.6	4.7	ND
4	4.5	7	133.0	–	–	–
			2.9	3.0	4.1	ND
5	12	12	759.0	–	–	–
			2.2	3.0	4.3	ND
6	4	1	129.0	–	–	–
			1.9	3.6	3.8	ND
7	13	9	120.0	–	–	–
			1.5	2.0	4.2	4.6
8	4	1	64.0	–	–	–
			1.2	3.4	4.5	ND
9	9	5	93.0	–	–	–
			2.4	4.0	4.5	ND
10	5	6	40.0	–	–	–
			2.2	2.3	3.4	ND
11	4	1	63.0	–	–	–
			1.6	1.8	4.2	ND
12	12	1	293.0	–	–	–
			1.8	3.8	ND	ND
13	11	6	140.0	–	–	–
			2.8	2.8	4.1	4.5
14	10	6	296.0	–	–	–
			3.4	4.0	ND	ND
15	13	12	187.0	–	–	–
			1.8	1.8	ND	3.8
16	4	3	244.0	–	–	–
			3.3	3.4	3.9	ND
17	4.5	4	160.0	–	–	–
			1.6	3.2	3.8	ND
18	10	9	407.0	–	–	–
			1.3	1.7	4.5	ND

I Relapse, before prednisone administration; II, disappearance of proteinuria; III, prednisone cessation; IV, remission at least 4 weeks after prednisone withdrawal; ND, not determined

Methods. Copper/zinc superoxide dismutase (CuZn SOD) activity in red blood cells was determined by the spectrophotometric method described by Misra and Fridovich [21]. CuZu SOD present in red blood cells inhibits spontaneous autooxidation of adrenaline to adrenochrome (wavelength 320 nm); 50% inhibition of adrenaline oxidation was taken as a unit of CuZn SOD activity.

Glutathione peroxidase (GPX) activity in red blood cells was measured by the method described by Sinet et al. [22], with an incubation temperature of 25° C instead of 37° C. The vitamin C level in blood was determined by electron spin resonance (ESR) with the use of ESR spectrometer SE/X 2544 Radiopan and resonance chamber RCX 660. Diphenyl-picryl-hydrazyl was employed as a standard to determine the *g* coefficient and the concentration of unpaired electrons. Lyophilized blood has a strong ESR in air, originating from the ascorbyl free radical whose precursor is L-ascorbic acid [23]; 10^{16} spin/g dry blood is approximately equal to 76.2 µmol vitamin C/l of blood.

Reduced glutathione (GSH) was estimated in blood using a colorimetric method with dithionitrobenzoic acid [24]. Serum vitamin E was measured by high-performance liquid chromatography (HPLC) [25, 26] on Merck Hitachi equipment with fluorometric detection (excitation 292 nm, emission 324 nm). Plasma malondialdehyde (MDA) was measured by HPLC (after reaction with thiobarbituric acid) and fluorometric detection (excitation 532 nm, emission 553 nm) [27].

Serum triglycerydes (TG), total cholesterol, low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol levels were estimated using kits produced by Boehringer Mannheim. Results and the ratio of vitamin E to lipid fractions were compared with the normal values of Polish children.

The results are expressed as means plus or minus standard deviation and were analyzed statistically by analysis of variance and Fisher test. A $P<0.05$ was considered statistically significant.

Results

The results in nephrotic patients obtained during selected stages of NS were compared with values found in healthy children. NS patients in relapse were also compared with those in full unmaintained remission (Table 2).

CuZn SOD activity was enhanced during the proteinuria stage ($P<0.05$) and thereafter normalized. CuZn SOD activity was normal in remission. GPX activity was decreased in relapse ($P<0.05$), was normal when proteinuria disappeared, and again decreased at termination of prednisone therapy and in remission ($P<0.05$). GPX activity was decreased both in relapse and in remission.

Serum vitamin C was low during relapse ($P<0.05$). When treatment was terminated (stage III) the vitamin C level increased, but was still lower than in remission (stage IV). The vitamin C level in remission (stage IV) was higher than in relapse ($P<0.05$). The GSH level was consistently and significantly elevated during all four stages. During remission the GSH level was still higher than in controls and during relapse ($P<0.05$).

Table 2. Parameters of indirect reactive oxygen species activity in various stages of steroid-sensitive nephrotic syndrome

Parameter	Normal value	I	II	III	IV
CuZn SOD_E (U/mg Hb)	0.97±0.27	1.17±0.39*[1]	1.16±0.37*[1]	0.98±0.43	1.07±0.38
GPX_E (nmol NADPH/mg Hb per min)	13.16±2.95	10.69±3.12*[1]	11.87±3.46	11.90±3.40 *[1]	9.78±3.80*[1]
Vitamin C_B (µmol/l)	114.3 ±16.8	67.06±22.9*[1,*3]	67.81±12.9*[1]	88.4 ±23.6*[1]	96.8 ±18.3
GSH_B (mg/dl)	26.06±4.99	33.60±5.98*[2,*3]	34.77±5.64*[2]	33.09±7.10*[2]	30.13±5.73*[1]
Vitamin E_S (µmol/l)	23.31±3.75	43.24±12.90*[2,*4]	40.27±11.57*[2]	29.23±18.75*[1]	26.10±9.83
MDA_P (µmol/l)	0.59±0.16	2.22±1.13*[2,*4]	1.79±0.56*[2]	1.14±0.46*[2]	0.95±0.42*[1]

E, Erythrocytes; B, blood; S, serum; P, plasma; CuZn SOD, copper/zinc superoxide dismutase; GPX, glutathione peroxidase; GSH, reduced glutathione; MDA, malondialdehyde; Hb, hemoglobin
*[1] $P<0.05$ vs. normal; *[2] $P<0.001$ vs. normal; *[3] $P<0.05$ group IV vs. group I; *[4] $P<0.001$ group IV vs. group I

The vitamin E level was elevated during the proteinuria stage ($P<0.001$) and at termination of treatment ($P<0.05$). During remission the vitamin E level was decreased compared with during relapse ($P<0.001$). The MDA concentration was significantly increased during all four stages (stages I–III, $P<0.001$; stage IV, $P<0.05$). The highest level was observed during the proteinuria stage, declining throughout the period of observation. The MDA concentration during remission was higher than in controls ($P<0.05$) and lower than during relapse ($P<0.001$).

Total cholesterol, LDL-cholesterol, and TG were elevated, the highest levels being noted during relapse and declining steadily throughout the subsequent stages of NS. The vitamin E/lipid ratio was elevated. Detailed lipid values are not shown in this paper.

Discussion

The term ''antioxidant'' is not uniformly interpreted. Some investigators restrict this term to chain-breaking inhibitors of peroxidation. We accept a broader definition of an antioxidant, as any substance that significantly delays or prevents oxidation of the substrate [28–30]. For this reason, in addition to vitamin C, vitamin E and glutathione, enzymes also active in ROS neutralization, have been included in the study.

The role of free radicals in inducing proteinuria experimentally has been well documented [5, 6, 8, 11]. However, in humans there is no direct evidence of a cause-effect relationship. Hyperlipidemia, which was observed throughout the stages of SSNS and which is known to be linked to oxidative reactions and generation of radicals, appears to be the most likely cause of the majority of findings reported in the present investigation.

Vitamin E is an important lipid-soluble antioxidant that acts synergistically with vitamin C, which regenerates vitamin E by reducing the tocopherol radical produced when vitamin E scavenges peroxyl radicals [31]. Vitamin E is transported in serum with serum lipids, and vitamin E levels correlate with serum lipid concentrations [32]. As the serum lipid level increases vitamin E appears to partition out of the cellular membranes into circulating lipoproteins [33]. In accordance with this observation, we found a decreased vitamin E concentration in erythrocyte membranes during relapse of NS when serum lipids were typically elevated [34]. The high ratio of serum vitamin E to lipid fractions observed during all four NS stages demonstrated sufficient vitamin E in nephrotic patients. However, a high serum vitamin E level does not imply an equally high content of this vitamin in lipid membranes, where it acts as a principal antioxidant.

Vitamin C is one of the most-efficient water-soluble antioxidants [35, 36]. After completing its role as a reducing agent, vitamin C is oxidized and forms the ascorbyl radical. The high disproportion rate constant of the ascorbyl radical allows for its conversion to dehydro-L-ascorbic acid and vitamin C before it interacts with substances liable to oxidation [37]. The low reduction potential of the ascorbyl radical/vitamin C compared with the reduction potential of several other one-electron reduction potential systems makes it a very effective antioxidant [37]. The low level of vitamin C during relapse, with increasing levels thereafter, indicates its consumption in the process of antioxidant activity.

GSH, besides being an independent antioxidant, is a key compound for regeneration of GPX and the oxidized form of vitamin C in a non-enzymatic reaction [38]. After completing its role as a reductant, GSH undergoes oxidation to GSSG, which in turn becomes reduced in the presence of glutathione reductase and NADPH. Since GSH-levels were high throughout period of observation, GSH-dependent element of antioxidant defense were sufficient in the patients examined.

In contrast to manganese SOD which is an inducible enzyme, CuZn SOD is considered a constitutive enzyme [39, 40]. Despite this, increased CuZn SOD activity was observed during NS relapse and subsidence of proteinuria ($P<0.05$). Interpretation of this findings is difficult. Increased CuZn SOD activity in stage I cannot be attributed to prednisone therapy, since measurement of enzyme activity during relapse preceded treatment.

Of particular interest and requiring further investigation is the behavior of GPX, which catalyzes decomposition of hydrogen peroxide and organic peroxides. GPX activity was decreased during the proteinuria stage ($P<0.05$ vs. controls), after cessation of prednisone therapy ($P<0.05$ vs. controls), and during unmaintained remission ($P<0.05$ vs. controls). The decreased GPX activity observed during relapse was also seen in remission. The possible impact of prednisone on the normal GPX activity observed at the time of disappearance of proteinuria (stage II), which coincided with the end of high-dose prednisone therapy, remains unclear. GPX is a selenium (Se)-containing enzyme, Se being localized to the active center of the enzyme. In this study neither blood Se level nor catalytic activity of GPX in terms of existing inhibitors were investigated. Se-deficient nephrotic rats had reduced activity of GPX and significantly greater proteinuria than animals with a normal Se supply [41]. Moderately but repeatedly reduced GPX activity during the course of NS – if confirmed – may be a feature of SSNS and a factor limiting antioxidant capacity in this disease.

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