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BRIEF REPORT

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Serum selenium level and glutathione peroxidase activity in steroid-sensitive nephrotic syndrome

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Abstract In our previous study the pattern of glutathione peroxidase (GPX) activity in the course of steroid–sensitive nephrotic syndrome (SSNS) in children suggested a defect in antioxidant defense. In the present report the serum selenium (Se) level, an essential component of GPX activity, was measured in a comparable group of children with SSNS at the same clinical stages at which GPX activity was determined in the previous study. Nephrotic children had normal serum Se levels during the edematous stage, at the end of prednisone treatment, and in remission. At the end of high-dose prednisone treatment, the serum Se level increased ($P<0.01$) simultaneously with enhanced activity of GPX. These results suggest that children with SSNS have a persistent defect in the antioxidant defense at the important stage of hydrogen peroxide and fatty acid hydroperoxide decomposition. This defect is transiently alleviated by high-dose prednisone treatment.

Keywords Steroid-sensitive nephrotic syndrome · Antioxidant defense · Glutathione peroxidase · Selenium

Introduction

The role of reactive oxygen species (ROS) in inducing proteinuria experimentally has been well documented [1, 2]. In humans there is no evidence of a cause-effect relationship between ROS and an increased protein excretion rate. In the clinical setting, direct evidence for 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 human free radical reactions.

Our previous study of the pattern of glutathione peroxidase (GPX) activity in the course of steroid-sensitive nephrotic syndrome (SSNS) in children suggested that low GPX activity may be a factor limiting the antioxidant capacity in this disease [3]. At that time we did not determine the serum selenium (Se) level at the same time as GPX activity. GPX activity is critically dependent upon Se [4]. Therefore, the aim of the present study was to examine serum levels of Se in a comparable group of children with SSNS at the same clinical stages at which GPX activity was determined in the previous study.

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Patients and methods

Patients

Children aged 4–14 years (mean 5.6±2.1 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). There were 22, 22, 7, and 12 patients in the four clinical stages of SSNS, respectively. All patients were infrequent relapsers, had normal blood pressure and serum creatinine level. Relapse, defined as daily urinary protein excretion ≥40 mg/h per m^2 was treated with daily prednisone (60 mg/m^2 per day) until the urine was protein free for 3–4 days, with subsequent alternate-day prednisone (40 mg/m^2 per day) in a single morning dose for 4 weeks. In addition to prednisone, some patients at an acute stage of SSNS were treated with blood volume expanders and furosemide. No other drugs were applied. Blood for investigations was drawn before any treatment was applied. Kidney biopsy was not performed. The control group comprised 20 healthy children (mean age 5.9±2.4 years). The study was approved by the local ethics committee.

Methods

The serum Se level was estimated by a fluorometric method after reaction with 2,3–diaminonaphthalene. Fluorescence of 4,5–benzodiazoselenole was measured with an LS 50 B Perkin Elmer

Table 1 Glutathione peroxidase (GPX) activity and serum levels of selenium (Se) in children with steroid-sensitive nephrotic syndrome compared with normal values

	Normals	I	II	III	IV
GPX[a] (±SD) (nmol NADPH/mg hemoglobin per min)	13.16±2.95	10.69±3.12*	11.87±3.46	11.9±3.4*	9.78±3.8*
Se (±SD) (ng/ml)	52.78±15.12	47.16±16.05	68.0±20.69**	52.92±17.94	52.82±15.26

*P<0.05, **P<0.01
[a] Data published in Fydryk et al. [3]

spectrofluorometer (excitation 378 nm, emission 519 nm) [5]. The coefficient of variation for 10 ng/ml was 5.93%, and for 70 ng/ml 2.02%. GPX activity in red blood cells was measured by the modified method described by Sinet et al. [3].

Statistical analysis

Results are expressed as means±standard deviation. Analysis of variance and Fisher's test were employed for statistical analysis. P values <0.05 were considered statistically significant.

Results

The serum Se level and GPX activity in children with SSNS and the control group are shown in Table 1. Serum Se levels at clinical stages I, III, and IV did not differ from the control group. Serum Se level at stage II was significantly higher than in control children (P<0.01). GPX activity was lower at stages I, III, and IV compared with the control group (P<0.05) and was normal at stage II.

Discussion

Reduced glutathione (GSH), which acts by destroying hydrogen peroxide and fatty acid hydroperoxides, plays an important role in the antioxidant defense. GPX is a seleno-enzyme that catalyzes the reaction of GSH to glutathione disulfide (GSSG):

$$2GSH + H_2O_2 \xrightarrow{GPX} GSSG + 2H_2O$$

GSH is regenerated in the presence of NADPH and glutathione reductase [6].

Our results show that nephrotic children with the same serum Se levels as controls exhibit reduced GPX activity during the edematous stage, at the end of prednisone treatment, and in remission. At the end of high-dose prednisone treatment, the serum Se level increases simultaneously with enhanced activity of GPX. Alternate-day prednisone treatment at a reduced dose does not influence GPX activity. The reason for this pattern is not known. It may be speculated that mechanism(s) related to nephrotic syndrome down-regulate GPX activity. The defect appears to be transiently alleviated by high-dose prednisone therapy. If confirmed, this observation suggests a defect in the antioxidant defense in SSNS at an important step of hydrogen peroxide and fatty acid hydroperoxide decomposition.

Treatment with glucocorticoids, depending on the dose, has been demonstrated to alter tissue levels of GPX in puromycin aminonucleoside (PAN) nephrosis in the rat [7]. Several studies provided evidence that glomerular injury induced by PAN is mediated by ROS [1, 8]. PAN nephrosis closely resembles human minimal change disease and lacks clear evidence for immunological or inflammatory changes. Glucocorticoids exert their impact on renal function in many ways, including renal hemodynamics, tubular protein reabsorption, and changes in the permselectivity. Interestingly, some effects of glucocorticoids depend on the dose. The relationship was documented both in experimental animals and in humans [6, 9]. The reason for this observation remains speculative and may be related to the influence of glucocorticoids on the breakdown of GPX enzyme protein [10, 11], regulation of the glucocorticoid receptor [12, 13], or some other cellular mechanisms.

Another interesting observation is the rise of serum Se level at the end of high-dose prednisone treatment. It is well known that an Se-deficient diet, both in humans and experimental animals, produces a reduction in GPX activity, and consequently decreases the antioxidant potential of tissues [14]. A diminished intake of Se in the diet of our patients seems unlikely. All of them remained on a diet appropriate for their age throughout the whole period of observation, the only limitation being the reduced salt and water intake during a period of edema. However, the impact of prednisone on the absorption and metabolism of Se cannot be excluded. The increase in GPX activity may be explained by the increased cellular GPX synthesis. Evidence was provided that Se influences the transcriptional regulation of GPX. A reduced intake of Se markedly impairs the expression of mRNA for the Se-dependent GPX [15].

Hyperlipidemia can generate oxidative reactions with consequent alterations in antioxidative defense. All our patients with exacerbation of SSNS (stage I) had typical patterns of serum lipids level. These alterations normalized during the course of observation. During remission after steroid withdrawal (stage IV), all patients had serum levels of lipids that were normal for age (results not shown). Since the serum Se level and GPX activity remained the same during stage I and IV of SSNS, an impact of lipid alterations on the Se level and enzyme activity seems unlikely.

A role for ROS in the pathogenesis of SSNS in humans remains unproven. For this reason, speculation about the influence of antioxidant defense on nephrotic

proteinuria appears premature. However, our findings support the hypothesis of a defect of the antioxidant defense in SSNS. If confirmed, this defect may be an important feature of SSNS. Its genesis and role remain to be elucidated.

References

1. Diamond JR, Bonventre JV, Karnovsky MJ (1986) A role for oxygen free radicals in aminonucleoside nephrosis. Kidney Int 29:478–483
2. Yoshioka T, Ichikawa J, Fogo A (1991) Reactive oxygen metabolites cause massive, reversible proteinuria and glomerular sieving defect without apparent ultrastructural abnormality. J Am Soc Nephrol 2:902–912
3. Fydryk J, Jacobson E, Kurzawska O, Małecka G, Gonet B, Urasiński T, Brodkiewicz A, Bukowska H (1998) Antioxidant status of children with steroid-sensitive nephrotic syndrome. Pediatr Nephrol 12:751–754
4. Burk RF (1983) Biologic activity of selenium. Annu Rev Nutr 3:53–70
5. Danch A, Drozdz M (1996) Uproszczona metoda oznaczania Se w materiale biologicznym. Diag Lab 32:529–534
6. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588–590
7. Kawamura T, Yoshioka T, Bills T, Fogo A (1991) Glucocorticoid activates glomerular antioxidant enzymes and protects glomeruli from oxidant injuries. Kidney Int 40:291–301
8. Kawaguchi M, Yamada M, Wada H, Okigaki T (1992) Roles of active oxygen species in glomerular epithelial cell injury in vitro caused by puromycin aminonucleoside. Toxicology 72:329–340
9. Wetzels JFM, Sluiter HE, Hoitsma AJ, Munster PJJ van, Koene RAP (1988) Prednisolone can increase glomerular permeability to proteins in nephrotic syndrome. Kidney Int 33:1169–1174
10. Goldberg A, Tischler M, Demartino G, Griffin G (1980) Hormonal regulation of protein degradation and synthesis in skeletal muscle. Fed Proc 39:31–36
11. Simmons PS, Miles JM, Gerich JH, Haymond MW (1984) Increased proteolysis. An effect of increases in plasma cortisol within the physiologic range. J Clin Invest 73:412–420
12. Svec F, Rudis M (1981) Glucocorticoids regulate the glucocorticoid receptor in the AtT-20 cell. J Biol Chem 256:5984–5987
13. Sapolsky RM, Krey LC, McEwen BS (1984) Stress down-regulates corticosterone receptors in a site-specific manner in the brain. Endocrinology 114:287–292
14. Baliga R, Baliga M, Shah SV (1992) Effect of selenium-deficient diet in experimental glomerular disease. Am J Physiol 263:F56–F61
15. Yoshimura S, Takekoshi S, Watanabe K, Fujii-Kuriyama Y (1988) Determination of nucleoside sequence of cDNA coding rat glutathione peroxidase and diminished expression of the m RNA in selenium deficient rat liver. Biochem Biophys Res Commun 154:1024–1028