Development of Oxidative Stress in Chronic Kidney Insufficiency following the Progression of Disease

P.		Bochev
N.		Tzvetkov
M.		Alexandrova
M.		Magrisso
B.		Bechev
O.		Monovich
V.		Markova

Biophysics Department, Medical University, Pleven, Bulgaria

Prof. P. Bochev, Head of Biophysics Department, Medical University, 1 St. Kliment Ochridsky Str., BG-5800 Pleven (Bulgaria)

Table 1. Mean values of some parameters of investigated groups

Control (n = 40)

Parameter

CKI-1

(n=11)

CKI-2

(n=13)

CKI-3

(n=12)

CKI-D

(n = 36)

Dear Sir,

There is not yet a commonly accepted explanation of the role of free-radical processes in the pathogenesis of many nephro-logical diseases and conditions, such as acute and chronic kidney insufficiency, glomerulo-nephritis, gentamicin nephrotoxicity, ischemic-reperfusion injury after kidney transplantation, etc. [1-3]. The oxidative stress and its consequences for the patients suffering from chronic kidney insufficiency (CKI) are the object of intensive investigations. Increased levels of lipoperoxidation products in serum and erythrocytes [4] of CKI patients on conservative treatment and especially on dialysis [5] have been found. In patients with uremia on conservative treatment a decrease of α -tocopherol in blood and catalase activity in erythrocytes [6] have been established. In dialysis patients super-oxide dismutase (SOD), catalase and glutathione peroxidase activities are found to be decreased [6]. CKI is also characterized by a higher risk of infections because of the alterations of phagocytosis [7]. Evaluating the oxidative activity of blood polymorphonuclear leukocytes (PMNL) with chemiluminescent methods, many authors have found a decreased stimulated chemiluminescence in cases of patients with CKI on conservative treatment [8] and on

dialysis [9]. In contrast, enhanced resting PMNL activity has been established in patients with CKI on conservative treatment and on dialysis [10].

To the best of our knowledge there is no published evaluation of relation between the level of free-radical processes in blood of CKI patients and the level of disease severity. We report here results obtained from such an investigation, following the disease progression from initial kidney insufficiency to terminated uremia on dialysis.

Whole blood samples were tested. They were collected in a fasting state at 08:00 h (for patients on hemodialysis, before dialysis procedure). The investigated group of patients suffering from CKI consisted of 72 individuals who did not present any other diseases. Thirty-six of them were on conservative treatment. They were divided in three subgroups depending on the severity of disease, based on their creatinine clearance: CKI-1 – from 0.66 to 0.33 ml/s, CKI-2 -from 0.33 to 0.16 ml/s, and CKI-3 – from 0.16 to 0.08 ml/s (table 1). The other 36 tested patients (CKI-D subgroup) were on dialysis 3 times/week (dialyzer ‘Unimat’ with cellulose-acetate membrane). Forty healthy individuals served as control. None of them took any drugs during the tests.

The level of lipid peroxidation was estimated by means of lipid hydroperoxides (ROOH) in serum [11] and thiobarbituric acid-reactive material in blood (TBARM) [12]. Since the major subject of peroxidation in blood is lipids of blood cell membranes, the initially obtained data were normalized according to the blood cell concentration. Blood antioxidant capacity was estimated by SOD activity in erythrocytes [13], total blood catalase activity (CTS) [14] and total concentration of blood thiol groups (RSH) [15]. Resting and stimulated chemiluminescent activity of PMNL, representing their oxidative activity, were measured by six-sample luminometer [16]. Each sample contained 15 ml blood and 3 ml luminol solution (6.6 mmol/l) in Krebs-Ringer phosphate buffer (pH 7.4). Zymosan (Sigma), preliminarily opsonized with pooled AB human serum, was used as stimulating agent for the evaluation of PMNL-stimulated activity -0.5 ml zymosan suspension (2 mg/ml) was added additionally to each sample. Since the hemoglobin concentration in erythrocytes and PMNL number in blood samples are important for the registered chemiluminescent intensity, the data acquired were normalized according to 104 PMNL and corrected as to the optic absorbency of hemoglobin by the method of Ristola and Repo [17]. The oxidative PMNL activity of each sam-

KARGER

E-Mail karger@karger.ch Fax + 41 61 306 12 34 <http://www.karger.ch>

© 1997 S. Karger AG, Basel 0028-2766/97/0772-0244\$ 12.00/0

This article is also accessible online at: <http://BioMedNet.com/karger>

Table 2. Levels of lipoperoxidation products, antioxidant capacity and PMNL activity

Control

CKI-1

CKI-2

CKI-3

CKI-D

1.28 ± 0.11* 0.68 ± 0.04* 1.09 ± 0.05* 1.69 ± 0.11* 3.06 ± 0.17* 62.8 ± 6.6*

ROOH, nmol/109 cells TBARM, nmol/109 cells SOD, hU/ml blood CTS, hU/ml blood RSH, mmol/ml blood Resting activity, RU Stimulated activity, RU

0.76 ± 0.03 0.48 ± 0.03 1.70 ± 0.05 3.88 ± 0.20 4.00 ± 0.06 36.3 ± 5.7 146.9 ± 11.1
 $0.99 \pm 0.12^*$ 0.55 ± 0.08 1.41 ± 0.14
 2.97 ± 0.42 4.08 ± 0.11 16.8 ± 1.5
 $205.3 \pm 32.2^*$

$1.41 \pm 0.11^*$
 $0.92 \pm 0.15^*$ $1.30 \pm 0.17^*$ $2.04 \pm 0.22^*$ $2.83 \pm 0.26^*$ $70.2 \pm 18.6^*$
 $1.30 \pm 0.15^*$ $0.95 \pm 0.15^*$ $1.33 \pm 0.12^*$ $2.30 \pm 0.30^*$ 3.11 ± 0.32 $63.9 \pm 11.8^*$
 $96.1 \pm 17.1^*$ $110.2 \pm 15.2^*$ 170.1 ± 23.3

*p < 0.05.

pie was estimated by the area under obtained kinetic chemiluminescent curve for 30 min and was presented in relative units (RU). Statistical processing of results was carried out by the methods of variation analysis. Results are presented as mean $\pm$ SE. Statistically reliable differences, compared to the control group, were accepted at p < 0.05 and marked with an asterisk.

We established a higher level of lipoperoxidation products (ROOH and TBARM) in the blood of patients suffering from CKI compared to the control group (table 2). It increases with the heaviness of disease. This is well expressed in dialyzed patients (CKI-D) as well, but to a lower extent, compared with CKI-3. The latter fact may be due to a loss of some water-soluble components of these peroxidation products during dialysis procedures, especially for TBARM, which contains both lipid- and water-soluble components.

Blood antioxidant capacity, evaluated by SOD activity, CTS activity and blood thiol groups (RSH) we found to decrease corresponding with the severity of CKI, reaching a minimum in patients under hemodialysis, with the exception of RSH. Increased resting PMNL oxidative activity is established for CKI patients with creatinine clearance < 0.33 ml/s. Stimulated PMNL activity is decreased for the patients in CKI-2 and CKI-3 groups.

Following the progression of disease from initial kidney insufficiency to terminated uremia on dialysis, we established a tendency for increase of lipid peroxidation and PMNL resting oxidative activity, and for decrease of blood antioxidant capacity. This disbalance between free-radical processes and antioxidant defense leads to the development of oxidative stress in blood.

Without sufficient blood antioxidant protection, erythrocytes of CKI patients, especially those of dialyzed patients, are probably the main target of free-radical damage. Increased resting PMNL oxidative activity contributes additionally to the development of lipid peroxidation in erythrocyte membranes and, as a consequence, to the anemic syndrome, characteristic for CKI.

References

- Baud L: Reactive oxygen species – Role in acute renal failure. *Presse Méd* 1992;21:2032-2036.
- Shah SV: The role of reactive oxygen metabolites in glomerular disease. *Annu Rev Physiol* 1995;57:245-262.
- Fauconneau B, Tallineau C, Huguet F, Priou A: Gentamicin-induced kidney damage and lipid peroxidation in rats. *Toxicol Lett* 1995; 76:127-134.
- Martino F, Lubrano R, Ruberto U, Mannario O, Galletti E, Enfemia D: Peroxidation of erythrocyte lipids and levels of vitamin E in red blood cells in children with chronic renal insufficiency. *Minerva Pediatr* 1989;41:315-317. Peuchant E, Carbonneau MA, Dubourg A,

Thomas MJ, Perromat A, Vallot C, Clerc C: Lipoperoxidation in plasma and red blood cells of patients undergoing haemodialysis – vitamin A, E and iron status. *Free Radic Biol Med* 1994;16:339-346.

Durak I, Akyol D, Busesme E, Caubolat O, Kavutcu M: Reduced erythrocyte defense mechanisms against free radical toxicity in patients with chronic renal failure. *Nephron* 1994;66:76-80.

Okada H, Miyazaki J, Kamidono S: Granulo-cyte functions of chronic hemodialysis patients. *Jpn J Urol* 1992;83:1506-1510.

Aparicio M, Vincendeau P, Gin H, Potaux L, Bouchet JL, Martin-Dupout P, Morel D, De Precigout V: Effect of a low-protein diet on chemiluminescence production by leukocytes from uremic patients. *Nephron* 1988;48:315-318.

9 Mori R, Triolo L, Ceriati F, Lippa S, Oradei A, De Sole P: Polymorphonuclear leukocyte activation during hemodialysis. *Acta Med Roma-na* 1984;22:200-203.

Ritchey EE, Wallin JD, Shah SV: Chemiluminescence and superoxide anion production by leukocytes from chronic hemodialysis patients. *Kidney Int* 1981;19:349-358.

Yagi K: Lipid peroxides and human diseases. *Chem Phys Lipids* 1987;45:337-351.

Asakawa T, Matsushita S: Thiobarbituric acid test for detecting lipid peroxides. *Lipids* 1980;14:401-406.

Maral J, Puget K, Michelson A: Comparative studies of SOD, catalase and glutathione peroxidase in erythrocytes of different animals. *Bio-chem Biophys Res Commun* 1977;77:1525-1535.

Sigma Chemie Catalog, 1996, p 235.

Ellman GL: Tissue sulphydryl groups. *Arch Biochem Biophys* 1959;82:70-77.

Bochev P, Bechev B, Margrisso M: Six-sample multiplexing computerized analyser for integral and spectral luminescence measurements. *Anal Chim Acta* 1992;256:29-32.

Ristola M, Repo H: Whole blood luminol-enhanced chemiluminescence: Statistical analysis of the responses of different subjects. *J Bio-lumChemilum* 1990;5:155-160.

Oxidative Stress in CKI
Nephron 1997;77:244-245
 245