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Erythrocyte Antioxidant Defense System in Patients with Chronic Renal Failure According to the Hemodialysis Conditions

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Background. Patients suffering from chronic renal failure (CRF) are exposed to increased oxidative stress generated by uremic toxins, factors connected with hemodialysis, chronic inflammatory state, lack of vitamins A, E and selenium, advanced age, and parenteral iron administration. Their antioxidative system is inefficient. In erythrocytes, hexosemonophosphate (HMP) cycle does not assure an adequate amount of reductive equivalents (NADPH) necessary to restore reduced glutathione (GSH), an important free radical scavenger. Hemodialysis treatment also causes a large loss of glucose, which is the basic substrate for that metabolic pathway. The object of the research was to establish the influence of glucose present in dialysate on antioxidant defense system in red blood cells.

Methods. A group of 51 patients undergoing regular hemodialysis using glucose (26 subjects, GL+ HD) or non-glucose fluid (25 subjects, GL− HD) was studied. The GSH concentration, glucose-6-phosphate dehydrogenase (G-6-P DH) and glutathione reductase (GSSG-R) activities were determined. Glucose concentration before and after the hemodialysis session was also measured.

Results. The activity of G-6-P DH was significantly higher in GL+ HD both before ($p = 0.000$) and after ($p = 0.0002$) the dialysis treatment compared to GL− HD, respectively, and to the healthy subjects ($p = 0.000$). The hemodialysis session caused a decrease in the activity of the enzyme in GL+ HD from 4.91 ± 1.53 to 4.42 ± 1.40 U/g Hb ($p = 0.004$) as well as in GL− HD from 3.97 ± 1.00 to 3.59 ± 0.87 U/g Hb ($p = 0.007$). The GSSG-R showed an increase in activity in CRF patients before HD (in GL+ HD to 2.57 ± 0.76 and in GL− HD to 2.82 ± 0.98 U/gHb). After dialysis, lower values were observed, particularly in GL+ HD (2.05 ± 0.59 U/g Hb, $p = 0.004$). GSH concentrations in the examined group were higher (in GL+ HD 0.0205 ± 0.008 and in GL− HD 0.0196 ± 0.008 mmol/g Hb) than in controlled subjects (0.0142 ± 0.002 mmol/g Hb) and decreased during dialysis treatment (considerably only in GL− HD to 0.0183 ± 0.007 mmol/g Hb, $p = 0.056$). Glucose concentrations in GL+ HD were significantly higher compared to GL− HD ($p < 0.002$).

Conclusions. Glucose presence in dialyzing fluid improves the HMP cycle activity as well as glutathione system reactions and determines a better antioxidant status of erythrocytes. It limits hemolysis and improves the hematological parameters in CRF.

Key Words: Chronic renal failure, Hemodialysis, Oxidative stress, Free radical scavengers, Hexosemonophosphate cycle, Reduced glutathione.

Introduction

Erythrocytes are equipped with an effective enzymatic and non-enzymatic free radical scavenger system. Superoxide dismutase (SOD), catalase (CAT) and glutathione

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peroxidase (GPX) contained in these cells show a high activity. Glutathione actions make them the most important removers of non-enzymatic antioxidant power to tissues and organs (1,2). In chronic renal failure patients (CRF), these processes do not provide adequate intensity because of increased oxidative stress. The creation of reactive oxygen species (ROS) is induced by uremic toxins and hemodialysis itself due to bioincompatible dialysis membrane, non-sterile dialysate, poor quality of dialysis water, and back-leak of contaminants across the dialysis membrane. In addition, these patients have reduced their anti-oxidant system, which manifests in deficiency of vitamins C, E and selenium. The other causes of enhanced oxidative stress are advanced age, high frequency of diabetes, chronic inflammatory state, and excessive parenteral iron administration. In these conditions the main erythrocyte metabolic pathways are inefficient. It is known that transketolase and glucose-6-phosphate dehydrogenase (G-6-P DH), the key enzymes of hexosemonophosphate (HMP) cycle, show decreased activity in patients undergoing regular hemodialysis (3). The HMP cycle plays a significant role in red blood cell antioxidant reactions because it is the only source of NADPH. This compound is indispensable in the process of restoring reduced glutathione (GSH). Uremic patients have a lower concentration of NADPH and GSH, compared to a higher amount of oxidized glutathione form (GSSG) (4–7). Moreover, an increased activity of glutathione reductase (GSSG-R) was established (4,8,9). The described disturbances are greater in hemodialyzed than in conservatively treated patients (10). The dialysis session leads to a decrease in plasma glucose concentration, which is the basic substrate in erythrocyte metabolism. Its deficit, particularly in CRF patients, can influence the efficiency of the antioxidant defense system.

This article reports the effect of the presence of glucose in dialysate on HMP cycle activity and glutathione reactions.

Materials and Methods

The research was performed at the Outpatient Dialysis Unit of Pomeranian Medical University, Szczecin, Poland. A group of 51 subjects with CRF undergoing regular hemodialysis were studied. The dialyses sessions were performed three times a week and lasted 4 h using polysulfone dialyzators (Fresenius, Homburg, Germany). Patients were divided into two subgroups: 26 subjects dialyzed with a fluid containing glucose with a concentration of 1 g/L (5.6 mmol/L) (GL+ HD) and 25 subjects dialyzed with a fluid not containing glucose (GL− HD). The other contents of the dialyzing fluid were Na^+ 138 mmol/L, K^+ 3.0 mmol/L, Ca^{2+} 1.75 mmol/L, Mg^{2+} 0.5 mmol/L, Cl^- 107.5 mmol/L, HCO_3^- 32 mmoL/L. Inclusion criteria were as follows: diagnosis of CRF, regular hemodialysis treatment for at least half a year before the study, lasting 12 h/week, with a fluid containing or not containing glucose at least 3 months before the research was performed, and hemodialysis adequacy defined by Kt/V ratio from 1:1.2. These patients did not have blood transfusions and iron therapy for 1 month before the study. Patients who suffered from diabetes, neoplasm and active inflammations were excluded from the study. Human recombinant erythropoietin (rHuEPO) supplementation at an average dose of 4000 IU/week was provided for the whole group. CRF was caused by chronic glomerulonephritis in 20 subjects, chronic pyelonephritis in 8 subjects, autosomal dominant polycystic kidney disease in 9 subjects, hypertensive nephropathy in 3 subjects, Alport's disease in just 1 subject and other or unknown causes in 10 subjects.

The control group consisted of 29 healthy volunteers of the same age and sex as the hemodialyzed patients. Those who abused alcohol (more than 30 g of alcohol daily) or were smokers were excluded.

The study lasted 12 months. In the examined group, blood was taken directly from arteriovenous fistula just before and after the hemodialysis session. Main data and biochemical characteristics of the patients and healthy controls such as urea, creatinine, glucose concentrations, total protein content in plasma, age, sex and time since first HD session (examined group) are presented in Table 1.

Activity of G-6-P DH was determined by the spectrophotometric method using the reaction of NADP reduction (11,12). GSH concentration was examined in the entire

Table 1. Chosen biochemical parameters, age and sex of hemodialyzed[a] patients and controls

	GL+ HD		GL− HD		
Group	Before HD	After HD	Before HD	After HD	Control
Urea (mg/dL)	135.04 ± 32.59	54.52 ± 21.88	145.73 ± 44.96	49.31 ± 19.77	—
Creatinine (mg/dL)	6.88 ± 1.87	3.50 ± 0.95	8.59 ± 2.46	3.97 ± 0.85	1.06 ± 0.13
Glucose (mg/dL)	102.86 ± 29.97	95.18 ± 46.26	68.96 ± 26.13	73.59 ± 26.54	87.41 ± 14.11
Protein (g/100 mL)	6.60 ± 0.78	6.87 ± 1.02	6.90 ± 0.54	7.72 ± 1.19	6.22 ± 0.59
Age (years)	60.54 ± 13.54		50.3 ± 13.79		49.7 ± 11.4
Sex (F/M)	12/14		13/12		15/14
Time since first HD (months)	24.43 ± 12.68		27.44 ± 15.87		—

[a]With glucose or non-glucose fluid.