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Short communication

The effect of lipoate on anaerobic cysteine metabolism in erythrocytes of patients treated with peritoneal dialysis

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ABSTRACT

Background: The studies aimed to evaluate the changes in cysteine sulfur metabolism in erythrocytes of end-stage renal failure (ESRF) patients treated with continuous ambulatory peritoneal dialysis (CAPD) caused by a one-month lipoate (LA) supplementation at a daily dose of 600 mg.
Methods: The level of sulfane sulfur and activity of sulfurtransferases were determined in erythrocytes of CAPD patients and in the control group.
Results: The sulfane sulfur level in erythrocytes of CAPD patients did not differ compared with healthy volunteers but LA supplementation increased the reactive sulfur concentration. LA elevated also cystathionase activity.
Conclusions: LA supplementation in ESRF patients treated with CAPD increases the sulfane sulfur level which indicates the augmentation of its antioxidant and regulatory properties.
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Introduction

End-stage renal failure is a pathological condition the pathogenesis of which is largely related to a harmful effect of reactive oxygen species and exaggerating oxidative stress leading to renal dysfunction. In addition, uremic toxins appearing in blood can be a source of oxidative stress in renal failure patients [15]. Oxidative stress was earlier defined as an imbalance between anti- and pro-oxidant processes. A newer definition of oxidative stress takes into account free radical-induced damage of cellular macromolecules on the one hand, and disturbance of thiol redox signaling on the other [11].

Erythrocytes are particularly exposed to oxidative damage due to their constant contact with oxygen, possibility to generate superoxide anion radical ($O_2^{\bullet -}$) in the oxidation reaction of Hb to metHb, and due to exposure to environmental toxins. Thus, erythrocytes are equipped with efficient both enzymatic and non-enzymatic antioxidant mechanisms [4]. A thiol tripeptide glutathione (GSH) plays also a significant role in antioxidant defense of erythrocytes. Cysteine is a rate-limiting amino acid in GSH synthesis. It is transformed *via* aerobic path to sulfates and *via* anaerobic route to sulfane sulfur-containing compounds (Scheme 1) [10]. Sulfane sulfur is a labile reactive sulfur atom in 0 or −1 oxidation state always bound to another sulfur atom. Sulfane sulfur-containing compounds are formed endogenously from cysteine in the reactions catalyzed by γ-cystathionase (CSE) and 3-mercaptopyruvate sulfurtransferase (MST). Sulfane sulfur-bearing compounds show regulatory and antioxidant actions [10]. Their regulatory properties are related with the ability to covalently modify protein –SH groups which influences their biological activity. Antioxidant activity of sulfane sulfur-containing compounds is linked with their capability of direct scavenging of free radicals. Perthiyl radicals ($RSS^{\bullet}$) formed in those reactions are more stable and less toxic than thiyl radicals ($RS^{\bullet}$). Moreover, sulfane sulfur can influence the activity of antioxidant enzymes. For these reasons, the compounds containing reactive sulfane sulfur are considered to belong to important elements of antioxidant defense of the cell.

Sulfane sulfur plays also an important role in cyanide detoxification processes in the reactions catalyzed by rhodanese (TST) or 3-mercaptopyruvate sulfurtransferase (MST) (Scheme 1). It is significant in uremic patients because they show an elevated level of cyanide and other toxins [7].

Lipoic acid (LA; 1,2-dithiolane-3-pentanoic acid) is a natural thiol compound playing in the cell the role of a cofactor of the multienzymatic complex catalyzing oxidative decarboxylation of α-ketoacids. Exogenous LA is reduced in the cell to dihydrolipoic

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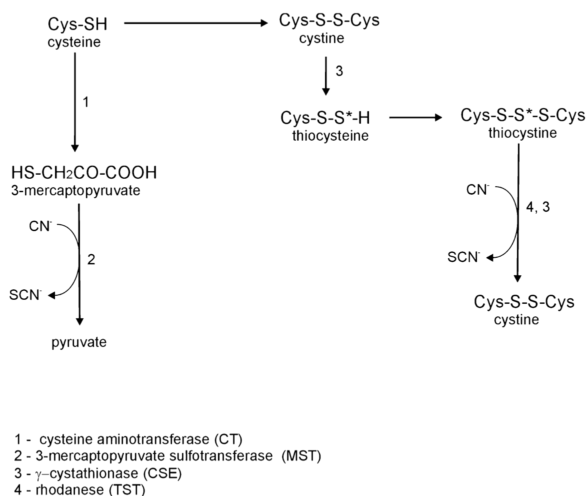

Scheme 1. Anaerobic transformation of cysteine to sulfane sulfur-containing compounds (S* – sulfane sulfur).

acid (DHLA) which is responsible for the antioxidant action. LA is a known antioxidant therapeutic used in humans particularly in diabetic neuropathy [3,6]. Studies conducted so far have confirmed a beneficial effect of LA also in aging, cancer therapy, obesity and inflammation [6].

The present study on erythrocytes of ESRF patients treated with CAPD investigated the level of sulfane sulfur and activity of sulfurtransferases: γ-cystathionase, 3-mercaptopyruvate sulfurtransferase and rhodanese in comparison with erythrocytes of healthy volunteers. In addition, the above determinations were performed in erythrocytes of CAPD patients after a one-month lipoate supplementation.

Materials and methods

Patients and control group

The studies were conducted in a group of 15 healthy volunteers (11 women, 4 men) with no clinical history of renal diseases and 14 patients (5 women, 9 men) with end stage renal failure (ESRF) undergoing continuous ambulatory peritoneal dialysis (CAPD).

The mean age of patients was 56.93 ± 13.06 years (range: 36–77). In the group of patients undergoing CAPD, the following indicators of renal function were determined: creatinine (761.59 ± 253.86 μmol/l), urea (22.45 ± 4.67 mmol/l), serum albumin (35.71 ± 2.59 g/l). Healthy control group comprised control subjects aged 41.67 ± 8.00 years (range 31–57). Their renal parameters were: creatinine (80.18 ± 13.02 μmol/l), urea (4.99 ± 1.2 mmol/l), serum albumin (45.88 ± 1.71 g/l).

The patients' blood was collected in the Rydygier's Hospital Fressenius Nephrocare II (Krakow). The control group was composed of employees of the hospital and Fresenius-Nephrocare II. These persons were healthy and received no pharmacological therapy. Blood of CAPD patients was collected twice: before the beginning of LA supplementation (CAPD group) and after a one-month therapy with lipoate at a daily dose of 600 mg (CAPD-LA group). Blood of CAPD patients and healthy control subjects was collected to tubes with EDTA and then it was centrifuged at $1500 \times g$ for 10 min. Erythrocytes were washed thrice with a twofold volume of physiological saline (after each washing, the suspension was centrifuged at $1500 \times g$ for 5 min). Finally, the whole supernatant was collected and discarded, while erythrocytes were gently mixed and frozen at −80 °C. Immediately before biochemical determinations, erythrocytes were thawed/frozen twice in order to achieve complete lysis of cells. The obtained hemolysates were used for determination of: the sulfane sulfur level and activities of enzymes involved in its formation and transport: γ-cystathionase (CSE), 3-mercaptopyruvate sulfurtransferase (MST) and rhodanese (TST).

All patients and healthy volunteers gave a written consent to participate in the study. The study protocol was approved by the Local Bioethics Committee in Krakow (nr. 127/KBL/OIL).

Chemicals

Lipoic acid (Neurolipon-MIP 600) was purchased from MIP PHARMA POLSKA. Thiosulfate, formaldehyde and sodium sulfite were obtained from the Polish Chemical Reagent Company (P.O.Ch, Gliwice, Poland). 3-Mercaptopyruvic acid (3-mp), N-ethylmaleimide (NEM), β-nicotinamide adenine dinucleotide reduced form (NADH), 3-methyl-2-benzo-thiazolinone hydrazone (MBTH), pyridoxal 5'-phosphate (PLP), homoserine, potassium cyanide (KCN), trichloroacetic acid (TCA) and lactic dehydrogenase (LDH) were provided by Sigma Chemical Co. (St. Louis, MO, USA).

Methods

Determination of sulfane sulfur level

The level of the compounds containing sulfane sulfur was determined by the method of Wood [22] based on cold cyanolysis. It consists in a nucleophilic attack of cyanide on sulfane sulfur-containing compounds in alkaline solution at room temperature. Thiocyanate formed in this reaction reacts with Fe^{3+} ions yielding red ferric thiocynate estimated spectrophotometrically at 460 nm.

Determination of γ-cystathionase activity

Enzymatic activity of CSE was determined according to Matsuo and Greenberg [12] with modifications. L-Homoserine was used as a substrate, while PLP was a coenzyme.

α-Ketobutyric acid formed from L-homoserine was assayed using 3-methyl-2-benzo-thiazolinone hydrazone (MBTH) according to the method of Soda [16].

Determination of 3-mercaptopyruvate sulfurtransferase activity

The activity of MST was determined by measuring the amount of pyruvate formed during 15-min incubation at 37 °C in accordance with the method of Valentine and Frankenfeld [19]. The assay has two stages: first, sulfur is transferred by MST from 3-mercaptopyruvate yielding pyruvate; and then pyruvate is reduced to lactate by LDH in the presence of NADH. This method utilizes the difference in absorbance between NADH and NAD^+ at 340 nm, which is a measure of the amount of pyruvate formed in MST-catalyzed reaction.

Determination of rhodanese activity

The activity of rhodanese was assayed according to the Sorbo's method [17] based on sulfane sulfur transfer from thiosulfate as a substrate to cyanide with thiocyanate formation. The absorbance of SCN^- formed during a 5-min incubation at 20 °C is measured at 460 nm.

Determination of hemoglobin content

Hemoglobin content was assayed by the Drabkin's method [5]. In this method Hb is oxidized by $K_3[Fe(CN)_6]$ to methemoglobin, which reacts with KCN yielding the stable derivative cyanomethemoglobin with the maximum absorbance at 540 nm.

Statistical analysis

The results are presented as the means ± SEM, and statistical significance of differences was evaluated using a one-way ANOVA.

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