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^-) 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-mercaptopypyruvate 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) formed in those reactions are more stable and less toxic than thyl radicals (RS^+). 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-mercaptopopyruvate 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...
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), 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 treatment. Blood of CAPD patients and healthy control subjects was collected 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 (Neurilipon-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 Fe3+ ions yielding red ferric thiocyanate 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 K4[Fe(CN)6] to methemoglobin, which reacts with KCN yielding the stable derivative cyanomethe-moglobin 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 one-way ANOVA.
The differences were considered statistically significant when \( p < 0.05 \).

**Results**

The studies demonstrated that the concentration of sulfane sulfur in ESRF patients treated with CAPD remained at the same level as in the group of healthy volunteers. After LA supplementation in the same group of CAPD patients, the sulfane sulfur level significantly rose (Fig. 1). In addition, in CAPD patients, erythrocytic activity of cystathionase, playing a crucial role in sulfane sulfur biosynthesis, increased (Fig. 2). It indicates that maintaining of the sulfane sulfur concentration at the control level in CAPD patients can be attributed to the elevated cystathionase activity. Further increase in the sulfane sulfur level caused by lipoate results from even greater increase in cystathionase activity. On the other hand, the activity of the second sulfurtransferase participating in the synthesis of sulfane sulfur-containing compounds, i.e. 3-mercaptopropionate sulfurtransferease (MST) was statistically significantly decreased in CAPD patients and did not change after LA supplementation (Fig. 3). The activity of rhodanese, an enzyme implicated in sulfane sulfur transfer to different acceptors, was slightly and insignificantly decreased in CAPD patients both untreated and treated with lipoate (Fig. 4).

**Discussion**

A common feature of patients suffering from end-stage renal failure (ESRF) patients is a progressive pro-inflammatory syndrome connected with aggravated oxidative stress [15]. Numerous reports demonstrated oxidative stress of the greatest intensity in patients treated with hemodialysis [8]. In CAPD-treated patients, due to continuous dialysis, toxic compounds do not accumulate, and, thus, severity of oxidative processes is lower. According to some authors, oxidative stress is linked with disturbances in thiol signaling in the cell [11,18]. Thiol compounds play a crucial role in maintaining normal redox status of cells. Our earlier studies confirmed disturbances in the thiol balance in chronic kidney disease patients [21].

These observations became an inspiration for the present studies on the effect of the thiol antioxidant lipoate on cysteine transformation to biologically active compounds of the sulfane sulfur pool in erythrocytes of CAPD patients. In our studies ESRF patients subjected to CAPD were administered p.o. the oxidized form of LA which is commonly used due to instability of the reduced form and capability of intracellular reduction.

Determinations performed in this work demonstrated that in CAPD patients, cystathionase activity was slightly increased while sulfane sulfur in erythrocytes remained at the control level. Our
earlier studies indicated a lowering of the sulfane sulfur level in erythrocytes of ESRF patients in pre-dialysis period [21] and in plasma of ESRF patients treated with hemodialysis, whereas a hemodialysis session further decreased the level of this reactive form of sulfur [20]. Possibly, preservation of the normal sulfane sulfur level in erythrocytes of CAPD patients resulted from continuous removal of uremic toxins which in non-dialyzed patients accumulate and lead to the inhibition of sulfane sulfur biosynthesis. No changes in the sulfane sulfur concentration in CAPD patients confirms also its antioxidant and regulatory actions. It also demonstrates the capability of detoxification of cyanide the concentration of which is elevated in ESRF patients [7]. It is commonly accepted that rhodanese and MST are the enzymes responsible for cyanide detoxification. However, the in vitro studies of Porter et al. [14] indicated that CSE also participates in this reaction. As cystathionase is a cytosolic enzyme, its role seems particularly important in erythrocytes which do not contain mitochondria. We observed that lipoate supplementation in patients significantly increased CSE activity and elevated the sulfane sulfur level which indicates much greater efficacy in detoxification of uremic toxins. A similar effect of LA on the enhancement of anaerobic cysteine metabolism leading to augmentation of the sulfane sulfur pool in vivo was also observed in the kidney and liver of healthy animals [2]. In addition, lipoate proved efficient in correcting the disturbances of anaerobic sulfur metabolism in cyanate (an uremic toxin)-induced peroxidative processes in the rat kidney [9].

In the present studies, generally lipoate did not affect MST activity. It means that the increase in the sulfane sulfur level is connected solely with the activation of the CSE-catalyzed pathway (the right side of Scheme 1).

In the light of the most recent reports, the hydrogen sulfide concentration is diminished in chronic kidney disease [1]. H2S is an endogenous signaling gas, which fulfills potent antioxidant, anti-inflammatory, antihypertensive and other regulatory functions. There is a close relationship between sulfane sulfur and hydrogen sulfide because sulfane sulfur-containing compounds can be direct precursors of H2S. According to Toohey, sulfurbased signaling depends not only on H2S but also on the sulfane sulfur level [18]. Modeling of the sulfane sulfur level, and consequently H2S concentration in the cell is difficult because of instability and tremendous reactivity of these compounds. For this reason, lipoate-induced increase in the sulfane sulfur level is crucial the more so that, as already mentioned, the hydrogen sulfide level was diminished in hemodialyzed ESRF patients [13].

In conclusion, supplementation with lipoate (LA) leads to increase in the sulfane sulfur level in erythrocytes of CAPD-patients, which preserves antioxidant and regulatory functions of this reactive sulfur.

Conflict of interest
There is no conflict of interest.

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References