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α-Lipoic acid increases the viability of nephrocytes and elevates sulfane sulfur level in plasma of patients with chronic kidney disease

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Abstract: Background: Kidney diseases are a major global health problem affecting millions of people. Despite this, there is as yet no effective drug therapy improving outcome in patients with renal disease. The aim of this study was to examine the nephroprotective effect of α-lipoic acid (ALA) in vitro and to examine the effect of ALA administered in vivo on the production of reactive sulfur species (RSS), including hydrogen sulfide (H_2S) and compounds containing sulfane sulfur.
Methods: The effect of ALA was studied in vitro by determining the viability of human embryonic kidney cells (HEK293) in normoxic and hypoxic conditions as well as in vivo in two groups of chronic kidney disease (CKD) patients: non-dialyzed (ND) and undergoing continuous ambulatory peritoneal dialysis (PD) after 30 days of ALA supplementation.
Results: The results revealed that the viability of HEK293 cells was significantly decreased by hypoxic conditions, while ALA administered during hypoxia increased the viability to the level observed in normoxic conditions. Studies performed in plasma of CKD patients after ALA supplementation suggested that ALA did not affect the parameters of oxidative stress, while significantly increased the level of reactive sulfane sulfur in both ND and PD patients suffering from CKD. The results suggest that ALA can exert nephroprotective effects which are related to sulfane sulfur production.

Keywords: chronic kidney disease, α-lipoic acid, sulfane sulfur, human embryonic kidney cells (HEK293), oxygen and glucose deprivation and next reoxygenation (OGD/R) procedure.

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Introduction

Kidney diseases are a major global health problem affecting millions of people in the world. However, there is as yet no effective drug therapy improving outcomes in patients with kidney diseases. Due to multiple causes of kidney failure, many experimental models both in vivo and in vitro have been developed to advance our understanding of kidney diseases. The main pathogenesis underlying acute kidney injury (AKI) is ischemia-reperfusion that induces significant oxidative injury and can be mimicked in vitro by an oxygen and glucose deprivation and next re-oxygenation (OGD/R) procedure applied to cultured kidney cells. In the present study, we exposed human embryonic kidney cells HEK293 to OGD/R by placing them in a hypoxia chamber containing 1%O_2, 5% CO_2 and 95% N_2. As already mentioned, the OGD/R model is a widely used in vitro model of ischemia/reperfusion (IR). In ischemic kidney and after subsequent reoxygenation, reactive oxygen species (ROS) generated in the reperfusion phase initiate a cascade of deleterious cellular responses leading to inflammation, cell death, and AKI, which in turn increases risk of developing chronic kidney disease (CKD) and cardiovascular disease (CVD) [1, 2]. So, well-known antioxidants, including α-lipoic acid (ALA; 1,2-dithiolane-3-pentanoic acid) and N-acetylcysteine (NAC) are proposed to be used to prevent or minimize I/R consequences in the kidneys.

ALA is a disulfide endogenously synthesized in the mitochondria. Amide of ALA acts as an essential coenzyme for certain dehydrogenase complexes including pyruvate dehydrogenase catalyzing oxidative decarboxylation of pyruvate, α-ketoglutarate dehydrogenase and dehydrogenase of branched-chain α-ketoacids, formed during transamination of leucine, isoleucine and valine. ALA is also an essential element of the mitochondrial glycine cleavage system. ALA can be easily reduced to 6,8-dimercaptooctanoic acid (dihydrolipoic acid, DHLA) and the reducing potential of ALA/DHLA couple is very low (–320 mV), thus, ALA can be considered as a universal and effective antioxidant. In pharmacologic doses, ALA scavenges ROS, and can chelate divalent transition metal ions forming stable complexes. Moreover, the low potential of ALA/DHLA system makes DHLA able to reduce the oxidized forms of other antioxidants including vitamins C and E and oxidized glutathione (GSSG). ALA can act as an antioxidant in both: the hydrophilic phase and in the hydrophobic membrane. Many experimental and clinical studies proved beneficial effect of ALA in diseases associated with an imbalance of redox status: diabetes and cardio-vascular disease [3, 4]. Literature data confirm the effectiveness of ALA also in kidney diseases [5, 6]. NAC is a prodrug of L-cysteine and glutathione (GSH) which is a non-enzymatic antioxidant. Nitescu *et al.* showed that NAC improved kidney function, and reduced renal interstitial inflammation in rats subjected to renal ischemia-reperfusion (IR) [7]. Many studies have also shown that NAC is beneficial for preventing contrast-induced nephropathy [8]. It is worth recalling that contrast-induced nephropathy is associated with dramatically increased mortality and morbidity that persists after hospital discharge, regardless of the need for renal dialysis [9]. On the other hand, some studies did not confirm the nephroprotective properties of NAC. Study of Webb *et al.* indicated that intravenous NAC was ineffective in preventing the contrast-induced nephropathy [10, 11].

Therefore in the light of the above-mentioned data, the present study was conducted to examine whether ALA can exert nephroprotective effects. Experiments were performed in vitro in HEK293 cell culture and were compared to the effect of NAC. Moreover, study in vivo in the plasma of CKD patients undergoing ALA supplementation aimed to assess the production of reactive sulfur species (RSS), including hydrogen sulfide (H_2S) and compounds containing sulfane sulfur were performed.

Materials and Methods

Cell culture and glucose deprivation/reoxygenation (OGD/R) model

Human embryonic kidney cells (HEK293) were kindly donated by Prof. Jolanta Jura (Jagiellonian University, Kraków). Cells were cultured in high-glucose DMEM (Life Tech-nologies, Poland, PL) containing 10% FBS (EURx, Poland, PL) and 100 units/ml penicillin (SigmaAldrich,), and 100 μg/ml streptomycin (Sigma-Aldrich). The cells were cultured in normoxic conditions at 37°C in an atmosphere containing 5% CO_2 and 21% O_2 in a humidified incubator. For cell viability tests, cells were cultured on 96 well plates.

HEK293 cells were seeded into 96-well plates in a final concentration of 3×10^4 cells in 200 μl of cell culture medium with glucose and FBS and subsequently were cultured for 24 hours. Then, media were removed and replaced by serum- and glucose-free DMEM with or without an appropriate ALA concentration. Next, the plates were placed in a hypoxia chamber and cultured in 1% O_2, 5% CO_2, 94% N_2 at 37°C for six hours. After this time, the plates were removed from anaerobic chamber and the medium was replaced with complete medium containing FBS and glucose. Reoxygenation was carried out for 16 hours.

MTT test

Cell viability was determined by the MTT assay analysis. In this assay, living cells reduce the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to insoluble purple formazan crystals. After reoxygenation 10 μL of MTT in PBS (5 mg/mL) was added to each well and the cells were left in a standard incubator (37°C degrees) for 3 h. Next, the cells were lysed by adding 100 μL of lysis buffer (10% SDS in 0.01 M HCl) and left for additional 24 h in a standard incubator. After that time, the absorbance was measured at 570 nm using a Synergy HT microplate reader (Biotek Instruments).

Lipoic acid and N-acetylcysteine

α-Lipoic acid (ALA) for in vitro study and N-acetylcysteine (NAC) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). ALA was solubilized in 3M NaOH solution and buffered to pH near 7 by adding 2 M HCl. Then the solution was diluted to the appropriate concentration of ALA in FBS-free DMEM. NAC was dissolved in FBS-free medium and diluted to the appropriate concentration.

ALA for treatment of CKD patients (Neurolipon-MIP 600) was obtained from MIP Pharma Poland.

Patients

A total of 28 CKD patients treated in the Rydygier's Hospital Fressenius Nephrocare II in Krakow and 15 healthy control subjects were recruited for this study. All participants in this study were Polish nationality. Study protocol was approved by Local Medical Ethics Committee and informed consent was obtained from all participants. The patients with CKD were divided into two groups: ND group — patients not treated with dialysis (non-dialyzed); PD group — patients undergoing

peritoneal dialysis. Parameters related to patients age, sex and renal function have been presented in Table 1. The control group comprised volunteers with no clinical history of renal diseases. Exclusion criteria in all groups included cancer, diabetes, liver disease, immune deficiency. All CKD patients participating in this study were supplemented orally for 30 days once a day after breakfast with ALA at a dose of 600 mg in the commercial preparation Neurolipon-MIP 600. Fasting blood samples from all participants were taken into EDTA tubes twice: one day before the first dose of ALA and one day after the last dose of ALA. Blood was centrifuged at 2500 rpm and plasma was divided into several samples and frozen at –80°C. The levels of sulfides, sulfane sufur and sulfates as the end products of oxidative RSS metabolism were assayed in the plasma. Moreover, the level of oxidative stress biomarkers: malonyl dialdehyde (MDA) and carbonyl protein groups were also estimated.

Biochemical assays

Sulfane sulfur. The level of sulfane sulfur in plasma was assayed according to the method of Wood [12], which is based on the reaction of cyanolysis. Details of the method were described previously [13].

Table 1. Characteristics of patients with chronic kidney disease (CKD) non-dialyzed (ND), undergoing peritoneal dialysis (PD) and healthy volunteers (Control) at the beginning of the experiment (before lipoic acid supplementation). Exclusion criteria in all groups included cancer, diabetes, liver disease, immune deficiency. Data are presented as the means ± SD.

Parameter	Control	Non Dialyzed (ND)	Peritoneal Dialysis (PD)*
Number of individuals	15	13	15
Sex	11 women 4 men	6 women 7 men	7 women 8 men
Mean age [years]	41.7 ± 8.0	70.5 ± 11.5	40.7 ± 9.4
Causes of kidney damage	No kidney damage	Glomerulonephritis — 2 Chronic pyelonephritis — 5 Polycystic kidney disease — 2 Hypertensive nephropathy — 3 Rheumatoid arthritis — 1	Glomerulonephritis — 6 Chronic pyelonephritis — 4 Hypertensive nephropathy — 4 Kidney stones — 1
Creatinine [µmol/l]	78.8 ± 9.1#	323.2 ± 128.7#	730.3 ± 261.2#
eGFR [ml/min]	71.7 ± 7.9#	18.6 ± 9.8#	7.0 ± 2.7#
Urea [mmol/l]	4.99 ± 1.20	19.35 ± 5.89#	22.45 ± 4.67#
Serum albumin [g/l]	45.88 ± 1.71#	39.27 ± 3.92#	35.71 ± 2.59#

Hydrogen sulfide. H_2S level was determined using a modification of the method of Shen *et al.* [14] with fluorometric detection. Details were described previously [15].

Inorganic sulfates. The level of inorganic sulfates was determined according Dogson's method [16]. The method is based on the precipitation reaction of barium sulfate in gelatin solution that stabilizes turbidity. Details of the method were described previously [13].

Carbonyl protein groups. Determination of the carbonyl group in plasma proteins was determined in reaction, in which 4-dinitrophenylhydrazine (DNPH) reacts with carbonyl groups of proteins forming yellow 2,4-dinitrophenylhydrazones [17]. Details of the method were described previously [18].

Lipid peroxidation. As a measure of lipid peroxidation, the level of MDA was determined in reaction with thiobarbituric acid (TBA) [19]. Briefly, to 250 µl of plasma, 500 µl of 15% TCA and 500 µl of 0.37% TBA were added. Then, samples were incubated at 100°C for 10 min, cooled and centrifuged at 12,000 × g for 10 min. The absorbance was assayed at λ = 535 nm and the level of MDA was evaluated using a standard curve prepared from 1,1',3,3'-tetraethoxypropane (TEP) at a concentration of 2.5 µM.

Statistical analysis

As for the viability assay (MTT test), the response to ALA concentration range was compared between normoxic and hypoxic cells. To verify the significance of the influence of ALA concentration and hypoxia on the viability of HEK293 cells, a two-way ANOVA with Fisher's post-hoc test was also used to test the significance of differences (Statistica 10, StatSoft Poland). The obtained results are presented as the means ± SD (standard deviations of the means) and were considered to be statistically significant when $p < 0.05$.

As for biochemical parameters in patient plasma, statistical analysis was performed using the Statistica 10, (StatSoft, Poland). Differences between the groups ND, PD and control were calculated using the analysis of variance and post-hoc comparisons or non-parametric Kruskal-Wallis test. The difference between the parameters before and after supplementation of ALA in the ND and PD groups was calculated using the Student's t-test when the distribution was normal or the Wilcoxon's test when the distribution differed from normal. p values <0.05 were considered statistically significant.

Results

The effect of ALA and NAC in various concentrations on viability of HEK293 cells

To establish the effect of ALA on the viability of HEK293 cells, we administrated ALA in the concentration range from 500 µM to 5 mM during hypoxia lasting 6 hours. After hypoxia conditions, medium with ALA was removed and replaced by complete medium containing FBS and glucose and cells were cultured in normoxic conditions for 16 hours (re-oxygenation). Simultaneously, the experiment was carried out with the same concentrations of ALA in normoxia. The obtained results revealed that the viability of HEK293 was significantly decreased by hypoxic conditions. ALA in concentration 2–5 mM administered during hypoxia increased the viability of HEK293 to the normoxic control level (Fig. 1A). Treatment of cells in normoxic conditions with the same

concentration of ALA did not affect the viability of the cells. Comparable studies with the same concentration range were conducted with NAC. The obtained results have shown that none of the tested concentrations of NAC increased cell viability (Fig. 1B). It suggested that positive effect of ALA on HEK293 viability is not only due to its antioxidant properties.

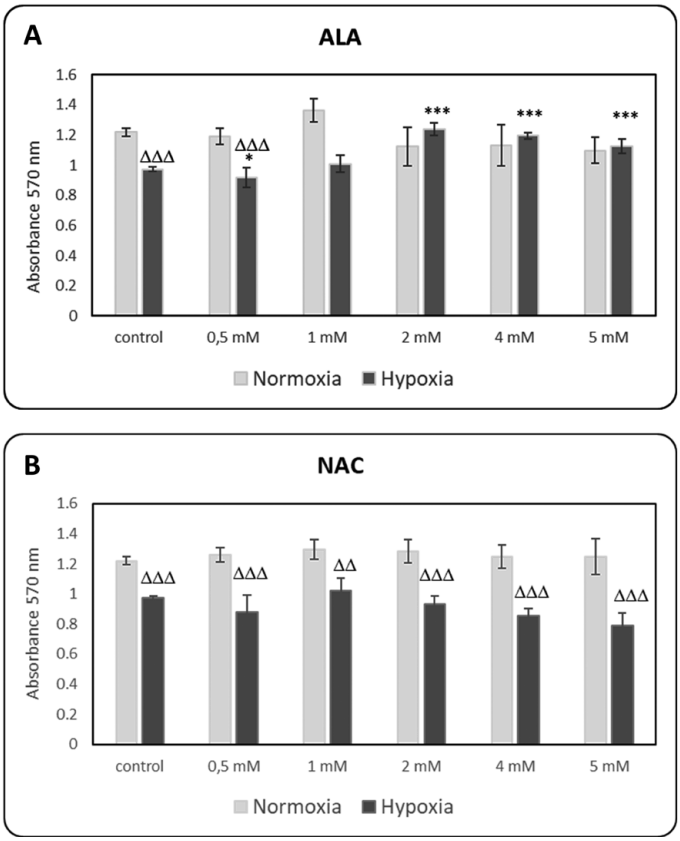

Fig. 1. The effect of α-lipoic acid (ALA) (A) and N-acetylcysteine (NAC) (B) on the viability of HEK293 cells. ALA and NAC were administrated in the concentration range from 500 μM to 5 mM. ALA in concentration 2–5 mM administered during hypoxia increased the viability of HEK293 cells to the normoxic level. The treatment was performed during hypoxia lasting 6 hours. In normoxic conditions cells were treated with ALA or NAC for the same time. Data are presented as mean from six samples ± SD. $^{\Delta\Delta\Delta}$ <0.001 compared to normoxic control; *p <0.05, ***p <0.001; compared to hypoxic control.

Biochemical parameters of CKD patients in relation to the control group

Table 2 presents the baseline plasma parameters associated with oxidative stress (MDA, protein carbonylation) and reactive sulfur species in the control group, in the group of CKD patients non-dialysed (ND) and CKD patients undergoing peritoneal dialysis (PD). In patients with CKD slightly elevated level of MDA compared to the control group was observed but only in PD patients this increase was statistically significant (p = 0.0056). In CKD patients an increase of protein carbonylation degree has also been found, however these changes were not statistically significant. A distinctive feature of CKD patients is significantly increased level of inorganic sulphates being the final product aerobic metabolism of cysteine (p = 0.000123 for ND and p = 0.000125 for PD). In the case of RSS formed during anaerobic transformations of cysteine, i.e. hydrogen sulfide and sulfane sulfur, only a decrease in the level of hydrogen sulfide in the ND vs. control was observed (p = 0.049).

Table 2. Biochemical parameters related to oxidative stress and reactive sulfur species determined in blood plasma in a group of healthy volunteers (control) and in CKD patients nondialysed (ND), as well in CKD patients undergoing peritoneal dialysis (PD) at the beginning of the experiment, i.e. before the start of LA supplementation. Data are presented as the means ± SD.

Parameter	Control mean ± SD	ND mean ± SD	PD mean ± SD	p
MDA [nmol/ml]	1.40 ± 0.38	1.62 ± 0.35	1.99 ± 0.57	0.0064
Carbonyl groups [nmol/ml]	5.60 ± 1.24	6.54 ± 1.66	5.96 ± 1.09	0.2230
Sulfates [nmol/ml]	0.77 ± 0.17	1.50 ± 0.31	1.40 ± 0.16	<0.0001
Sulfane sulfur [nmol/ml]	97.57 ± 24.28	94.97 ± 26.79	89.24 ± 16.79	0.6000
Hydrogen sulfide [μmol/ml]	6.53 ± 1.31	5.29 ± 1.81	6.88 ± 2.11	0.0459

The effect of 30-day ALA treatment at a dose of 600 mg on oxidative stress-related parameters and biochemical markers of sulfur metabolism in plasma of non-dialyzed (ND) patients diagnosed with CKD

In ND patients, 1-month ALA supplementation did not change the MDA level (Fig. 2A). Also, protein carbonylation level was not reduced after treatment of the patients with ALA. Hence, the obtained results indicated that ALA, known for its antioxidant properties, did not significantly affect the MDA level or degree of protein carbonylation (Fig. 2B). Thirty-day ALA supplementation did not significantly affect neither the level of hydrogen sulfide and the level of sulfates ($p > 0.05$) (Fig. 3A, 3B). In turn, it is noteworthy that ALA treatment in ND patients significantly raised the level of reactive sulfane sulfur ($p = 0.0063$) (Fig. 4).

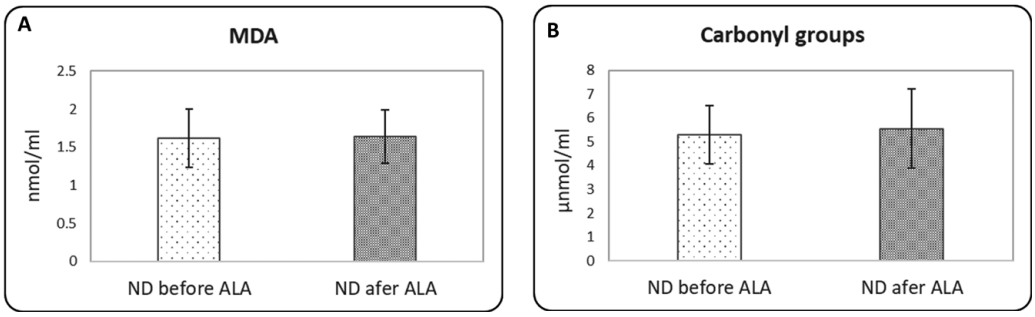

Fig. 2. The effect of 30-day ALA supplementation at a dose of 600 mg on oxidative stress parameters: malonyldialdehyde (MDA) (A) and carbonyl groups (B) in plasma of non-dialyzed (ND) patients diagnosed with CKD. Data are presented as mean ± SD (n = 13).

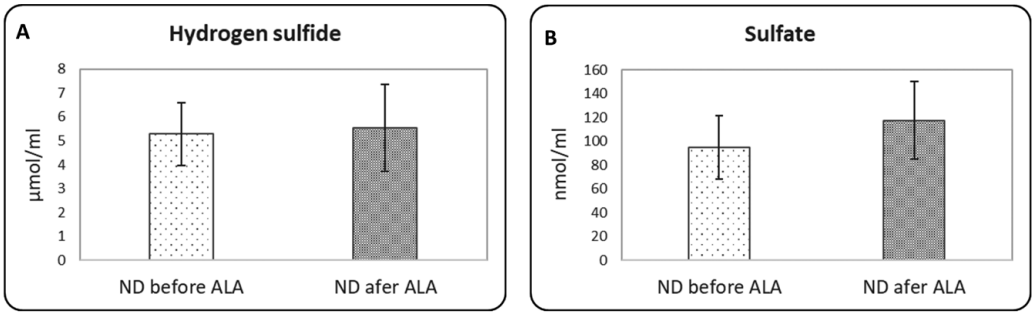

Fig. 3. The effect of 30-day ALA supplementation at a dose of 600 mg on hydrogen sulfide level (A) and sulfates concentration (B) in plasma of non-dialyzed (ND) patients diagnosed with CKD. Data are presented as mean ± SD (n = 13).

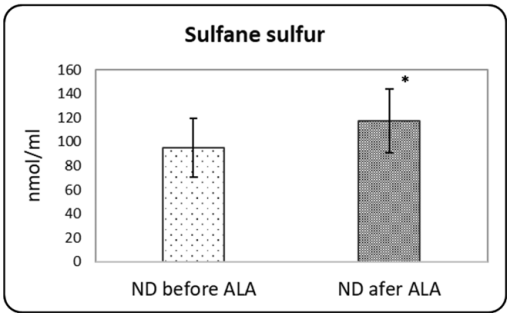

Fig. 4. The effect of 30-day ALA supplementation at a dose of 600 mg on sulfane sulfur level in plasma of non-dialyzed (ND) patients diagnosed with CKD. Data are presented as mean ± SD (n = 13). *p <0.05 compared to the same group before ALA treatment.

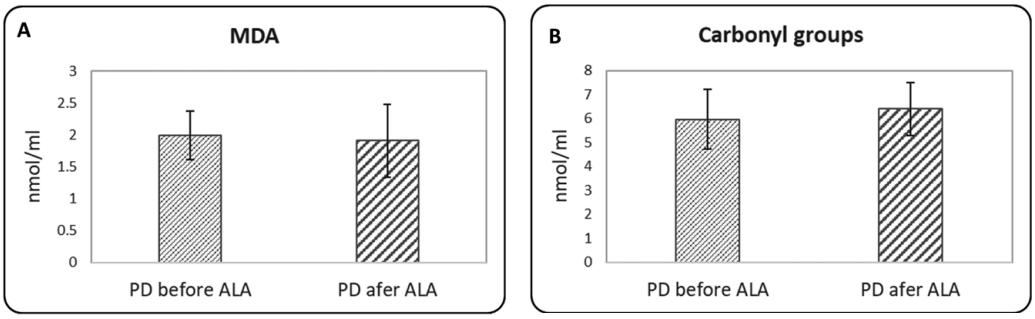

Fig. 5. The effect of 30-day ALA supplementation at a dose of 600 mg on oxidative stress parameters: malonyldialdehyde (MDA) (A) and carbonyl groups (B) in plasma of patients diagnosed with CKD treated with peritoneal dialysis (PD). Data are presented as mean ± SD (n = 15).

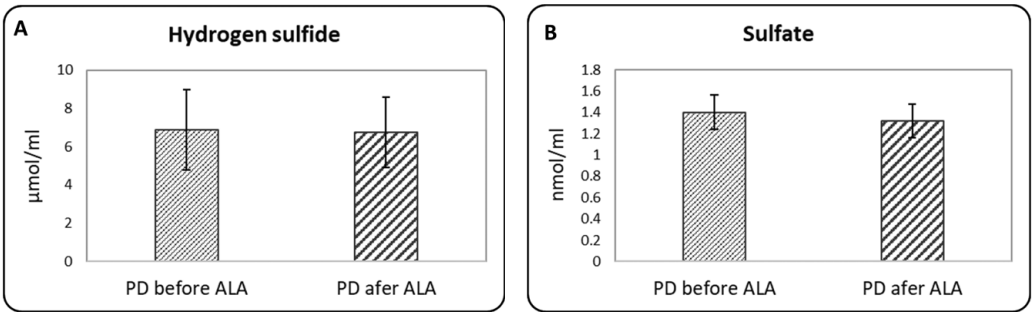

Fig. 6. The effect of 30-day ALA supplementation at a dose of 600 mg on hydrogen sulfide level (A) and sulfates concentration (B) in plasma of patients diagnosed with CKD treated with peritoneal dialysis (PD). Data are presented as mean ± SD (n = 15).

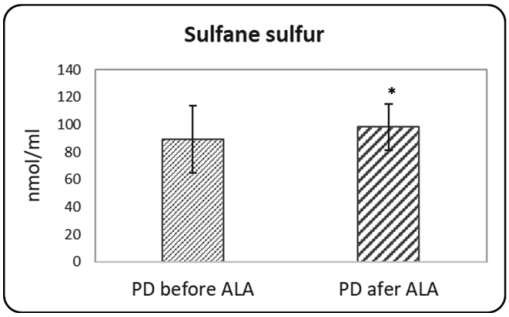

Fig. 7. The effect of 30-day ALA supplementation at a dose of 600 mg on sulfane sulfur level in plasma of patients diagnosed with CKD treated with peritoneal dialysis (PD). Data are presented as mean ± SD (n = 15). *p <0.05 compared to the same group before ALA treatment.

The effect of 30-day ALA supplementation at a dose of 600 mg on oxidative stress-related parameters and biochemical markers of sulfur metabolism in plasma of patients diagnosed with CKD and treated with peritoneal dialysis (PD)

In PD patients, after 1-month ALA supplementation, MDA level was not reduced. (Fig. 5A). Likewise, no changes in protein carbonylation were noted (Fig. 5B). Therefore, the obtained results indicate that ALA administration to dialyzed patients does not significantly affect lipid peroxidation level or degree of protein carbonylation ($p > 0.05$). ALA supplementation did not significantly alter hydrogen sulfide and sulfate levels ($p > 0.05$) (Fig. 6A, 6B). However, just as in ND patients, a significant increase in sulfane sulfur level was observed after 30-day ALA supplementation (Fig. 7).

Discussion

The obtained results indicated that the viability of HEK293 cells cultured in OGD/R environment was significantly decreased. At the same time, we have shown that ALA in concentrations 2–5 mM administered during hypoxia increased the viability of HEK293 cells to the normoxic control level (Fig. 1A). This result appears to be in line with our expectations, because it seems obvious that compounds capable of scavenging ROS are ideal protective agents against IR injury. The an-

tioxidant properties of ALA have been repeatedly confirmed by many authors on various renal IR injury models, both in vitro and in vivo [20–22]. However, the results of our next experiment turned out to be surprising. Namely, the NAC administered during hypoxia did not affect the viability of HEK293 cells (Fig. 1B). This may mean that the positive effect of ALA on HEK293 cells we observed is not directly related to its antioxidant properties. In order to better understand the mechanisms responsible for the beneficial effect of ALA on kidney function, we also conducted research in clinically stable CKD patients. The obtained results showed that ALA, known for its antioxidant activity, produced no significant effect on the level of MDA or the degree of protein carbonylation in both groups (ND and PD) of CKD patients (Figs. 2A and 2B, and 3A and 3B, respectively). Similarly, Khabbazi *et al.* demonstrated that high sensitive C-reactive protein (hsCRP), as an inflammation biomarker and other oxidative stress biomarkers including MDA as well as total antioxidant status were not altered in the ALA-treated groups of CKD patients [23]. Other studies performed in the serum of patients undergoing hemodialysis (HD) also did not reveal changes in oxidative stress biomarkers after ALA treatment [23–25]. This confirms the hypothesis that the positive effect of ALA on kidney cells is not related to its antioxidant properties.

In 2008, we showed for the first time in vivo that biological activity of ALA might be connected with anaerobic metabolism of cysteine to sulfane sulfur compounds [26]. Then, in 2017, we demonstrated that H_2S and sulfane sulfur were formed in vitro from ALA non-enzymatically in the presence of environmental light [27]. It has been also documented in in vivo conditions that the level of sulfane sulfur was elevated in the rat kidney, heart and liver after intraperitoneal administration of ALA [26, 28, 29].

Sulfane sulfur is a labile reactive sulfur atom in the 0 or –1 oxidation state, which is covalently bound to another sulfur atom (R-S-S*-). Sulfur with such features can easily leave the compound's structure and can be transferred to acceptors, such as thiols or cyanide [30]. Compounds containing this reactive sulfur include persulfides, polysulfides, thiosulfate, thiosulfinates, polythionates, and elemental sulfur. Sulfane sulfur appears in a number of biologically important compounds, including thiocysteine, thiocystine and thiotaurine, products of the anaerobic cysteine metabolism, as well as in glutathione persulfide [31]. At present, interrelations between the existing deposits of sulfane sulfur in cells and endogenously produced H_2S are increasingly indicated. It is generally assumed that sulfane sulfur is a form of storage and transport of H_2S, from where — depending on the needs of the cell — it can be released back as H_2S in the presence of reducing agents [32]. H_2S, until recently commonly treated only as a toxic gas, is now perceived as an important endogenous metabolite, crucial for biochemical and physiological processes taking place in the cells of living organisms. The emerging importance of H_2S and its derivatives as signaling molecules is also confirmed by coining the term „reactive sulfur species (RSS)" analogous to the already existing „reactive oxygen species (ROS)" and "reactive nitrogen species (RNS)" [33]. RSS include mainly sulfane sulfur compounds (persulfides and polysulfides) as well as H_2S. A recent research by Griffiths *et al.* also is noteworthy because the authors demonstrated that endogenous cysteine persulfide (CysSSH) played an important role as a "redox preconditioning" agent to combat the oxidative insult in myocardial IR [34].

Therefore in the light of the above-mentioned data, the present in vivo study was conducted to examine whether nephroprotective effects of ALA are connected with RSS production. The obtained results indicated that CKD patients in both PD and ND groups showed a significant increase in plasma sulfane sulfur levels after 30 days of ALA supplementation ($p = 0.0043$ and $p = 0.0063$, respectively) (Figs. 4A and 7).

It has been quite well documented that many diseases, including CKD are associated with a disturbance of the endogenous RSS pool, most often with its decrease. Kuang *et al.* indicated that the plasma H_2S level (μmol/L) in CKD patients was significantly lower than in healthy controls (7.32 ± 4.02 vs. 14.11 ± 5.24 μmol/L, $p < 0.01$) [35]. Similarly, studies by Li *et al.* showed that low plasma H_2S level was associated with impaired renal function [36]. In the present study, we observed a decrease in the H_2S level in plasma of ND patients but not in PD patients. Our previous studies showed a statistically significant decrease of sulfane sulfur concentration in erythrocytes of ND patients with CKD [18]. Sulfane sulfur in the cells and tissues of CKD patients is important because of its significance for detoxification of cyanide, increased level of which has been observed in uremic patients [37]. The increase in the sulfane sulfur level was also found in erythrocytes of patients undergoing PD supplemented with ALA after 30 days of treatment [38]. Olson *et al.* showed that ALA increased H_2S and sulfane sulfur in a concentration-dependent manner in vitro in HEK293 cells [39, 40]. In turn, Dugbartey *et al.* indicated that ALA in diabetic rats significantly increased hepatic expression of H_2S-producing enzymes as well as hepatic sulfane sulfur and H_2S levels compared to healthy control rats [41].

Thus, the increase in plasma sulfane sulfur levels we found after supplementation with ALA in CKD patients seems to suggest that ALA may prove to be an effective drug supporting the treatment of CKD patients. The suggestion seems all the more justified as our previous research showed that in the CKD patients in ND group supplemented with ALA for 30 days, the serum creatinine concentration decreased by 17.9 μmol/L on average and thus eGFR increased by 2 mL/min per 1.73 m^2 [42]. It would seem that the increase in eGFR is relatively small. However, taking into account that the placement on the waiting list for a donor kidney transplant occurs when patient's eGFR is 20 mL/min per 1.73 m^2 or less and that most patients do not receive a transplant until their eGFR is less than 15 mL/min per 1.73 m^2, this value is a significant improvement of kidney function [43]. This means that the use of ALA in CKD patients, especially in the stadium of predialysis (ND), slows down the progression of the disease and postpones the need for renal replacement therapy.

The protective potential of ALA in kidney disease have been reported in some previous studies conducted on humans [44–46] but none of them relates the biological activity of ALA to reactive sulfur species. Our study shows that administration of ALA can elevate sulfane sulfur level in plasma of CKD patients. Moreover, our in vitro study showed that ALA increases the viability of kidney cells. Thus, we propose ALA as a nephroprotective agent able to improve kidney function by increasing the viability of kidney cells and by raising the level of reactive sulfane sulfur in patients suffering from CKD.

Strengths and limitations

Our study provides strong evidence that ALA in vitro increases the viability of the tested kidney HEK293 cells undergoing hypoxia. Moreover, we showed that ALA supplementation of CKD patients leads to an increase in their plasma sulfane sulfur level without affecting oxidative stress parameters. At the same time we are aware that there are existing limitations that should be taken into consideration. The major limitation of this study is the small number of patients in both CKD groups and healthy volunteers (13 to 15 individuals). Another drawback of the study may be that the individuals in ND group were older than subjects in the PD group and in the control group. Theses limitations result from the difficulties in finding at the same time more patients suffered

from chronic kidney disease non-dialyzed or undergoing peritoneal dialysis, who meet certain criteria, including age, sex and the lack other comorbidities. Despite the above limitations our study showed the protective potential of ALA against kidney cells what suggests ALA as a nephroprotective agent. Our study provide evidence that biological effect of ALA in kidney disease is related to sulfane sulfur production rather than its antioxidant potential.

Author contributions

A.B.W., M.I. and B.M. contributed to conceptualization, methodology and investigation. M.G., K.G. and K.K.P. were involved in investigation and formal analysis. A.B.W. and M.I. wrote, prepared and revied the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

None declared.

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