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THE EFFECT OF LIPOIC ACID ADMINISTRATION ON THE URINARY EXCRETION OF THIOCYANATE IN RATS EXPOSED TO POTASSIUM CYANIDE

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Abstract: The oxidation of cyanide (CN^-) to a much less toxic thiocyanate (SCN^-) is the main *in vivo* biochemical pathway for CN^- detoxification. SCN^- is excreted mainly in urine. This study was performed to investigate the effect of lipoic acid (LA) on the urinary excretion of thiocyanate (SCN^-; rhodanate) in rats. Groups of the animals were treated intraperitoneally (*i.p.*) as follows: group 1: potassium cyanide (KCN) (1 mg/kg); group 2: KCN (1 mg/kg) + LA (100 mg/kg). Urine was collected for 24 h and the pooled samples were examined for SCN^- levels. The obtained results indicated that the treatment of animals with potassium cyanide and LA in combination significantly increased the urinary excretion of SCN^- in comparison with the respective values in the KCN-alone-treated group. It indicates that LA increased the rate of CN^- detoxification in rats.

Keywords: lipoic acid, dihydrolipoic acid, cyanides, thiocyanates (rhodanates), sulfane sulfur

Cyanides (CN^-), salts of hydrogen cyanide (HCN), and HCN itself have always been feared as poisons because of their toxic properties. Cyanides are synthesized, excreted and degraded in nature by many species of bacteria, plants and insects. Cyanides have also been used for various purposes in industry. Toxic level of cyanides in the body is generated after administration of certain drugs, like laetrile and sodium nitroprusside. Cyanide targets primarily mitochondrial cytochrome oxidase, thereby causing the inhibition of cellular respiration, acceleration of anaerobic glycolysis, and consequent tissue hypoxia and metabolic lactic acidosis.

The oxidation of CN^- to a much less toxic thiocyanate (the oral LD_{50} values of cyanide and thiocyanate in rats are 3 and 854 mg/kg, respectively) (1) is the main *in vivo* biochemical pathway for CN^- detoxification according to the following reaction:

$$CN^- + S \rightarrow SCN^-$$

The conversion of CN^- to SCN^- is catalyzed by sulfurtransferases: rhodanese (EC. 2.8.1.1; TST), 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2; MST) and cystathionine γ-lyase (EC 4.4.1.1; CSE). The role of these enzymes consists in sulfane sulfur transfer to CN^-.

Sulfane sulfur is a divalent sulfur atom (-S-) covalently bound to another sulfur atom (R-S-S-H). The elemental sulfur (S_8) and outer sulfur atom of thiosulfate ($S{=}SO_3^-$) exhibit also sulfane sulfur properties. The sulfane sulfur-containing compounds are endogenous metabolites formed from cysteine. Cysteine is synthesized from the essential amino acid methionine to a limited extent and for this reason, it is called a semi-essential amino acid. Cysteine and methionine are only two of the twenty amino acids normally present in proteins and belong to the sulfur-containing amino acids (SAA). Thiocyanate is excreted mainly in urine.

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Lipoic acid (R)-5-(1,2-dithiolan-3-yl)pentanoic acid, LA, $C_8H_{14}O_2S_2$), a naturally occurring dithiol compound, is a cofactor for a number of multienzymatic complexes involved in energy metabolism. Dihydrolipoic acid (6,8-dimercaptooctanoic acid, DHLA, $C_8H_{16}O_2S_2$) is the reduced form of LA. Exogenous LA has been shown to exhibit pharmacological activities (2–6). Literature data indicate that the LA/DHLA system participates also in sulfane sulfur metabolism. Namely, it was shown that DHLA serves as a sulfane sulfur acceptor in the TST- and MST-catalyzed sulfane sulfur transfer. In these reactions DHLA hydropersulfide is formed, from which sulfane sulfur is released in the form of hydrogen sulfide (H_2S) and LA is produced concomitantly (7, 8).

Thus, it can be assumed that LA may be involved in cyanide detoxification reactions in the body. This study was undertaken to investigate for the first time the effect of LA administration on the urinary excretion of SCN^- in rats exposed to CN^-.

EXPERIMENTAL

Animals

The experiments were carried out on male Wistar rats (300–350 g). Animals were housed in metabolic cages for urine collection, in a room at a constant temperature of 20 ± 2°C with a natural light-dark cycle. They had free access to standard pellet diet and water. Groups consisted of 6 animals each. The pharmaceutical with the commercial name Thiogamma, which contains LA as an active substance was used in our experiments as a source of LA. All procedures were approved by the Ethics Committee for Animal Research in Kraków (license no. 44/2012).

Groups were treated as follows: Group 1: KCN (1 mg/kg b.w., *i.p.*). Group 2: KCN (1 mg/kg b.w.; *i.p.*) + LA (100 mg/kg b.w.; *i.p.*).

Urine was collected for 24 h after drug injection and the pooled samples were examined for SCN^- levels.

Methods

Determination of thiocyanate level in the urine of rats

The content of SCN^- was determined by the method of Goldstein (9). SCN^- present in the urine reacts with Fe^{3+} ions to form the colored compound $Fe(SCN)_3$, the concentration of which is measured spectrophotometrically at a wavelength of $\lambda = 450$ nm. SCN^- concentration is calculated based on a standard curve prepared for potassium thiocyanate (KSCN).

Statistical analysis

Data are presented as the means ± SEM. The statistical significance of the differences between the means was analyzed using Student's *t*-test. A level of $p \leq 0.001$ was adopted to indicate statistical significance.

RESULTS

The results presented in Table 1 indicated that 24-h urinary excretion of SCN^- was significantly higher in rats which were administered KCN and LA in combination in comparison with the group treated with KCN alone (4.86 µmol/24 ± 0.23 h *vs.* 2.5 µmol/24 ± 0.37 h, respectively).

DISCUSSION AND CONCLUSIONS

The obtained results showed that LA increased CN^- detoxification rate in rats, which indicates that this compound can act as a CN^- antidote. It was confirmed also by studies of other authors. Müller and Krieglstein reported that one hour of preincubation with 1 µM DHLA reduced damage of neurons from chick embryo telencephalon caused by 1 mM sodium cyanide (10). Abdel-Zaher et al. demonstrated that intraperitoneal injection of KCN into mice produced clonic and tonic seizures. The severity of convulsions was dose-dependent. The ED_{50} value of KCN was 4.8 mg/kg. In contrast, animals administered LA at a dose of 100 mg/kg before KCN injection showed the increase in CN^- ED_{50} values from 4.8 mg/kg to 9.4 mg/kg (10). The same authors also demonstrated that LA (25, 50 and 100 mg/kg) given to mice 15 min before KCN injection increased the CN^- LD_{50} values (based on 24 h mortality) from 6 mg/kg to 6.2, 7.2 and 11.2 mg/kg, respectively (11). It has also been reported that LA protects cholinergic cells against sodium nitroprusside neurotoxicity (12).

Therefore, it is a plausible proposal that LA plays a role in cyanide detoxification process. However, further studies are necessary to explain the mechanisms of these processes. Swenne et al. exposed normal and protein-malnourished rats to the cyanogenic compound acetonitrile (CH_3CN); that study indicated that urinary excretion of SCN^- was lower in rats fed a low-protein diet compared to rats fed a normal-protein diet (13). Analysis of the above data suggests that there may be some analogy with the present results. Namely, when rats are exposed to CN^- the urinary SCN^- excretion is higher in animals, which were fed a normal protein diet and in those which were administered LA. It indicates that

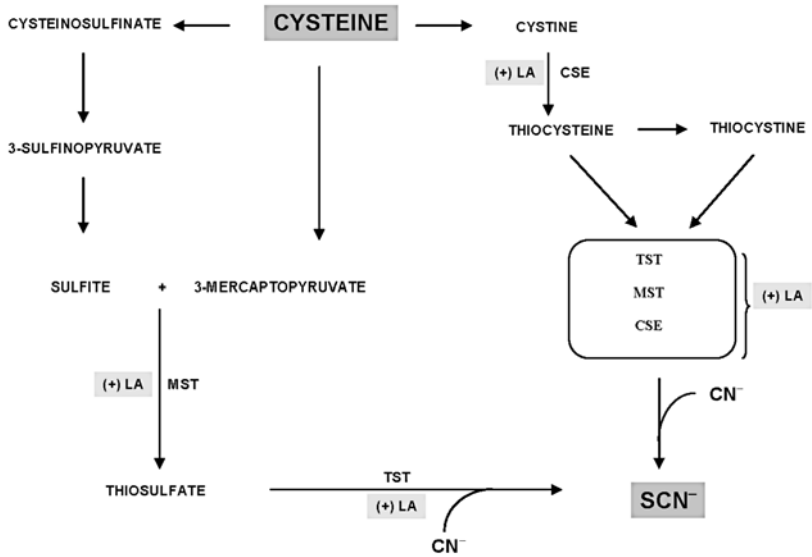

Figure 1. A proposal of the mechanism of LA action in processes leading to the formation of sulfane sulfur compounds: thiosulfate, thiocysteine and thiocystine, and in the CN^- to SCN^- transformation. TST – thiosulfate: cyanide sulfurtransferase (EC 2.8.1.1; rhodanese). MST – 3-mercaptopyruvate:cyanide sulfurtransferase (EC 2.8.1.2). CSE – cystathionine γ-lyase (EC 4.4.1.1; cystathionase)

Table 1. The urinary excretion of SCN^- in rats treated with KCN in the absence or presence of LA.

Treatment	SCN^- [µmol/24 h]
KCN	2.50 ± 0.37
KCN + LA	4.86[a] ± 0.23

[a]Student's *t*-test, significance $p < 0.001$ in comparison with KCN group

LA acted in that case like a normal-protein diet. The literature data indicate that a low-protein diet increases CN^- toxicity, because the concentrations of sulfane sulfur compounds are dependent on the availability of SAA from dietary protein (14). Tor-Agbidye et al. proposed that SAA deficiency might be a risk factor for human neurological diseases among protein-poor populations subsisting on cassava (*Manihot esculenta),* a cyanophoric plant (15). In 1981, Westley found that purified bovine liver TST had a high turnover rate of almost 20,000/min *in vitro*, which meant that 1 mole of TST could convert 20,000 moles of CN^- to SCN^- per 1 minute. This high turnover rate means that the availability of sulfane sulfur compounds, rather than the TST activity, is the rate limiting factor for CN^- conversion to SCN^- (16).

Therefore, it appears that all the data warrant hypothesis that LA increases CN^- to SCN^- transformation rate by elevation of the level of sulfane sulfur compounds in the body. It was confirmed also by data from Bilska et al. studies, which demonstrated for the first time *in vivo* that the level of sulfane sulfur compounds in the heart, liver and kidney of rats given LA at a dose of 100 mg/kg/24 h for 8 days was significantly higher than in the control LA-free group (17). The same experiments indicated that LA elevated also TST activity in several tissues of rats (17). However, in the light of the present knowledge, LA sulfur is not a sulfane sulfur, and TST does not participate in its formation. However, on the other hand, Frankenberg indicated that the administration of TST together with sulfane sulfur compounds had a good antidotic effect against CN^- poisoning in mice, yet, when sulfane sulfur compounds were given without TST, the antidotic effect was weaker (18).

So, based on literature data, it can be hypothesized that, apart from TST, LA also elevates activity of MST and/or CSE (Fig. 1). It is known that CSE and MST belong to enzymes which are directly involved in the generation of sulfane sulfur compounds. MST is also known to be implicated in the CN^- to SCN^- conversion reaction. As for CSE, *in vivo* studies indicated that the inhibition of this enzyme by L-propargylglycine increased the toxicity of CN^- (19). It can indicate that, like for MST, the CSE-catalyzed reactions lead both to the production of sulfane sulfur compounds, and to CN^- to SCN^-

conversion. Already in the 1960s, it was documented that the products of CSE-catalyzed cleavage of cystine (Cys-S-S-Cys) produced sulfane sulfur donors for TST (20, 21).

In summary, this paper clearly demonstrated that the treatment of rats with KCN and LA in combination significantly increased the urinary excretion of SCN^- in comparison with the respective values in the KCN-alone-treated group, which confirms the hypothesis suggesting that LA plays a role in the CN^- detoxification process. These results suggest that biological activity of LA may be connected with sulfane sulfur metabolism (Fig. 1). It points to potential new pharmacological properties of LA. However, confirmation of this hypothesis requires further studies.

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