Pharmacological Reports 66 (2014) 87–92

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

The effect of lipoic acid on cyanate toxicity in the rat heart

Maria Sokołowska [a,*], Maciej Kostański [a], Elżbieta Lorenc-Koci [b], Anna Bilska [a], Małgorzata Iciek [a], Lidia Włodek [a]

[a] The Chair of Medical Biochemistry, Jagiellonian University, Collegium Medicum, Kraków, Poland
[b] Department of Neuropsychopharmacology, Institute of Pharmacology, Polish Academy of Science, Kraków, Poland

ARTICLE INFO

Article history:
Received 12 February 2013
Received in revised form 26 June 2013
Accepted 2 August 2013
Available online 1 February 2014

Keywords:
Cyanate
Lipoic acid
Cardiovascular diseases
Ur(a)emia
Sulfane sulfur

ABSTRACT

Background: Cyanate is a uremic toxin formed principally *via* spontaneous urea biodegradation. Its active isoform, isocyanate, is capable of reaction with proteins by –N and –S carbamoylation, which influences their structure and function. Sulfurtransferases implicated in anaerobic cysteine transformation and cyanide detoxification belong to the enzymes possessing –SH groups in their active centers. The present studies aimed to demonstrate the effect of cyanate and lipoic acid on the activity of these enzymes as well as on the level of antioxidants and prooxidants in the rat heart.
Methods: Wistar rats, which received intraperitoneal injections of cyanate and lipoic acid alone and in combination were sacrificed 2.5 h after the first injection. The hearts were isolated and homogenized in phosphate buffer and next biochemical assays were performed comprising determination of the level of glutathione, malondialdehyde and sulfane sulfur and the activity of antioxidant enzymes as well as glutathione S-transferase and gamma glutamyl transferase.
Results: Sulfurtransferases and glutathione S-transferase were deactivated by cyanate treatment. It was accompanied by the decreased level of glutathione and sulfane sulfur and the increased level of reactive oxygen species and malondialdehyde. In parallel, antioxidant enzymes: catalase, glutathione peroxidase and gamma glutamyl transferase were activated under such circumstances. Lipoic acid, administered in combination with cyanate prevented the decrease in the level of glutathione and reduction of a pool of sulfane sulfur-containing compounds, concomitantly preserving the activity of antioxidant enzymes.
Conclusions: Since uremia, characterized by the elevated cyanate/isocyanate level, is accompanied by frequent cases of cardiovascular diseases, the addition of lipoic acid to the therapy seems promising in prophylaxis of heart diseases in uremic patients.

Introduction

Isocyanate, an active isoform of cyanate, is capable of reaction with proteins by –N and –S carbamoylation, thereby influencing their structure and function. In biological systems cyanate shows the highest reactivity with sulfhydryl (SH) groups of peptides and proteins [2,42]. Since the enzymes participating in anaerobic cysteine transformation, namely cystathionase (CSE EC 4.2.1.15) and mercaptopyruvate sulfurtransferase (MST EC 2.8.1.2), as well as the sulfane sulfur transporting enzyme, thiosulfate sulfurtransferase (TST EC 2.8.1.1) possess –SH groups in their active centers [33], isocyanate can potentially influence the activity of these enzymes. Consequently, it can influence cysteine transformation to hydrogen sulfide (H_2S) and sulfane sulfur compounds, *i.e.* polysulfides (R-S-S_n*-S-R), thiosulfate ($S_2O_3^{2-}$), persulfides (R-S-S*H) and others, which contain a labile highly reactive sulfur atom (S*) in 0 or −1 oxidation state, covalently bound to another sulfur atom. Sulfane sulfur compounds are formed by biodegradation of mixed disulfide of homocysteine and cysteine and β-elimination of L-cysteine, and both processes are catalyzed by CSE. On the other hand, H_2S is formed by decomposition of cysteine (β- and α,β-elimination) in the presence of CSE, desulfuration of 3-mercaptopyruvate in the presence of MST and during reactions of persulfides with an excess of thiols [8,15,32]. S*-containing compounds play an important role in cyanide (CN^-) to thiocyanate (SCN^-) detoxification catalyzed by TST and MST, as well as by CSE [22,32].

Results of our previous studies demonstrated peroxidative character of cyanate remaining in balance with isocyanate, its ability to lower glutathione and sulfane sulfur levels and to inhibit sulfurtransferase activities in the rat liver. On the other hand, these

Abbreviations: OCN^-, cyanate; NCO^-, isocyanate; DHLA, dihydrolipoic acid; S*, sulfane sulfur; GSH, glutathione; ROS, reactive oxygen species; GPx, glutathione peroxidase; TST, rhodanese; MST, mercaptopyruvate sulfurtransferase; CSE, cystathionase; CN^-, cyanide; SCN^-, thiocyanate; γGT, γ-glutamyl transferase; cLDL, carbamoylated LDL.

* Corresponding author.
E-mail addresses: mbsokolo@cyf-kr.edu.pl (M. Sokołowska), lorenc@if-pan.krakow.pl (E. Lorenc-Koci), mbbilska@cyf-kr.edu.pl (A. Bilska), miciek@cm-uj.krakow.pl (M. Iciek), mbwlodek@cyf-kr.edu.pl (L. Włodek).

1734-1140/$ – see front matter
http://dx.doi.org/10.1016/j.pharep.2013.08.009

studies demonstrated a stimulating effect of lipoic acid on the glutathione (GSH) production and activity of sulfurtransferases, and its antioxidant properties confirmed by a number of reports [5,6,28,29]. Since cyanate/isocyanate are the spontaneous biodegradation products of urea [4,10], the level of which is extremely high in uremic patients (higher than other substances), the results of our studies can to some extent be applicable to uremia. Uremia is characterized by retention of fluid and electrolytes, the considerable part of which affects the organism inducing negative biological changes. These are the so-called uremic toxins and urea is one of them [36,37]. Currently used defense strategies against uremic toxins entail intensification of their removal or attenuation of their toxic effects [36]. Since uremia is accompanied by frequent cases of cardiovascular diseases, we decided to perform similar investigations on the rat heart [9,36,44].

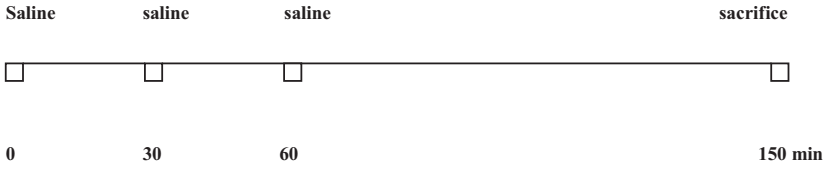
The present studies aimed to demonstrate *in vivo* the effect of the combined treatment with cyanate and lipoic acid both on the anaerobic cysteine transformation, and peroxidative processes in the rat cardiomyocytes in relation to cyanate and lipoic acid alone-treated animals. Lipoic acid, which can react with cyanate due to the presence of two –SH groups in its molecule [5] seems to be an agent which can diminish cyanate toxicity and potentially decrease the risk of heart diseases in uremic patients.

Materials and methods

Animals

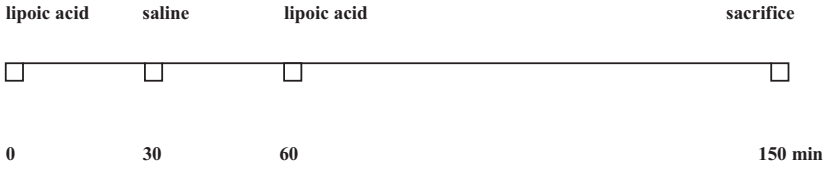
The experiments were carried out on male Wistar rats weighing approximately 250 g. The animals were kept under standard laboratory conditions and were fed a standard diet. All procedures were approved by the Ethics Committee for Animal Research in Kraków. Animals were assigned to 4 groups, containing 6–7 animals each. Groups were treated as follows:

Group 1



Group 2

Group 3



Group 4