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The influence of a nitric oxide synthase inhibitor and endothelin receptor blocker on the free sulfhydryl groups content in lung homogenates*

Wpływ inhibitora syntazy tlenku azotu oraz blokera receptora endotelinowego na zawartość wolnych grup sulfhydrylowych w homogenatach płuc

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Summary

Introduction: The aim of the study was to assess the influence of the nitric oxide synthase inhibitor L-NAME and the endothelin receptor blocker BQ123 on the free sulfhydryl (-SH) groups content in rat lung homogenates.

Material and methods: Experiments were performed on Wistar-Kyoto rats divided into the following groups: group I (control) received (*i.v.*) saline; group II (ET-1) received (*i.v.*) endothelin 1 (3 g μg/kg b.w.); group III (BQ123+ET-1) received (*i.v.*) ET_A receptor blocker (1 mg/kg b.w.) + endothelin 1 (3 g μg/kg b.w.); group IV (L-NAME+ET-1) received (*i.v.*) nitric oxide synthase inhibitor (5 mg/kg b.w.) + endothelin 1 (3 μg/kg b.w.).

Results: Administration of BQ123 at a dose of 1 mg/kg b.w. resulted in a statistically significant increase in the concentration of -SH groups ($p<0.001$ vs. ET-1). L-NAME (5 mg/kg b.w.) also significantly increased the level of -SH groups in the lungs of rats during oxidative stress induced ET-1 ($p<0.001$).

Discussion: The nitric oxide synthase inhibitor L-NAME at a dose of 5 mg/kg b.w. and the endothelin receptor blocker BQ123 at a dose of 1 mg/kg b.w. showed a significant increase in the concentration of -SH groups in the lungs, which may be associated with an increase in synthesis of proteins containing sulfhydryl groups.

Key words: **BQ123 • L-NAME • oxidative stress**

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Abbreviations: **BQ123** – ET_A receptor blocker; **eNOS** – nitric oxide synthase; **ET-1** – endothelin 1; **L-NAME** – NG--nitro-L-arginine methyl ester; **ROS** – reactive oxygen species; **VSMC** – vascular smooth muscle cell.

Introduction

Endothelin 1 (ET-1) is a 21-amino acid-length peptide that has powerful vasoconstrictor properties [32,39]. Peptide at physiological concentrations plays an important biological role in the body; e.g. it regulates blood pressure, maintains acid/base balance and electrolyte-water regimes, and participates in the growth and differentiation of cells. However, overproduction of this peptide may lead to the development of many diseases, especially diseases of the cardiovascular system [5,8,12,13,21]. ET-1 has proinflammatory properties, causes increased synthesis of many cytokines, and leads to neutrophil activation, peroxide formation and leukocytosis [3,17]. There are 3 main isoforms of endothelin: endothelin 1 (ET-1), endothelin 2 (ET-2) and endothelin 3 (ET-3). Peptides are composed of the two disulphide bridges Cys1-Cys15 and Cys3-Cys11 and highly hydrophobic, C-terminal fragment molecules. ET-1 is secreted by endothelium [30,38], cardiomyocytes, the respiratory system, kidneys, liver Kupffer cells, the mucosal lining of the intestines, the endometrium, macrophages and neurons [31]. ET-2 is synthesized in the kidneys, intestines and in small quantities in the uterus and heart [24]. ET-3 is produced in the digestive tract, the kidneys and the central nervous system [32]. The biologically active form of ET-1 is formed as a result of transformation of the two inactive precursors preproendothelin (prepro-ET) and proendothelin (proET), with the participation of specific enzymes [9]. Factors affecting the synthesis of this peptide are: hypoxia, angiotensin II, vasopressin, thrombin, insulin, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). However, nitric oxide, prostaglandin (PGE_2), bradykinin, heparin and glucocorticoids inhibit the production of endothelin [20]. Two types of receptors for endothelin 1 have been described: ET_A and ET_B (built with a similar number of amino acids: 415 to 442) [25]. In addition, within the ET_B receptors 2 subtypes have been described: ET_B (endothelial) and ET_{B1} (muscular). The ET_A receptor is located on vascular smooth muscle cells (VSMC), and its activation causes the contraction of the smooth muscle cells. ET_B receptors are present on endothelial cells and VSMC, and their activation induces vasodilation by activating NO synthase and prostacyclin PGI_2. Endothelin receptors, which are located on the VSMC, are responsible for vasoconstriction in the coronary, portal and renal circulation [6,22,26].

It was found that intravenous administration of ET-1 causes ischemia of the internal organs, vascular endothelial function disorder, ROS generation [36] and the development of oxidative stress [2]. Endothelin receptor blockade has found application in the clinical therapy of hypertension [15,16,18].

The aim of the present study was to assess the impact of a nitric oxide synthase inhibitor (L-NAME), and the endothelin receptor blocker ET_A (BQ123) on the content of free sulfhydryl groups in rat lung homogenates.

Material and methods

Experiments were performed on Wistar-Kyoto rats, aged 2-3 months. Animals were divided into the following groups: group I (control) received (*i.v.*) saline; group II (ET-1) received (*i.v.*) endothelin 1 (3 g µg/kg b.w.); group III (BQ123+ET-1) received (*i.v.*) ET_A receptor blocker (1 mg/kg b.w.) + endothelin 1 (3 g µg/kg b.w.); group IV (L-NAME+ET-1) received (*i.v.*) nitric oxide synthase inhibitor (5 mg/kg b.w.) + endothelin 1 (3 µg/kg b.w.).

All chemicals were administered via the femoral vein. Doses of the compounds were selected on the basis of literature. BQ123 and L-NAME were obtained from Sigma-Aldrich (Poland, ul. Szelągowska 30, 61-626 Poznań).

Measurement of -SH concentration in the lung homogenates

The total -SH groups content in lung homogenates was determined using the Ellman method [10] based on the reaction of 5,5'-dithio-bis (2-nitrobenzoic acid) with thiol groups of proteins. The absorbance of the obtained solution was measured at 412 nm using a Pharmacia LKB-Ultrospect III UV/VISIBLE spectrophotometer. 40 mg scraps of lungs were homogenized in a cold solution of 6% TCA. Then, the following were added to the measuring cuvette: 0.5 ml of the supernatant, 0.5 ml of 0.3 M Na_2HPO_4 and 0.5 ml of 0.04% Ellman reagent (DTNB) – freshly dissolved in a solution of 10% sodium citrate.

The data (µM) are presented as mean ± SEM (standard error of the mean) from 6 animals in each group. The statistical significance was evaluated by ANOVA followed by Duncan's multiple range test as post-hoc. The differences between the results in each group were evaluated using Student's t-test. A P value of less than 0.05 was considered significant.

The study was conducted with the consent of the Local Ethical Committee for Experiments on Animals, resolution no. 28/ŁB 520/2010.

Results

As shown in Figure 1, in the control group the concentration of free -SH groups was 18.12 ± 0.12 μM. Intravenous injection of endothelin 1 resulted in a statistically significant decrease in the concentration of -SH groups compared to the respective control group (13.79 ± 0.47 μM *vs.* 18.12 ± 0.12 μM, p<0.01). The administration of ET_A receptor blocker (BQ123) before ET-1 infusion resulted in an increase in -SH groups in comparison with ET-1 (20.43 ± 0.6 μM *vs.* 13.79 ± 0.47 μM, p<0.001). In the group receiving L-NAME + ET-1 the concentration of -SH groups was also significantly increased vs. ET-1 (18.18 ± 0.18 *vs.* 13.79 ± 0.47 μM, p<0.001).

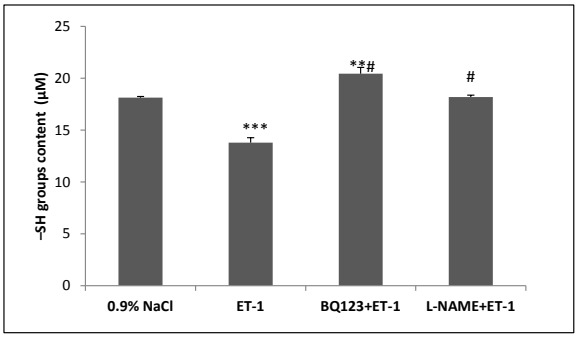

Figure 1. The influence of a nitric oxide synthase inhibitor and endothelin receptor blocker on the free sulfhydryl groups content in lungs homogenates; ET-1 – endothelin 1 (3 μg/kg b. w.); BQ 123+ET-1 - ETA receptor blocker (1 mg/kg b. w.) + ET- 1 (3 g μg/kg b. w.); L-NAME+ET-1 - nitric oxide synthase inhibitor (5 mg/kg b. w.) + ET-1 (3 μg/kg b. w.); *** p<0.001; **p<0.001 *vs*. 0.9% NaCl; # p<0.001 *vs*. ET-1

Discussion

Reactive oxygen species (ROS) participate in many important physiological processes, but their overproduction can lead to a cascade of reactions, resulting in degradation of cell ingredients and the development of oxidative stress, which is involved in the pathogenesis of many diseases.

In the present investigation, intravenous administration of ET-1 led to the development of oxidative stress. Similar results were obtained by Bohm and Pernow [2] and Thakali et al. [36]. Li et al. [23] and Elmarakby et al. [11] observed increased production of ROS after administration of ET-1 in experimental rats. Hynynen et al. [18] found that ROS increased the production of ET-1 and led to the development of oxidative stress. In our study, a decrease in the concentration of total free -SH groups in ET-1-treated rats may be attributed to the increase in ROS concentration in the lung homogenates. Proteins are the main object of attack by reactive oxygen species. It was reported that ROS (superoxide anion, hydrogen peroxide) oxidized thiol groups, which in turn can influence the structure and function of numerous proteins [28]. The reduction in the content of -SH groups in our study may result from a decrease in the synthesis of proteins containing -SH groups, as well as a decrease in glutathione synthesis. Research of Scalera et al. [34] confirms an increase in lipid peroxidation and decrease in intracellular glutathione and -SH groups during oxidative stress induced by ET-1. Viswanatha Swamy et al. [37] reported that ROS generation leads to reduction of the concentration of glutathione.

In recent years, it was found that the ET_A receptors [11,23] mediate in ROS production.

In our study intravenous application of ET_A receptor blocker during oxidative stress induced by ET-1 resulted in a statistically significant increase in the concentration of -SH groups. This fact can be explained by an increased synthesis of proteins containing thiol groups and increased glutathione synthesis. Glutathione is an important endogenous antioxidant responsible for free radical scavenging in all cell types. Antioxidant mechanisms also depend to a large extent on the presence of compounds containing -SH groups [1].

Therefore, the results obtained show an increase in antioxidant defense after administration of this compound. Briyal et al. [4] stated that BQ123 increases the level of superoxide dismutase (SOD) and contributes to a significant increase in the concentration of total glutathione. Ozdemir et al. [27] also confirmed that the use of BQ123 in oxidative stress increased the activity of antioxidant enzymes such as SOD and catalase (CAT).

Nitric oxide synthase (eNOS) is one of the sources of ROS (mainly superoxide anion) and reactive nitrogen (mostly $ONOO^-$) in the body [14]. Increased levels of NO can react with $O_2^{\bullet-}$, leading to the formation of $ONOO^-$, which in turn oxidizes sulfhydryl groups and generates hydroxyl radicals. Skalska et al. [35] showed that elevated concentrations of ET-1 can lead to overproduction of $ONOO^-$ and to decreased potential of antioxidant immune cells. One of the inhibitors of nitric oxide synthase, L-NAME, was applied in this study. In the present work, intravenous administration of L-NAME significantly increased the concentration of free-SH groups in the lung tissue after administration of ET-1. Therefore, this result may indicate that the oxidation of proteins containing -SH groups was inhibited. The results also show that L-NAME administration increases synthesis of antioxidant enzymes [7]. Jakovljevic et al. [19] proved that L-NAME reduces the lipid peroxidation. However, some authors [32,33] have observed a decrease in the concentration of glutathione and antioxidant enzymes after the application of this compound.

In conclusion, this report demonstrates that endothelin 1 causes a decrease in the concentration of free -SH groups, while nitric oxide synthase inhibitor and endothelin receptor blocker significantly increase the content of -SH groups in the lungs of rats during oxidative stress induced ET-1. We can assume that intravenous injection of BQ123 and L-NAME leads to inhibition of oxidation of proteins containing the -SH groups and thus to an increase in antioxidant capacity and a significant reduction in the generation of ROS in this organ.

Conclusions

1. The ET_A receptor blocker BQ123 given at a dose of 1 mg/kg b.w. and the nitric oxide synthase inhibitor L-NAME given at a dose of 5 mg/kg cause a significant increase in the concentration of free -SH groups in the lungs of rats during oxidative stress induced ET-1 (3 μg/kg b.w.).

2. Both BQ123 and L-NAME inhibit oxidation of proteins containing -SH groups.

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