

Received: 2014.03.13
Accepted: 2015.09.25
Published: 2015.31.12

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

Paulina Kleniewska^{B, C, D, E, F, G}, Anna Gorąca^{A, C, D, E, F, G}

Medical University of Lodz, Poland, Chair of Experimental and Clinical Physiology, Department of Cardiovascular Physiology

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 µg/kg b.w.); group III (BQ123+ET-1) received (i.v.) ET_A receptor blocker (1 mg/kg b.w.) + endothelin 1 (3 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

Full-text PDF:

<http://www.phmd.pl/fulltxt.php?ICID=1192623>

Word count:

1558

Tables:

–

Figures:

1

References:

39

*This study was supported by grant 503/0-079-03/503-01 and 502-03/0-079-03/502-04-017 from the Medical University of Lodz.

Author's address: prof. Anna Gorąca, Medical University of Lodz, Department of Cardiovascular Physiology, ul. Mazowiecka 6/8, 92-215 Lodz; e-mail: anna.goraca@umed.lodz.pl

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 (preproET) 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 (PGE₂), 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 PGI₂. 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 endo-

thelial 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. Szczęśliwowska 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-UV-trospect 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 Na₂HPO₄ 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 $18.12 \pm 0.12 \mu\text{M}$. Intravenous injection of endothelin 1 resulted in a statistically significant decrease in the concentration of -SH groups compared to the respective control group ($13.79 \pm 0.47 \mu\text{M}$ vs. $18.12 \pm 0.12 \mu\text{M}$, $p < 0.01$). The administration of ET_A receptor blocker (BQ123) before ET-1 infusion resulted in an increase in -SH groups in comparison with ET-1 ($20.43 \pm 0.6 \mu\text{M}$ vs. $13.79 \pm 0.47 \mu\text{M}$, $p < 0.001$). 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. $13.79 \pm 0.47 \mu\text{M}$, $p < 0.001$).

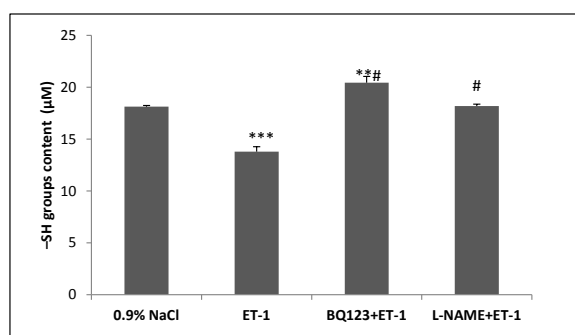


Figure 1. The influence of a nitric oxide synthase inhibitor and endothelin receptor blocker on the free sulfhydryl groups content in lungs homogenates; ET-1 – endothelin 1 (3 μg/kg b. w.); BQ 123+ET-1 - ETA receptor blocker (1 mg/kg b. w.) + ET-1 (3 g μg/kg b. w.); L-NAME+ET-1 - nitric oxide synthase 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 O₂⁻, 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.

REFERENCES

[1] Balcerczyk A., Bartosz G.: Thiols are main determinants of total antioxidant capacity of cellular homogenates. *Free Radic. Res.*, 2003; 37: 537-541

[2] Böhm F., Pernow J.: The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. *Cardiovasc. Res.*, 2007; 76: 8-18

[3] Boros M., Massberg S., Baranyi L., Okada H., Messmer K.: Endothelin 1 induces leukocyte adhesion in submucosal venules of the rat small intestine. *Gastroenterology*, 1998; 114: 103-114

[4] Briyal S., Philip T., Gulati A.: Endothelin-A receptor antagonists prevent amyloid- β -induced increase in ETA receptor expression, oxidative stress, and cognitive impairment. *J. Alzheimers Dis.*, 2011; 23: 491-503

[5] Comellas A.P., Briva A.: Role of endothelin-1 in acute lung injury. *Transl. Res.*, 2009; 153: 263-271

[6] Curtis T.M., Scholfield N.: Evidence for two endothelin E_{TA} receptor subtypes in rabbit arteriolar smooth muscle. *Br. J. Pharmacol.*, 2001; 134: 1787-1795

[7] Djukic M., Jovanovic M.D., Ninkovic M., Stevanovic I., Curcic M., Topic A., Vujanovic D., Djurdjevic D.: Intrastriatal pre-treatment with L-NAME protects rats from diquat neurotoxicity. *Ann. Agric. Environ. Med.*, 2012; 19: 666-672

[8] Dobrek Ł., Thor P.: Endothelin in cardiovascular diseases pathophysiology. *Pol. Merkur. Lekarski*, 2010; 28: 289-292

[9] Dorfman D.M., Wilson D.B., Bruns G.A., Orkin S.H.: Human transcription factor GATA-2. Evidence for regulation of preproendothelin-1 gene expression in endothelial cells. *J. Biol. Chem.*, 1992; 267: 1279-1285

[10] Ellman G.L.: SH group determination in biological fluids. *Anal. Biochem.*, 1970; 46: 233-235

[11] Elmarakby A.A., Loomis E.D., Pollock J.S., Pollock D.M.: NADPH oxidase inhibition attenuates oxidative stress but not hypertension produced by chronic ET-1. *Hypertension*, 2005; 45: 283-287

[12] Ergul A.: Endothelin-1 and endothelin receptor antagonists as potential cardiovascular therapeutic agents. *Pharmacotherapy*, 2002; 22: 54-65

[13] Feldstein C., Romero C.: Role of endothelins in hypertension. *Am. J. Ther.*, 2007; 14: 147-153

[14] Fleming I., Busse R.: Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2003; 284: R1-R12

[15] Hoepfer M.M., Halank M., Marx C., Hoeffken G., Seyfarth H.J., Schauer J., Niedermeyer J., Winkler J.: Bosentan therapy for portopulmonary hypertension. *Eur. Respir. J.*, 2005; 25: 502-508

[16] Hoepfer M.M., Seyfarth H.J., Hoeffken G., Wirtz H., Spiekerkoetter E., Pletz M.W., Welte T., Halank M.: Experience with inhaled iloprost and bosentan in portopulmonary hypertension. *Eur. Respir.*

CONCLUSIONS

1. The E_{TA} 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 μ g/kg b.w.).
2. Both BQ123 and L-NAME inhibit oxidation of proteins containing -SH groups.

J., 2007; 30: 1096-1102

[17] Huribal M., McMillen M.A.: Role of endothelin in ischemia-reperfusion injury. *Ann. N.Y. Acad. Sci.*, 1994; 723: 484-485

[18] Hynynen M.M., Khalil R.A.: The vascular endothelin system in hypertension - recent patents and discoveries. *Recent Pat. Cardiovasc. Drug Discov.*, 2006; 1: 95-108

[19] Jakovljevic V.L., Djordjevic D.Z., Djuric D.M.: The effects of vitamin C and nitric oxide synthase inhibition on coronary flow and oxidative stress markers in isolated rat heart. *Gen. Physiol. Biophys.*, 2011; 30: 293-300

[20] Janas J., Sitkiewicz D., Januszewicz A., Szczesniak C., Grenda R., Janas R.M.: Endothelin-1 inactivating peptidase in the human kidney and urine. *J. Hypertens.*, 2000; 18: 475-83

[21] Kulbacka J., Saczko J., Chwiłkowska A.: Oxidative stress in cells damage processes. *Pol. Merkur. Lekarski*, 2009; 27: 44-47

[22] Kun T., Dąbrowski R.: Endoteliny w regulacji funkcji układu krążenia. *Pol. Przegl. Kardiol.*, 2002; 4: 149-155

[23] Li L., Fink G.D., Watts S.W., Northcott C.A., Galligan J.J., Pagano P.J., Chen A.F.: Endothelin-1 increases vascular superoxide via endothelin(A)-NADPH oxidase pathway in low-renin hypertension. *Circulation*, 2003; 107: 1053-1058

[24] Masaki T., Miwa S., Sawamura T., Ninomiya H., Okamoto Y.: Subcellular mechanisms of endothelin action in vascular system. *Eur. J. Pharmacol.*, 1999; 375: 133-138

[25] Motte S., McEntee K., Naeije R.: Endothelin receptor antagonists. *Pharmacol. Ther.*, 2006; 110: 386-414

[26] Ohlstein E.H., Elliott J.D., Feuerstein G.Z., Ruffolo R.R.jr.: Endothelin receptors: receptor classification, novel receptor antagonists, and potential therapeutic targets. *Med. Res. Rev.*, 1996; 16: 365-390

[27] Ozdemir R., Parlakpınar H., Polat A., Colak C., Ermis N., Acet A.: Selective endothelin a (ETA) receptor antagonist (BQ-123) reduces both myocardial infarct size and oxidant injury. *Toxicology*, 2006; 219: 142-149

[28] Ponczek M.B., Wachowicz B.: Interaction of reactive oxygen and nitrogen species with proteins. *Postępy Biochem.*, 2005; 51: 140-145

[29] Ramprasath T., Kumar P.H., Puhari S.S., Murugan P.S., Vasudevan V., Selvam G.S.: L-Arginine ameliorates cardiac left ventricular oxidative stress by upregulating eNOS and Nrf2 target genes in alloxan-induced hyperglycemic rats. *Biochem. Biophys. Res. Commun.*, 2012; 428: 389-394

[30] Resink T.J., Hahn A.W., Scott-Burden T., Powell J., Weber E., Bühler F.R.: Inducible endothelin mRNA expression and peptide secretion in cultured human vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, 1990; 168: 1303-1310

[31] Rosen B., Barg J., Zimlichman R.: The effects of angiotensin II, endothelin-1, and protein kinase C inhibitor on DNA synthesis and



intracellular calcium mobilization in vascular smooth muscle cells from young normotensive and spontaneously hypertensive rats. *Am. J. Hypertens.*, 1999; 12: 1243-1251

[32] Rubanyi G.M., Polokoff M.A.: Endothelins: molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacol. Rev.*, 1994; 46: 325-415

[33] Saravanakumar M., Raja B.: Veratric acid, a phenolic acid attenuates blood pressure and oxidative stress in L-NAME induced hypertensive rats. *Eur. J. Pharmacol.*, 2011; 671: 87-94

[34] Scalera F., Dittrich R., Beckmann M.W., Beinder E.: Effect of endothelin-1 on intracellular glutathione and lipid peroxide availability and on the secretion of vasoactive substances by human umbilical vein endothelial cells. *Eur. J. Clin. Invest.*, 2002; 32: 556-562

[35] Skalska A.B., Pietrzycka A., Stepniewski M.: Correlation of endothelin 1 plasma levels with plasma antioxidant capacity in elderly patients treated for hypertension. *Clin. Biochem.*, 2009; 42: 358-364

[36] Thakali K., Demel S.L., Fink G.D., Watts S.W.: Endothelin-1-induced contraction in veins is independent of hydrogen peroxide. *Am. J. Physiol. Heart Circ. Physiol.*, 2005; 289: H1115-H1122

[37] Viswanatha Swamy A.H., Wangikar U., Koti B.C., Thippeswamy A.H., Ronad P.M., Manjula D.V.: Cardioprotective effect of ascorbic acid on doxorubicin-induced myocardial toxicity in rats. *Indian J. Pharmacol.*, 2011; 43: 507-511

[38] Weitzberg E., Rudehill A., Modin A., Lundberg J.M.: Porcine intrinsic pulmonary and systemic vascular tone is endothelin-dependent. *Acta Physiol. Scand.*, 1994; 152: 433-434

[39] Yanagisawa M., Kurihara H., Kimura S., Tomobe Y., Kobayashi M., Mitsui Y., Yazaki Y., Goto K., Masaki T.: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, 1988; 332: 411-415

The authors have no potential conflicts of interest to declare.