Influence of endothelin 1 receptor blockers and a nitric oxide synthase inhibitor on reactive oxygen species formation in rat lungs

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Abstract

This study was designated to estimate protective role of ET_A and ET_B receptor antagonist against endothelin 1 (ET-1)-induced oxidative stress in lungs and determine whether these effects are mediated by nitric oxide (NO) synthase. Experiments were performed on Wistar rats divided into the following groups: I – saline (0.9% NaCl); II - ET-1 (3 μg/kg b. w.), III - BQ123 (1 mg/kg b. w.) + ET-1 (3 μg/kg b. w.), IV - BQ788 (3 mg/kg b. w.) + ET-1 (3 μg/kg b. w.), V - N-nitro-L-arginine methyl ester (L-NAME) (5 mg/kg b. w.) + ET-1 (3 μg/kg b. w.). ET_A and ET_B receptor antagonist or L-NAME were administered 30 min before ET-1 injection. The levels of the following substances were measured in the lungs homogenates: thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H_2O_2), reduced glutathione (GSH) and tumor necrosis factor–alpha (TNF-α). The results showed that ET-1 significantly increased TBARS, H_2O_2 (respectively: $p<0.001$, $p<0.02$) and TNF-α levels ($p<0.02$) and decreased the GSH level ($p<0.01$) *vs.* control group. On the other hand, prior administration of ET_A receptor blocker (BQ123) significantly attenuated TBARS ($p<0.01$), H_2O_2 ($p<0.02$), TNF-α ($p<0.02$) and increased GSH ($p<0.02$) levels *vs.* ET-1. However, prior administration of ET_B receptor blocker BQ788 did not cause significant changes in the: TBARS, H_2O_2 and TNF-α ($p>0.05$) levels, but significantly increased the GSH level and GSH/GSSG ratio ($p<0.05$). Administration of L-NAME significantly attenuated TBARS ($p<0.001$), H_2O_2 ($p<0.05$), TNF-α ($p<0.01$) and increased GSH ($p<0.05$) levels *vs.* ET-1. In conclusion, we demonstrated that ET-1 induced oxidative stress in the lungs is mediated by ET_A receptors. ET_A receptor blockage inhibited generation of free radicals and TNF-α and ameliorated antioxidant properties. Moreover, generation of reactive oxygen species is mediated by NOS in the lungs.

Key words: endothelin 1, endothelin receptor blockers, L-NAME, lungs

Introduction

Endothelins are a family of peptides (ET-1, ET-2, and ET-3) which have different biological activities in both vascular and non-vascular tissues. Endothelin-1 (ET-1) is a 21-amino-acid polypeptide produced primarily by vascular endothelial cells and is characterized as a powerful smooth muscle vasoconstrictor and mitogen (Galie *et al.* 2004). In mammals, ET-1 binds to specific G protein-coupled membrane receptors, ET_A and ET_B. ET_A receptors are found on smooth muscle cells and mediate contraction, cell growth, adhesion, fibrosis and thrombosis. ET_B receptors are localized on endothelial cells and to some extent in smooth muscle cells and macrophages and mediate vasodilatation via nitric oxide (NO) and prostacyclin generation (Schiffrin 2001). Increasing evidence demonstrates that this peptide stimulates superoxide anion production in vascular smooth muscle cells (VSMC) (Laplante *et al.* 2005), isolated arteries (Galie *et al.* 2004, Loomis *et al.* 2005, Sedeek *et al.* 2003), veins (Li *et al*. 2003, Thakali *et al.* 2005), lungs (Hsu *et al.* 2010), and heart (Lund *et al.* 2005). Some studies have shown that chronic intravenous ET-1 infusion increased vascular superoxide anions production and plasma TBARS level (Yao *et al.* 2004). Also “in vitro” studies have demonstrated that ET-1 increases ROS formation in endothelial cells (Li *et al.* 2003, Sedeek *et al.* 2003). Another authors also confirmed that an increase in the superoxide level was associated with a decrease in NOS activity (Hsu *et al.* 2010) and developed hypertension (Sullivan *et al.* 2006, Thakali *et al.* 2005)

Nitric oxide (NO) is a key regulator of cardiovascular function and is generated by a family of nitric oxide synthase (NOS) enzymes or by non-enzymatic reduction in nitrite. All three isoforms of NOS, neural nNOS, inducible iNOS, and endothelial eNOS, which produce NO from L-arginine are expressed in the cardiovascular tissues (Schulz *et al.* 2005). NO can act as an antioxidant by inhibiting activation of xanthine oxidase (XO) (Hassoun *et al.* 1995) and NADPH oxidase (Yao *et al.* 2004), and maintaining normal $O_2{\bullet}^-$ / NO homeostasis. NO can interact with superoxide anion to form peroxynitrite $ONOO^-$. Peroxynitrite can be decomposed to nitrate or can trigger an array of cytotoxic processes including lipid peroxidation and protein modification, thus modulating biological processes (Zhang *et al.* 2012). Under elevated superoxide anion level conditions peroxynitrite can lead to formation of hydroxyl radicals (Schulz *et al.* 2005). ROS and reactive forms of nitrogen (RFN) causing oxidative damage biomolecules ultimately lead to oxidative stress and cell injury.

The mechanism effect of ET_A and ET_B receptor blockade on generation of ROS in the lungs has not been well understood yet. In this context, we wanted to investigate protective role of endothelin receptor antagonists against ET-1 induced oxidative stress in lungs and determine whether the effects of ET-1 are mediated by NOS in the lungs.

Material and methods

Chemicals

Endothelin 1 (powder; a synthetic peptide with the sequence of human and porcine ET-1; powerful vasoconstrictor properties); BQ123 (cyclo-(D-Asp-Pro-D-Val-Leu-D-Trp; selective ET-A receptor blocker - a drug that blocks endothelin A receptors.); BQ788 (2,6 Dimethylpiperidinecarbonyl-γ-Methyl-Leu-Nin-(Methoxycarbonyl)-D-Trp-D-Nle,N-[N-[N-[(2,6-Dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-(methoxycarbonyl)-D-tryptophyl]- D-norleucine sodium salt; selective ET-B receptor blocker - a drug that blocks endothelin B receptors) L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride; an analog of arginine that inhibits NO production); thiobarbituric acid (TBA); butylated hydroxytoluene (BHT); sodium acetate trihydrate, triethanoloamine hydrochloride (TEA); 5-sulfosalicylic acid hydrate (5-SSA); 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB); ß-NADPH (b-nicotinamide adenine dinucleotide phosphate); glutathione reductase (GR) and 2-

vinylpyridine were obtained from Sigma Aldrich Chemical Co. (30 Szelagowska St, 61-626) Poland). All other reagents were obtained from POCH (Gliwice, Poland) and were of analytical grade.

Animals

Experiments were performed on male Wistar rats weighing 200-220g, aged 2-3 months. The animals were housed 6 per cage under standard laboratory conditions in a 12/12 h light-dark cycle (lights on at 7.00 a.m.) at 20 ± 2^{o}C ambient temperature and air humidity of 55 ± 5%. All animals received a standard laboratory diet and water ad libitum. All animals were given a one-week acclimation period before the onset of the experiment. The experimental procedures followed the guidelines for the care and use of laboratory animals, and were approved by the Medical University of Lodz Ethics Committee (28/LB 520/2010).

Experimental protocol

Animals were randomly divided into five groups as follows:

1st group (control group, n= 6) received two doses of 0.2 ml of saline, at 30 min interval;

2nd group (ET-1 group, n=6) received 0.2 ml of saline, and half an hour later the rats were injected with a single dose of ET-1 (3 μg/kg);

3rd group (BQ123 +ET-1 group, n=6) were given 0.2 ml of BQ123 (1 mg/kg), and half an hour later the rats were injected with a single dose of ET-1 (3 μg/kg);

4th group (BQ788 + ET-1, n=6) received 0.2 ml BQ788 (3 mg/kg) and 0.5 h later ET-1 (3 μg/kg);

5th group (L-NAME + ET-1, n=6) received 0.2 ml L-NAME (5 mg/kg) and 30 min later ET-1 (3 μg/kg).

All reagents were injected intravenously into the femoral vein between 8.00 a.m. and 9.00 a.m. After the administration of compounds, each group of animals was observed for a period of 5h. The doses used in this study were selected on the basis of reports of previous studies (Piechota-Polanczyk *et al.* 2012).

Animal preparations

Animals were anaesthetized by an intraperitoneal injection of 10% urethane (2 ml/100 g b. w.). When a sufficient level of anesthesia was achieved, a 2-cm-long polyethylene tube (2.00 mm) was inserted into the trachea. A polyethylene catheter PE-10 was inserted into the femoral vein for administration of experimental drugs.

Tissue preparation and collection of samples:

At the end of the experimental period, the animals were euthanized. The lungs were surgically removed and cleaned of extraneous tissue. They were rinsed with cold isotonic saline, dried by blotting between two pieces of filter paper and weighed on an electronic balance to estimate lungs edema. The lungs were stored at -80^{o}C for further measurement of their oxidative parameters and TNF-α level.

Preparation of homogenates

An accurately-weighed portion of the lungs (50 mg) was homogenized in either 0.15 M KCl for estimation of lipid peroxidation and concentration of H_2O_2 or 5% SSA for estimation of glutathione or HEPES buffer for estimation of TNF-α level. The resulting supernatant was immediately used for biochemical analyses.

Measurement of TBARS in the lungs homogenates

To determine the degree of oxidative damage in the lungs, lipid peroxidation was measured in lungs homogenates. The lipid peroxidation product content in lungs homogenates was assayed as TBARS, previously described by Yagi (1998). Briefly, 50 mg of the lungs tissue was homogenized with 2 ml of 1.15% potassium chloride. Then, 4 ml of 0.25% hydrochloric acid containing 0.375% thiobarbituric acid (TBA), 15% trichloroacetic acid (TCA) and 0.015% butylated hydroxytoluene (BHT) were added. The samples were boiled for 30 min at 100°C in tightly closed tubes. After cooling to 10°C, 2.5 ml of butanol was added to each tube and the samples were centrifuged for 10 min (3800 x *g*, 20°C). TBA-reactive substances in the butanol layer were measured spectrofluorometrically using an LS-50 Perkin Elmer Luminescence Spectrometer (Norwalk, CT, U.S.A.). Excitation was set at 515 nm and emission was measured at 546 nm. Sample TBARS concentrations were calculated by the use of the regression equation as follows: $y=0.43(x-x_o) – 2.43$, where y=TBARS concentration (μM); x, x_o = fluorescence intensity of the samples and control, respectively (arbitrary units; AU). The regression equation was prepared from triplicate assays of six increasing concentrations of tetramethoxypropane (range 0.01- 50 μM) as a standard for TBARS. A mixture of 2 ml of 1.1% potassium chloride and 4 ml of 0.25 N hydrochloric acid was used as a control. Finally, the results were calculated for 50 mg of the lungs tissue.

Measurement of H_2O_2 in the lungs homogenates

The lungs tissue fragments were washed in ice-cold saline and stored at –80°C for no longer than 2 weeks. Generation of H_2O_2 in lungs homogenates was determined according to Ruch *et al.* (1983). Briefly, 50 mg of the lungs tissue fragments were homogenized with 2 ml of 1.15% potassium chloride. Then, 10μl aliquot of tissue homogenate was mixed with 90 μl of PBS (pH 7.0) and 100 μl of horseradish peroxidase (1 U/ml) containing 400 μmol homovanilic acid (HRP + HVA assay) or with 90 μl of PBS and 100 μl of 1 U/ml horseradish peroxidase only (HRP assay). Both homogenates were incubated for 60 min at 37°C. Subsequently, 300 μl of PBS and 125 μl of 0.1 M glycine - NaOH buffer (pH 12.0) with 25 mM EDTA were added to each homogenate sample. Excitation was set at 312 nm and emission was measured at 420 nm (Perkin Elmer Luminescence Spectrometer, Beaconsfield UK). Readings were converted into H_2O_2 concentration using the regression equation: $y= 0.0361x – 0.081$, where $y=H_2O_2$ concentration in homogenate (μM); x = intensity of light emission at 420 nm for HRP + HVA assay reduced by HRP assay emission (arbitrary units, AU). The regression equation was prepared from three series of calibration experiments with 10 increasing H_2O_2 concentrations (range 10-1000 μM). The lowest H_2O_2 detection was 0.1 nM, with intraassay variability not exceeding 2%.

Determination of GSH levels:

Total glutathione (GSHt), reduced glutathione (GSH) and oxidized glutathione (GSSG) were measured in the lungs homogenates. Briefly, the lungs were homogenized in cold 5% 5-SSA and centrifuged (10000 x *g*, 10 min, 4°C). The GSHt content of the supernatant was measured in a 1 ml cuvette containing 0.7 ml of 0.2 mM NADPH, 0.1 ml of 0.6 mM DTNB, 0.150 ml of H_2O and 50 μl of the sample. The cuvette with the mixture was incubated for 5 min at 37°C and then supplemented with 0.6 U/l of GR. The reaction kinetics was followed spectrophotometrically at 412 nm for 5 min by monitoring the increase in absorbance.

GSSG concentration was determined in supernatant aliquots by the same method after optimization of pH to 6-7 with 1 M TEA and derivatization of endogenous GSH with 2-vinylpyridine (v:v). The reduced glutathione level in the supernatant was calculated as the difference between GSHt and GSSG. The increments in absorbance at 412 nm were converted to GSHt and GSSG concentrations using a standard curve (3.2 - 500 μM glutathione for GSHt and 0.975 – 62 M for GSSG). The results were expressed in μM.

Tumor necrosis factor-assay

TNF-α in the lungs tissues was assayed by specific enzyme linked immunosorbent assay using a commercially-available ELISA test kit (R&D Systems) containing a monoclonal antibody specific for rat TNF-α. The results were read using a TEK Instruments EL340 BIO-spectrophotometer (Winooski VT, USA) (λ=45 nm). The sensitivity of the kit was 10 pg/ml. The TNF-α concentration was read from standard curves and expressed in pg/ml.

The experiments were repeated twice.

Statistical analysis

The results are presented as mean ± S.E.M. The statistical analysis was done by ANOVA followed by the Duncan's multiple range test as post-hoc. A p-value less than 0.05 was considered significant.

Results

Evaluation of lipid peroxidation, H_2O_2 and glutathione levels

A significant increase in the homogenate level of a marker of the lipid peroxidation TBARS and H_2O_2 level (respectively: $p<0.001$, $p<0.02$) was observed in ET-1 treated rats in comparison to that in the control rats. A significant decrease in TBARS ($p<0.01$) and H_2O_2 ($p<0.02$) levels was observed in rats of the BQ123 and ET-1-treated group in comparison to rats in the ET-1 group. There was also a significant decrease in lungs TBARS ($p<0.001$) and H_2O_2 levels ($p<0.05$) in the L-NAME + ET-1-treated group in comparison to the ET-1 group. However, no significant changes in TBARS and H_2O_2 levels were observed in BQ788 + ET-1–treated rats compared to those in ET-1-treated rats.
(Fig 1[a, b])

Levels of GSHt and GSH in the lungs of ET-1 treated animals were significantly lower than those of the control group ($p<0.01$). BQ123 treatment significantly improved levels of GSHt and GSH (respectively: $p<0.001$, $p<0.02$) as compared to ET-1 treated rats. Also BQ788 and L-NAME treatment cause significant changes in GSHt and GSH levels - respectively: $p<0.001$, $p<0.05$ and $p<0.001$, $p<0.05$ (Fig 2[a]).

There was a significant decrease in lungs GSH/GSSG ratio in the ET-1-treated group in comparison to the control group ($p<0.001$). Treatment with BQ123 and BQ788 before ET-1 administration resulting in an increase in the GSH/GSSG ratio compared with the ET-1 group ($p<0.05$). L-NAME did not lead to a significant increase in GSH/GSSG when compared with ET-1 group (Fig 2[b])

Evaluation of TNF-α

Fig.1[c] shows that following ET-1 administration, the level of TNF-α was markedly increased in lungs homogenates when compared to the control group ($p<0.02$). However, a significant decrease in TNF-α level was observed in BQ123 + ET-1 treated group in comparison to that in ET-1 treated rats ($p<0.02$). Significant change was found in L-NAME + ET-1- treated rats in comparison to ET-1-treated rats ($p<0.01$). The level of TNF-α decreased also in the BQ788 group. However, no significant changes were observed.

Discussion

In the present study, we have demonstrated that ET-1 administration increased the levels of TBARS, H_2O_2, and TNF-α and decreased the glutathione level in the lungs homogenates. These parameters were improved significantly after the ET_A receptor blockade, whereas the ET_B receptor blockade had a slight effect. Similarly, in accordance with our results, several other studies have reported that ET-1 produces ROS (Elmarakby *et al.* 2008, Lee *et al.* 2010, Li *et al.* 2003). Increased ROS generation in our study is supported by the increased TBARS lungs levels, which reflects an elevation of enzymatic lipoperoxidation. Lipid peroxidation of unsaturated fatty acids impairs cell membrane fluidity and alters activity of membrane-bound enzymes and receptors. The high level of the lipid peroxidation marker TBARS reflects ROS-mediated damage of cells. The toxic effect of ROS on cells in our study was indicated by the enhanced level of H_2O_2 in lungs homogenates. H_2O_2 is produced from superoxide anion by superoxide dismutase (SOD). H_2O_2 is more toxic than oxygen-derived free radicals and is capable of producing the most toxic hydroxyl radical (OH^o). Catalase (CAT) and peroxidase (GPx) convert H_2O_2 to water and oxygen. The increase in H_2O_2 level in our study may be caused by enhanced activity of SOD and reduced CAT activity in the lungs tissue after ET-1 administration as it has been indicated in hemorrhagic rats (Korzonek-Szlacheta and Gwóźdź 2007).

Glutathione is a primary non-enzymatic intracellular antioxidant. It mainly appears in a reduced state (GSH) but is oxidized to disulfide glutathione (GSSG) in order to inactivate free radicals. In this study, the decreased glutathione level and GSH/GSSG ratio seen after ET-1 administration reflect the antioxidant status of the tissue and was associated with the increased TBARS level. These results are in line with previous reports, which demonstrated that ET-1 may lead to oxidative stress by reducing glutathione, diminishing the antioxidant GSH/GSSG ratio and stimulating lipid peroxidation in a time-dependent manner (Viswanatha Swamy *et al.* 2011).

TNF-α is an inflammatory cytokine that acts mainly through the activation of nuclear factor -*k*B (NF-*k*B) (Elmarakby *et al.* 2008). In experimental models of inflammatory diseases, NF-*k*B is activated and regulates the expression of genes involved in tissue inflammation such as TNF-α (Therrien *et al.* 2012). ROS can increase gene expression of this inflammatory mediator from macrophages, alveolar and bronchial epithelial cells. Generation of TNF-α at high levels leads to the development of inflammatory responses that are hallmarks of many pulmonary diseases (e.g. asthma, chronic bronchitis - CB, chronic obstructive pulmonary disease - COPD, acute lung injury - ALI and acute respiratory distress syndrome - ARDS). In our study, the ET-1 administration resulted in an increase in TNF-α level in the lungs tissue. These findings are in agreement with those showing that ET-1 stimulates monocytes and macrophages to release TNF-α and increased plasma or tissue concentration of TNF-α (Jesmin *et al.* 2011, Tonari *et al.* 2012).

In the present study we observed that the decreased TBARS and H_2O_2 levels in ET-1 challenged rats were significantly ameliorated by BQ123 or L-NAME pretreatment, consistent with our previous report (Piechota-Polanczyk *et al.* 2012). This shows that BQ123 prevents lipid peroxidation. These findings are in accordance with studies of other investigators who observed reduction in lipid peroxidation in different organs after blocking ET_A (Briyal *et al.* 2012, Goyal *et al.* 2010, Ozdemir *et al.* 2006), or ET_B receptors (Dai *et al.* 2004, Leonard *et al.* 2011, Li *et al.* 2010). The decrease in H_2O_2 level in our study indicates the decrease in ROS formation and lipid peroxidation in the lungs tissue. It was shown that the use of ET_A receptor antagonist provided beneficial effects in chronic heart failure as evidenced by a reduction of infarct size, improved reperfusion, coronary flow or protection during ischemic/reperfusion injury (Ozdemir *et al.* 2006). In our study, BQ123 not only prevented ET-1-induced lungs injury but also increased the GSH levels and GSH/GSSG ratio. The increase in reduced glutathione and GSH/GSSG ratio within lungs tissue demonstrates the ability of ET-1 receptor antagonist to combat oxidative damage. These results are consistent with previous reports showing that blockade of ET_A receptor increases glutathione level in heart (Ozdemir *et al.* 2006) and other tissues (Briyal *et al.* 2012). In our study pretreatment with BQ788 had significant influence on the level of GSH in the lungs tissue and caused the increased GSH/GSSG ratio. However, other authors obtained that pretreatment with BQ788 had slight influence on the level of GSH in the brain but caused the increased GSH/GSSG ratio (Briyal *et al.* 2012). Studies on blocking the ET_B receptors are ambiguous. Wedgwood *et al.* (2005) demonstrated that ET_B receptor antagonist (RES-701-3) increased production of H_2O_2 in the cell culture of the pulmonary artery smooth muscle, but not in the endothelial cells. However, results of some study indicated that the administration of ET_B receptor blocker - BQ788 reduces the production of reactive oxygen species in the various tissues (Dai *et al.* 2004; Piechota-Polanczyk *et al.* 2012). The use of ET_B receptor blockers binds both to the preferred (a decrease in contractility of the blood vessels) as well as harmful (worsening hemodynamics and renal system) effects. (Tostes and Muscara 2005). ET_B receptors are involved in vascular remodeling after injury so their blockade impairs this process (Murakoshi *et al.* 2002). Blockade of the ET_B receptor is also associated with impaired clearance of ET-1 in the pulmonary vasculature. Moreover, development of hypertension after chronic administration of ET_B receptor antagonist was observed (the administration of the ET_A receptor blocker abolished this effect) (Reinhart *et al.* 2002).

In the current study we found that TNF-α levels were significantly lower in the BQ123 and L-NAME-treated groups than in the ET-1 group. The decrease in TNF-α level indicates the reduction in lugs inflammation. This result is consistent with other authors who demonstrated diminished TNF- α level after ET_A blockade in the different tissues. Tonari *et al.* (2012) have reported that treatment with BQ123 antagonist of ET_A receptors decreased TNF-α in the optic nerve crush. However, Verri *et al.* (2004) have presented that inhibition of ET_B receptors by BQ788 may be beneficial in controlling inflammation hypernociception of diseases in which IL-18 plays a role in their pathogenesis.

In our study, we have shown that inhibition of NO synthesis by L-NAME, a compound inhibiting both the constitutive and inducible NO synthases, led to a reduction of TBARS and H_2O_2 concentrations in lung homogenates. However, Saravanakumar and Raja (2011) have showed an increase in lipid peroxidation in plasma rats after L-NAME administration. Ramprasath *et al.* (2012) have reported an increase in the TBARS level in left ventricles after L-NAME administration in diabetic rats. In this study, L-NAME administration resulted in a slight increase in the GSHt and GSH concentration and enhanced the GSH/GSSG ratio as compared to the ET-1 group. However, other authors demonstrated decrease in glutathione level and antioxidant enzymes (SOD, CAT) in the plasma (Saravanakumar and Raja, 2011) and in the heart (Ramprasath *et al.* 2012) after L-NAME administration. These results suggest that other alternative signaling pathways different than NOS take part in the ROS generation.

We have also found that inhibition of NO synthase by L-NAME, significantly ameliorated the lungs inflammation (assessed by tissue TNF-α level) caused by exogenous ET-1 administration. It is believed that TNF-α initiates the inflammatory reaction by releasing other inflammatory mediators, increasing the expression of cell adhesion factor, and promoting neutrophil adhesion to endothelial cells. Some other studies have shown that blockade of NOS with L-NAME resulted in significant increase in TNF-α in heart (Sojitra *et al* 2012), liver (Guo *et al.* 2011), and aortas (Sukhanov *et al.* 2011). Conversely, L-NAME had a protective effect against injury induced by the deposition of immune complexes (Mulligan *et al.* 1992) and against alveolar injury caused by smoke inhalation (Ischiropoulos *et al.* 1994).

Moreover, some studies on ET-1 treated rats demonstrated a significant increase in the heart/body weight ratio. This may be attributed to albumin extravasculation in the vascular bed (Filep *et al.* 1994, 1996) increase in vascular permeability and myocardial water content (Murray *et al.* 2004) or with increased VEGF release and TNF-α in the heart after ET-1 injection (Shimojo *et al.* 2007). This finding clearly demonstrated the development of heart injury due to acute inflammation and interstitial edema in rat heart. Reduced endothelin-1 induced increase in HW/BW ratio may be associated with significant reduction in ROS generation and inflammatory response or decreased VEGF content in the heart (Goyal *et al.* 2010, Labrutto *et al.* 2007, Oz *et al.* 2012, Shimojo *et al.* 2007). Recently reports have demonstrated that the inhibition of nitric oxide synthase (NOS) activity by L-NAME caused increase in HW/BW (Sojitra *et al.* 2012).

Conclusion

We have demonstrated that ET-1 induced oxidative stress in lungs is mediated by ET_A receptors. ET_A receptor blockage inhibited free radical generation and TNF-α and ameliorated antioxidant properties; ET_B receptor blockage was no effective. Moreover, NOS play role in generation of ROS in the lungs because L-NAME administration significantly reduced the TBARS, H_2O_2 and TNF-α level and slightly increased the GSH level.

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Fig. 1. The influence of endothelin-1 (ET-1), its receptor blockers and L-NAME on the concentrations of: **(a)** thiobarbituric acid reactive substance (TBARS), **(b)** hydrogen peroxide (H_2O_2), and **(c)** TNF-α levels in lungs.

ET-1 (3 μg/kg) was injected 30 min after saline administration. BQ123 - endothelin-A receptor blocker (1 mg/kg), BQ788 - endothelin-B receptor blocker (3 mg/kg), and L-NAME - Nω-Nitro-L-arginine methyl ester hydrochloride (5 mg/kg) were administered 30 min prior to the injection of ET-1. The results are mean ± S.E.M. The data was statistically evaluated by one-way ANOVA.

Fig. 2. Changes in **(a)** the total glutathione (GSHt), oxidized glutathione (GSSG), reduced glutathione (GSH) and **(b)** reduced to oxidized glutathione ratio (GSH/GSSG) in experimental groups of rats.

ET-1 – endothelin 1 (3 μg/kg) was injected 30 min after saline administration. BQ123 - endothelin-A receptor blocker (1 mg/kg), BQ788 - endothelin-B receptor blocker (3 mg/kg), and L-NAME - Nω-Nitro-L-arginine methyl ester hydrochloride (5 mg/kg) were administered 30 min prior to the injection of ET-1. The results are mean ± S.E.M. The data was statistically evaluated by one-way ANOVA.

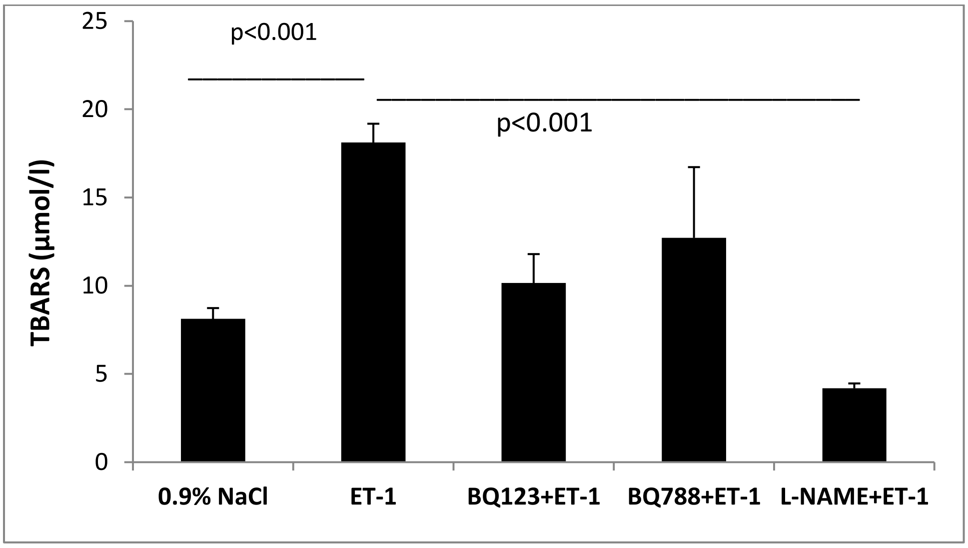

Fig. 1a

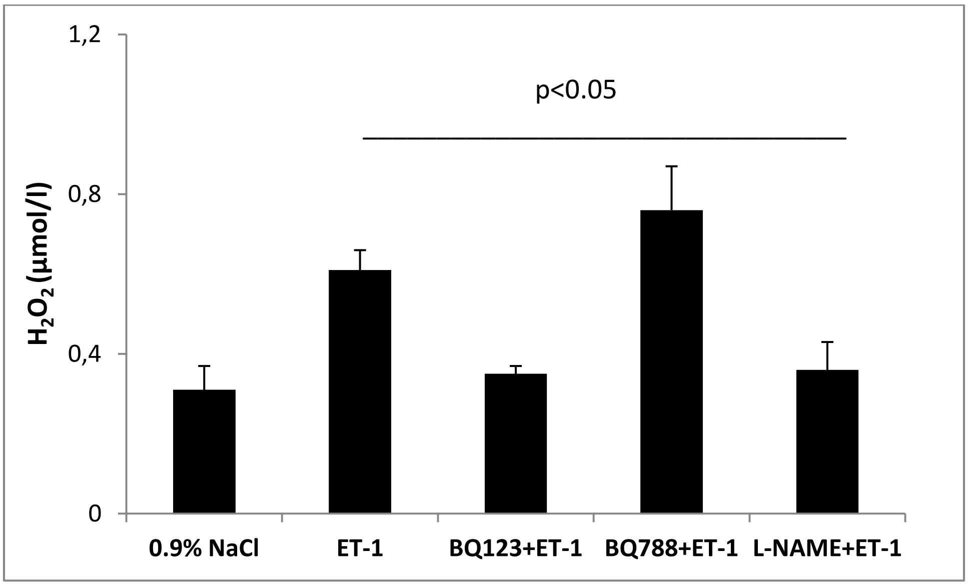

Fig. 1b

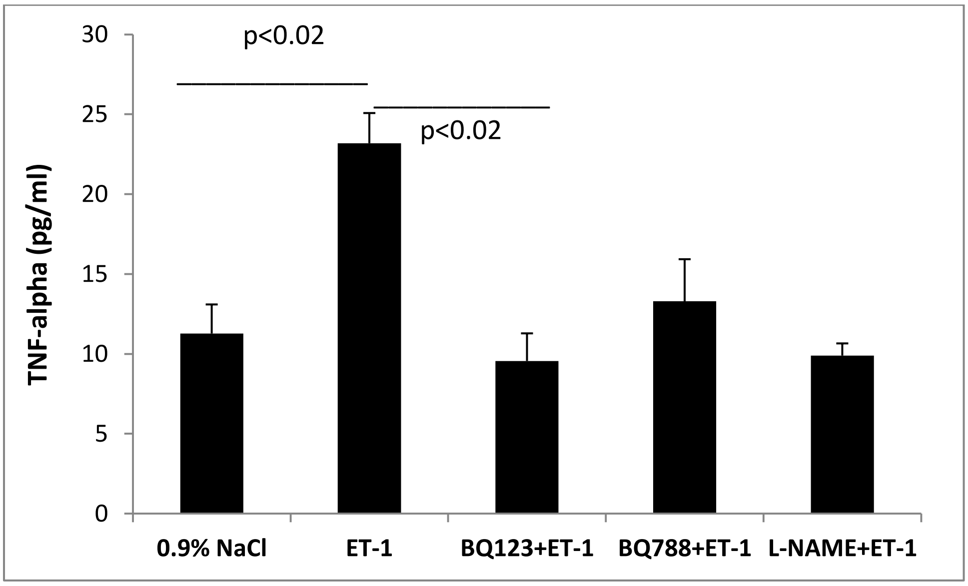

Fig. 1c

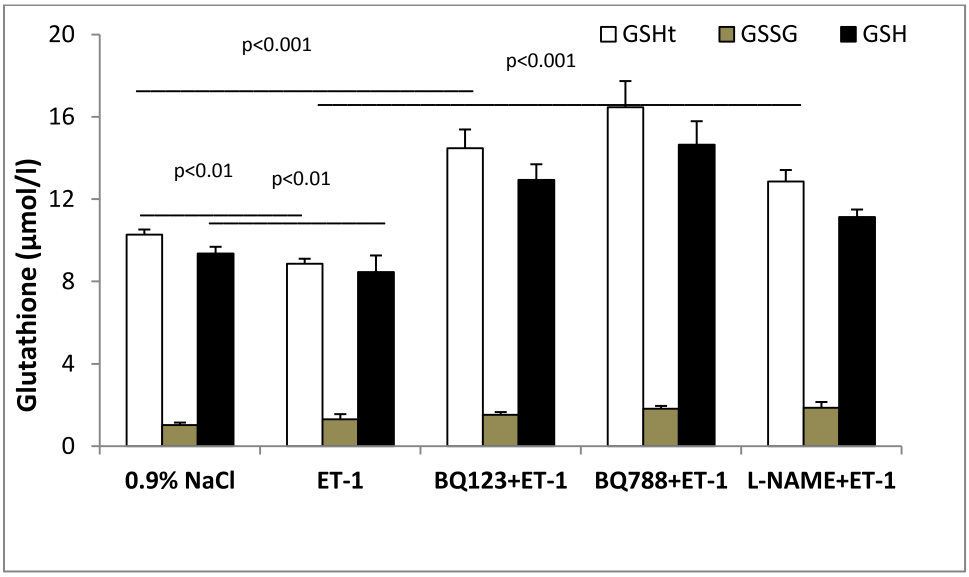
☐ GSHt
▨ GSSG
■ GSH
p<0.001
p<0.001
p<0.01
p<0.01
20
16
12
8
4
0
Glutathione (µmol/l)
0.9% NaCl
ET-1
BQ123+ET-1
BQ788+ET-1
L-NAME+ET-1

Fig. 2a

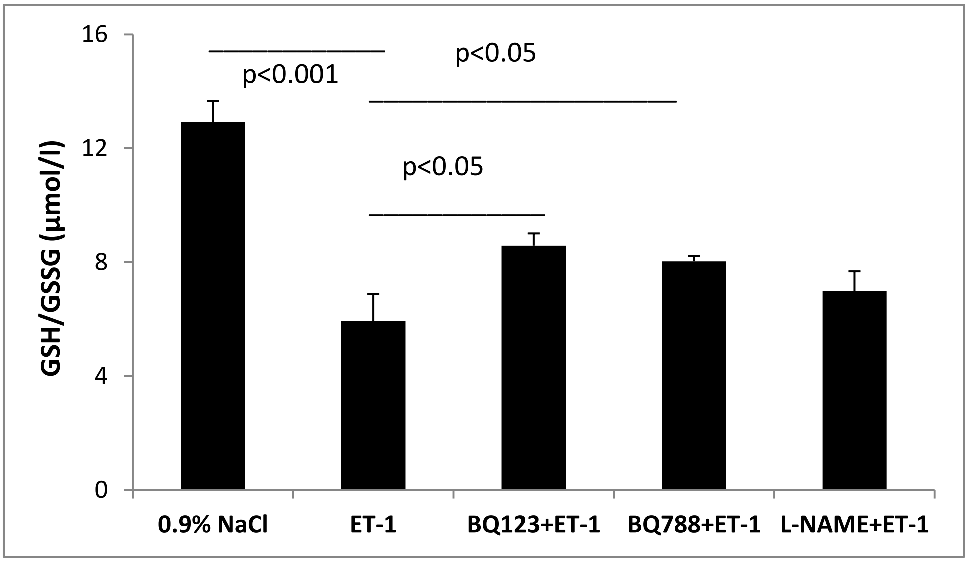
16
12
8
4
0
GSH/GSSG (µmol/l)
p<0.001
p<0.05
p<0.05
0.9% NaCl
ET-1
BQ123+ET-1
BQ788+ET-1
L-NAME+ET-1

Fig. 2b