Inhalation of endothelin receptor blockers in pulmonary hypertension

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Inhalation of endothelin receptor blockers in pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 294: L772–L777, 2008. First published February 22, 2008; doi:10.1152/ajplung.00405.2007.—Endothelin 1 (ET-1) is a potent pulmonary vasoconstrictor and mediator of lung diseases. Antagonism of the ET-1-mediated effects has become an important therapeutic approach. ET-1 (A and B) receptors are differentially distributed in the lung vasculature. Whereas the ET\(_A\) receptors mainly mediate vasoconstriction, the endothelial ET\(_B\) receptor seems to have vasodilative properties. We sought to determine if antagonism of ET receptors can be achieved by inhalation of specific blockers in a model of ET-1-mediated pulmonary hypertension.

ENDOTHELIN-1 (ET-1) is a potent pulmonary vasoconstrictor and mediator of lung diseases. Antagonizing ET-1-mediated effects in the lungs has become an important therapeutic approach. ET-1 causes elevated pulmonary pressures in acute (5, 10, 15) and chronic pulmonary hypertension (PH) (4). Blocking the ET-1 pathway is an established therapy in PH. Since pulmonary vasoconstriction is an important component of the pathophysiology of PH, the application of vasoactive substances has been in focus to attenuate pulmonary artery pressures in pulmonary hypertensive states (21, 22). To restrict the action of these substances to the lungs and maximize their deposition, the inhalative route of application has been suggested. ET-1 is one of the most potent endogenous vasoconstrictors in systemic and pulmonary circulation. It is predominantly released from endothelial cells and mediates its vasoactive properties via two different G protein-coupled receptors, namely the ET\(_A\) and the ET\(_B\) receptor (4). With regard to the pulmonary vasculature, the ET\(_A\) receptor is localized on vascular smooth muscle cells, whereas under normal conditions the ET\(_B\) receptor is preferentially found on endothelial cells and only to a minor extent on smooth muscle cells of pulmonary vessels (12, 17). Both receptors expressed on vascular smooth muscle cells mediate vasoconstriction (9, 19) via activation of phospholipase C. Stimulation of ET\(_B\) receptors on endothelial cells leads to a release of vasodilating factors, such as nitric oxide and prostacyclin. These opposite ET-1-mediated effects suggest an equilibrium between the receptors under normal conditions (12, 16).

Besides its acute vasoactive properties, ET-1 is an important smooth muscle and fibroblast mitogen, chemoattractant, and stimulant of collagen synthesis and is a mediator of different lung diseases (3). Consequently, ET-1 is in the scope of extensive work on lung diseases. ET-1 has been shown to be crucially involved in the remodeling in models of chronic proliferating diseases (6) and PH (2, 7, 26, 27). Moreover, systemic administration of ET-1 blockers has been proven beneficial in experimental and clinical PH (12). However, despite extensive earlier studies on chronic systemic administration of endothelin receptor antagonists (ETRA), the inhalational route has not been investigated so far. Due to the differential distribution of the endothelin receptors on smooth muscle and endothelial cells, we sought to investigate if aerosolization of two selective (BQ-123 for the ET\(_A\) receptor and BQ-788 for the ET\(_B\) receptor) and one dual ETRA (Tezosentan) would lead to a different pattern of effects compared with their systemic administration. We used the bolus application of ET-1 (24) as a model of ET-1-mediated lung disease in an ex vivo study in the isolated ventilated and blood-free perfused rabbit lung model, allowing an aerosol approach to the intact organ.

MATERIALS AND METHODS

Study Design

Sixty lung preparations were randomly assigned to one of the intervention groups, and another six were used as a control group without any intervention. In separate experiments, a single dose of ET-1 (0.1 \(\mu\)M; Calbiochem) was found to achieve a sustained increase in mean pulmonary artery pressure (PAP) without a significant lung edema formation. After a steady-state period of 30 min, ET-1 was administered into the buffer fluid. After a constant rise over 60 min, the PAP reached a plateau for another 60 min (Fig. 1).

Experimental Groups

Control group (6). After a steady-state period of 30 min, all main parameters were observed and registered for at least 180 min without further intervention.

ET-1-induced PH (ET solo or control, n = 6). After termination of a steady-state period of 30 min, ET-1 (0.1 \(\mu\)M) was bolus injected into the recirculating buffer fluid. All main parameters were observed and registered for another 120 min without further intervention.

Dose/response curve for BQ-788, BQ-123, and Tezosentan (each n = 4). After establishing a stable ET-1-induced PH (plateau phase, 60 min after ET-1), increasing doses (0.1, 1, and 10 \(\mu\)M) of the respective ETRA were added to the buffer fluid in an incremental manner. The maximum effect on each parameter was read out 30 min after the respective application.

Systemic administration of BQ-788, BQ-123, and Tezosentan application during ET-1-induced PH (each n = 6). Fifteen minutes after ET-1 administration, ETRA were added into the buffer fluid (each 1 \(\mu\)M). All main parameters were observed and registered for another 105 min without further intervention.

Aerosol groups. NaCl 0.9% (ET NaCl-aer) (0.01 ml kg\(^{-1}\) min\(^{-1}\)) or the respective ETRAs were aerosolized over 15 min, beginning 15
Animal experimentation was performed according to the Helsinki convention for the use and care of animals. Animal experiments were approved by the governmental review board for the use and care of animals. Animal experiments were approved by the governmental review board for the use and care of animals. Animal experiments were approved by the governmental review board for the use and care of animals. Animal experiments were approved by the governmental review board for the use and care of animals.

The isolated lung model has been described previously (20, 23). Briefly, rabbits (New Zealand White bastards) of either sex, weighing 2.2–2.7 kg, were anesthetized with intravenous ketamine (Ketamin HCl, Serag Wiesner, Naila, Germany) and anticoagulated with heparin (1,000 U/kg body wt). Tracheotomy was performed, and the animals were artificially ventilated with room air using a Harvard respirator (tidal volume 30 ml and frequency 30/min). Room air, supplemented with 3% CO2, was delivered to the lungs at a constant ventilator setting (tidal volume 30 ml and frequency 30/min). Out of this, we detected 0.13 g of aerosol distal to the tracheal cannula, according to an absolute deposition fraction of ~0.22.

To use comparable nebulized doses as during the intravenous application, we used the fourfold dose of the intravenous administered dosage for the aerosol experiments.

**Data Analysis**

All data are shown as means ± SE. For primary data analysis, comparison was performed between the ET solo group and interventional groups with intravascular administration of ETRA. Interventions with inhaled ETRA were compared with the ET NaCl-aer group. Further comparison between interventional groups was conducted as stated. Comparison between groups was performed at the end of inhalation (time 45 min) and at the end of the experiment. For comparison of statistical difference between groups, we performed a one-factorial analysis of variance with the Bonferroni correction. Significance was assumed when P ≤ 0.05.

**RESULTS**

**Steady State Conditions**

After completion of the steady-state period, all lungs displayed a mean PAP of 6–8 mmHg without any lung weight gain and a mean ventilation pressure of 2–4 mmHg. In the control group, no significant changes were observed with regard to PAP, ventilation pressure, and lung weight gain during the whole observation period of at least 180 min (data not shown).

**ET-1-Induced PH**

Bolus application of ET-1 into the recirculating buffer fluid evoked a rapid and constant increase of mean PAP to 22.94 ± 0.89 mmHg within 60 min, followed by a constant plateau over another 60 min (mean PAP 23.62 ± 0.88 mmHg, 120 min after ET-1 injection). The total lung weight gain was 1.25 ± 0.1 g after 120 min, while no significant lung edema formation was observed and ventilation pressures were unaffected (see Figs. 1 and 3A).

**Dose/Response Curves For Different ETRAs**

Using a concentration of 0.1 μM, none of the ETRA evoked a significant effect on any of the registered parameters. Administration of 1 and 10 μM of BQ-788 led to a dose-dependent increase, whereas BQ-123 and Tezosentan significantly decreased mean PAP (Fig. 2).

In these dose/response experiments, ventilation pressure or lung weight did not change significantly (data not shown).

**Intravascular Application of ETRAs**

Application of 1 μM BQ-788 into the buffer fluid 15 min after ET-1 administration augmented the PAP increase compared with the ET solo group at time point 45 min (P < 0.05). PAP was not different compared with the ET solo group at the...
end of the experiment. The maximum difference of mean PAP was $+6.45 \pm 1.35$ mmHg ($P < 0.01$) (Fig. 3, A–C).

An equimolar dose of BQ-123 significantly attenuated PAP increase compared with the ET solo group at time point 45 min and at the end of the experiment ($P < 0.001$). The maximum difference of mean PAP was $-13.98 \pm 0.69$ mmHg ($P < 0.001$).

Finally, intravascular application of Tezosentan reduced the ET-1-induced PAP increase significantly at 45 min and still at the end of the experiment ($P < 0.001$). The maximum PAP difference was $-11.96 \pm 1.92$ ($P < 0.001$). A tendency to a more pronounced effect of BQ-123 on PAP compared with Tezosentan was observed.

Lung weight increased slightly in all experiments and was comparable to that in the ET solo group. Ventilation pressures were not affected.

Aerosolization of NaCl and ETRAs

Aerosolization of $0.51 \pm 0.02$ ml of NaCl 0.9% over 15 min further increased PAP and ventilation pressures compared with the ET solo group ($P > 0.05$) (Fig. 4, A–C).

During and after application of the BQ-788 (1.14 μM) aerosol, there was a tendency to an attenuation of PAP compared with the ET NaCl-aer group (maximum PAP difference $-3.04 \pm 1.85$ mmHg; $P > 0.05$). Comparing the inhalational effects with the intravenous administered BQ-788 revealed opposite effects ($P < 0.05$).

When BQ-123 (1.14 μM) was applied as an aerosol, PAP was significantly reduced after the inhalation was stopped (time 45 min, $P < 0.05$). However, this effect ceased at the end of the experiment. The maximum effect on PAP was $-7.3 \pm 1.7$ mmHg ($P < 0.01$). Ventilation pressures were not significantly different compared with the other aerosol groups.

Application of a Tezosentan-aerosol (1.16 μM) caused a reduction of the ET-1 effect on PAP at the end of the inhalation.
period (time 45 min) and still at the end of the experiment (each P < 0.01). The maximum effect on PAP was −11.4 ± 2.97 mmHg (P < 0.01).

In all aerosol groups, lung weight increased with a slight acceleration during application of the substances (Fig. 4C). However, after stopping the inhalation, lung weight gain returned to levels that were observed before inhalation and in other treatment groups. There was no significant difference between the groups, and the slight acceleration could be attributed to the amount of the aerosol.

DISCUSSION

This study demonstrates for the first time that pulmonary vasoconstriction, as an example of ET-1-mediated lung disease, can be attenuated by aerosolization of a selective ETA (BQ-123) and a dual selective ETA and ETB receptor blocker (Tezosentan). While the effect of the BQ-123 aerosol ceased at the end of the experiment, the Tezosentan effect was long acting and caused a significant mitigation of the ET-1 effect throughout the observation period.

These effects may be explained by the fact that both (the ETA and ETB) receptors are differentially distributed and exert opposite effects within the lung vasculature. ET-1 mediates its actions via the ETA and ETB receptors, members of a G protein-coupled superfamily with a high conservation of each type across mammalian species (85–90%) (11). With regard to the vascular bed, these receptors coexist on vascular smooth muscle cells in the pulmonary vessels and mediate ET-1-induced vasoconstriction (1, 9). However, ETB receptors are also located on endothelial cells and induce the release of vasodilating substances, such as nitric oxide and prostacyclin, which in turn results in pulmonary vasodilation (1, 5a, 13). Nevertheless, the net effect of ET-1 on the pulmonary vessels is a sustained vasoconstriction. In line with this, we are able to demonstrate that the vasoconstrictive effect of ET-1 in the lung is mainly mediated via ETA receptors. These detrimental effects can be alleviated not only by intravascular but also by aerosol application of a specific ETA receptor blocker (BQ-123), leading to an attenuation of the ET-1-induced vasoconstriction. In contrast, intravascular, but not inhalative, administration of a selective ETB receptor blocker (BQ-788) led to an augmented ET-1-mediated vasoconstriction. One explanation for this observation during inhalation of BQ-788 may be that even the effect of intravascular BQ-788 was too small to be preserved during inhalation of the substance. Indeed, compared with the BQ-123 and Tezosentan effects, BQ-788 effects were only small. In our opinion, this underlines the fact that ETB receptor-mediated vasodilation has only a minor role compared with the vasoconstrictive properties. Since the fourfold intravascular dosage of each substance was administered as an aerosol, we would like to exclude a dosing problem. However, since we did not perform a formal dose response curve, we might have missed some effect at higher aerosol doses. But this
could have been true for BQ-123 and Tezosentan. It seems reasonable to speculate that BQ-788 did not reach the \( \text{ET}_B \) receptor on the endothelial cell layer preventing its detrimental effects. However, although we know that it is hard to discuss nonsignificant results, we would like to point out the tendency of BQ-788 aerosol to reduce PAP. Moreover, direct comparison of the intravascular and the aerosol effects of BQ-788 significantly manifest the opposite effects. Interestingly, inhalation of the dual \( \text{ETRA} \) (Tezosentan) evoked the most prominent effect of all aerosol groups. One attempt to explain this phenomenon is that all aerosols reached smooth muscle, but not endothelial, ET receptors in our isolated lung model. Although unproven, this could provide an explanation for the difference in response to intravascular and aerosolized \( \text{ETRAs} \), namely the lack of an effect of the BQ-788 aerosol and the superiority of the Tezosentan over the BQ-123 aerosol. Again, we are not able to prove this interpretation, but it seems to fit in previous observations and aspects of the pathophysiological concept of \( \text{ET-1-mediated} \) effects. Consequently, inhalation of a specific \( \text{ET}\_A \) (BQ-123) or a dual selective \( \text{ETRA} \) (Tezosentan) may be an alternative therapeutic approach in \( \text{ET-1-mediated} \) lung disease.

The isolated whole lung model enables an investigation of some of the pathophysiological aspects of \( \text{ET-1} \) within this organ as it allows interaction of the lung vasculature with the adjacent compartments (epithelium and interstitium) in a quasi-in vivo setting. In addition, due to a blood-free perfusion, \( \text{ET-1} \) effects on peripheral blood cells can be neglected. Since some superiority of aerosolized vasoactive substances has been shown before (14, 28), we have chosen this route of application into the target organ to maximize benefit/risk ratio of the substances. We aimed to establish an \( \text{ET-1-induced} \) PH without lung edema formation, giving us the chance to describe the “pure” vascular action of \( \text{ET-1} \) in the pulmonary circulation. However, the actions of \( \text{ET-1} \) are manifold (8, 18), with a very prominent disease-mediating role within the lung (3).

In our experiments, intravascular administration of the specific \( \text{ET}_B \) receptor blocker BQ-788 enhanced pulmonary vasoconstriction, whereas the selective \( \text{ET}_A \) receptor blocker BQ-123 and the blockade of both receptors reduced this \( \text{ET-1} \) effect. The endothelial \( \text{ET}_B \) receptor plays a significant role in the clearance of \( \text{ET-1} \) from pulmonary circulation. This could have led to an increased activation of the \( \text{ET}_A \) receptor and consequently enhanced pulmonary vasoconstriction. Another interpretation may be that BQ-788 blocked the vasodilating properties of the endothelial \( \text{ET}_B \) receptor leading to disturbed counterregulation. This observation is in accordance with earlier studies. An augmented pulmonary vasoconstriction has been described after blockade of the \( \text{ET}_B \) receptor in systemic circulation, isolated pulmonary arteries, and in an isolated lung model (19, 24). In addition, it is in accordance with this pathophysiological concept that antagonizing the \( \text{ET}_A \) receptor alone attenuates experimental PH, as well as blocking both \( \text{ET}_1 \) receptors.

Our study clearly had limitations. Besides its vasoconstrictive effects, \( \text{ET-1} \) also mediates cardiac and vascular remodeling, including proliferation of vascular smooth muscle cells in chronic disease states. The latter has successfully been treated in models of PH by inhibition of the \( \text{ET}_A \) receptor or dual blockade of both receptors (12). However, in this model we are not able to draw any conclusions on these chronic aspects of \( \text{ET-1} \). In addition, we did not investigate the effects on microcirculation, e.g., fluid filtration coefficient, and we did not measure concentrations of any \( \text{ETRAs} \) or \( \text{ET-1} \). The latter may be of importance to conclude on the bioavailability and influence on \( \text{ET-1} \) clearance. However, since we observed a significant biological effect on pulmonary vasculature, we only lack a formal dose response for the aerosol group. Nevertheless this could give incentive for further studies in chronic models.

We conclude that antagonism of \( \text{ET-1-induced} \) vasoconstriction as an indicator of \( \text{ET-1-mediated} \) lung disease is possible via aerosolization of a selective \( \text{ET}_A \) receptor and even stronger by a dual endothelin receptor blocker. The superiority of the dual blockade over the selective \( \text{ET}_A \) receptor blockade may be achieved by avoiding antagonism of potentially beneficial \( \text{ET} \) effects on endothelial \( \text{ET}_B \) receptors.

This inhalational route could restrict the \( \text{ET-1}-\)antagonizing effects to the lung and thereby optimize the beneficial effects of \( \text{ETRAs} \). Consequently, inhalation of \( \text{ETRA} \) may be a new concept for the treatment of \( \text{ET-1-mediated} \) diseases of the lung.

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