British Journal of Pharmacology (2000) 131, 1129–1134

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Effect of endothelin antagonists, including the novel ET_A receptor antagonist LBL 031, on endothelin-1 and lipopolysaccharide-induced microvascular leakage in rat airways

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1 The effect of the novel ET_A receptor antagonist LBL 031 and other selective and mixed endothelin receptor antagonists on endothelin-1 (ET-1)-induced and lipopolysaccharide (LPS)-induced microvascular leakage was assessed in rat airways.

2 Intravenously administered ET-1 (1 nmole kg^{-1}) or LPS (30 mg kg^{-1}) caused a significant increase in microvascular leakage in rat airways when compared to vehicle treated animals.

3 Pre-treatment with the selective ET_A receptor antagonists, LBL 031 or PD 156707, or the mixed $ET_{A/B}$ receptor antagonist, bosentan (each at 30 mg kg^{-1}), reduced ET-1-induced leakage to baseline levels. ET-1-induced leakage was not reduced by pre-treatment with the ET_B selective antagonist BQ 788 (3 mg kg^{-1}).

4 Pre-treatment with the selective ET_A receptor antagonist, LBL 031 (0.1 mg kg^{-1}) or PD 156707 (10 mg kg^{-1}), or the mixed $ET_{A/B}$ receptor antagonist, bosentan (30 mg kg^{-1}), reduced LPS-induced leakage by 54, 48 and 59% respectively. LPS-induced leakage was not affected by pre-treatment with the ET_B selective antagonist BQ 788 (3 mg kg^{-1}).

5 The data suggests that ET-1-induced microvascular leakage in the rat airway is ET_A receptor mediated and that part of the increase induced by LPS may be due to the actions of ET-1. Therefore, a potent ET_A receptor selective antagonist, such as LBL 031, may provide a suitable treatment for inflammatory diseases of the airways, especially those involving LPS and having an exudative phase, such as the septic shock-induced adult respiratory distress syndrome.

British Journal of Pharmacology (2000) **131,** 1129–1134

Keywords: Endothelin; lipopolysaccharide; endothelin antagonists; microvascular leakage; airways; adult respiratory distress syndrome

Abbreviations: ARDS, adult respiratory distress syndrome; EGF, epidermal growth factor; ET, endothelin; ET-1, endothelin-1; ET_A endothelin A; ET_B endothelin B; LPS, lipopolysaccharide; N.E., no effect

Introduction

The leakage of plasma proteins from the microvasculature into airways tissue is seen as an important factor in the development of inflammatory diseases of the lung such as the adult respiratory distress syndrome (ARDS). The early stages of ARDS present with acute and diffuse injury to the endothelial and epithelial lining of the lungs and are characterized by increased vascular permeability with protein-rich exudative oedema (Meduri, 1996).

Endothelin-1 (ET-1) has been implicated in the pathophysiology of ARDS in man (Filep *et al*., 1993). ARDS patients often present with sepsis (Montgomery *et al*., 1985). During sepsis large amounts of lipopolysaccharide (LPS) are released from bacterial cell walls. LPS causes the release of inflammatory mediators such as ET-1. ET-1 is a potent vasoactive agent (Yanagisawa *et al*., 1988). ET-1 and the ET_A and ET_B receptors have been shown to be widely distributed in the lung (Goldie *et al*., 1995). Circulating levels of ET-1 have been shown to be elevated in ARDS (Sanai *et al*., 1996). ET-1 has been shown to cause vascular remodelling and pulmonary hypertension and is known to remain elevated in patients where ARDS progresses (Langleben *et al*., 1993). ET-1 has been shown to increase vascular permeability in the bronchi and intra pulmonary airways in rats (Filep *et al*., 1994). The administration of LPS has been shown to cause the release of ET-1 in rats (Morise *et al*., 1994) and pigs (Weitzberg *et al*., 1993), and to increase the haematocrit in rats by promoting loss of plasma volume from the capillaries, an effect which was blocked by an ET_A receptor antagonist (Allcock & Warner, 1997).

In these studies we compared the effects of two ET_A receptor antagonists, the novel ET_A antagonist, LBL 031 (Astles *et al*., 2000) and PD 156707 (Reynolds *et al*., 1995) and the mixed ET_A/ET_B receptor antagonist, bosentan (Clozel *et al*., 1994) and the ET_B receptor antagonist, BQ 788 (Ishikawa *et al*., 1994), against ET-1-induced and LPS-induced microvascular leakage in rat airway tissue. Preliminary data from this study has been presented in abstract form (Hele *et al*., 1999).

Methods

Effect of ET-1 on microvascular leakage in rat airways

Plasma leakage was measured using a modification of the technique employed by Bernareggi *et al*. (1997). Experiments were conducted using male Wistar rats (250–350 g, $n=6-8$)

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housed at 21±1°C under a 14:10 h light-dark cycle. Animals were allowed free access to standard laboratory chow and water. They were anaesthetized with isofluorane (3.5% in oxygen) and given Evans blue dye (20 mg kg^{-1} i.v.) *via* the tail vein. One minute later while still anaesthetized they received ET-1 (0.25, 0.5, 1 or 2 nmole kg^{-1} i.v.) or vehicle (10 mM sodium bicarbonate in sterile saline). They were allowed to recover and 10 min after ET-1 administration were killed by overdose of pentobarbitone (200 mg kg^{-1} i.p.). ET-1 (1 nmole kg^{-1}) evoked a significant increase in microvascular leakage which was reduced at 2 nmole kg and so ET-1 (1 nmole kg^{-1}) was the dose selected for subsequent experiments (Figure 1a). A substantial leakage response was obtained 10 min after ET administration which was not significantly different at the later time points evaluated and therefore this time point was selected for the subsequent experiments (Figure 1b). The chest was opened and an incision made in the left ventricle, a cannula was inserted through the left ventricle and into the ascending aorta and approximately 150 ml of sterile saline (0.9%) was perfused at a pressure of 100 mmHg. The heart and lungs were removed *en bloc*. The trachea, bronchi and lungs were dissected free and the parenchyma was scraped from the intra pulmonary airways. The bronchi and intra pulmonary airways combined and the trachea were each placed in 2 ml of formamide for 18 h at 37°C to facilitate the extraction of Evans blue dye. The absorbances of the resulting extracts were determined against standard concentrations of Evans blue at 620 nm using a Cecil 2000 spectrophotometer.

Effect of LBL 031, PD 156707, bosentan and BQ 788 on ET-1-induced microvascular leakage in rat airways

LBL 031 (1, 3, 10 and 30 mg kg^{-1}), PD156707 (30 mg kg^{-1}), bosentan (30 mg kg^{-1}) or vehicle (sterile saline 0.9%) or BQ 788 (0.3, 1 and 3 mg kg^{-1}) or vehicle (3% Pluronic F68 in 5% sodium bicarbonate) were administered intravenously under isofluorane anaesthesia 30 min prior to ET-1 (1 nmole kg^{-1} i.v.) administration. Evans blue (20 mg kg^{-1} i.v.) was given 1 min prior to ET-1. Animals were killed 10 min after ET-1 administration. Tissues were removed, extracted and analysed as described above. The time and dose for ET-1 were determined in preliminary studies (Figure 1a,b). A dose of ET-1 (1 nmole kg^{-1} i.v.) which produced a significant leakage response was selected to investigate the effects of ET receptor antagonists on ET-1-induced plasma extravasation (Figure 1a). The earliest time point (10 min) was selected as the leakage response evoked by ET-1 was already significant

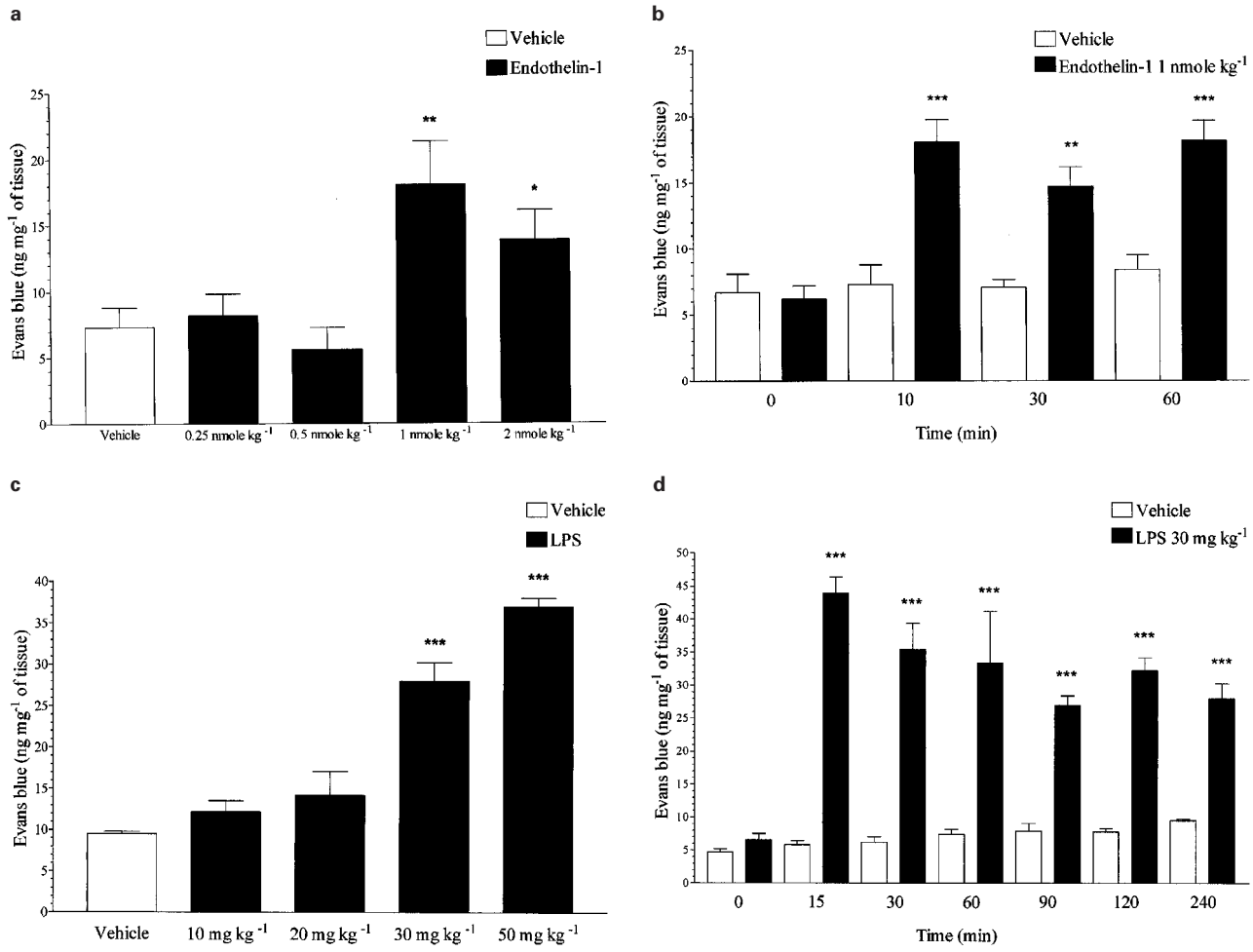

Figure 1 Effect of intravenous ET-1 or LPS on microvascular leakage into rat airways. (a) ET-1 dose–response study; (b) ET-1 time course study; (c) LPS dose–response study; (d) LPS time course study. Rats were anaesthetized with isofluorane (3.5% in oxygen) and received Evans blue (20 mg kg^{-1} i.v.). One minute later they received ET-1 or LPS or vehicle i.v. In the dose–response studies rats were killed 10 min after ET-1 or vehicle administration or 15 min after LPS or vehicle and tissues removed and placed in formamide for Evans blue dye extraction. Values for the dose–response studies are presented as data minus basal leak at time 0 and all data is expressed as mean±s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). $n=4-8$. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

by 10 min and did not change with subsequent time points investigated (Figure 1b).

Effect of endothelin antagonists on bacterial lipopolysaccharide-induced microvascular leakage in rat airways

The experimental protocol for the LPS studies is as for the ET-1 studies described above except that animals were killed 15 min after LPS (30 mg kg^{-1} i.v.). The time and dose for LPS were determined in preliminary studies (Figure 1c,d). A submaximal dose of LPS (30 mg kg^{-1}) was selected to investigate the effects of ET receptor antagonists on LPS-induced plasma extravasation (Figure 1c). The earliest time point (15 min) was selected as the leakage response evoked by LPS appeared to be reduced at all subsequent time points investigated (Figure 1d). Dose–response studies were carried out with LBL 031 (0.001–30 mg kg^{-1} i.v.), PD 156707 (0.001–30 mg kg^{-1} i.v.), bosentan (0.001–30 mg kg^{-1} i.v.), or BQ 788 (0.01–3 mg kg^{-1} i.v.) or their relevant vehicles administered 30 min prior to LPS administration.

Data analysis

Results are presented as data minus basal leak at time 0 and expressed as mean ± s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). Statistical analysis was carried out by one way analysis of variance with a correction for multiple comparisons using Dunnett's critical values. All experiments were conducted in accordance with UK Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) Act 1986.

Chemicals

ET-1 (Peptide and Protein Research, Washington Singer laboratories, University of Exeter, U.K.), Pluronic F68, LPS from *E. Coli* 0111 : B4 and Evans Blue Dye (Sigma Chem. Co., St. Louis, MO, U.S.A.). LBL 031, PD 156707 and BQ 788 were supplied by Aventis Pharma (Dagenham, Essex, U.K.), Sodium bicarbonate by Rhône Poulenc Ltd. (Manchester, U.K.), Sterile saline 0.9% (Fresenius Kabi Ltd., Warrington, U.K.) and Isofluorane by Abbott Laboratories (Queenborough, Kent, U.K.).

Results

Effect of endothelin-1 on microvascular leakage in rat airways

ET-1 (1 nmole kg^{-1} i.v.) significantly increased the leakage of Evans blue labelled plasma proteins into the bronchi and intra pulmonary airways (7.3 ± 1.5 increased to 18.09 ± 3.29, $P<0.01$, see Figure 1a). ET-1 did not cause significant leakage into the trachea. ET-1 when administered at 2 nmole kg^{-1} i.v. proved to be a near lethal dose and consequently caused less leakage than ET-1 at 1 nmole kg^{-1} i.v. ET-1 caused no significant effects at the lower doses tested (0.25 and 0.5 nmole kg^{-1} i.v.).

Effect of LBL 031, PD 156707, bosentan and BQ 788 on endothelin-1-induced microvascular leakage in rat airways

LBL 031, PD 156707 or bosentan (all at 30 mg kg^{-1} i.v.) reduced ET-1-induced microvascular leakage in the bronchi and IPA to basal levels (8.96 ± 0.87 reduced to 3.74 ± 0.43, $P<0.01$, 5.05 ± 1.45, $P<0.05$ and 1.36 ± 1.25, $P<0.01$, respectively, see Figure 2). Although this dose of bosentan reduced the dye extravasation to a level below that attained with the vehicle injection this difference was not statistically significant. In the dose response study LBL 031 inhibited ET-1-induced leakage at both 10 and 30 mg kg^{-1} i.v. (8.1 ± 1.02 reduced to 2.78 ± 0.71 and 2.32 ± 0.81 respectively, $P<0.01$, see Figure 3). BQ 788 had no effect at the lower doses tested (0.3 and 1 mg kg^{-1} i.v.) but significantly potentiated ET-1-induced microvascular leakage in the bronchi and IPA at 3 mg kg^{-1} i.v. (8.1 ± 1.07 increased to 14.56 ± 2.83, $P<0.01$, see Figure 4). It should also be noted that when BQ 788 (3 mg kg^{-1} i.v.) was given in the absence of ET-1 it caused a similar degree of leakage to that elicited by ET-1 at 1 nmole kg^{-1} i.v. (7.11 ± 1.49 and 8.1 ± 1.07 respectively).

Effect of endothelin antagonists on bacterial LPS-induced microvascular leakage in rat airways

LBL 031 evoked a statistically significant inhibition of microvascular leakage into the airways at all doses tested between 0.01 and 30 mg kg^{-1} i.v. (0.1 mg kg^{-1} i.v reduced 31.91 ± 1.9 to 14.54 ± 2.09, $P<0.001$), PD 156707 caused significant inhibition at 10 and 30 mg kg^{-1} i.v. (34.91 ± 4.69 reduced to 24.9 ± 2.78 and 25.72 ± 3.2 respectively, $P<0.05$,), whereas bosentan only caused significant inhibition at 30 mg kg^{-1} i.v. (34.5 ± 3.34 reduced to 20.97 ± 0.99, $P<0.001$) (See Table 1 and Figures 5a,b and 6). BQ 788 had no effect on LPS-induced microvascular leakage at doses up to 3 mg kg^{-1} i.v. (see Figure 7), a dose shown to potentiate ET-1-induced increases in microvascular leakage (see Figure 4).

Discussion

Inflammatory mediators such as ET-1 have been implicated in the pathophysiology of ARDS and have been shown to be

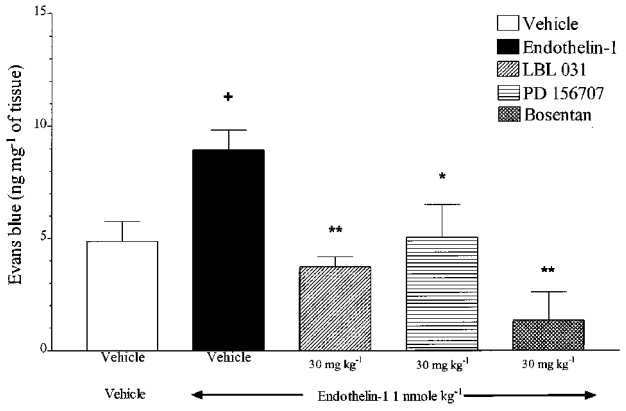

Figure 2 Effect of LBL 031, PD 156707 and bosentan (each 30 mg kg^{-1}) on ET-1-induced microvascular leakage into rat bronchi and intra-pulmonary airways. Rats were anaesthetized with isofluorane (3.5% in oxygen) and received vehicle or compound i.v. 30 min prior to Evans blue (20 mg kg^{-1} i.v.). One minute after Evans blue administration they received ET-1 (1 nmole kg^{-1} i.v.) or vehicle i.v. The rats were killed 10 min after ET-1 or vehicle administration and tissues removed and placed in formamide for Evans blue dye extraction. Values are presented as data minus basal leak at time 0 and expressed as mean ± s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). $n=6$. *$P<0.05$, **$P<0.01$ when compared to the Vehicle/ET-1 group, +$P<0.05$ when compared to Vehicle/Vehicle group.

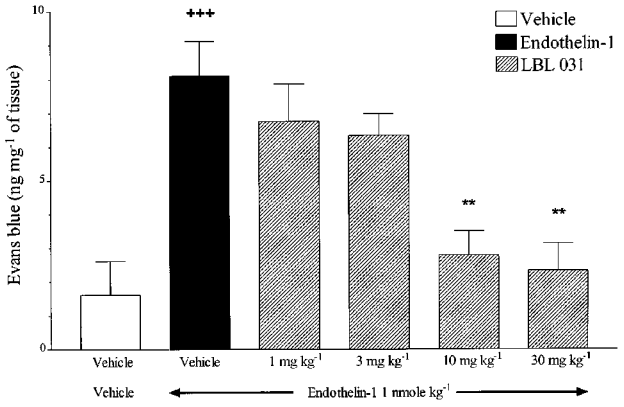

Figure 3 Effect of LBL 031 ($1-30$ mg kg^{-1}) on ET-1-induced microvascular leakage into rat bronchi and intra-pulmonary airways. Rats were anaesthetized with isofluorane (3.5% in oxygen) and received vehicle or LBL 031 i.v. 30 min prior to Evans blue (20 mg kg^{-1} i.v.). One minute after Evans blue administration they received ET-1 (1 nmole kg^{-1} i.v.) or vehicle i.v. The rats were killed 10 min after ET-1 or vehicle administration and tissues removed and placed in formamide for Evans blue dye extraction. Values are presented as data minus basal leak at time 0 and expressed as mean$\pm$s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). $n=6$. $^{**}P<0.01$ when compared to the Vehicle/ET-1 group, $+++P<0.001$ when compared to Vehicle/Vehicle group.

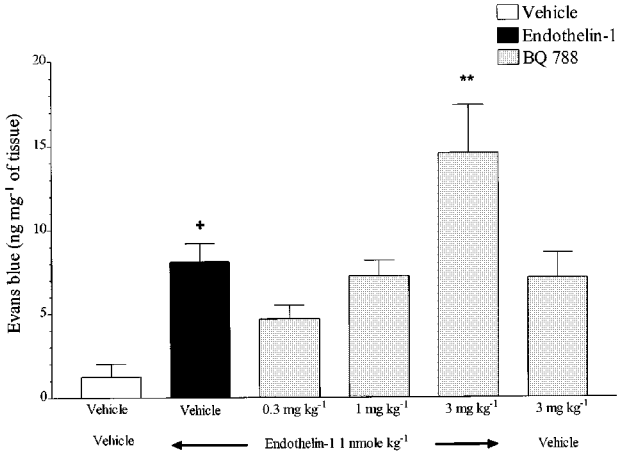

Figure 4 Effect of BQ 788 ($0.3-3$ mg kg^{-1}) on ET-1-induced microvascular leakage into rat bronchi and intra-pulmonary airways. Rats were anaesthetized with isofluorane (3.5% in oxygen) and received vehicle or BQ 788 i.v. 30 min prior to Evans blue (20 mg kg^{-1} i.v.). One minute after Evans blue administration they received ET-1 (1 nmole kg^{-1} i.v.) or vehicle i.v. The rats were killed 10 min after ET-1 or vehicle administration and tissues removed and placed in formamide for Evans blue dye extraction. Values are presented as data minus basal leak at time 0 and expressed as mean$\pm$s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). $n=6$. $^{**}P<0.01$ when compared to the Vehicle/ET-1 group, $+P<0.05$ when compared to Vehicle/Vehicle group.

released after the administration of LPS. ARDS is known to be an acute pulmonary inflammatory syndrome often induced by systemic infection (sepsis). LPS is produced in abundance during sepsis. ARDS is a disease of three phases, the exudative or oedematous phase lasting 7–10 days, the fibroproliferative phase and the fibrotic phase (Meduri, 1996). A major contributor to the oedematous phase is the leakage of plasma proteins from the vasculature into the surrounding airways tissue. In these studies we have examined the effect of endothelin antagonists on microvascular leakage elicited by ET-1 and LPS which were

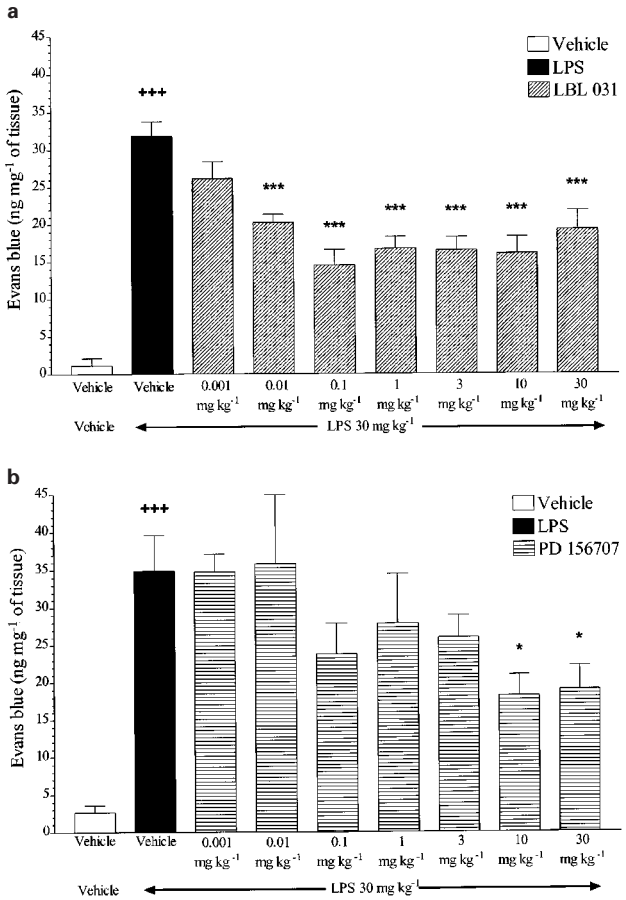

Figure 5 Effect of ETA receptor antagonists ($0.001-30$ mg kg^{-1}) on LPS-induced microvascular leakage into rat bronchi and intra-pulmonary airways. (a) LBL 031, (b) PD 156707. Rats were anaesthetized with isofluorane (3.5% in oxygen) and received vehicle, LBL 031 or PD 156707 i.v. 30 min prior to Evans blue (20 mg kg^{-1} i.v.). One minute after Evans blue administration they received LPS (30 mg kg^{-1} i.v.) or vehicle i.v. The rats were killed 15 min after LPS or vehicle administration and tissues removed and placed in formamide for Evans blue dye extraction. Values are presented as data minus basal leak at time 0 and expressed as mean$\pm$s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). $n=6$. $^{***}P<0.001$ when compared to the Vehicle/LPS group, $+++P<0.001$ when compared to Vehicle/Vehicle group.

Table 1 Effect of ET receptor antagonists on LPS-induced microvascular leakage in rat airways

Compound	*Endothelin receptor*	*Minimal effective dose* (mg kg^{-1}, i.v.)	*Percentage inhibition*	*Dose range tested* (mg kg^{-1}, i.v.)
LBL 031	A	0.01	36***	0.001–30
PD 156707	A	10	48*	0.001–30
Bosentan	A/B	30	59***	0.001–30
BQ 788	B	>3	N.E.	0.01–3

All percentage changes are from control groups. $^{*}P<0.05$, $^{***}P<0.001$, $n=6-8$, N.E., no effect.

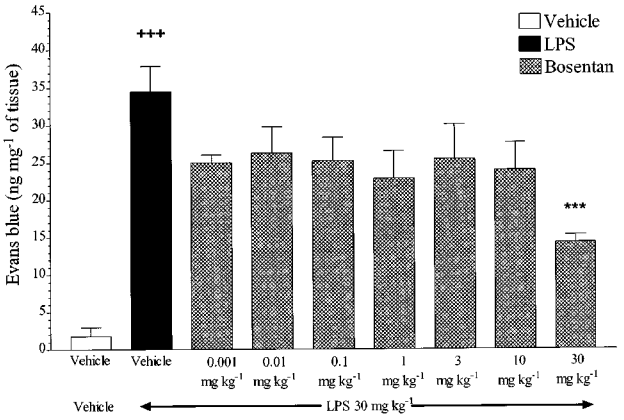

Figure 6 Effect of the ET_A/ET_B receptor antagonist, bosentan (0.001–30 mg kg^{-1}), on LPS-induced microvascular leakage into rat bronchi and intra-pulmonary airways. Rats were anaesthetized with isofluorane (3.5% in oxygen) and received vehicle or bosentan i.v. 30 min prior to Evans blue (20 mg kg^{-1} i.v.). One minute after Evans blue administration they received LPS (30 mg kg^{-1} i.v.) or vehicle i.v. The rats were killed 15 min after LPS or vehicle administration and tissues removed and placed in formamide for Evans blue dye extraction. Values are presented as data minus basal leak at time 0 and expressed as mean ± s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). $n=6$. ***$P<0.001$ when compared to the Vehicle/LPS group, +++$P<0.001$ when compared to Vehicle/Vehicle group.

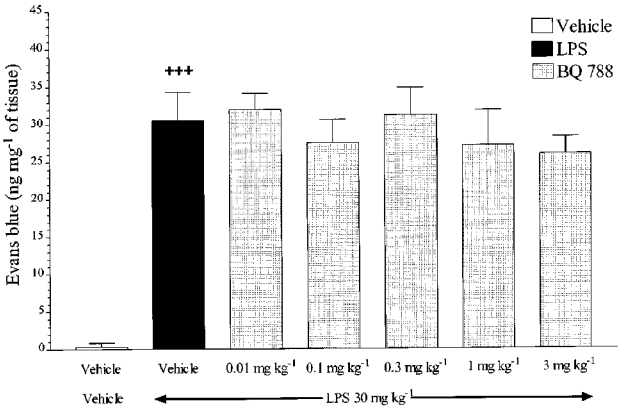

Figure 7 Effect of the ET_B receptor antagonist, BQ 788 (0.01–3 mg kg^{-1}), on LPS-induced microvascular leakage into rat bronchi and intra-pulmonary airways. Rats were anaesthetized with isofluorane (3.5% in oxygen) and received vehicle or BQ 788 i.v. 30 min prior to Evans blue (20 mg kg^{-1} i.v.). One minute after Evans blue administration they received LPS (30 mg kg^{-1} i.v.) or vehicle i.v. The rats were killed 15 min after LPS or vehicle administration and tissues removed and placed in formamide for Evans blue dye extraction. Values are presented as data minus basal leak at time 0 and expressed as mean ± s.e.mean of the concentration of Evans blue dye (ng mg^{-1} of tissue). $n=6$. +++$P<0.001$ when compared to Vehicle/Vehicle group.

administered in an attempt to model the exudative phase of the disease.

In this study we have shown that ET-1 induces microvascular leakage in rat airways and that the selective ET_A receptor antagonists, LBL 031 and PD 156707, or a mixed $ET_{A/B}$ receptor antagonist, bosentan, can inhibit this action. The lack of any inhibitory effect by the selective ET_B receptor antagonist, BQ 788, at a dose greater than that shown to abolish the ET_B receptor mediated depressor response to ET-1 in rats (Ishikawa *et al*., 1994), suggests that this is an ET_A-receptor mediated response.

The apparent potentiation of ET-1-induced leakage by BQ 788 may be explained by the fact that ET_B receptors in the pulmonary circulation are thought to be responsible for the clearance of ET-1 through receptor-mediated endocytosis (Dupuis *et al*., 1996). Therefore inhibition of ET_B receptors by BQ 788 may result in an increase in plasma ET-1 concentration which when allied to exogenously administered ET-1 may explain the enhanced leakage response seen in the presence of BQ 788. However, when a higher dose of ET-1 was given (2 nmol kg^{-1} i.v., see Figure 1) this did not result in greater leakage than the dose of ET-1 (1 nmol kg^{-1} i.v.) administered in the presence of BQ 788. It is therefore possible that the increase in ET-1-induced leakage caused by BQ 788 may not be mediated *via* ET_B receptor antagonism or that the BQ 788-induced increase in plasma ET-1 concentration was sufficient to enhance ET-1-induced leakage without attaining the same plasma level of ET-1 as that reached when a dose of 2 nmol kg^{-1} i.v. is given.

LBL 031 (ET_A), PD 156707 (ET_A), and bosentan ($ET_{A/B}$) partially inhibited LPS-induced microvascular leakage. BQ 788 (ET_B) had no effect, again suggesting an ET_A receptor involvement. Interestingly, the dose–response relationship obtained with LBL 031 on LPS-induced leak is only evident at the lower doses utilized and a maximum response was obtained at doses >0.1 mg kg^{-1}. The greater potency of LBL 031 when compared to PD 156707 may be explained by the increased antagonistic activity of LBL 031 at ET_A receptors. PD 156707 inhibited ET-1-induced vasoconstriction in the isolated rabbit femoral artery with a pA_2 of 7.5 (Reynolds *et al*., 1995) whereas LBL 031 inhibited ET-1 induced contraction of isolated rat aortic rings (denuded of endothelium) with a pK_B of 8.1 (Astles *et al*., 2000). The advantage of LBL 031 over bosentan is probably due to its selectivity and greater affinity for the ET_A receptor. The greater potency of LBL 031 against LPS-induced micro vascular leakage compared to ET-1-induced microvascular leakage is more difficult to explain. However, it may be due to the increased difficulty in inhibiting the all or nothing leakage response evoked by exogenous ET-1 rather than the local and directed release of ET-1 induced by LPS.

The lack of a dose–response relationship with bosentan and PD 156707 on the LPS-induced microvascular leakage response and the fact that an inhibitory response was only achieved at the higher doses may suggest that these effects may not be due to their ET_A receptor antagonist activity. However, we believe that there is strong evidence presented in this manuscript that ET-1 does mediate, in part, the increased microvascular leakage into the airways observed after LPS administration. The fact that exogenously administered ET-1 evokes increases in microvascular leakage in the airways and that two ET_A receptor antagonists and one mixed $ET_{A/B}$ receptor antagonist all reduce LPS-induced leakage by approximately 50% is evidence supporting a role for ET-1 in LPS-induced increases in microvascular extravasation into the airways.

There are several possible advantages to using a selective ET_A receptor antagonist in the treatment of ARDS apart from their ability, demonstrated in this study, to limit the leakage of plasma proteins from the vasculature into the airway tissue. One other advantage may be their ability to reverse the pressor effects of circulating ET-1 which are mediated predominantly *via* vascular smooth muscle ET_A receptors (Webb & Strachan, 1998). One of the clinical signs of ARDS is raised pulmonary artery pressure often associated with right ventricular dysfunction (Murray *et al*., 1988). ET-1 may have a role in this process as circulating

levels of ET-1 are known to be raised in ARDS and ET-1 is known to preferentially increase pulmonary artery pressure (Langleben *et al*., 1993). Therefore, the use of a selective ET_A receptor antagonist may be beneficial in normalizing vascular pressure in the pulmonary vascular bed thereby improving oxygen exchange and prognosis. ET-1 has also been shown to potentiate the effect of mitogens, such as epidermal growth factor (EGF), on human airway smooth muscle cells and selective ET_A antagonists have been shown to inhibit the proliferation of these cells (Panettieri *et al*., 1996). ET-1 has also been shown to contribute to fibroblast proliferative activity in man (Cambrey *et al*., 1994), a process which occurs during the second phase of ARDS and may also be attenuated by endothelin antagonists. Endothelin antagonists may also impact on the fibrotic or third phase of ARDS as there is strong evidence to suggest that ET-1 plays a role in the pathogenesis of pulmonary fibrosis in man (Uguccioni *et al*., 1995) and in animal models (Mutsaers *et al*., 1998) and the antagonists have been shown to reduce fibrosis in an airway model of fibrosis (Park *et al*., 1997).

These studies demonstrate a role for ET-1 in microvascular leakage and suggest that LPS-induced microvascular leakage is in part caused by ET-1 and that this effect may be ET_A receptor mediated. We have demonstrated that an ET_A or a mixed ET_A/ET_B receptor antagonist can inhibit LPS-induced microvascular leakage and would suggest that a potent ET_A receptor antagonist, LBL 031, may provide an ideal treatment for the initial exudative phase of sepsis-induced ARDS as well as for the later fibroproliferative and the fibrotic phases.

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(Received March 8, 2000
Revised September 4, 2000
Accepted September 8, 2000)