Endothelin\textsubscript{A}-receptor antagonism attenuates pulmonary hypertension in porcine endotoxin shock

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ABSTRACT: Porcine endotoxin shock is characterized by pulmonary hypertension, decreased mean arterial pressure and deteriorated cardiac performance. These pathophysiological findings are accompanied by increased plasma endothelin-1 levels. Previous studies have shown that both the pulmonary and systemic circulation are improved by combined endothelin\textsubscript{A}- and endothelin\textsubscript{B}-receptor antagonism. This study was designed to evaluate further the specific involvement of the endothelin\textsubscript{A}-receptor mechanism in cardiopulmonary pathophysiology during endotoxin shock.

In a porcine endotoxin shock model, the endothelin\textsubscript{A}-receptor antagonist PD 155080 was administered after the onset of endotoxaemia. Cardiopulmonary vascular changes, dynamic lung compliance, oxygen-related variables and plasma levels of endothelin-1-like immunoreactivity were compared with a control group receiving only endotoxin.

PD 155080 counteracted the increase in pulmonary artery pressure seen in control animals. In contrast, cardiac performance was not improved. Dynamic lung compliance was not affected by PD 155080. Plasma levels of endothelin-1-like immunoreactivity increased to a similar degree in both groups.

These findings indicate that the endothelin system is involved in the pathophysiology of endotoxin shock. Furthermore, the change in pulmonary circulation seen in the present shock model is to a large extent mediated by the endothelin\textsubscript{A}-receptor mechanism. In view of previous findings, the deterioration of cardiac performance may involve the endothelin\textsubscript{B}-receptor, either alone or in combination with the endothelin\textsubscript{A}-receptor.


Since the discovery of the endothelins in 1988 an impressive number of studies concerning their multiple biological functions has been published [1]. The most potent endogenous vasoconstrictor known [2], endothelin (ET)-1, is the most studied of the three 21-amino acid isopeptides in the ET family. ET-1 is produced mainly by the endothelium, but also by a broad variety of other cell types, including airway epithelium, endocardium and myocardium [1] and macrophages [3]. Two other isomers, ET-2 and ET-3, are also known but, in contrast to ET-1, are not produced by the endothelium. Under normal circumstances ET-1 is suggested to work as a paracrine factor [4], mainly secreted abluminally [5], but under conditions with increased production, ET-1 also constitutes a circulating mediator [6]. The biological effects of ET-1 in mammals are mediated by at least two different receptors, ET\textsubscript{A} and ET\textsubscript{B}, both of which have been characterized and cloned [7, 8]. Both types of receptor are located on the vascular smooth muscle cell and have constitutive properties. In contrast, a subpopulation of ET\textsubscript{B}-receptors, located on the endothelium, mediate vasodilation through the release of nitric oxide and prostacyclin [9].

Septic shock is characterized by profound haemodynamic changes including pulmonary hypertension, systemic hypotension and impaired cardiac performance as well as maldistribution of regional blood flow. Similar changes are seen in response to endotoxin administration in various animal models as well as in humans. The aetiology of these disturbances has been sought in the large number of cytokines released in response to bacteria and endotoxin and also in endothelium-derived substances such as NO [10] and ET-1 [11]. Tumour necrosis factor (TNF)\texttextgreek{\textalpha}, a cytokine produced in excessive amounts in response to endotoxin, is known to stimulate the production of ET-1 from the endothelium [12]. Sepsis in humans is associated with a three- to four-fold increase in plasma ET-1 levels [13] and elevated ET-1 plasma levels are associated with poor prognosis in patients with septic shock [14]. Furthermore, complications of septic shock, such as acute respiratory distress syndrome and disseminated intravascular coagulation, are associated with increased plasma levels of ET-1 [15, 16]. Exogenous ET-1 administered to generate circulating plasma levels comparable to those found in human septic shock produces cardiopulmonary changes, in part similar to those seen in human sepsis [6, 17].

The development of ET receptor antagonists has provided useful tools in evaluating the involvement of ETs in the pathogenesis of endotoxin shock. It has previously
been shown that the nonpeptide mixed ET\textsubscript{A}/ET\textsubscript{B} antagonist bosentan (Ro-47-0203, Hoffman La Roche, Basel, Switzerland), when administered both before and during established porcine endotoxin shock, markedly improves a deteriorated cardiopulmonary condition [18, 19].

The present study was designed to evaluate specifically the role of the ETA-receptor in the disturbances in pulmonary circulation and function as well as in systemic haemodynamics during porcine endotoxin shock by use of the nonpeptide selective ETA-receptor antagonist PD 155080 (Parke Davis, Morris Plains, NJ, USA).

**Materials and methods**

**Animals**

Sixteen landrace pigs of both sexes, weighing between 18.0–26.2 kg, were fasted overnight with free access to water. The experimental protocol for the study was approved by the Ethics Committee for experimental animal research at the Karolinska Institute (Stockholm, Sweden).

**Preparations**

The animals were premedicated with \textit{i.m.} injections of ketamine 20 mg-kg\textsuperscript{-1} and atropine 25 \textmu g-kg\textsuperscript{-1} before being transported from the animal department. Anaesthesia was induced by an \textit{i.v.} bolus injection of pentobarbital 12 mg-kg\textsuperscript{-1} and sustained by a continuous infusion of 3–6 mg-kg\textsuperscript{-1}h\textsuperscript{-1}. Incremental bolus doses of pentobarbital were given when needed. After the reaction to pain from a forehoof stimulation was extinguished, to prevent shivering and to allow measurements of dynamic lung compliance, muscle paralysis was achieved by an infusion of pancuronium bromide 0.5 mg-kg\textsuperscript{-1}h\textsuperscript{-1}. After tracheotomy the animals were mechanically ventilated with a gas mixture of oxygen in air (inspiratory oxygen fraction (\textit{F}\textsubscript{I,O2}) 0.30, Servo 900 ventilator; Siemens Elema, Solna, Sweden). The respiratory frequency was set to 18 breaths min\textsuperscript{-1} and the minute volume was initially adjusted to keep the oxygen in air (inspiratory oxygen fraction (\textit{F}\textsubscript{I,O2}) 0.30, Servo 900 ventilator; Siemens Elema, Solna, Sweden). The respiratory frequency was set to 18 breaths min\textsuperscript{-1} and the minute volume was initially adjusted to keep the oxygen in air (inspiratory oxygen fraction (\textit{F}\textsubscript{I,O2}) 0.30, Servo 900 ventilator; Siemens Elema, Solna, Sweden).

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The arterial and pulmonary artery catheters were connected to a position in the pulmonary artery for measurements of pressures and cardiac output. By means of a minor lower midline laparotomy a catheter placed in the urinary bladder was used to monitor urinary output. At the end of preparation the animals were fully turned to the left lateral position.

**Study design**

The arterial and pulmonary artery catheters were connected to pressure transducers (Statham P23 Ac, Statham Instruments, Oxnard, CA, USA) and cardiac frequency (\textit{fC}), mean arterial blood pressure (MAP) and mean pulmonary arterial blood pressure (MPAP) were measured continuously, while pulmonary capillary wedge pressure (\textit{PCW}) and central venous pressure (CVP) were measured intermittently. All parameters were registered on a polygraph (Grass 7B; Quincy, MA, USA). Cardiac index (\textit{CI}; indexed to body weight) was measured by thermodilution (Edwards Lab 9520A, St Ana, CA, USA) and determined as the mean of a triplicate of 10 mL of ice-cold saline injections. Stroke volume index (SVI) was calculated as \textit{CI}/\textit{fC}. Systemic vascular resistance index (SVRI) was calculated as MAP-CVP/\textit{CI} and pulmonary vascular resistance index (PVRI) as MPAP-\textit{PCW}/\textit{CI}. Blood was collected from the arterial and pulmonary artery catheters for analysis of blood gases on an ILS 1610 blood gas analyser (Instrumental Laboratories, Warrington, Cheshire, UK). Systemic oxygen delivery index (\textit{DO}_{2}\textsubscript{I}) was calculated as arterial oxygen saturation (\textit{Sa,O2}) \times haemoglobin concentration (Hb) \times 0.0139 \times \textit{CI}, and systemic oxygen consumption index (\textit{VO}_{2}\textsubscript{I}) as \textit{Sa,O2}-mixed venous oxygen saturation (\textit{Sv,O2}) \times Hb \times 0.0139 \times \textit{CI}. To measure tracheal pressure, the outlet of the tracheal tube was connected to a Statham PM 131 TC pressure transducer. Airflow was measured with a heated pneumotachygraph (Model 3500A; Hans Rudolph, Kansas City, USA) connected to a different pressure transducer (Kent Scientific, Litchfield, CT, USA) and signals were sent to an AP 200 Pulmonary Computer (ConMcTech AB, Uppsala, Sweden) for online calculations of pulmonary dynamic compliance (tidal volume/pressure at maximal volume). Before termination of the experiment a bronchoalveolar lavage (BAL) with two instillations of 50 mL physiological saline each was performed under the guidance of a fiberoptic bronchoscope. In the BAL fluid the total protein concentration was analysed using the Pierce bicinchoninic acid protein assay reagent (Pierce Chemical Company, Rockford, IL, USA).

**Biochemical analysis**

Arterial plasma levels of ET-1-like immunoreactivity were analysed using a radioimmunoassay as described previously [20]. Hb was measured spectrophotometrically (Haemoglobin photometer; LEO, Helsingborg, Sweden).

**Methods**

After surgical preparation the animals were allowed 1 h of stabilization before baseline measurements were made (-1 h and 0 h). An \textit{i.v.} endotoxin infusion, \textit{Escherichia coli} lipopolysaccharide (serotype 0111:B4; Sigma, St Louis, MO, USA), dissolved in saline and heated, was started at 0 h at a rate of 2.5 \textmu g-kg\textsuperscript{-1}h\textsuperscript{-1} and was increased stepwise during 30 min until reaching 20 \textmu g-kg\textsuperscript{-1}h\textsuperscript{-1}. The endotoxin infusion was discontinued after 3 h. After 2 h of endotoxin infusion, eight animals received an \textit{i.v.} bolus injection of PD 155080 (10 mg-kg\textsuperscript{-1}) followed by a continuous \textit{i.v.} infusion of 5 mg-kg\textsuperscript{-1}h\textsuperscript{-1}, maintained throughout the experiment (5 h). PD 155080 was dissolved in a total of 100 mL saline. The eight animals in the control group received only endotoxin and saline vehicle. MAP, \textit{fC} and MPAP were followed continuously, while cardiac output,
Results

Apart from base excess no significant differences were found in any of the measured or calculated parameters between the control group and the PD 155080-treated group before the onset of the endotoxin challenge (0 h). Before the start of intervention (2 h) no significant differences were found between the groups in any parameter.

Pulmonary parameters

Endotoxin infusion induced a biphasic increase in MPAP and PVRI with an initial peak at approximately 30 min followed by a second gradual increase in these parameters. Administration of PD 155080 induced an instant decrease in MPAP which was maintained throughout the experiment with a significant difference from time-matched contrasts between groups at 5 h. At this time point, MPAP in the PD 155080-treated animals did not differ from baseline values (fig. 1a). Analogously, PD 155080 administration resulted in a significant reduction in PVRI. However, no significant difference was observed from time-matched contrasts at 5 h (fig. 1b). A decrease in arterial oxygen tension (PaO₂), SaO₂ and SvO₂ was seen in response to endotoxin (2 h). This decline continued in both groups and was not affected by PD 155080 treatment (table 1). Arterial carbon dioxide tension (PaCO₂) did not change significantly in either group during the experiment. In both groups, dynamic lung compliance gradually declined in the first 2 h of endotoxaemia, but no further change was observed during the last 3 h of the experiment, with no significant differences between the groups at 5 h (table 1). The total protein concentration in BAL fluid, obtained at 5 h, did not differ significantly between the groups (table 1).

Acid–base status and haemoglobin concentration

Both arterial pH and base excess decreased markedly in both groups during endotoxaemia. PD 155080 did not significantly affect these parameters, although base excess tended to be lower at the end of the experiment among the
Endothelin-1

The initial 2 h of endotoxaemia resulted in a three-fold increase in arterial plasma ET1-like immunoreactivity levels. This increase continued in both groups, but plasma ET-1-like immunoreactivity levels were without significant differences between groups at 5 h (fig. 3).

**Discussion**

The results presented here show that during established porcine endotoxin shock, pulmonary hypertension is abolished by treatment with the ETₐ-receptor antagonist PD 155080. In contrast, cardiac performance was not improved by PD 155080. These results differ considerably from those achieved by the mixed ETₐ/ETₐ-receptor antagonist bosentan, where both pulmonary hypertension and cardiac performance were improved [19]. This suggests that the ETₐ-receptor mediated events are of large importance in endotoxin-induced pulmonary hypertension.

Endotoxin infusion caused a biphasic increase in MPAP and PVRI. This is a well-described response to endotoxin in animal models observed by this group [21] and others [22]. The early increase in MPAP and PVRI can be prevented by cyclooxygenase inhibitors and is associated with an increase in thromboxane metabolites [23], but is unaffected by mixed ET-receptor antagonism [18], suggesting a cyclooxygenase-dependent mechanism. The second, more prolonged increase is probably multifactorial [24]. In the present study, PD 155080 administered after 2 h of endotoxaemia completely abolished the late increase in MPAP. PVRI decreased in a similar way but was not restored to pre-endotoxin values. High ET-1-like immunoreactivity concentrations are found in both porcine [25] and human lung tissues, which also contain large amounts of ET receptors [20]. In addition, under non-endotoxic conditions i.v. administered ET-1 increases pulmonary vascular tone in pigs and humans [17, 26]. In the present study the late increase in MPAP coincided in time with the elevation of plasma ET-1-like immunoreactivity. A previous study from this group, utilizing the mixed ETₐ/ETₐ-receptor antagonist bosentan in an identical model, showed a decrease in MPAP and PVRI to levels below basal values [19]. A moderate reduction in MPAP and PVRI in response to bosentan is also seen under non-endotoxic conditions [18]. This indicates that ET participates in the regulation of intrinsic pulmonary vascular tone and in endotoxin-induced pulmonary hypertension. The lack of effects on CI by PD 155080 in the present study contributes to the moderate reduction seen in PVRI compared with the mixed ETₐ/ETₐ-receptor antagonist bosentan, where a marked increase in CI was observed [19]. As the decrease in MPAP and PVRI in response to PD 155080 is almost instant and similar responses are seen under noninflammatory states using the mixed ET-receptor antagonist bosentan [18], this is probably due to vasodilation. However, ET-1 infusion given to rats promotes activation of platelets and neutrophils, with resulting microembolism in the pulmonary circulation [27].

**Table 1.** Effects of endotoxin infusion and PD 155080 in pigs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>0 h</th>
<th>2 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min⁻¹)</td>
<td>Controls</td>
<td>158±10</td>
<td>200±10</td>
<td>197±16</td>
</tr>
<tr>
<td>Arterial oxygen tension (kPa)</td>
<td>PD 155080</td>
<td>181±12</td>
<td>198±11</td>
<td>209±13</td>
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<td>Arterial carbon dioxide tension (kPa)</td>
<td>PD 155080</td>
<td>19.7±0.5</td>
<td>17.9±0.5</td>
<td>17.0±1.2</td>
</tr>
<tr>
<td>Arterial oxygen saturation (%)</td>
<td>PD 155080</td>
<td>99±0.01</td>
<td>98±0.4</td>
<td>98±0.8</td>
</tr>
<tr>
<td>Mixed venous oxygen saturation (%)</td>
<td>PD 155080</td>
<td>78±1.4</td>
<td>60±2.9</td>
<td>50±4.1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>PD 155080</td>
<td>7.38±0.02</td>
<td>7.16±0.05</td>
<td>7.15±0.06</td>
</tr>
<tr>
<td>Base excess (mmol/L⁻¹)</td>
<td>PD 155080</td>
<td>2.0±0.9</td>
<td>-12.6±2.5</td>
<td>-13.2±2.6</td>
</tr>
<tr>
<td>Haemoglobin concentration (g/L)</td>
<td>PD 155080</td>
<td>121±5</td>
<td>134±7</td>
<td>124±6</td>
</tr>
<tr>
<td>Systemic oxygen delivery index (mL/min⁻¹·kg⁻¹)</td>
<td>PD 155080</td>
<td>19.0±3.47</td>
<td>12.0±2.1</td>
<td>8.9±3.3</td>
</tr>
<tr>
<td>Systemic oxygen consumption index (mL/min⁻¹·kg⁻¹)</td>
<td>PD 155080</td>
<td>21.3±1.3</td>
<td>11.6±1.3</td>
<td>8.3±0.4</td>
</tr>
<tr>
<td>Lung compliance (%)</td>
<td>PD 155080</td>
<td>18±1.4</td>
<td>16±1.2</td>
<td>16±1.2</td>
</tr>
<tr>
<td>Bronchoalveolar lavage protein (g/L⁻¹)</td>
<td>PD 155080</td>
<td>17±1.4</td>
<td>14±1.2</td>
<td>14±0.9</td>
</tr>
<tr>
<td>Diuresis (mL/kg⁻¹·min⁻¹)</td>
<td>PD 155080</td>
<td>2.4±0.76</td>
<td>0.8±0.31</td>
<td>0.3±0.14</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. After baseline measurements at time zero (0 h) an endotoxin infusion was started and after 2 h of endotoxaemia (2 h) PD 155080 was administered (n=5–8). Data are compared to animals (n=5–8) not receiving PD 155080. Groups are compared at 0, 2 and at end 5 h. *: p<0.05 for differences between groups in time-matched between-group contrasts.
findings of decreased white cell count and total protein concentration in BAL fluid in response to mixed ETA/ETB-receptor antagonism after endotoxin challenge, suggesting that ET-receptor activity promotes migration of leukocytes and protein leakage. In the present study, neither BAL fluid protein content nor dynamic lung compliance was affected by PD 155080, indicating that ET A-mediated events are not of major importance in this context. Despite a decrease in MPAP and PVRI no significant changes in blood gas parameters after PD 155080 treatment were observed.

Endotoxin infusion resulted in a profound decrease in CI, SVI and MAP. The impact on these parameters by PD 155080 was restricted to a further decrease in MAP. Since CI was unchanged by PD 155080 treatment the observed reduction in MAP was due to a decreased SVRI. Cardiac dysfunction is a feature of human sepsis and several aetiopathological factors have been suggested. Cytokines such as TNF-α and interleukin-1β and NO have all been found to have cardiodepressant effects. The coronary constrictive effect of TNF-α can be prevented by ET antagonism, suggesting an ET-receptor-mediated mechanism [28]. Another indication of the involvement of ET-1 in sepsis-induced cardiac dysfunction is the inverse correlation between plasma ET-1-like immunoreactivity levels and cardiac index in septic patients [14]. Mixed ETA/ETB-receptor antagonism in porcine endotoxaemia [19] and human congestive heart failure [29] has been demonstrated to improve cardiac output as well as SVI and may be explained by afterload reduction [19]. However, in the present study the selective ETA-receptor antagonist PD 155080 caused significant reductions in both right and left ventricular afterload without positive effects on cardiac performance. These findings could suggest a direct effect of ET-1 on myocardial contractility or coronary perfusion, possibly involving the ETB-receptor. Another explanation for the absence of improved haemodynamics in response to selective ETA antagonism could be that the dose of PD 155080 was insufficient, although a study on basilary artery spasm has shown an equal degree of reduction in vessel spasm comparing similar doses of bosentan and PD 155080 [30]. To evaluate the effect of increased doses of PD 155080, two pigs in an identical model were given doses of PD 155080 double those used in the present study. Both animals developed severe hypotension; one animal died within 30 min after the bolus administration and the other developed a profound metabolic acidosis (unpublished observations). A possible mechanism for the hypotension in these two pigs could be that drug occupancy of the ETA-receptor may have allowed a further increase in the activation of the unopposed vasodilatory ETB-receptor. Altogether, data are conflicting and further studies are needed to evaluate the effect of endogenous ET-1 on cardiovascular function during septic-like conditions.

The absence of changes in systemic oxygen consumption despite a marked reduction in systemic oxygen delivery suggests that an oxygen supply-dependent state was not present. The concept of a pathological supply dependency of systemic oxygen uptake during septic shock
ceptor-mediated events are of importance in porcine endo-
toxin shock, started at 0 h. At 2 h, treated pigs (n=8, 
\( \square \)) received an i.v. bolus of PD 155080 (10 mg kg\(^{-1}\) i.v.) followed by a continuous infusion (5 mg kg\(^{-1}\) h\(^{-1}\)). Control pigs (n=8, \( \bigcirc \)) received only endotoxin infusion.

has been questioned as the changes seen may be the result of therapeutic interventions such as volume loading [31] and the use of inotropic agents.

In keeping with a prior study, where bosentan had no impact on renal blood flow [32], no effect on diuresis following intervention with PD 155080 could be demonstrated. The absence of effect of these ET-receptor antagonists could indicate that other mechanisms or mediators are more important for renal homeostasis during endotoxemia.

Arterial ET-1-like immunoreactivity plasma levels increased progressively during endotoxemia, in accordance with previous studies in humans and pigs [14, 19]. Both endotoxin [33] and TNF-\( \alpha \) [34], a cytokine released in large amounts during endotoxin challenge, have been shown to stimulate the production of ET-1 from cultured endothelial cells. No differences were seen in arterial plasma ET-1-like immunoreactivity levels when comparing the two groups at the end of this study. This is in line with other studies where selective ETA-receptor antagonism has been shown not to affect plasma ET-1-like immunoreactivity levels [35]. In contrast, in studies using mixed ETA/ETB-receptor antagonism significantly higher plasma ET-1-like immunoreactivity levels were seen among the treated animals [19]. Pulmonary clearance of ET-1 in dogs has been shown to be exclusively mediated by the ETB-receptor [36]. Drug occupancy of the ETB-receptor would, therefore, prevent ET-1 plasma clearance, leading to increased plasma ET-1-like immunoreactivity levels.

In conclusion, the nonpeptide endothelin\(_A\)-receptor antagonist PD 155080 given during established porcine endotoxin shock antagonized pulmonary hypertension but had no effect on the deteriorated cardiac performance. In a previous study in an identical animal model it was shown that mixed endothelin\(_A\)/endothelin\(_B\)-receptor antagonism markedly improved both pulmonary hypertension and cardiac performance. This suggests that endothelin\(_A\)-receptor-mediated events are of importance in porcine endotoxin-induced pulmonary hypertension, while the reduced cardiac performance probably involves other mechanisms, possibly the endothelin\(_B\)-receptor. However, further studies on the activation profile of the endothelin-receptor subtypes in this condition are needed.

**Fig. 3.** Arterial endothelin-1-like immunoreactivity (ET-1-LI) during endotoxin infusion: started at 0 h. At 2 h, treated pigs (n=8, \( \square \)) received an i.v. bolus of PD 155080 (10 mg kg\(^{-1}\)) followed by a continuous infusion (5 mg kg\(^{-1}\) h\(^{-1}\)). Control pigs (n=8, \( \bigcirc \)) received only endotoxin infusion.

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**References**


