Chronic Endothelin-A Receptor Blockade

in Lambs with Increased Pulmonary Blood Flow and Pressure

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Running head: ETA block and increased pulmonary blood flow

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ABSTRACT

Endothelin-receptor blockade is an emerging therapy for pulmonary hypertension. However, hemodynamic and structural effects and potential changes in endogenous NO-cGMP and endothelin-1 signalling of chronic endothelin$_A$-receptor blockade in pulmonary hypertension secondary to congenital heart disease are unknown. Therefore the objectives of this study were to determine hemodynamic and structural effects and potential changes in endogenous NO-cGMP and endothelin-1 signalling of chronic endothelin$_A$-receptor blockade in a lamb model of increased pulmonary blood flow following in-utero placement of an aortopulmonary shunt. Immediately after spontaneous birth shunt-lambs were treated lifelong with either an endothelin$_A$-receptor antagonist (PD156707) or placebo. At four weeks age, PD156707-treated shunt-lambs (n=6) had lower pulmonary vascular resistance and right atrial pressure than placebo-treated shunt-lambs (n=8) (p<0.05). Smooth muscle thickness or arterial number per unit area was not different between the two groups. However, the number of alveolar profiles per unit area was increased in the PD156707-treated shunt-lambs (190.7±5.6 vs. 132.9±10.0, p<0.05). Plasma endothelin-1 and cGMP levels, and lung NOS activity, cGMP, eNOS, preproendothelin-1, endothelin-converting-enzyme-1, endothelin$_A$ and endothelin$_B$-receptors protein levels were similar in both groups. We conclude that chronic endothelin$_A$-receptor blockade attenuates the progression of pulmonary hypertension and augments alveolar growth in lambs with increased pulmonary blood flow.

Key Words: Endothelin; Pulmonary Heart Disease; Congenital Heart Defect
INTRODUCTION

Altered pulmonary vascular reactivity and pulmonary hypertension is a common accompaniment of congenital heart disease with increased pulmonary blood flow\(^1\). Normal pulmonary vascular tone and vascular smooth muscle cell proliferation is regulated by a complex interaction of vasoactive substances that are locally produced by the vascular endothelium, such as nitric oxide (NO) and endothelin-1 (ET-1)\(^2\)\(^-\)\(^4\). Endothelial injury secondary to increased pulmonary blood flow and/or pressure disrupts these regulatory mechanisms, and is a potential factor in the development of pulmonary hypertension secondary to congenital heart disease\(^5\)\(^,\)\(^6\).

ET-1 is a 21 amino acid polypeptide produced by vascular endothelial cells that has potent vasoactive properties, and is co-mitogenic for vascular smooth muscle cells\(^4\)\(^,\)\(^7\). Recent data suggest a role for ET-1 in the pathophysiology of pulmonary hypertension. For example, ET-1 concentrations are increased in children with increased pulmonary blood flow and pulmonary hypertension, and preproET-1 gene expression is increased in adults with advanced pulmonary vascular disease\(^6\)\(^,\)\(^8\)\(^,\)\(^9\). In addition, chronic endothelin receptor blockade has demonstrated clinical improvement in adults with advanced pulmonary vascular disease\(^10\). However, the early role of ET-1 in the pathophysiology of pulmonary hypertension secondary to congenital heart disease with increased pulmonary blood flow remains unclear\(^11\). In addition, the potential effects of chronic ET receptor blockade on endogenous endothelial signaling are unknown.

To better define the role of ET-1 in the pathogenesis of pulmonary hypertension, we established a model of pulmonary hypertension with increased pulmonary blood flow in the lamb, following in utero placement of an aorta-to-pulmonary vascular graft. At one month of age, these lambs (shunt-lambs) have a pulmonary to systemic blood flow ratio of approximately 2:1, a mean pulmonary arterial pressure that is 50% of mean systemic arterial pressure, and pulmonary vascular remodeling characteristic of children.
with pulmonary hypertension and increased pulmonary blood flow\textsuperscript{12}. Previously, we demonstrated that shunt lambs display alterations in the ET-1 cascade at 4 weeks of age. These include increased plasma ET-1 levels, decreased ET\textsubscript{B} receptor protein with loss of ET\textsubscript{B} receptor-mediated vasodilation, and increased ET\textsubscript{A} receptor protein with augmentation of ET\textsubscript{A} receptor-mediated vasoconstriction, suggesting a role for ET-1 in the pathophysiology of pulmonary hypertension secondary to increased pulmonary blood flow\textsuperscript{13,14}.

The objectives of this study were to determine the role of ET\textsubscript{A} receptor activity in the early progression of pulmonary hypertension secondary to congenital heart disease with increased pulmonary blood flow, and determine the potential effects of ET receptor blockade on endothelial signaling. Therefore, we studied the effects of chronically administered PD156707 (an ET\textsubscript{A} receptor antagonist), or placebo, in our lamb model of congenital heart disease. Four weeks after spontaneous delivery of the lambs, hemodynamic variables, ET-1-induced vasoactive responses, and pulmonary artery morphology were determined and compared. In addition, potential alterations in the endogenous NO-cGMP and ET-1 pathways induced by chronic ET\textsubscript{A} receptor blockade were determined by comparing plasma ET-1, plasma and lung tissue NOx and cGMP levels, cGMP levels, tissue nitric oxide synthase [NOS] activity, and tissue endothelial NOS, preproET-1, ECE-1, and ET\textsubscript{A} and ET\textsubscript{B} receptor protein levels in lambs with and without ET receptor blockade.
METHODS

Surgical Preparation and Care

Ewes

Twenty mixed-breed Western pregnant ewes (135-141 days gestation, term = 145 days) were operated on as previously described\textsuperscript{12-14}.

Lambs

Before spontaneous delivery, the lambs were randomly divided into a treatment and a placebo group. The treatment group (n=12) received PD156707, an \textit{ET}_{A} receptor antagonist diluted in water (150 mg/kg/d). The placebo group (n=8) received daily water. All treatments were started within 12 hours of spontaneous delivery, and continued for 4 weeks. The lambs were weighed daily and the respiratory rate and heart rates were obtained weekly by an animal care nurse who was blinded to the therapy. Furosemide (1 mg/kg IM) was administered twice daily. At 4 weeks of age, vascular pressures and flow was measured as previously described\textsuperscript{13,14}.

Experimental Protocol

After baseline measurements of the hemodynamic variables were obtained, ET-1 (250 µg/kg) was injected into the pulmonary artery over 1 minute. The hemodynamic variables were measured continuously.
ETA block and increased pulmonary blood flow

Tissue preparation

Blocks of tissue of the left lung were removed and stored for later Western Blot analysis. Additionally, in five of the PD156707-treated shunt-lambs and five of the placebo-treated shunt-lambs, the pulmonary arterial bed of the right lung was distended with a barium gelatin suspension and the lungs were inflated by way of the trachea with formalin and placed in a bath of formalin for fixation.

Six random blocks for routine light microscopic examination were taken from each left lung. The sections were then examined for the characteristic structural changes of chronic pulmonary hypertension by the use of well-established quantitative techniques\textsuperscript{15,16}. Briefly, external diameter of at least 100 arterial profiles was measured as well as medial thickness of the muscular and partially muscular arteries. Medial thickness was then related to arterial size using the calculation: percent medial thickness = 2 X medial thickness/external diameter X 100. The structure of each artery was also noted -- muscular, partially muscular, nonmuscular -- as was the structure of the accompanying airway -- bronchus, bronchiolus, terminal bronchiolus, respiratory bronchiolus, alveolar duct, and alveolar wall. The density of the barium filled intraacinar arteries was also assessed. Using a X 25 objective and an eyepiece reticule, the number of barium filled arteries of less than 200 \( \mu m \) external diameter was counted and related to the number of alveolar profiles in these same fields. Lastly, the number of alveolar per unit area were counted. At least 25 consecutive microscopic fields were counted for each animal.

Plasma and Tissue Determinations

Blood samples were assayed for immunoreactive endothelin (irET-1) as previously described\textsuperscript{13}.

Blood samples and tissue were assayed with a cGMP \([125\text{I}]\) assay kit (Amersham International plc, Amersham UK) according to manufacturers instructions\textsuperscript{17}.
Utilizing a vanadium (III) and hydrochloric acid reduction, total NO, nitrite, and nitrate (NO$_x$) were detected by chemiluminescence (NOA 280, Sievers Instruments Inc. Boulder CO) in blood samples and lung tissue$^{18}$. NOS activity in the lung tissue was determined by using the conversion of $^3$H-arginine to $^3$H-citrulline as previously described$^{19}$.

**Western Blot Analysis**

Western blot analysis (ET$_A$ and ET$_B$ receptor, prepro-Endothelin-1, ECE-1, endothelial NOS) was performed as previously described$^{14}$.

**Statistical Analysis**

The mean ± standard deviation was calculated for the hemodynamic variables, systemic arterial blood gases and pH and ET-1 levels, structural changes, cGMP, NO$_x$, and NOS activity. Comparisons were made by the paired t-test using the Bonferroni correction, the unpaired t-test or ANOVA for repeated measures with multiple comparison testing.

Quantitation of autoradiographic results was performed by scanning the bands of interest into an image editing software program (Adobe Photoshop, Adobe Systems, Mt. View, CA). The means ± standard deviation was calculated for the relative protein. Protein and structural data were compared by the unpaired $t$-test. A $P < 0.05$ was considered statistically significant.
RESULTS

Spontaneous delivery occurred 2-19 days after fetal surgery. Six of the PD156707-treated shunt-lambs died between day 1 and 21 of life. Two lambs died from presumed sepsis, one from necrotic enterocolitis, and one sudden death from unknown causes. Two additional lambs were sacrificed because of respiratory distress following an aspiration event. All of the placebo-treated shunt-lambs survived the 4-week study period. In general, we observed that the lambs in the treatment group were clinically sicker, with increased work of breathing and decreased activity level. However, this could not be quantified and there were no differences in body weight, respiratory rate, or heart rate between the 2 groups (data not shown). A total of 14 lambs (n=6 treated and n=8 placebo) completed the 4-week study period, and underwent the hemodynamic study and biochemical and morphometric analysis.

Hemodynamic Study

All lambs had an audible continuous murmur and an increase in oxygen saturation between the right ventricle and distal pulmonary artery. The baseline systemic arterial pCO₂, pO₂, and hemoglobin, were similar between the two groups and within the normal limits of the laboratory. There were no differences in systemic arterial pH between the two groups (7.46±0.04 vs. 7.52±0.06), however both were greater than historical controls for the laboratory\textsuperscript{13,14}. Left pulmonary vascular resistance, mean right atrial pressure, and systemic pulse pressure were decreased in PD156707-treated shunt-lambs compared to placebo-treated shunt-lambs (p<0.05, Table 1). All other hemodynamic variables measured were not different between the two groups. However, mean pulmonary arterial pressure in PD156707-treated shunt-lambs was similar to values previously noted in age-matched control lambs\textsuperscript{13,14}. 
In placebo-treated shunt-lambs, the intrapulmonary injection of ET-1 increased left pulmonary vascular resistance (18.5±26.0) and mean systemic arterial pressure (9.4% ± 5.0) (p<0.05). However, in all PD156707-treated shunt-lambs both the pulmonary and systemic vasoconstriction to ET-1 was blocked.

Biochemical and Protein Determinations

Lung tissue NOx levels, plasma and lung tissue cGMP levels, plasma ET-1 (P=0.06), and tissue NOS activity were all similar between the two groups. Plasma NOx levels were decreased in PD156707-treated shunt-lambs when compared to placebo-treated shunt-lambs (P<0.05, Table 2). Protein levels of eNOS, preproET-1, ECE-1, ET_A and ET_B receptors were similar between the two groups (Table 3).

Structural Studies

Morphometric analysis revealed no differences in the percent medial thickness of pulmonary arteries between the two groups (Figure 1). However, the presence of muscular arteries appearance in the intraacinar region at the alveolar duct and alveolar wall levels was significantly increased in the PD156707-treated shunt-lambs compared to the placebo-treated shunt-lambs (Table 4). In addition, while there was no difference in the number of barium-filled small arteries per unit area between the two groups (3.8 ± 0.2, n = 5 vs. 4.0 ± 0.3, n = 5), the number of alveolar profiles per unit area was increased by 43.5% in PD156707-treated shunt-lambs (p<0.05) (Figure 2). This led to a significant reduction in number of small barium-filled arteries per 100 alveolar profiles in the PD156707-treated shunt-lambs (2.1 ± 0.3 vs. 2.9 ± 0.3 arteries / 100 alveoli, p = 0.002).
DISCUSSION

Activation of the ET-1 cascade has been demonstrated in a variety of pulmonary hypertensive disorders including children and animals with congenital heart disease and increased pulmonary blood flow. The present study is the first to demonstrate that chronic endothelin receptor blockade lowers pulmonary vascular resistance in a model of increased pulmonary blood flow secondary to congenital heart disease, suggesting a role for ET-1 in the early pathophysiology of this disorder. Following in utero aortopulmonary graft placement in the lamb, we found that chronic postnatal ET\textsubscript{A} receptor blockade lowered pulmonary vascular resistance and right atrial pressure without affecting basal endogenous NO-cGMP or ET-1 signalling. We also demonstrate the novel finding that ET\textsubscript{A} receptor blockade amplified alveolar growth, suggesting a role for ET-1 in the regulation of postnatal alveolar development in this setting.

Although chronic ET receptor blockade has demonstrated symptomatic improvement in adults with advanced primary pulmonary hypertension, this would not be expected in the setting of increased pulmonary blood flow\textsuperscript{10}. Since it is well established that pulmonary vasodilator therapy in the setting of a left-to-right shunt worsens congestive heart failure and mortality, the objective of this study was to utilize PD156707 as a pharmacologic tool to further delineate the role of ET-1 in the pathophysiology of this disorder, rather than a potential therapy in this setting\textsuperscript{20}. As expected, PD156707-treated shunt-lambs had increased work of breathing, decreased activity, and a greater mortality (50% vs. 0%, \(P<0.05\)) than placebo-treated shunt-lambs. Postmortem investigation of the deaths suggested that two occurred from aspiration events, one from an intrauterine infection, one from a postnatal urachus infection, and one sudden death of unclear etiology. In an attempt to prevent the increased symptomatology of increased pulmonary blood flow, both groups of lambs were treated with furosemide twice a day, beginning on the
first day of life. It is noteworthy that both PD156707 and placebo-treated shunt-lambs had lower pulmonary arterial pressure, higher systemic arterial pH, and less vascular remodelling than historical shunt controls, which only received furosemide on an intermittent basis in response to increased symptomatology\textsuperscript{12}. The effects of the aggressive diuretic regimen, in addition to the high mortality of the treatment group were unavoidable, but significant limitations of the current study. We cannot exclude the possibility that the high mortality selected out lambs with differential hemodynamics and vascular morphology, and that a shorter treatment course with a less aggressive diuretic regimen may have demonstrated greater differences between the PD156707 and placebo-treated shunt-lambs. Additionally, we also cannot exclude the possibility that the 50\% mortality has introduced other unknown serious biases to the study. Further studies are needed to better delineate these potential factors.

In previous animal models of pulmonary hypertension, chronic ET receptor blockade attenuated the progression of vascular smooth muscle remodeling. For example in rats exposed to either hypoxia or monocrotaline, ET receptor blockade resulted in decreased medial thickness of the small pulmonary arteries compared to controls\textsuperscript{21,22}. Similarly, in postnatal piglets with increased pulmonary blood flow, ET receptor blockade prevented the increase in medial thickness of the small pulmonary arteries\textsuperscript{23}. However, ETA receptor blockade did not prevent pulmonary vascular remodelling in a rat model of myocardial infarction\textsuperscript{24}. In the present study, PD156707-treated shunt-lambs displayed no differences in percent medial thickness of the pulmonary arteries compared to controls. In fact, the abnormal presence of muscularized pulmonary arteries in the region of the alveolar duct and wall was greater in PD156707-treated shunt-lambs (Table 4). These surprising findings are not completely understood, but a few potential explanations are noteworthy. First, as previously mentioned, the degree of vascular remodeling was not generally significant in neither the PD156707-treated shunt-lambs nor the placebo-treated shunt-lambs, and much less than historical shunt controls, which is likely due to the aggressive diuretic regimen
ETA block and increased pulmonary blood flow utilized in this study. Second, the high mortality in the PD156707-treated shunt-lambs may have selected for a group of survivors with more vascular remodelling, which could limit pulmonary blood flow and clinical symptomatology. Lastly and most importantly, this study is unique in that the abnormal hemodynamic state was present from birth, and treatment was started immediately following birth. Since the early morphologic abnormalities in children with increased pulmonary blood flow are similar to the normal fetal vascular morphology, it is likely that the early vascular changes represent a failure of the fetal morphologic state to regress rather than remodelling. Therefore, the abnormal extension of muscle present in the PD156707-treated shunt-lambs may represent a failure of the normal regression of fetal morphology, and may suggest a role for ET-1 in this process.

Previously, we demonstrated that increased pulmonary blood flow induces significant alterations in the endogenous ET-1 and NO-cGMP cascades. In the present study, we demonstrate that chronic ET_A receptor blockade does not significantly alter these changes. For example, there were no differences in plasma ET-1 levels, plasma and tissue cGMP levels, NOS activity, and protein levels of eNOS, preproET-1, ECE-1, ET_A and ET_B receptors. Plasma NOx levels were increased in PD156707-treated shunt-lambs. However, plasma NOx levels may be altered by differences in extracellular volume and renal clearance. Since lung tissue NOx levels were unchanged, differences in plasma NOx are unlikely to represent differences in basal pulmonary NO production.

To achieve ET_A receptor blockade treated lambs received 150mg/kg/dy of PD156707, an orally active, selective ET_A receptor antagonist. The dose of PD156707 was chosen after several previous studies showed that this dose completely blocked the vasoconstricting effects of exogenous ET-1 and resulted in steady-state plasma concentrations that blocked ET_A receptors in vivo. To insure adequate ET_A receptor blockade with PD156707 in this study, the effect of exogenous ET-1 was studied in both groups of lambs. Similar to our previous studies ET-1 induced both pulmonary and systemic
vessel and increased pulmonary blood flow. However, in all PD156707-treated lambs, the vasoconstricting effect of ET-1 was blocked suggesting adequate ET_A receptor blockade. Interestingly, ET-1 did not elicit pulmonary vasodilation. These physiologic data in conjunction with the protein data demonstrating no change in ET_B receptor protein, suggest that ET_B receptors were not up-regulated during ET_A receptor blockade.

Lastly, this study demonstrates the novel finding that chronic ET_A receptor blockade increases alveolar, and presumably capillary, growth in lambs with increased pulmonary blood flow. As seen in Figure 2, the number of alveolar-capillary profiles per unit area was increased by 44% in PD156707-treated shunt-lambs (P<0.05). Although ET-1 is known to have mitogenic actions on cardiovascular tissues, and is co-mitogenic with a variety of growth factors and vasoactive substances, there is very limited data on the role of ET-1 in the developing lung. However, ET-1 is expressed in the airway epithelium of the perinatal mouse, suggesting a role for ET-1 in lung development. In the current study, we have uncovered a previously unknown role for ET-1 in postnatal lung development during the stimulus of increased pulmonary blood flow. These novel observations suggest that ET-1 may play an important role in regulating lung growth in both normal and pathologic situations. Although age-matched control lambs with lower pulmonary arterial pressures have similar alveolar growth as vehicle-treated shunt lambs, our results cannot rule out that the effect on alveolarization in the ET antagonist group was not secondary to some indirect, hemodynamic effect. Therefore, further investigations will be required to elucidate the role of ET-1 in regulating lung development. It is likely that such studies will yield important biologic and clinically relevant information.
GRANTS

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DISCLOSURES

There are no potential conflict of interests.
REFERENCES


FIGURE LEGENDS

Figure 1:
PD156707 treatment has little effect on percent medial thickness of pulmonary arteries up to > 10,000 um external diameter. Values are mean±SD, n=5 placebo-treated shunt-lambs and n=5 PD156707-treated shunt-lambs.

Figure 2:
TOP: Light micrographs showing an increase in alveolar profiles in PD156707-treated shunt-lambs (right) as compared to placebo-treated shunt-lambs (left). Verhoeff’s elastin stain/ Van Gieson.

BOTTOM: Bar graph showing that the number of alveolar profiles per unit area in increased in PD 156707-treated shunt lambs compared to placebo-treated shunt lambs. Values are Mean±SD, n=8 placebo-treated shunt-lambs and 6 PD156707-treated shunt-lambs. *p = 0.002 vs. placebo.
Figure 1:
Figure 2:

Top

Bottom

ETA block and increased pulmonary blood flow
TABLE 1: GENERAL HEMODYNAMIC VARIABLES

<table>
<thead>
<tr>
<th>Hemodynamic Variable</th>
<th>Placebo (n=8)</th>
<th>PD156707 (n=6)</th>
</tr>
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<tbody>
<tr>
<td>mPAP (mmHg)</td>
<td>25.0 ± 7.4</td>
<td>18.3 ± 8.5</td>
</tr>
<tr>
<td>mSAP (mmHg)</td>
<td>55.9 ± 8.5</td>
<td>54.5 ± 6.5</td>
</tr>
<tr>
<td>pulse pressure SAP (mmHg)</td>
<td>93.1 ± 21.8</td>
<td>55.0 ± 17.3 *</td>
</tr>
<tr>
<td>mPAP : mSAP</td>
<td>0.45 ± 0.1</td>
<td>0.33 ± 0.1</td>
</tr>
<tr>
<td>mRAP (mmHg)</td>
<td>5.4 ± 0.8</td>
<td>4.0 ± 1.2 *</td>
</tr>
<tr>
<td>mLAP (mmHg)</td>
<td>9.9 ± 2.9</td>
<td>7.3 ± 1.9</td>
</tr>
<tr>
<td>Q_{lpa} (ml/min/kg)</td>
<td>0.13 ± 0.1</td>
<td>0.14 ± 0.1</td>
</tr>
<tr>
<td>PVR_{left} (mmHg/ml/min/kg)</td>
<td>118.1 ± 32.0</td>
<td>76.5 ± 34.1 *</td>
</tr>
<tr>
<td>SVR (mmHg/ml/min/kg)</td>
<td>416.3 ± 229.6</td>
<td>450.3 ± 205.0</td>
</tr>
<tr>
<td>Qp : Qs</td>
<td>2.43 ± 0.8</td>
<td>2.85 ± 0.6</td>
</tr>
</tbody>
</table>

mRAP: mean right atrial pressure, mPAP: mean pulmonary arterial pressure, mLAP: mean left atrial pressure, mSAP: mean systemic arterial pressure, Q_{lpa}: blood flow through the left pulmonary artery, PVR_{left}: left pulmonary vascular resistance, SVR: systemic vascular resistance, Qp:Qs: ratio of pulmonary to systemic blood flow. Values are mean±S.D., * p < 0.05 vs. placebo.
**TABLE 2: BIOCHEMICAL DETERMINATIONS**

<table>
<thead>
<tr>
<th></th>
<th>Plasma NOx (µM)</th>
<th>Tissue NOx (µM)</th>
<th>Plasma cGMP (pmol/ml)</th>
<th>Tissue cGMP (pmol/mg)</th>
<th>Plasma ET-1 (pg/ml)</th>
<th>NOS Activity (pmols/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLACEBO</td>
<td>11.09±1.5</td>
<td>0.89±0.4</td>
<td>415.0±203</td>
<td>147.5±80.7</td>
<td>14.4±6.6</td>
<td>0.43±0.09</td>
</tr>
<tr>
<td>PD156707</td>
<td>6.73±1.8*</td>
<td>0.59±0.5</td>
<td>672.8±418</td>
<td>148.1±63.5</td>
<td>23.3±9.5</td>
<td>0.45±0.04</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=8 placebo-treated shunt-lambs and n=6 PD156707-treated shunt-lambs. * p < 0.05 vs. placebo.
TABLE 3: *PROTEIN DETERMINATIONS (DENSITOMETRIC VALUES)*

<table>
<thead>
<tr>
<th></th>
<th>eNOS</th>
<th>PreproET-1</th>
<th>ECE-1</th>
<th>ET(_A) Receptor</th>
<th>ET(_B) Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLACEBO</td>
<td>63.2±15.7</td>
<td>21.9±9.6</td>
<td>4130.6±1148.0</td>
<td>224585.7±478183</td>
<td>27401.6±14094</td>
</tr>
<tr>
<td>PD 156707</td>
<td>52.2±11.8</td>
<td>30.7±14.7</td>
<td>3988.3±1273.8</td>
<td>232175.7±375440</td>
<td>33196.6±19536</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=8 placebo-treated shunt-lambs and n=6 PD156707-treated shunt-lambs. * p < 0.05 vs. placebo.
TABLE 4: Percentage of Fully Muscular and Nonmuscular Pulmonary Arteries Related to Intraacinar Airway Level in PD156707 and Placebo-Treated Shunt-Lambs

<table>
<thead>
<tr>
<th></th>
<th>Respiratory Bronchiole</th>
<th>Alveolar Duct</th>
<th>Alveolar Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degree of Muscularization</td>
<td>% Distribution</td>
<td>Degree of Muscularization</td>
</tr>
<tr>
<td>PD156707</td>
<td>muscular</td>
<td>98 ± 5.6</td>
<td>muscular</td>
</tr>
<tr>
<td></td>
<td>non-muscular</td>
<td>2 ± 5.6</td>
<td>non-muscular</td>
</tr>
<tr>
<td>Placebo</td>
<td>muscular</td>
<td>85 ± 14.6</td>
<td>muscular</td>
</tr>
<tr>
<td></td>
<td>non-muscular</td>
<td>15 ± 14.6</td>
<td>non-muscular</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 5 PD156707-treated shunt-lambs and n = 5 placebo-treated shunt-lambs. *p < 0.05 vs. placebo.