Endothelin receptor blockade does not improve hypoxemia following acute pulmonary thromboembolism

John Y. C. Tsang,1 Wayne J. E. Lamm,3 Blazej Neradilek,4 Nayak L. Polissar, and Michael P. Hlastala2,3
1James Hogg iCAPTURE Research Laboratory, University of British Columbia, Vancouver, British Columbia, Canada; Departments of 2Physiology and Biophysics and 3Medicine, University of Washington, and 4The Mountain-Whisper-Light Statistical Consulting, Seattle, Washington

Submitted 12 September 2005; accepted in final form 10 October 2006

Tsang JY, Lamm WJ, Neradilek B, Polissar NL, Hlastala MP. Endothelin receptor blockade does not improve hypoxemia following acute pulmonary thromboembolism. J Appl Physiol 102: 762–771, 2007. First published November 2, 2006; doi:10.1152/japplphysiol.01139.2005.—We studied the roles of endothelins in determining ventilation (V̇A) and perfusion (Q̇) mismatch in a porcine model of acute pulmonary thromboembolism (APTE), using a nonspecific endothelin antagonist, tezosentan. Nine anesthetized piglets (~23 kg) received autologous clots (~20 g) via a central venous catheter at time 0 min. The distribution of V̇A and Q̇ at five different time points (~30, ~5, 30, 60, 120 min) was mapped by fluorescent microspheres of 10 different colors. Five piglets (group 1) received tezosentan (courtesy of Actelion) starting at time = 40 min for 2 h, and four piglets (group 2) received only saline and served as control. Our results showed that, in all of the animals at 30 min following APTE but before tezosentan, the mean V̇A/Q̇ was increased, as was V̇A/Q̇ heterogeneity (log SD V̇A/Q̇), which represented a widening of its main peak. Afterwards, tezosentan attenuated the pulmonary hypertension in group 1 but also produced moderate systemic hypotension. However, it did not improve arterial PO2 or V̇A/Q̇ mismatch. We concluded that endothelin antagonism had minimal impact on gas exchange following APTE and confirmed our earlier observation that the main mechanism for hypoxemia in APTE was due to the mechanical redistribution of pulmonary regional blood flow away from the embolized vessels, resulting in the creation of many divergent low and high V̇A/Q̇ regions.

ENDOTHELINS (ET) are 21-amino acid peptides, which belong to a family of very potent vasoconstrictors (19). At least three isoforms of ET have been identified, i.e., ET-1, ET-2, and ET-3, with ET-1 being the most common (6). They are found abundantly in the lung and are synthesized in both the airway endothelium and some smooth muscle cells of the muscular pulmonary arteries, as opposed to the capillaries and other vessels. These observations suggested that ET receptor blockade could play an additional role in affecting gas exchange.

Sophia et al. (26) reported that there was an increased release of ET from the lung of patients following acute pulmonary thromboembolism (APTE). Dschietzig et al. (9) showed that big ET, the immediate precursor of ET-1, was released during pulmonary air embolism, and its conversion to ET in the cardiac tissue resulted in coronary vasoconstriction and subsequent myocardial depression. Our laboratory also found similar increases in ET-1 plasma levels in two porcine models of APTE (29) and air embolism (25), respectively. Furthermore, several other studies reported that the pulmonary hypertension following acute pulmonary embolism could be attenuated by the use of ET antagonists (24, 28). However, the physiological impact of ET on gas exchange after APTE has not been systematically studied.

Our laboratory previously demonstrated that changes in V̇A/Q̇ mismatch after APTE could be explained by a redistribution of regional blood flow, as a consequence of the mechanical obstruction by the thromboemboli in the pulmonary vasculature (30). The lower V̇A/Q̇ regions, created by higher regional Q̇, were found in the less obstructed regions and contributed significantly to the acute hypoxemia after APTE. On the other hand, higher V̇A/Q̇ regions, created by reduced Q̇, were found distal to the clots and in the periphery of the lung. They behaved physiologically like dead space. But it was not clear whether ET, a family of mediators found to be released in APTE, might play an additional role in affecting gas exchange.

Lee et al. (17) recently reported that, following APTE, arterial and venous plasma levels of ET-1 were doubled. There was also an increase in immunostaining of ET-1 in the endothelium and some smooth muscle cells of the muscular pulmonary arteries, as opposed to the capillaries and other vessels. Observations suggested that there might be heterogeneous release of ET in the lung after an acute embolic injury.

With that premise, we proposed that hypoxemia due to V̇A/Q̇ mismatch in APTE might be worsened by ET release. In addition to the V̇A/Q̇ mismatch resulting from mechanical diversion of blood flow by the obstructing thrombi in the pulmonary vasculature, ET-mediated vasoconstriction would result in further V̇A/Q̇ mismatch and worsen the hypoxemia. We reasoned that the negative effect of ET on gas exchange during APTE would be represented experimentally by the amount of hypoxemia and V̇A/Q̇ mismatch that could be mitigated by a nonspecific ET antagonist.

Most of the previous studies on ET in pulmonary embolism have been focused on pulmonary hemodynamics. Melot et al.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
(21) showed that pulmonary arterial pressure (Ppa) could be predicted by the degree of angiographic obstruction in an experimental setting of acute pulmonary embolism, whereas others have reported that vasodilators have little effect on pulmonary vascular resistance in an embolized lung (8). Mechanical obstruction, rather than vasoactive mediators, explained most of the increase in pulmonary vascular resistance. Similarly, we were interested to study whether the mechanical effect of embolic obstruction also had a dominant effect in causing hypoxemia following APTE, compared with that of a humoral factor, such as ET-1.

In our established porcine model, we serially marked the regional VA and Q before and after APTE at five different time points during the experiment, using the aerosolized and injected fluorescent microspheres (FMS) technique. We planned to show how the regional VA and Q could be affected after APTE, with or without the treatment of a nonspecific ET antagonist. The results also tested our hypothesis by examining the arterial oxygenation and various gas exchange parameters in these two experimental groups.

METHODS

Surgical preparations and physiological measurements. The experimental protocol was approved by the University of Washington Animal Care Committee. Nine piglets (23 ± 3 kg) were premedicated with ketamine 20 mg/kg im and xylazine 2 mg/kg im. They were maintained under general anesthetic for the entire experiment using intravenous pentothal, initially set at 100 mg/h, and the dose was only occasionally titrated afterwards. These animals were ventilated with room air in the supine posture, while the tracheostomy and vascular line insertions were completed.

One femoral arterial line and two femoral venous lines were inserted for the purposes of monitoring systemic blood pressure (BP), fluid infusion, and FMS injection, respectively. A Swan Ganz catheter (Edwards Laboratory) was inserted in the right external jugular vein for the measurement of Ppa, wedge pressure, and cardiac output (Qr), while a large-bore catheter (0.5-cm internal diameter) was inserted in the left external jugular vein for the rapid infusion of preformed blood clots (see below). Generally, these animals received normal saline at 100 ml/h during the experiments. They were kept warm using a warming blanket so that the body temperature was maintained at ~38°C. No heparin was used.

After the insertion of the femoral arterial line, 80 ml of blood were withdrawn and mixed with 2,500 units of thrombin-JMI at room temperature, so that clots were allowed to form and fibrinized over the next 2 h.

Upon completion of the surgical procedures, the animals were placed in the prone posture and received stacking of at least three consecutive breaths to remove residual atelectasis. Their control ventilatory settings were adjusted according to the arterial blood-gas results, such that arterial PCO2 was maintained at 35 ± 2 Torr, positive end-expiratory pressure was 0 cmH2O, tidal volume was 12–15 ml/kg, respiratory rate was 18–20/min, and inspired oxygen was at 21% or room air. Once they were set, there was no further adjustment of these settings for the remaining part of the experiment. At each of the subsequent data collection time points, hemodynamic parameters, such as BP, Ppa, wedge pressure, heart rate, and Qr, were measured, along with hemoglobin, arterial and venous blood gases, Fowler dead space (MacLab at 100 mm/s), tidal volume, airway pressure, and respiratory rate.

The time for the induction of APTE was defined as time = 0 min, and all of the events before and following that point would be recorded in relation to that time. To establish reproducibility of the distribution of aerosolized and injected microspheres, which marked the regional VA and Q in the lung, respectively, there were two control runs at 30 and 5 min before the induction of APTE (or time = −30 and −5 min). In each experiment, 10 different FMS of distinct colors (Molecular Probes, Eugene, OR) were chosen and used in random orders: five for aerosolization and five for injection. The details of the FMS techniques have been well described (12).

After the two control runs were performed and all the physiological measurements were recorded, APTE was induced at time = 0 min. Approximately 12–16 pieces of preformed fibrinized clots (1.5 × 0.5 × 0.5 cm3 per piece) were suspended in normal saline in a large catheter tip syringe and injected into the left external jugular vein over the next 10–15 min, until Ppa was 2.5 times the baseline value. Upon completion, there were no further injections of clots. On some occasions, ~200 ml of normal saline were given to restore BP. At time = 30 min after the induction of APTE, physiological measurements and blood samplings were similarly done as in the control runs, followed by FMS aerosolization and injection using FMS of different colors. This would mark the regional VA and Q in the initial phase following APTE.

The animals were then randomly divided into two groups. In group 1 (n = 5), the animals received the treatment of a nonspecific ET antagonist (tezosentan, Actelion) (7), by an intravenous bolus of 10 mg/kg over 20 min, followed by an infusion of 2 mg/min for the duration of the experiment. Group 2 (n = 4) received only normal saline at 100 ml/h and served as control.

Later, at time = 60, 90, and 120 min, all the measurements were again done according to the protocol, except that, at time = 90 min, no FMS were administered. Stacking of breaths would be routinely done before data collection at each designated time point to minimize atelectasis.

Postmortem lung preparations. At the end of the experiment, the animals from groups 1 and 2 were similarly treated. They were deeply anesthetized with intravenous pentothal, heparinized with 5,000 units, and exsanguinated. The lungs were extracted after gentle saline flush, suspended vertically, and inflated to no more than 25 cmH2O. The lobes were kept in their resting anatomical position by a small amount of cyanoacrylate glue and blown dry with warm air through the lungs for 72 h. The injected thromboemboli were readily seen in the major pulmonary arteries and not macerated. Small puncture holes were made to allow good airflow through the lungs during the drying process.

After the thorough drying of the harvested lungs, they were sectioned into 2-cm3 sized cubes, with each sample carefully assigned a three-dimensional (3D) coordinate, according to a preestablished grid pattern. Approximately 1,000 pieces were analyzed per animal (see RESULTS). For each lung sample, its spatial location, weight, amount of airway tissue, and the presence or absence of blood clots in arteries >1 mm were recorded.

We determined the effect of tezosentan (administered 40 min after APTE) on subsequent measurements of gas exchange and hemodynamics. The fluorescent intensities of all 10 FMS embedded in each sample, which marked the regional VA and Q at five different time points (i.e., time = −30, −5, 30, 60, and 120 min), were measured as previously described (12, 23). Briefly, the fluorescent signal, which reflected the number of a given microsphere trapped in a lung piece, was determined by measuring its intensity in a spectrophotometer (Perkin-Elmer LS-50B), following 4 days of soaking in 2 ml of organic solvent (Cellosolve, Sigma-Aldrich, MO). Overlaps from adjacent colors were then corrected using a matrix inversion method (12).

Statistical methods. The spectrophotometric data were used to calculate regional VA, Q, and V/A/Q of each lung piece. A value of 50 was assigned to V/A/Q for lung pieces with V/A/Q >50 (usually due to very low flow) to include them in our analysis. By solving the mass balance equation, the corresponding regional P2O (Pr2O) of each lung sample could also be estimated (1). The weight-normalized relative VA (WNRVA) and weight-normalized relative Q (WNRQ) of each
lungs of each lung sample were calculated in order that these lung samples could be compared with each other at the same time point and at different time points, when total V\(A\) and Qt (or total pulmonary blood flow) became somewhat different. The steps used were summarized in the following paragraphs (30).

After the regional V\(A\) and Q of each lung sample were obtained from the FMS data for a given animal and at a given time, the V\(A\) and Q values were first adjusted by the weight of the sample by dividing V\(A\) or Q for each piece by the weight of the piece. The adjusted values were then weight-normalized by dividing all of the weight-adjusted values by the grand mean of all weight-adjusted values, separately for V\(A\) and Q. The lung samples could then be expressed as WNRV\(A\) and WNRQ, relative to that mean for V\(A\) and Q, respectively, which were represented as either 1 or 100%. Since large airway and blood vessels added substantially to the weight, pieces designated as containing >20% large airways/vessels, which, on the average, accounted for 10% of the original total number of lung pieces, were omitted from the dataset before all normalizations and subsequent analyses.

Afterwards, we allocated the lung samples to three clusters, according to the change in WNRQ from the second of two control measurements (at time \(-5 \text{ min}, \text{ before APTE}\)) to the first measurement after APTE (at time \(30 \text{ min}\)). Namely, the three clusters were denoted as: cluster 1 included lung pieces that had an absolute decrease of 0.5 in WNRQ from time \(-5 \text{ min} \) to 30 min after APTE; cluster 2 included lung pieces that had an absolute increase of 0.5 in WNRQ from time \(-5 \text{ min} \) to 30 min after APTE; cluster 3 included lung pieces in which WNRQ was relatively unchanged and included all the remaining samples. It is important to note that the clusters were created before the initiation of tezosentan or saline treatment and thus are independent of subsequent treatment effects. The absolute increase or decrease of 0.5 in WNRQ was chosen because this would clearly differentiate the three cluster patterns, particularly when WNRQ of most samples was \(-1\) before embolization.

Since cluster 3 represented lung regions with higher blood flow and presumably lower V\(A\)/Q, it had potentially the most impact on global hypoxemia after APTE in both groups, whereas the contrary was true in cluster 1. Thus cluster 3 warranted more focused attention in our analysis (see RESULTS and DISCUSSION).

We also tested whether the mean temporal changes of the physiological parameters after 30 min depended on tezosentan administration, which was started at 40 min. Among them, arterial PO2 (Pao2) was chosen as the primary variable, since it was the key factor in our hypothesis. Other cardiovascular parameters were considered as secondary variables to minimize false positive results due to multiple testings.

The mean temporal changes in Pao2, Ppa, mean arterial pressure, Qt, mean V\(A\)/Q, log SD V\(A\)/Q, and percent dead space from 30 min to later time points (60, 90, and 120 min) were compared between the two treatment groups using repeated-measures ANOVA. The repeated measurements were the change from 30 min to each of the available later times at 60, 90, and 120 min. Differences in change between the tezosentan and control animals and the corresponding statistical significance were estimated from a linear mixed model (using REML).

For each outcome variable, the estimated treatment effect on the post-AFTE change was presented with a normal-based 95% confidence interval for the treatment effect. The treatment effect on post-AFTE changes in WNRQ, WNRV, mean V\(A\)/Q, and Pao2 from 30 min to later time points (60 and 120 min) was also calculated separately for each of the three clusters. The residuals from the fitted models and the individual animal effects were examined in Q-Q plots for consistency, with the assumption of normally distributed random effects.

Fisher’s paired least-significant difference post hoc tests were performed within repeated-measures ANOVA to test for pairwise differences in gas exchange parameters (see below: Table 1) at two different times within each treatment group.

The unpaired \(t\)-test was used to detect differences between the two experimental groups at specific time points. The Bonferroni adjustment of \(P\) values was used to adjust for the multiple tests over time.

The spatial locations of all the lung pieces in the same cluster, labeled by the same color, were presented in a 3D lung image, using the JMP software (SAS Institute, Cary, NC). The positions of the thromboemboli seen within arteries (\(\geq 1 \text{ mm diameter}\)) were also marked. Thus the anatomical location of these three clusters could be visualized, particularly in terms of their proximity to the clots.

To assess the reproducibility of the FMS measurements under similar experimental conditions, i.e., during the two control runs before APTE, the intracluster correlation coefficient was calculated for V\(A\) and Q of the same samples using the raw spectrophotometric data. All data were expressed as means \(\pm \text{SE}\), unless indicated otherwise. \(P < 0.05\) was used to designate statistical significance.

### RESULTS

**Physiological data.** Figure 1A showed that Pao2 abruptly decreased from \(-110\) Torr before APTE to \(-50\) Torr afterwards, but gradually recovered over time in both groups to \(-75\) Torr at similar rates, even after the infusion of tezosentan. Since this parameter was central to our hypothesis, it was designated as our primary variable. Figure 1B–D, showed the hemodynamic changes of Ppa, BP, and Qt, respectively, during all phases of the experiments. The data showed that, after APTE at time \(=0\) min, there were substantial increases in Ppa (Fig. 1B), while the Qt remained comparable (Fig. 1D). However, after the treatment of tezosentan at 40 min, moderate systemic hypotension was observed (Fig. 1C). These data

---

**Table 1. V\(A\)/Q and gas exchange parameters**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>(-30) min Pre-AFTE</th>
<th>(-5) min Pre-AFTE</th>
<th>30 min Post-AFTE</th>
<th>60 min Post-AFTE</th>
<th>120 min Post-AFTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean V(A)/Q (^{f})</td>
<td>Control</td>
<td>1.05 (\pm) 0.32</td>
<td>1.06 (\pm) 0.30</td>
<td>2.90 (\pm) 1.77*</td>
<td>2.20 (\pm) 1.19*</td>
<td>1.99 (\pm) 1.03</td>
</tr>
<tr>
<td></td>
<td>Tezosentan</td>
<td>0.93 (\pm) 0.22</td>
<td>1.08 (\pm) 0.23</td>
<td>2.47 (\pm) 0.53*</td>
<td>2.03 (\pm) 0.26*</td>
<td>2.27 (\pm) 0.65*</td>
</tr>
<tr>
<td>Log SD V(A)/Q (^{g})</td>
<td>Control</td>
<td>0.34 (\pm) 0.04</td>
<td>0.33 (\pm) 0.09</td>
<td>1.83 (\pm) 0.21*</td>
<td>1.41 (\pm) 0.58*</td>
<td>1.50 (\pm) 0.23*</td>
</tr>
<tr>
<td></td>
<td>Tezosentan</td>
<td>0.36 (\pm) 0.08</td>
<td>0.33 (\pm) 0.09</td>
<td>1.79 (\pm) 0.26*</td>
<td>1.80 (\pm) 0.34*</td>
<td>1.62 (\pm) 0.36*</td>
</tr>
<tr>
<td>%Shunt (^{h})</td>
<td>Control</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
</tr>
<tr>
<td></td>
<td>Tezosentan</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
</tr>
<tr>
<td>%Dead space (^{i})</td>
<td>Control</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>9.5 (\pm) 5.2*</td>
<td>6.8 (\pm) 5.8*</td>
<td>5.4 (\pm) 5.6</td>
</tr>
<tr>
<td></td>
<td>Tezosentan</td>
<td>0 (\pm) 0</td>
<td>0 (\pm) 0</td>
<td>8.8 (\pm) 6.8*</td>
<td>9.8 (\pm) 4.0*</td>
<td>8.5 (\pm) 3.4*</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SD. Acute pulmonary thromboembolism (APTE) at time \(= 0\). \(^{f}\) Ventilation-perfusion (V\(A\)/Q) ranges from 0.01 to 100. \(^{g}\) V\(A\)/Q < 0.01. \(^{h}\) PaO\(_{2}\). *P < 0.05 compared with pretreatment runs at \(-30\) and \(-5\) min, indicating statistically significant differences over time within group based on repeated-measure ANOVA and Fisher’s paired least significant difference post hoc test. There was no significant difference between groups at any time by unpaired \(t\)-test.

---

J Appl Physiol • VOL 102 • FEBRUARY 2007 • www.jap.org
indicated that a sufficient dose of tezosentan had been given, so that its impact on gas exchange, if any, could be readily observed. Ppa in both groups was not statistically different (P = 0.08). These latter parameters described the cardiovascular status of the animals and were considered as secondary variables.

Reproducibility of the FMS technique. To establish the reproducibility of the FMS labeling technique, two control runs were done under similar experimental conditions at time = −30 and −5 min. The intraclass correlations for V̇A and Q̇ measurement within sample were 91 ± 8% and 91 ± 3%, respectively. These results showed that there was excellent reproducibility of regional markings by the FMS technique. Thus the changes in V̇A and Q̇ after APTE at time = 0 min could be confidently attributed to the induced embolic injury in the lung.

V̇A/Q̇ analyses. Figure 2 showed the FMS data on V̇A/Q̇ distributions at different times during a typical control experiment. The results consistently showed that regional V̇A and Q̇ were closely matched before APTE, when the vast majority of regions had V̇A/Q̇ ratio near 1. However, at 30, 60, and 120 min, the main peak moved to the right and widened, i.e., there was more scattering of V̇A/Q̇ ratios, ranging from ~0.2 to 100. This heterogeneity, estimated by a computer program as the standard deviation of the main peak, was represented by log SD V̇A/Q̇. Table 1 showed the data on V̇A/Q̇ heterogeneity in both groups. After the induction of APTE, there were some recovery of mean V̇A/Q̇ and log SD V̇A/Q̇ toward the preembolized condition, as seen in our laboratory’s previous study (30). However, tezosentan did not improve or worsen these changes to any notable degree. While many high and low V̇A/Q̇ units were created after acute embolic injury, physiological shunt, defined as V̇A/Q̇ < 0.01, remained at 0% for all animals.

Cluster analysis. Using cluster analysis, three subpopulations of lung pieces were identified according to the three patterns of WNRQ̇ at 30 min after APTE (see METHODS), namely, cluster 1 in which there was an absolute decrease of 0.5 in WNRQ̇; cluster 2 in which WNRQ̇ remained relatively unchanged (cluster 2); and cluster 3 in which there was an absolute increase of 0.5 in WNRQ̇ over time.

Table 2 showed the number of pieces in each cluster in all of the experiments for both groups. Note that cluster 1, which...
represented ~50% of the regions in the embolized lung, received much less blood flow due to the thrombotic obstruction in the pulmonary vasculature. On the other hand, cluster 3, which represented roughly 25% of lung regions, received the largest portion of the diverted flow while the Qt or total pulmonary flow remained comparable (Fig. 1C). The percentage of total blood flow to cluster 1 dropped from ~50 to ~10% after APTE, while flow to cluster 3 increased from ~25% before APTE to ~75% at 30 min after APTE (Fig. 3).

Figure 4 showed the changes of WNRQ from these three clusters over time. Tezosentan treatment began at time = 40 min. There were no significant differences in WNRQ for each cluster between groups before APTE and at 30 min after APTE. In both groups, WNRQ in cluster 3 remained high

Table 2. Cluster distribution among all animals (as number of lung pieces per cluster and as percentage of total lung pieces)

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal No.</th>
<th>Total</th>
<th>Cluster 1</th>
<th></th>
<th>Cluster 2</th>
<th></th>
<th>Cluster 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>686</td>
<td>314</td>
<td>46</td>
<td>191</td>
<td>28</td>
<td>181</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>895</td>
<td>448</td>
<td>50</td>
<td>236</td>
<td>26</td>
<td>211</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,043</td>
<td>515</td>
<td>49</td>
<td>304</td>
<td>29</td>
<td>224</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>812</td>
<td>398</td>
<td>49</td>
<td>234</td>
<td>29</td>
<td>180</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>859</td>
<td>419</td>
<td>49</td>
<td>241</td>
<td>28</td>
<td>199</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>150</td>
<td>85</td>
<td>2</td>
<td>47</td>
<td>1</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Tezosentan</td>
<td>1</td>
<td>954</td>
<td>574</td>
<td>60</td>
<td>103</td>
<td>11</td>
<td>277</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>782</td>
<td>417</td>
<td>53</td>
<td>172</td>
<td>22</td>
<td>193</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>971</td>
<td>581</td>
<td>60</td>
<td>201</td>
<td>21</td>
<td>189</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1,166</td>
<td>562</td>
<td>48</td>
<td>295</td>
<td>25</td>
<td>309</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1,205</td>
<td>453</td>
<td>38</td>
<td>410</td>
<td>34</td>
<td>342</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1,016</td>
<td>517</td>
<td>52</td>
<td>236</td>
<td>23</td>
<td>262</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>172</td>
<td>77</td>
<td>9</td>
<td>119</td>
<td>8</td>
<td>69</td>
<td>4</td>
</tr>
</tbody>
</table>
Having identified these three clusters according to their initial change in WNRQ after APTE, the corresponding WNRV\(\lambda\) in these same clusters were calculated and presented in Fig. 5. The results showed that WNRV\(\lambda\) in each cluster increased or decreased in the same direction as WNRQ in both groups, but these changes were more modest. There was no statistical significant difference in WNRV\(\lambda\) in each cluster between groups before APTE. Furthermore, tezosentan did not appear to have any discernible effect on regional \(V_{A}/Q\), as the WNRV\(\lambda\) in each of the three clusters in both groups showed little change between 30, 60, and 120 min. In these analyses, the treatment effect was differentiated from the effect of time. They included formal tests for treatment effect, either at a single time (\(t\)-test) or at multiple times (with a time/treatment interaction). In any case, all treatment effects control for time.

Figure 6 shows that there were no significant differences in \(V_{A}/Q\) between groups before tezosentan treatment. After its infusion at 40 min, tezosentan had little effect on all three clusters. In cluster 3 (green), which had the lowest \(V_{A}/Q\) due to its highest WNRQ and only a modest increase in WNRV\(\lambda\), its impact on hypoxemia should be the most significant. Furthermore, it also received the most blood flow diverted there from clot obstruction in the other parts of the pulmonary vasculature and contributed most to the overall venous admixture in terms of blood flow. However, the impact of tezosentan on this cluster was minimal. Similarly, the very high \(V_{A}/Q\) values after 30 min in cluster 1 in both groups were not significantly different. The very high standard error seen in cluster 1 was the result of some samples with very high \(V_{A}/Q\) values in some animals. Cluster 1 in either group acted physiologically like dead space and was unlikely to have a direct effect on the overall hypoxemia (see DISCUSSION). Cluster 2 in both groups also behaved very similarly at 60 and 120 min.

Using the mass balance equation, the PrO\(_2\) could be calculated when the \(V_{A}/Q\) was known (1). As a result, the compo-

---

Fig. 3. Values are means ± SE for percentage of total pulmonary blood flow in each cluster vs. time. Cluster 1 had the highest initial flow due to the large number of samples included. There was no significant effect of tezosentan on the recovery of any cluster over time, as calculated from the comparison of the mean absolute difference between the control and tezosentan groups by the linear mixed model with random animal intercept (comparing 60 and 120 min together).

Fig. 4. Values are means ± SE for weight-normalized relative perfusion (WNRQ) in each cluster vs. time. There was no statistically significant effect of tezosentan on the recovery of any cluster over time, as calculated from the comparison of the mean absolute difference between the control and tezosentan groups by the linear mixed model with random animal intercept (comparing 60 and 120 min together).

Fig. 5. Values are means ± SE. There was no statistical difference in weight-normalized relative ventilation (WNRV\(\lambda\)) between groups for all three clusters at 30 min (unpaired \(t\)-test, \(P > 0.05\)). There was no significant effect of tezosentan on the recovery of any cluster over time, as calculated from the comparison of the mean absolute difference between the control and tezosentan groups by the linear mixed model with random animal intercept (comparing 60 and 120 min together).
site PrO2 of all three preidentified clusters could also be estimated and plotted over time (Fig. 7). The PrO2 in clusters 1 and 2 tended to be higher after tezosentan treatment (calculated from the corresponding higher V\textsubscript{A}/Q\textsubscript{O}2), but they both received less blood flow than cluster 3. Physiologically, the PaO2 of the animal was influenced mainly by the PrO2 of cluster 3, which received close to 55–75% of the total pulmonary blood flow after APTE. However, tezosentan did not appear to have much effect on the PrO2 of this cluster. Thus these data also explained the lack of difference of the arterial blood-gas data in Fig. 1A after treatment began.

**DISCUSSION**

The purpose of our present study was to investigate the role of ET in mediating hypoxemia following APTE. In our data, tezosentan, a nonspecific ET antagonist, demonstrated its potent hemodynamic effects, such as the attenuation of pulmonary hypertension and systemic hypotension (Fig. 1, B and C). However, there was no substantial difference between the two experimental groups in terms of V\textsubscript{A}/Q\textsubscript{O}2 distribution, reflected by mean V\textsubscript{A}/Q\textsubscript{O}2 or log SD V\textsubscript{A}/Q\textsubscript{O}2, during its treatment at 60 and 120 min (Table 1). Figure 1A showed that the measured PaO2 also did not differ during the same time interval. We concluded that tezosentan’s effect on gas exchange was minimal.

In cluster 3 (green), WNRQ was persistently high in both groups (Fig. 4), while Qt remained relatively unchanged (Fig. 7). Cluster 3 represented the least embolized part of the lung, remote from the sites of the clots (Fig. 8). This particular cluster is critical in determining the extent of hypoxemia in the embolized lung, not only because of its highest WNRQ and lowest corresponding V\textsubscript{A}/Q\textsubscript{O}2 (Fig. 6), but it also received a large proportion of pulmonary blood flow at ~75% (Fig. 3), which ultimately affected significantly the venous admixture. Note that the calculated PrO2 from this cluster was only ~60–70 Torr (Fig. 7), which was much lower than the values in the other two clusters. Furthermore, we observed that V\textsubscript{A}/Q\textsubscript{O}2 in this cluster, with or without the treatment of tezosentan, remained relatively constant over time between the treatment groups (Fig. 6). These data confirmed our laboratory’s previous work (30), which showed that the main mechanism for hypoxemia was the mechanical redistribution of pulmonary regional blood flow away from the embolized vessels, resulting in the creation of low V\textsubscript{A}/Q\textsubscript{O}2 regions and indicated that ET did not have much notable impact on gas exchange following APTE.

In cluster 3, we also noted that there was a slower recovery trend of WNRQ toward the preembolized level after the treatment with tezosentan (Fig. 4), even though the difference in trends between groups was not statistically significant. We speculated that this might be the result of some moderate vasodilatation in this less obstructed part of the vasculature, mediated by tezosentan, to maintain this continually higher regional flow. Theoretically, ET receptor blockade might even worsen hypoxemia by reducing the driving pressure in the pulmonary circulation, which normally would redistribute blood flow back to the more embolized vessels over time. As a result, the well-perfused regions continued to receive high WNRQ, which would only render the corresponding V\textsubscript{A}/Q\textsubscript{O}2 persistently low. In the control group, there might be slightly more redistribution of blood flow toward the preembolized state in all three clusters after 60 min as the WNRQ gradually recovered. However, in the final analysis, the difference of
mean $\dot{V}A/Q\dot{A}$ and log SD $\dot{V}A/Q\dot{A}$ reported in Table 1. However, in creation after APTE accounted for most of the increases in 6). This cluster behaved physiologically like dead space. Its standard deviation of the calculated $\dot{V}A/Q\dot{A}$. However, since this cluster, resulting in some samples receiving reduced flow and causing the increases in the corresponding mean and this cluster only increased modestly with higher increasing 4 and 5). However, the corresponding $\dot{V}A/Q\dot{A}$ in after the tezosentan treatment, at 60 and 120 min (Figs. APTE by the definition of this cluster, but did not change much either after the tezosentan treatment, at 60 and 120 min (Figs. 4 and 5). However, the corresponding $\dot{V}A/Q\dot{A}$ in cluster 2 became slightly greater in both groups after APTE (Fig. 6), presumably because of some internal redistribution of Q within this cluster, resulting in some samples receiving reduced flow and causing the increases in the corresponding mean and standard deviation of the calculated $\dot{V}A/Q\dot{A}$. However, since most of the changes in the $\dot{V}A/Q\dot{A}$ of this cluster ranged only from approximately 1 to 2, these changes did not have much impact on overall hypoxemia from this cluster. Cluster 2 in both groups was comparable, and tezosentan did not demonstrate a statistically significant effect.

Cluster 1 (red) was mainly located in the periphery of the lung, distal to the emboli (Fig. 8). Because of its very low WNRQ (Fig. 4) and only a modestly reduced corresponding WNRV\textsubscript{A} (Fig. 5), the resultant mean $\dot{V}A/Q\dot{A}$ was quite high (Fig. 6). This cluster behaved physiologically like dead space. Its creation after APTE accounted for most of the increases in mean $\dot{V}A/Q\dot{A}$ and log SD $\dot{V}A/Q\dot{A}$ reported in Table 1. However, in terms of its effect on gas exchange, the impact was less than that in the other two clusters. This was because, even though the $PrO_2$ (Fig. 7) was higher in this cluster due to the higher $\dot{V}A/Q\dot{A}$, it had little impact on improving the overall oxygenation in the blood, because they received only $\sim$10% of total $\dot{Q}$ after APTE (Fig. 3), and the oxygen content of the blood from this cluster remained modestly higher increasing $PrO_2$, according to the sigmoid shape of the hemoglobin-oxygen dissociation curve.

Figure 6 showed the persistently higher $\dot{V}A/Q\dot{A}$ in cluster 1 in both groups. Again, since many samples in this cluster had very low blood flow, the calculated $\dot{V}A/Q\dot{A}$ in the same regions became quite high. While these high $\dot{V}A/Q\dot{A}$ units resulted in a corresponding high mean and standard deviations of $\dot{V}A/Q\dot{A}$, their effect on gas exchange, as it was mentioned earlier, was comparatively small because of their limited contribution to venous admixture. The $\dot{V}A/Q\dot{A}$ of cluster 1 in the control group gradually decreased after APTE without treatment, and the same was also seen after tezosentan treatment. The difference in trend between the two groups was small and not statistically significant.

Our data showed that tezosentan did not notably improve the WNRQ of cluster 1, possibly due to the limited delivery of this drug beyond the obstructing clots. Locally released ET in these regions, if any, would likely have their vasoconstrictive effect unopposed. This might explain the persistently low flow there after tezosentan treatment, while the other less obstructed part of the vasculature became more dilated during its infusion.

While cluster 1 (red) might not affect the resultant $PrO_2$, for reasons mentioned above, its high $\dot{V}A/Q\dot{A}$ (Fig. 6) could affect the regional $PCO_2$. Levy and Simmons (18) reported that, following acute pulmonary embolism, reduced alveolar $PCO_2$ ($P_{ACO_2}$) in high $\dot{V}A/Q\dot{A}$ regions might cause local airway constriction and reduce regional $\dot{V}A$. Our data supported their observation, i.e., the higher the $\dot{V}A/Q\dot{A}$ (or the lower the regional $PCO_2$), the more decreased was the corresponding WNRV\textsubscript{A} (Figs. 5 and 6). In contrast, in cluster 3 (green), which had low $\dot{V}A/Q\dot{A}$ from high $\dot{Q}$, its corresponding $P_{ACO_2}$ would be expected to be higher. These local $P_{ACO_2}$ changes appeared to result in ventilatory shifting from cluster 1 toward cluster 3 (Figs. 5 and 6), resulting in higher WNRV\textsubscript{A} in cluster 3 and less in cluster 1. This was exactly what was proposed by Levy and Simmons (18). Consequently, these secondary redistributions of regional $\dot{V}A$ might serve to mitigate the initial changes in $\dot{V}A/Q\dot{A}$ due to the changes in WNRQ alone. However, tezosentan did not appear to have any notable impact on WNRV\textsubscript{A} after the initial shifting of $\dot{V}A$ at 30 min.

Figure 8 shows that, following APTE, there was not a visually notable difference in the 3D distribution of the three
clusters to animals assigned with or without the tezosentan treatment, in terms of their overall relationship to the hilar and pleural areas. These distributions were comparable to those previously reported by our laboratory (30). We did not demonstrate any significant effect on gas exchange with ET antagonism following APTE, as the minor redistributions of blood flow or \( V_{A} \) between clusters after treatment were not sufficient to alleviate hypoxemia. Furthermore, from the standpoint of gas exchange, we suggested caution in this approach because of the potentially negative effect of maintaining high regional blood flow to the less obstructed regions, as the \( V_{A}/Q \) there would remain low for a longer period of time.

There were also previous studies that reported that inhalational NO might be beneficial in treating hypoxemia in massive pulmonary embolism (4, 5, 11, 13). Presumably NO interacted closely with ETs to maintain vascular tone under different conditions and generally had an opposing effect against them in vivo (10). In some way, NO was not unlike that of an ET antagonist, except that it was delivered therapeutically via the airway. However, these few reports were anecdotal in nature, and some have reported deterioration with the same treatment (31).

Our chosen dosage of tezosentan was relatively high, to the extent that some systemic hypotension was observed. At a more moderate dose, this adverse effect on the systemic BP could be understandable less. \( Q_{r} \) did not change significantly. However, this higher dosage was chosen to ensure that sufficient antagonism of ET was achieved, and its effect on gas exchange would not be missed.

In summary, we conclude that the primary mechanism for hypoxemia in APTE is the mechanical diversion of regional blood flow to the least embolized regions, thus creating low \( V_{A}/Q \) units. Using a nonspecific ET antagonist tezosentan, our data do not support the hypothesis that ET receptor blockade affects gas exchange after APTE, even though there were minor redistributions of blood flow within the embolized lung after its treatment. Regional \( V_{A} \) also remained relatively unchanged after the tezosentan.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the receipt of tezosentan from Actelion Pharmaceutical, Switzerland, for the completion of this research project. Furthermore, they express sincere appreciation for technical assistance from Ian R. Starr and Sucheol Gil.

GRANTS

The authors thank the funding support from the British Columbia Lung Association and National Heart, Lung, and Blood Institute Grant HL-69868.

REFERENCES


