Role of Endothelium in the Maintenance of Low Pulmonary Vascular Tone in Normal Children

David S. Celermajer, PhD, FRACP; Clare Dollery, MB, BS, MRCP; Michael Burch, MD, MRCP; John E. Deanfield, MB, BChir, FRCP

Background Resting vascular tone is low in the normal pulmonary circulation, and experimental studies have suggested that this may be due to the continuous release of endothelium-derived nitric oxide (NO), a locally acting vasodilator. We have investigated whether NO contributes to the normal control of pulmonary vascular tone and resistance in children.

Methods and Results We studied the hemodynamic effects of N⁰-monomethyl-L-arginine (L-NMMA), a specific inhibitor of NO synthesis, on the pulmonary circulation of six children 2 to 17 years old (mean, 9 years) with congenital heart disease but normal pulmonary blood flow, pressure, and resistance (all had isolated left heart obstructive lesions). The diameter of a segmental pulmonary artery and pulmonary blood flow velocity were measured by quantitative angiography and intrapulmonary Doppler catheters. There was a consistent, dose-dependent fall in pulmonary blood flow velocity in response to three increasing doses of L-NMMA (compared with baseline, flow velocity fell to 75±7%, 62±8%, and 40±10%, P<.01). Flow velocity returned to control values with subsequent infusion of L-arginine, the substrate for NO. Thereafter, acetylcholine, an endothelium-dependent dilator, produced an increase in flow velocity (56±10% greater than baseline, P<.01). Arterial diameter was unchanged during L-NMMA and L-arginine infusions, indicating that the major effect of each agent is to alter vascular tone distal to the segmental pulmonary arteries.

Conclusions The dilator action of endothelium-derived NO contributes to the maintenance of low resting pulmonary tone in normal children. Impairment of NO production may contribute to the elevated pulmonary vascular resistance that complicates some cases of congenital heart disease. (Circulation. 1994;89:2041-2044.)

Key Words • circulation • endothelium-derived factors • lung

The vascular endothelium produces a labile substance, endothelium-derived relaxing factor (EDRF), that mediates the vasodilator effects of certain pharmacological agents (such as acetylcholine and substance P).1 There is increasing evidence from experimental studies that continuous release of EDRF is important in maintaining low pulmonary vascular tone.2-4 In lambs, infusing a specific antagonist of EDRF produces pulmonary hypertension.5 EDRF is now known to be nitric oxide (NO),6 which is synthesized from the amino acid L-arginine or a closely related compound. Arginine analogues, such as N⁰-monomethyl-L-arginine (L-NMMA), cause specific inhibition of NO formation in vascular endothelial cells7 and inhibit endothelium-dependent relaxation. Valance et al8 demonstrated that L-NMMA causes a fall in basal forearm blood flow and therefore suggested that NO is constantly secreted by systemic vascular endothelial cells. Since endothelial dysfunction may be an important early event in pulmonary vascular disease complicating certain congenital heart lesions, we used intrapulmonary infusions of L-NMMA and L-arginine to assess whether intact pulmonary endothelium maintains low vascular tone in the lungs of children with normal pulmonary hemodynamics.

Received September 11, 1993; revision accepted January 14, 1994.

From the Cardiothoracic Unit, Hospital for Sick Children, London, England.

Correspondence to Dr J.E. Deanfield, Cardiothoracic Unit, Hospital for Sick Children, Great Ormond St, London WC1N 3JH, England.

Methods

Patients

We studied six children (three boys and three girls) 2 to 17 years old (mean, 9±2 years) who were undergoing cardiac catheterization as part of the routine clinical evaluation of congenital heart disease. We were unable to study children without heart disease, who do not usually undergo catheterization, but selected subjects with normal pulmonary hemodynamics. All had isolated left heart obstructive lesions (aortic valve stenosis in three, subaortic stenosis in two, and recoarctation of the aorta in one). None had had previous bypass surgery, parenchymal lung disease, or any chromosomal or extracardiac abnormalities, and none were taking medications. Balloon dilatations were performed in four, and two were catheterized for diagnostic purposes only. Informed consent was obtained in all cases, and the study was approved by the local committee on ethical practice.

Study Protocol

After benzodiazepine premedication, all children had cardiac catheterization via the femoral approach under general anesthesia. During all studies, FiO₂ was 0.21, general anesthesia was maintained with an intravenous agent (propofol) rather than volatile gas inhalation, and care was taken to avoid anesthesia-related hypotension. Arterial blood gases were taken at the beginning, during, and at the end of each study to exclude carbon dioxide retention and to document respiratory stability throughout the infusion protocol. At least 30 minutes after any intervention, pulmonary hemodynamics were measured, and a long biopsy sheath was placed in the left lower lobe pulmonary artery. Through this sheath, a 20-MHz pulsed Doppler crystal sidemounted on a 3F catheter (Wessex Medical, Midhurst, UK) was positioned into a straight segment of a branch of the lower lobe artery, as previously described.9 Serial infusions were made via
Clinical and Hemodynamic Characteristics of the Patients Studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>PAP, mm Hg</th>
<th>PVRI, U · m⁻²</th>
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<td>M</td>
<td>AVS</td>
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<tr>
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<td>5</td>
<td>F</td>
<td>SubAS</td>
<td>20/11/15</td>
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<tr>
<td>3</td>
<td>17</td>
<td>F</td>
<td>ReCoA</td>
<td>27/12/18</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>M</td>
<td>AVS</td>
<td>16/8/10</td>
<td>0.7</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>F</td>
<td>SubAS†</td>
<td>26/9/14</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>M</td>
<td>AVS, mild AR†</td>
<td>30/12/18</td>
<td>1.2</td>
</tr>
</tbody>
</table>

PAP indicates systolic/diastolic mean pulmonary artery pressure; PVRI, pulmonary vascular resistance index; AVS, aortic valve stenosis; SubAS, subaortic stenosis; ReCoA, recoarctation of the aorta; and AR, aortic regurgitation. Values are mean±SEM.

*Before the study protocol.
†Did not undergo balloon dilatation.

Statistics

All data are expressed as mean±SEM. Since the six children were of different sizes, results for each condition were expressed as a percentage relative to the first control value for each subject. Each child served as his or her own control; therefore, the t test for paired values was used to assess the significance of changes observed during the protocol. Statistical significance was inferred at a value of P≤.01. This figure was obtained using Bonferroni’s method to correct for multiple t tests and corresponds to an actual significance level for each test of P≤.05. In addition, a one-way ANOVA was performed to assess the relation between flow velocity and the increasing doses of L-NMMA given.

Results

Hemodynamic Data

All subjects had normal pulmonary hemodynamics; mean pulmonary artery pressure was 15±1 mm Hg, and pulmonary vascular resistance index was 1.1±0.1 U · m⁻² (Table). All patients were stable throughout the protocol, including arterial PCO₂. The pulmonary and systemic artery pressures and heart rate were monitored continuously, and no parameter changed by ≥10% during any of the studies.

Experimental Protocol

In every patient, L-NMMA caused a fall in pulmonary flow velocity to 75±7% (range, 46% to 88%), 62±8% (range, 35% to 81%), and 40±10% (range, 10% to 72%) of the baseline value for the three increasing doses given (P<.01 by ANOVA) (Figure). The lower dose of L-arginine restored these values to baseline within 3 minutes (106±8% [range, 92% to 122%] compared with control), and there was no further effect from the higher dose. In the three subjects given a 5-minute control infusion between L-NMMA and L-arginine, the flow velocity values remained decreased during the control period (50±14%, P=NS compared with the final infusion of L-NMMA) but rose with subsequent L-arginine infusion. Acetylcholine caused an increase in flow velocity of 36±10% (range, 20% to 84%) compared with baseline (P<.01). Quantitative angiography showed no significant changes in artery diameter compared with control (L-NMMA, 98±1%; L-arginine, 99±2%; repeat control, 99±2%; P=NS).

Discussion

This study of children with normal pulmonary hemodynamics supports in vitro and animal data showing that resting pulmonary vascular tone is mediated in part by the release of NO. The first study in humans on the effects of inhibition of NO synthesis in the pulmonary circulation. NO is a potent vasodilator, and our observations suggest that its continuous release from pulmonary endothelium plays a major role in the maintenance of normal basal pulmonary vasorelaxation.

We investigated the role of endothelium in pulmonary vascular control in children by administering an inhibitor of EDRF synthesis (L-NMMA), the substrate for the synthetic pathway (L-arginine), and a pharmacological stimulator of EDRF production (acetylcholine). In this study, all were used as transient diagnostic tools rather than as anticipated therapeutic agents. Arterial diameter was unchanged during each of the infusions, indicating that the major effect of each agent was to alter vascular tone distal to the segmental pulmonary artery. The pattern of response was very similar in all the children studied. In each case, L-NMMA produced distal vasoconstriction in a dose-related manner, with the vasoactive...
effects occurring within 2 minutes. In experimental animal work, intrapulmonary administration of L-NMMA raises pulmonary artery pressure in the lamb.\textsuperscript{5} These data suggest that the normal pulmonary endothelium releases NO in the basal state, and the rapid inhibitory effect of L-NMMA is consistent with the known short half-life of NO.

After L-NMMA, L-arginine at the lower dose caused pulmonary flow velocity to return to control values within minutes, indicating that L-NMMA is a competitive antagonist of NO synthesis, which may be overcome by increasing substrate availability. Higher doses of L-arginine, however, did not lead to further pulmonary vasorelaxation. Similar results have been reported in normal lambs; L-arginine had no pulmonary hemodynamic effects, even at high doses.\textsuperscript{15} In the setting of pulmonary hypertension, however, in which endothelial function may be impaired, both L-arginine and NO may be important therapeutic agents, acting as pulmonary vasodilators.\textsuperscript{15-17}

In contrast to the response to L-arginine, acetylcholine did produce significant increases in pulmonary flow above baseline, suggesting that EDRF production can still be stimulated after L-arginine counteracts the effects of L-NMMA infusion. Acetylcholine has previously been shown to produce pulmonary vasorelaxation in normal subjects\textsuperscript{6,18} by pharmacological stimulation of endothelial cell muscarinic receptors.\textsuperscript{19} Although the precise mechanism whereby acetylcholine causes dilation of the resistance vessels is unknown, at least part of its effect is independent of NO,\textsuperscript{20,21}

The physiological role of NO in the pulmonary circulation has been difficult to investigate until recently because of its labile nature, the lack of specific antagonists that could be safely reversed, and the lack of a method to study pulmonary endothelial function in vivo. In this study, we observed no systemic effects from giving L-NMMA, L-arginine, or acetylcholine into a small segment of one lung. The doses chosen were similar to those used by Vallyance et al\textsuperscript{8} in human brachial artery studies, in which L-NMMA caused decreased forearm blood flow in normal subjects, adjusted for estimated flow rates in the segmental pulmonary arteries. In the forearm studies, L-NMMA reduced blood flow by \textsuperscript{22} This suggests that either the latter group of patients had a degree of endothelial dysfunction despite their angiographically normal coronary arteries, as has been found in similar older patients undergoing catheterization,\textsuperscript{23} or that basal release of NO is variable in different vascular beds.

Our data suggest that EDRF/NO has a significant role in the regulation of pulmonary vascular tone in the normal lung. Damage to the endothelium with consequent loss of this mechanism may be an important early event in children at risk of pulmonary vascular disease. Histological studies have shown structural endothelial damage in children with abnormal pulmonary hemodynamics secondary to congenital heart disease,\textsuperscript{24} and we have recently reported impairment of endothelium-dependent pulmonary artery relaxation in children with high pulmonary blood flow and normal resistance as well as those with established pulmonary vascular disease.\textsuperscript{8} A functional consequence of endothelial dysfunction may be failure to produce or release EDRF.\textsuperscript{1,21} Given that EDRF is important in maintaining low pulmonary tone, endothelial dysfunction secondary to elevated pulmonary flow and/or pressure may have a pathogenetic role in the process of pulmonary hypertension.

Acknowledgments

This work was supported by a grant from the British Heart Foundation. The authors would like to thank Mary Jane Potter for her assistance in the preparation of this manuscript.

References


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Circulation. 1994;89:2041-2044
doi: 10.1161/01.CIR.89.5.2041

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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