Original Article
Effect of nucleoprotein factor-kB (NF-κB) in endothelial cells during high blood flow-associated pulmonary vascular remodeling on vasoactive substances adrenomedullin and prostacyclin

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Abstract: The aim of this study was to investigate the role of nucleoprotein factor-kB (NF-κB) on the production and secretion of vasoactive substances adrenomedullin (ADM) and prostacyclin (PGI2) by endothelial cells in a high blood flow, pulmonary hypertension in vivo model. Fifty male Wistar rats were randomly divided into four groups: 15 rats received shunt surgery (Tn group); 15 rats received shunt surgery + NF-κB inhibitor [pyrrolidine dithiocarbamate (PDTC)] (Ti group); 10 rats received sham surgery (Co group); and 10 rats were negative controls (Cn group). A left to right shunt pulmonary hypertension model was established in groups Tn and Ti. Rats in the Ti group received an intraperitoneal injection of PDTC (120 mg/kg·d) one hour before the operation for 2 weeks, and rats in the Co group were processed in the same fashion as that of the experimental groups, except that they did not undergo surgery. After 12 weeks, pulmonary artery systolic pressure was measured by cardiac catheterization, pulmonary arterial endothelial cells were isolated, and NF-κB, ADM and PGI2 protein expressions were measured in the endothelium using immunohistochemistry. ADM and PGI2 expressions were significantly lower in the Tn group relative to those of the Cn group (P<0.01) but no difference in the Ti group (P>0.05). Expressions in the Co and Cn groups were not significantly different (P>0.05). Heightened NF-κB activity in pulmonary arterial endothelial cells during high blood flow can suppress the synthesis and secretion of ADM and PGI2, potentially leading to vascular remodeling and pulmonary hypertension.

Keywords: PDTC, NF-κB, vascular endothelial cell, pulmonary vascular remolding

Introduction

Increased blood flow volume associated with left to right shunt congenital heart disease can lead to increased pulmonary arterial shear stress and changes in pulmonary endothelial structure and function, resulting in significant damage to the vascular endothelium [1, 2]. When the endothelial cells are damaged, the barrier function of the endothelium and of the muscle-endothelium interface are destroyed, and the concomitant loss of endothelial cell regulation of smooth muscle cells leads to increased smooth muscle cell proliferation and pulmonary vascular reconstruction [3, 4]. The nucleoprotein factor-kB (NF-κB) activation pathway is present in the vascular endothelium, smooth muscle cells and cardiomyocytes [5], and pulmonary vasoconstriction and structural remodeling induced by high pulmonaryocytes [6]. This study employed a high blood flow, pulmonary hypertension animal model to measure the effects of NF-κB on the production and secretion of vasoactive substances adrenomedullin (ADM) and the prostaglandin, prostacyclin (PGI2) [7-9].

Materials and methods

Animals

Fifty male Wistar rats (purchased from the Experimental Animal Center of Shandong
University School of Medicine), aged 4 weeks and having an average weight of 120 g, were randomly divided into four groups, as follows: 15 rats were included in the shunt surgery group (Tn); 15 rats were included in the shunt surgery + NF-κB inhibitor [pyrrolidine dithiocarbamate (PDTC)] group (Ti); 10 rats were included in the sham surgery group (Co); and 10 rats were included in the negative control group (Cn). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shandong University.

Reagents

A nucleoprotein extraction kit was purchased from Active Motif (USA), a digoxin-labeled NF-κB kit was purchased from Roche (USA), and immunohistochemistry kits were purchased from Pfizer (USA).

Establishment of animal models

Using extracorporeal carotid-jugular shunting, rats in the Tn and Ti groups were established the left to right shunt, pulmonary arterial hypertension model. Rats in the Ti group were also received an intraperitoneal injection of PDTC (120 mg/kg·d) one hour before surgery, and last for 2 weeks after operation. Rats in the Co group were processed in the same manner as Tn group except that they did not undergo surgery. Rats in all groups were fed continuously for 12 weeks with SPF-grade food.

Measurement of pulmonary artery pressure

Ten-percent chloral hydrate (0.3 ml/100 g) was used to anesthetize the rats, then right-heart catheterization (size 3 F catheter) through the right external jugular vein to the right ventricle (guided by fluoroscopy) was used to record right ventricular systolic pressure, which is theoretically equal to pulmonary artery systolic pressure (PASP), through the pressure sensor channel.

Pulmonary tissue paraffin sections

Pulmonary tissue was prepared, washed with 0.85% saline to remove any blood and rapidly fixed in 10% neutral-buffered formalin at a fixative-to-tissue volume ratio of 20:1. At 48 hours, tissue sections were washed, dehydrated with stepwise 50%, 70%, 95% and 100% ethanol, hardened with chloroform and embedded in paraffin.

Immunohistochemical measurement of NF-κB, ADM and PGI2

Paraffin sections were deparaffinized, incubated for 5-10 minutes with 3% H2O2 at room temperature to deactivate endogenous peroxidase and then washed with distilled water and placed in PBS for 5 minutes.

Sections were incubated with 5-10% normal goat serum in PBS to block the antigen at room temperature for 10 minutes, then the serum was decanted without washing. Primary antibodies against NF-κB, ADM and PGI2 (Pfizer) were added at the appropriate dilution ratios to independent sections, and they were left to incubate at 37°C for 1-2 hours. Lastly, sections were washed three times with PBS for 5 minutes each.

Biotin-labeled secondary antibody diluted with 1% BSA-PBS at the appropriate dilution ratio was added to the sections, and they were incubated at 37°C for 10-30 minutes. Sections were then washed three times with PBS for 5 minutes each. Horseradish peroxidase-labeled streptavidin diluted with PBS was then added to the sections, and they were left to incubate at 37°C for 10-30 minutes.

Chromogenic reagent was added to visualize sections under a microscope, and Image-Pro Plus software (Media Cybernetics, Rockville, MD, USA) was used for image analysis.

Statistical analysis

Data are expressed as mean ± standard deviation (S). SPSS 17.0 statistical software was used to perform the student’s t-tests. All tests were two-sided with significance level α set to 0.05.

Results

Surgical results and pulmonary blood flow (Qp)/systemic blood flow (Qs) average

During shunt surgery, one rat from Tn and Ti groups died respectively. During the observa-
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Table 1. Pulmonary artery systolic pressure in the four groups (mean ± s)

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>PASP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn (13)</td>
<td>41.4±2.7</td>
</tr>
<tr>
<td>Ti (12)</td>
<td>22.5±5.9</td>
</tr>
<tr>
<td>Cn (10)</td>
<td>16.1±3.6</td>
</tr>
<tr>
<td>Co (9)</td>
<td>15.7±3.1</td>
</tr>
</tbody>
</table>

Table 2. ADM and PGI2 content in the four groups (mean ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>ADM</th>
<th>PGI2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn</td>
<td>26.5±11.6</td>
<td>16.1±10.3</td>
</tr>
<tr>
<td>Ti</td>
<td>58.5±16.3</td>
<td>70.3±17.2</td>
</tr>
<tr>
<td>Cn</td>
<td>67.1±15.7</td>
<td>83.5±15.9</td>
</tr>
<tr>
<td>Co</td>
<td>73.8±24.5</td>
<td>92.7±18.7</td>
</tr>
</tbody>
</table>

Discussion

Left to right shunt congenital heart disease in children is a clinical problem that has attracted increasing attention over the years, specifically in regards to the availability and effectiveness of therapeutic interventions for pulmonary hypertension. With the increased pulmonary blood flow associated with left to right shunting, the pulmonary vasculature undergoes an adaptive remodeling, forming the pathophysiological basis of pulmonary hypertension.

The vasoactive substances ADM and PGI2, which are present in many tissues and organs, including the adrenal gland, heart, lung, kidneys and blood vessels, are primarily synthesized and secreted by vascular endothelial cells and smooth muscle cells [8, 9]. A large number of molecules, however, are involved in the pathogenesis of pulmonary hypertension, and endothelial dysfunction plays a key role in this process [10]. In vitro studies have demonstrated that the increased shear stress associated with high pulmonary blood flow can induce the synthesis and secretion of vasoactive substances, such as endothelin (ET), angiotensin (AngII), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and the newly discovered vasoactive peptide U-II [11-15], from the endothelium. Additionally, shear stress can promote the proliferation of smooth muscle cells and coordinate decrease of the synthesis and secretion of cytokines that suppress the proliferation of smooth muscle cells, such as prostaglandins (PGI2), atrial natriuretic peptide (ANP), ADM, nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H2S) [16-21]. Pulmonary vascular endothelial cells can regulate the quality and quantity of vasoactive substances in the blood via transformation and uptake, thus affecting the pulmonary vascular smooth muscle tone. Under normal physiological conditions, endothelial cells produce mainly a variety of growth inhibitory factors to maintain normal vascular structure. However, under pathological conditions, certain stimuli can induce endothelial cells to synthesize and secrete growth factors, which promote pulmonary vascular structural remodeling.
NF-κB regulates the expressions of a variety of genes, particularly those involved in the body’s early defense response. Notably, the NF-κB activation pathway exists in the vascular endothelium, smooth muscle cells and cardiac cells, and NF-κB family members are known to regulate the expression of certain vascular genes [22-24]. In vivo and in vitro studies have shown that the transcriptional regulation of many genes plays an important role in the formation of the vascular intima, which acts to adjust the hemodynamics of the vasculature. This information was used as a theoretical basis for development of this study.

We established a high blood flow, pulmonary hypertension rat model to examine the activity of NF-κB and the expression of vasoactive substances ADM and PGI2 during left to right shunting. NF-κB activity in the Tn experimental group was found to be significantly higher than that in the Cn negative control group, implying that the shear stress associated with high blood flow acted on the vascular endothelium and activated the NF-κB signaling pathway. In the Ti experimental group, the potent NF-κB inhibitor PDTC was added one hour prior to surgery. As a result, NF-κB activity was significantly lower than that in the Cn group, and there was no significant increase in PASP, further indicating that NF-κB may contribute to the course of pulmonary artery hypertension. ADM and PGI2 expressions in the Tn group were significantly lower than those in the Cn group, while there was no statistical difference between their expressions in the Ti group and Cn or Co groups. According to above study, the increased endothelial cell activity of NF-κB during high blood flow inhibited the synthesis and secretion of vasoactive substances ADM and PGI2, potentially leading to the subsequent promotion of pulmonary vascular remodeling and associated pulmonary hypertension.

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Disclosure of conflict of interest

None.

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