Changes of Vasoactive Peptides and Effects of Inhaled Nitric Oxide after Pneumonectomy

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Summary: To clarify the role of vasoactive peptides in the physiologic response to pneumonectomy, we investigated the changes of atrial (A-type) natriuretic peptide (ANP), C-type natriuretic peptide (CNP), and endothelin-1 (ET-1) levels in the lung and blood after pneumonectomy and the effects of inhaled nitric oxide (NO; 5 ppm) after pneumonectomy in beagle dogs. The concentrations of these peptides in the lung and blood were measured by radioimmunoassay. The dogs in group A (n=10) were observed without NO inhaling after right pneumonectomy, and the dogs in group B (n=5) were observed with NO inhaling from 120 to 180 min after right pneumonectomy. After the thoracotomy, right lung tissue was resected for the pre-operative histological control. Tissue from the left lung was obtained at 120 min (5 dogs in group A), at 180 min (5 dogs in group A), and after 60 min of NO inhalation (group B) for the post-operative histological material. Peripheral blood was collected from the femoral artery. The pulmonary arterial pressure (PAP) was significantly increased after pneumonectomy, but rapidly decreased to the same level as the pre-operative stage after NO inhalation. Increases of plasma ANP, lung ANP and lung CNP levels occurred after pneumonectomy, while the ET-1 level was unchanged. Inhaled NO rapidly reduced the plasma ANP, lung ANP and lung CNP. These results indicate that both ANP and CNP act to maintain normotensive homeostatic balance in the pulmonary circulation.

Key words pneumonectomy, pulmonary artery pressure, atrial natriuretic peptide, C-type natriuretic peptide, endothelin-1, inhaled nitric oxide

Introduction

Since the initial discovery of the potent diuretic, natriuretic, and vasorelaxant activities in extracts from rat atria (de Bold et al. 1981), some forms of the natriuretic polypeptide have been isolated. Atrial (A-type) natriuretic peptide (ANP), the prototype of the natriuretic peptide family, was originally identified in mammalian atrial tissue (Kangawa and Matsuo, 1984). Brain natriuretic peptide (BNP) was identified in the porcine brain (Sudoh et al. 1988) and was also present in the heart. C-type natriuretic peptide (CNP), identified in

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295
the porcine brain, (Sudoh et al. 1990) was distributed mainly in the central nervous system and vascular tissues. These peptides are considered to form the natriuretic peptide family and involved in the regulation of blood pressure and volume homeostasis, both as cardiac hormones and as neuropeptides.

Nitric oxide (NO), an endothelium-derived relaxing factor, is a selective and effective pulmonary vasodilator in pulmonary hypertension (Pepke-Zeba et al. 1991). Inhalation of low levels of NO reverses pulmonary vasoconstriction caused by hypoxemia or the infusion of a thromboxane endoperoxide analogue in the lamb (Frostell et al. 1991; Robers et al. 1993).

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide originally isolated from vascular endothelial cells (Yanagisawa et al. 1988). Onizuka et al. (1992) observed that plasma ET-1 concentrations were significantly increased after pulmonary surgery.

In thoracic surgery, pneumonectomy is the major procedure and postoperative pulmonary hypertension is one of the severe complications. However, the acute phase of postoperative changes in circulatory kinetics are not clear and the mechanism of the vasoactive peptides in pulmonary hypertension is obscure. Furthermore, the value of inhaled NO after pneumonectomy is unknown. In this study, the changes of pulmonary arterial pressure, the changes of vasoactive peptide (ANP, CNP, and ET-1) levels in the lung and blood, and the effects of inhaled NO were investigated in dogs after pneumonectomy.

Materials and Methods

Animals

The study was performed on 15 beagle dogs weighting 12.3±1.7 kg. They were kept in automatically controlled rooms (temperature: 24±1℃; humidity: 55±5%; automatic lighting: 6:00 a.m. to 8:00 p.m.) and provided with a commercial diet LABO D STOCK (Nihon Nosan K.K., Kanagawa, Japan) and water ab libitum. The experiments were approved by the Committee of Animal Experimentation, Kurume University School of Medicine.

Experimental design

The animals were anesthetized with intramuscular injections of ketamine hydrochloride (10 mg/kg) and intravenous injections of thiamyal sodium (10 mg/kg). The dogs were intubated and ventilated with 40% oxygen at a tidal volume of 25 ml/kg, and at 14 breaths per minute. Normal saline was administered, intravenously, during the operation (4 ml/kg/hr). The anesthesia was maintained with a continuous infusion of pentobarbital calcium (5 mg/kg/hr) and pancronium bromide (0.1 mg/kg/hr). A pressure and blood sampling line was inserted into the femoral artery. A Swan-Ganz catheter was inserted into the femoral vein and advanced to the pulmonary artery for the measurement of pulmonary arterial pressure (PAP) and cardiac output (CO).

Fifteen dogs were divided into two groups. The dogs in group A (n=10) were observed without NO inhaling after right pneumonectomy and the dogs in group B (n=5) were observed with NO inhaling (5 ppm) from 120 to 180 min after right
pneumonectomy. Immediately after the thoracotomy, right resected lung tissue was obtained for the pre-operative histological control. After pneumonectomy, the tidal volume was reduced to 12.5 ml/kg. Tissue from the left lung was obtained at 120 min (5 dogs in group A), 180 min (5 dogs in group A) and after 60 min of NO inhaling (group B) for the post-operative histological material. In group A, PAP and CO were measured before the thoracotomy and at 0, 60, 120, 135, 150 and 180 min after right pneumonectomy. Peripheral blood was collected from the femoral artery at the same times. In group B, the measurement of PAP and CO and the blood sampling were performed before the thoracotomy and at 0, 60 and 120 min after right pneumonectomy and at 15, 30 and 60 min after NO inhaling. The tissue and blood samples were stored at -80°C for radioimmunoassay (RIA).

Immunohistochemistry

The lung tissue blocks were fixed in Zamboni's solution for 24 hs at 4°C. After the blocks were washed with 0.15 M phosphate-buffered saline (PBS) at pH 7.3, they were dehydrated, embedded in paraffin and sectioned at 5 μm thickness using a micrometer. Prior to immunohistochemical staining, the deparaffinized sections were incubated in absolute methanol containing 3% H2O2 for 20 min. Immunohistochemical staining was performed according to the modified avidin-biotin-peroxidase complex (ABC) technique. Following incubation in normal swine serum, the sections were incubated with primary antibodies, overnight at 4°C. For ANP, CNP and ET-1 immunohistochemical studies, rabbit antiserum against synthesized human ANP/cardiodilatin 99-126 [Code: NAW 160], human CNP-22 (Peptide Institute Inc., Japan) and ET-1 (Peptide Institute Inc., Japan) were used as the primary antibodies respectively, and were diluted 1:1000 with PBS containing 0.02% Triton ×100. As a secondary antibody, biotinylated swine anti-rabbit immunoglobulin was used.

RIA

Blood samples were drawn from the femoral artery into a syringe containing 1 mg of EDTA and 1000 units of the Kallikrein-inhibitor, aprotinin (Bayer, Germany). The plasma samples were rapidly frozen and stored at -80°C for the measurement of plasma ANP, CNP, and ET-1 levels. The tissue samples were boiled for 5 min in 10 volumes of 0.1 M acetic acid to abolish intrinsic proteolytic activity and then homogenized with a polytron homogenizer for 60 sec. The homogenates were centrifuged at 18,000×g for 30 min at 4°C and the supernatants were stored at -80°C for the RIA. Plasma and lung ANP, CNP and ET-1 levels were measured using the RIA kit (Peninsula, USA).

Statistical Analysis

All values are reported as the mean± standard deviation and comparisons were performed with a paired t test. Significance was set at a p value of less than 0.01.

Results

Immunohistochemical studies

The immunoreactivities for ANP, CNP, and ET-1 were detected in the
endothelial cells of the pulmonary artery, but were not detected in the smooth muscle tissue (Table 1).

**PAP and CO**

After pneumonectomy, the PAP increased significantly from 6.07±1.95 mmHg to 13.4±12.64 mmHg at 0 min (p<0.001). At 60 min or more after the operation the PAP decreased to 11.2±1.97 mmHg, but was still higher than before pneumonectomy. After inhalation of 5 ppm NO, the means of the PAP were 5.6±0.88, 5.2±1.44, and 5.0±0.8 mmHg at 15, 30 and 60 min, respectively. These were significantly lower than the means of the PAP without inhalation of NO (p<0.001) (Fig. 1). Neither pneumonectomy nor inhalation of NO affected the CO (Fig. 2).

**Concentrations of plasma peptides**

The concentration of plasma ANP increased significantly from 99.9±13.6 pg/ml to 135.9±28.8 pg/ml at 0 min (p<0.01) and increased gradually for 180 min after pneumonectomy (Fig. 3). After inhalation of 5 ppm NO at 15, 30 and 60 min, the concentrations of plasma ANP were 98.6±21.0, 95.7±22.5, and 98.5±13.4 pg/ml, respectively. These were significantly decreased in comparison with the control plasma ANP level at 120 min.

**Fig. 1.** Changes of pulmonary arterial pressure after pneumonectomy.

The PAP was significantly increased by pneumonectomy. After 60 min or longer, the PAP was slightly reduced, but was still higher than before pneumonectomy. After inhalation of NO, the mean PAP was significantly decreased to the level before pneumonectomy.

<table>
<thead>
<tr>
<th>Endothelial cells of the pulmonary artery</th>
<th>ANP</th>
<th>CNP</th>
<th>ET-1</th>
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<td>Smooth muscle tissue of the pulmonary artery</td>
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**TABLE 1.**

*Immunoreactivity of Natriuretic Peptides in Lung Specimens*
Fig. 2. Changes of cardiac output after pneumonectomy.
The CO was not affected by pneumonectomy or inhalation of NO.

Fig. 3. Concentration of plasma ANP after pneumonectomy.
The concentration of plasma ANP was significantly increased by pneumonectomy and was further increased for 180 min after pneumonectomy. Inhaled NO significantly decreased the concentration of plasma ANP in comparison with the plasma ANP concentration at 120 min.
**Fig. 4.** Concentration of plasma CNP after pneumonectomy.

The concentration of plasma CNP was not changed by pneumonectomy or inhalation of NO.

**Fig. 5.** Concentration of plasma ET-1 after pneumonectomy.

The concentration of plasma ET-1 was not affected by pneumonectomy or inhalation of NO.
**Fig. 6.** Concentration of lung ANP after pneumonectomy.

The concentration of lung ANP was significantly increased at 120 min and was further increased at 180 min after pneumonectomy. Inhalation of NO for 60 min decreased the lung ANP to the level before thoracotomy.

**Fig. 7.** Concentration of lung CNP after pneumonectomy.

The concentration of lung CNP was significantly increased at 120 min and was further increased at 180 min after pneumonectomy. Inhalation of NO for 60 min decreased the lung CNP.
The concentrations of lung ANP increased significantly from 1723.2±311.5 pg/g to 2455.9±374.41 pg/g at 120 min after pneumonectomy (p<0.01) and increased gradually to 2840.2±576.7 pg/g at 180 min after pneumonectomy. The inhalation of 5 ppm NO for 60 min decreased the concentration of lung ANP to 1795.8±311.8 pg/g. This level was significantly decreased from the level before NO inhaling (p<0.01) (Fig. 6).

The concentration of lung CNP increased significantly from 335.3±53.7 pg/g to 461.4±42.2 pg/g at 120 min after pneumonectomy (p<0.01) and increased gradually to 505.0±67.7 pg/g at 180 min after pneumonectomy. The inhalation of 5 ppm NO for 60 min significantly decreased the concentration of lung CNP to 346.8±49.1 pg/g (p<0.01) (Fig. 7).

Neither pneumonectomy nor inhalation of NO affected the concentration of lung ET-1 (Fig. 8).

**Discussion**

After the first report of the use of pneumonectomy for lung cancer by Evarts Graham (1933), it became one of the common treatments for lung cancer. Pneumonectomy is a general procedure to treat a localized advanced lung cancer which is centrally located, invades across fissures and involves both lobes on the left, or the upper and lower lobes on the right. Many retrospective analyses on the morbidity after pneumonectomy,
such as pneumonia, pulmonary edema, pulmonary hypertension, dysrhythmia, and cardiac failure have been reported (Harmon et al. 1976; Ginsberg et al. 1983; Wahi et al. 1989). Some hemodynamic studies have been performed after pneumonectomy. Crouch et al. (1987) studied the short-term physiological effects of pneumonectomy in 10 mongrel dogs. The pulmonary vascular resistance (PVR) and PAP were significantly increased with no change in the mean left atrial pressure after pneumonectomy. The CO and heart rate remained unchanged. In this study, the PAP was significantly increased with no change of cardiac output after pneumonectomy. The increase of PAP was considered to be an acute effect from a reduced compliance after the removal of one-half of the vascular bed. The unchanged CO in the present study supports the results by Crouch et al. (1987), although pneumonectomy should cause dramatic changes in the pulmonary vascular bed.

NO, an endothelium-derived relaxing factor (Palmer et al. 1987), is produced from L-arginine by constitutive endothelial NO synthase and may play an important regulatory role in the developing pulmonary circulation. Frostell et al. (1991) observed that inhalation of a gas mixture containing 40-80 ppm NO could reverse the acute pulmonary vasoconstriction induced by severe hypoxia in eight awake lambs. In clinically, Pepke-Zaba et al. (1991) studied the effects of inhaled NO (40 ppm) on PVR and systemic vascular resistance (SVR) in 8 patients with severe pulmonary hypertension and 10 cardiac patients with normal values for PVR. Inhaled NO did act as a selective local pulmonary vasodilator in the patients without causing systemic vasodilation. Since NO is rapidly inactivated by combining with hemoglobin, the vasodilating effect of inhaled NO must be selectively exerted on the pulmonary vasculature, and both systemic arterial pressure and vascular resistance are unchanged. Five ppm NO inhalation significantly decreased the PAP after pneumonectomy. Inhaled NO would be useful for patients with pulmonary hypertension after pneumonectomy.

ANP, the prototype of the natriuretic peptide family, was originally identified in mammalian atrial tissues. It is synthesized in the heart and secreted into the circulation as a cardiac hormone, primarily in response to atrial stretch (Lang et al. 1985). CNP is distributed mainly in the central nervous system and vascular tissues, and acts as a local regulator for vasoactive peptide (Suga et al. 1992). Both of these peptides are members of a family of vasodilatory hormones involved in the regulation of blood pressure and volume homeostasis. The present results show that the lung ANP and CNP levels were increased by an elevation of pulmonary arterial pressure after pneumonectomy. The concentration of plasma ANP increased but the plasma CNP level was not changed after pneumonectomy. On the other hand, the plasma and lung ET-1 levels were not affected. These results indicate that the lung ANP and CNP are extremely important factors for local pulmonary vaso-regulation, and that ANP may participate in the homeostatic balance of pulmonary arterial pressure after pneumonectomy.

In conclusion this study demon-
strates that increases of plasma ANP and lung ANP and CNP levels are induced by pneumonectomy, while the ET-1 level was not affected. Inhaled NO rapidly reduced the plasma ANP, lung ANP and lung CNP concentrations. This indicates that ANP and CNP act to maintain the normotensive homeostatic balance in the pulmonary circulation.

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References


