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Endothelin-1 Content of Bronchoalveolar Lavage in Allografted Lungs during Acute Rejection*

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= **Abstract** =

Objective : The aim of this study was to investigate whether or not endothelin-1 content of bronchoalveolar lavage was elevated in allografted lungs during acute rejection.

Methods : After single lung allotransplantation, dogs were immunosuppressed with triple standard therapy and divided into 2 groups. Group 1(Immunosuppression ; n=4) was maintained on immunosuppression as controls. In group 2(Rejection ; n=13), triple therapy was discontinued to induce acute rejection from postoperative day 5.

At postoperative day 9, broncholaveolar lavage was done through bronchoscopy in native unoperated lung and transplanted lung in group 1. Bronchoalveolar lavage was repeated in group 2 in the same way. Endothelin-1 content of bronchoalveolar lavage was measured by radioimmunoassay.

Endothelin-1 content in transplanted lung of group 2 was compared to that of transplanted lung of group 1 and to that of native unoperated lung of group 2.

Results : Endothelin-1 content of bronchoalveolar lavage in transplanted lung of group 2 was comparable to that of group 1(42.18±26.39 vs 3.08±3.08pg/ml ; p=0.08). Endothelin-1 content of bronchoalveolar lavage in transplanted lung of group 2 was comparable to that of native unoperated lung of group 2(42.18±26.39 vs 3.74±2.62pg/ml ; p=0.07)

Conclusion : Endothelin-1 content of bronchoalveolar lavage in transplanted lung was altered during acute rejection, but without statistical significance.

KEY WORD : Lung allograft · Acute rejection · Bronchoalveolar lavage · Endothelin-1.

Introduction

Endothelin-1 is a 21-residue peptide and isolated from vascular endothelium. It has potent vasoconstriction and mitogenic properties[1]. Endothelin-1 can cause bronchoconstriction and increase pulmonary vascular resistance(PVR)[2]. It is released from pulmonary endothelial and epithelial cells in conditions with increased PVR and cellular proliferation, such as pulmonary hypertension and fibrosis.

Lung transplantation is the therapeutic option for end-stage lung disease. Acute rejection is one of the main problem after lung transplantion[3][4][5].

Endothelin-1 content of bronchoalveolar lavage

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fluid was elevated in some chronic inflammatory pulmonary disease including asthma[6]. Tissue content of endothelin-1 was elevated in allografted lung during acute rejection[7]. Endothelin-1 levels of bronchoalveolar lavage and plasma were elevated early after lung allograft[8]. These may suggest that endothelin-1 content of bronchoalveolar lavage may be elevated in rejecting allografted lungs[9][10][11].

This study was designed to investigate whether or not endothelin-1 content of bronchoalveolar lavage fluid was elevated during acute rejection after lung allotransplantation.

Materials and Methods

1. Operative procedure

Male mongrel dogs of similar weight(20 – 25kg) were used as donors and recipients. In donors, under general anesthesia and mechanical ventilation, midsternotomy was done in supine position. Azygos vein was ligated and venae cavae, aorta and trachea were encircled with umbilical tape. Heparin and methylprednisolone acetate were given intravenously. Lungs were flush-perfused with cold(4℃) modified Euro-Collins solution(60ml/kg) through main pulmonary artery. Simultaneously topical cooling was obtained by irrigation of cold saline into the thoracic cavity and lungs. Prostaglandin E_1 was infused intravenously before perfusion. Trachea was clamped with full inflation of lung and heart-lung block was excised.

In recipient dogs, the fifth intercostal space was opened and extrapericardial pneumonectomy was done. Left atrial cuff and pulmonary artery were anastomosised with Prolene 5 – 0 continuously. Bronchus were anastomosised with interrupted 4 – 0 Prolene using telescoping technique. During above procedure, transplanted lung was protected by wrapping with cold soaked sponges and continuous irrigation with cold saline. Before reperfusion, heparin and methylprednisolone acetate were given intravenously.

All dogs received standard triple immunosuppressive therapy(cyclosporine, 10mg/kg/day ; azathioprine, 2.5mg/kg/day ; methylprednisolone acetate, 1mg/kg/day) and antibiotics(gentamycin sulfate 40mg twice a day ; clindamycin phosphate 300mg twice a day ; cafazolin sodium 250mg twice a day).

2. Induction of rejection

At postoperative day 5, chest radiography was taken and open lung wedge biopsy was done under general anesthesia. Dogs without pathological findings in chest radiography and biopsy, were randomly assigned into one of the following two groups. **Group 1(Immunosuppression ; n=4)** was maintained triple therapy. In **group 2(Rejection ; n=13)**, triple therapy was discontinued from postoperative day 5 and induced acute rejection.

3. Measurement of Endothelin-1

At postoperative day 9, general anesthesia was induced with sodium methohexital(12.5mg/kg i.v.) and maintained with halothane(inspired 1 – 2% concentration) under endotracheal intubation. Fiberoptic bronchoscopy was performed through tracheostomy and bronchoalveolar lavage fluid was obtained. The tip of bronchoscope was wedged into the segmental bronchus of the lower lobe of native unoperated lung and 25 – 40ml of saline was flushed into the bronchus. Approximately 20ml of the fluid was aspirated through the suction channel of the bronchoscope and kept at 4℃. The bronchoscopic lavage was repeated in the same way in the transplanted lung. Bronchoscopic lavage was done both in group 1 and group 2 respectively.

Samples were centrifuged at 500g for 10minutes and 10ml of supernatant was prepared. Aprotinin (300U/ml) and EDTA(2.5mg/ml) were added to supernatant and kept at −70℃. Content of endothelin-1 was measured by radioimmunoassay. Lowest detectable concentration was 0.5pg/ml.

4. Statistical analysis

All results were reported as the mean and standard error of mean and analyzed by statistical program Microsoft Excel. Endothelin-1 content of bronchoalveolar lavage in transplanted lung of group 2 were compared to that of group 1 by unpaired t-test.

Endothelin-1 content of bronchoalveolar lavage in transplanted lung of group 2 were compared to that of native unoperated lung of group 2 by paired t-test. P value was regarded as significant when less than 0. 05.

Results

Endothelin-1 content of bronchoalveolar lavage in transplanted lung of group 2 was comparable to that of group 1(42.18±26.39 vs 3.08±3.08pg/ml ; p=0. 08). Endothelin-1 content of bronchoalveolar lavage in transplanted lung of group 2 was comparable to that of native unoperated lung of group 2(42.18±26. 39 vs 3.74±2.62pg/ml ; p=0.07)(Table 1 and Fig 1).

Discussion

In 1988, Yanagisawa et al. first isolated and cloned a new vasoconstrictor peptide endothelin(Et) from supernatant of porcine endothelial cells[12]. Endothelin has three distinct isoforms ; endothelin-1, endothelin-2 and endothelin-3[13][14]. Endothelin-1 was initially isolated from porcine endothelium and most common in human, pig, dog, rat and probably all mammals. Endothelin was synthesized form the large vessel endothelial cell and also from smooth muscle cells, epithelial cells and circulating cells. In kidney, endothelial cells, mesangial cells, glomerular cells and tubular epithelial cells are the site of synthesis[15][16].

Table 1. Endothelin-1 content(pg/ml) of bronchoalveolar lavage fluid

	Native lung	Transplanted lung
Group 1 (Immunosuppression)	0	3.08± 3.08
Group 2(Rejection)	3.74±2.62	42.18±26.39

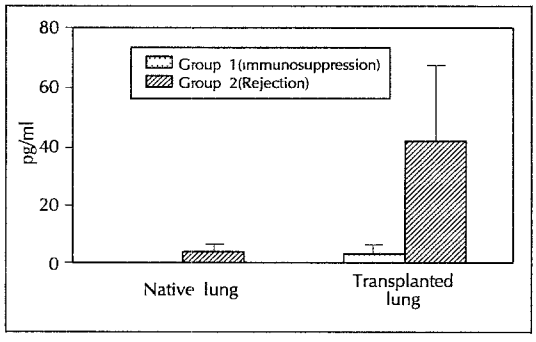

Fig. 1. Endothelin-1 content(pg/ml) of bronchoalveolar lavage fluid.

Endothelin react not only on the cardiovascular system but also the central nervous system, kidney and lung. Besides long strong vasoconstriction, endothelin modulates inotropy, chronotropy, bronchoconstriction and neurotransmission, hyperplastic and hypertropic effect. Endothelin maintain vasoconstriction in various pathophysiologic conditions like acute myocardial infarction, hypertension, renal failure, hemolytic uremic syndrome, sepsis, vasculitis and preeclampsia[17][18].

Besides bloodstream, endothelin was reported to be found and increased in bronchoalveolar lavage fluid of asthma and rejecting allografted lung[6][9][10][11]. In this study, endothelin-1 was found in rejecting(group 2) and non-rejecting(group 1) transplanted lung, native unoperated lung of rejection group(group 2), but couldn't be detected in native unoperated lung of non-rejecting lung(group 1). This may suggest that endothelin in bronchoalveolar lavage was found only in pathological state, which may be different from endothlin in plasma or urine.

Endothelin level of rejecting transplanted lung (group 2) was comparably elevated but failed to show statistical significance. P values were 0.08 and 0.07 and close to 0.05. This may be simply from too small sample size.

Endothelin in bronchoalveolar lavage fluid may be from vascular endothelium, bronchial epithelium and other inflammatory cells including polymorphonuclear leukocyte, lymphocyte and alveolar macrophage[12][19][20][21]. During acute rejection, proportion of polymorphonuclear cell and lymphocytes were increased and these can be the source of increased endothelin level.

Pathophysiological effect of endothelin in rejecting lung was thought to increased airway resistance after lung allograft by bronchoconstriction[22]. And the mitogenic effect in bronchi, by increased expression of proliferating cell nuclear antigen, can contribute to

the development of bronchiolitis obliterance in allografted lungs[23)24)].

Bronchoalveolar lavage is a technique to evaluate the cellular and molecular components of the epithelial lining fluid of the lower respiratory tract. It is based on the concept that saline was infused through the bronchoscope, mixed with epithelial lining fluid and its components were recovered along with it. However it has difficulty to estimate the actual concentration of recovered molecules and cells in the epithelial lining fluid, due to dilution by different volume of infused saline. Normalization may be necessary to quantify accurate concentration[25)]. In this experiment, normalization was tried with protein content and aspirated volume, but without statistical significance.

Bronchoalveolar lavage level of endothelin-1 can be used as the marker of acute rejection after lung allograft, if further study confirmed the elevation of endothelin-1 level.

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폐이식후 급성 거부반응시 기관지폐포 세척액에서의 Endothelin-1 농도의 변화

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박 영 식

= 국 문 초 록 =

본 실험은 황견에서 동종 일측 폐이식후에 나타난 급성 거부반응시 기관지폐포 세척액에서의 Endothelin-1 농도의 변화를 조사하기위하여 계획되었다.

황견에서 일측 폐이식을 시행한뒤, 제1군(대조군 ; 황견 4마리)은 면역억제제의 투여를 계속하여 대조군으로 삼았다. 제2군(거부반응군 ; 황견 13마리)은 수술후 5일째부터 면역억제제의 투여를 중단하여 급성 거부반응을 유도하였다. 먼저 제1군에서 수술후 9일에 기관지경을 통하여 이식받지않은 쪽과 이식을 받은 쪽에서 각각 기관지폐포 세척액을 흡입하여, Endothelin-1의 농도를 Radioimmunoassay로 측정하였다. 다시 제2군에서 같은 방법을 되풀이하였다.

제2군(거부반응군)의 이식을 받은 폐의 기관지폐포 세척액의 Endothelin-1의 농도는 제1군(대조군)의 이식을 받은 폐에 비하여 상승 되었으나, 통계학적 의의를 나타내는데에는 실패하였다(42.18±26.39pg/ml vs 3.08±3.08pg/ml ; p=0.08). 제2군(거부반응군)의 이식을 받은 폐의 기관지폐포 세척액의 Endothelin-1의 농도는 제2군(거부반응군)의 이식을 받지않은 폐에 비하여 상승되었으나, 통계학적 의의를 나타내는 데에는 역시 실패하였다(42.18±26.39pg/ml vs 3.74±2.62pg/ml ; p=0.07).

폐이식후 거부반응시 기관지폐포 세척액에서의 Endothelin-1의 농도는 상승되었지만, 통계학적 의의는 나타내지 못하였다.