梨花醫大誌 : 第 20 卷 第 4 號 1997
Ewha Med J Vol. 20, No. 4, 1997

Nitric Oxide Content of Bronchoalveolar Lavage during Infection or Acute Rejection after Lung Allotransplantation*

Young-Sik Park

Department of Thoracic and Cardiovascular Surgery, College of Medicine, Ewha Womans University

=국문초록=

폐이식후에 합병된 감염증과 거부반응시 기관지폐포 세척액에서의 Nitric Oxide 농도의 변화*

이화여자대학교 의과대학 흉부외과학교실
박 영 식

본 실험은 황견에서 폐이식 후에 합병된 감염증과 급성 거부반응시 기관지폐포 세척액에서의 Nitric oxide농도의 변화를 조사하기 위하여 계획되었다.

황견에서 일측 폐이식을 시행한뒤, 제1군(대조군 ; 4마리)은 면역억제제의 투여를 계속하여 대조군으로 삼았다. 제2군(감염증군 ; 6마리)은 수술 후 5일째에 기관지경을 통하여, 대장균을 이식된 폐에 주입하여 감염을 유발시켰다. 제3군(거부반응군 ; 15마리)은 수술 후 5일째부터 면역억제제의 투여를 중단하여 급성 거부반응을 유도하였다. 각군에서 수술 후 9일째에 기관지경을 통하여 이식받지않은 폐와 이식받은 폐에서 각각 기관지폐포 세척액을 흡입하여, Nitric oxide의 농도를 Chemiluminescence 방법으로 측정하였다.

이식받지 않은 폐의 기관지폐포 세척액의 Nitric oxide의 농도는 세 군사이에서 의의있는 차이는 없었다(제1군, 3.350.35uM/L ; 제2군, 4.691.92uM/L ; 제3군, 2.710.26uM/L ; $p>0.05$). 이식받은 폐의 기관지폐포 세척액의 Nitric oxide의 농도도 세 군사이에서 의의있는 차이는 없었다(제1군, 2.600.27uM/L ; 제2군, 3.110.56uM/L ; 제3군, 3.180.74uM/L ; $p>0.05$). 또한 각군에서 이식받지 않은 폐와 이식받은 폐의 기관지폐포 세척액에서의 Nitric oxide농도의 의의있는 차이는 없었다.

폐이식 후에 합병된 감염증이나 거부반응시 기관지폐포 세척액에서의 Nitric oxide농도의 상승은 관찰되지 않았다.

KEY WORDS : Lung · Allotransplantation · Infection · Acute rejection · Bronchoalveolar Lavage · Nitric oxide.

---

*본 논문은 1997년 이화여자대학교 교내연구비에의하여 연구되었음.
**본 논문의 요지는 1997년 대한흉부외과 추계학회에서 구연발표되었음.

Introduction

Lung transplantation is an accepted treatment for end-stage lung disease. Infection and acute rejection are the two main causes of morbidity and mortality after transplantation. However, it is not easy to get differential diagnosis by clinical symptoms, chest radiography, bronchoalveolar lavage or steroid "pulse" therapy[1)2)3)]. Plasma concentration of nitric oxide has been shown to be elevated during infection or acute rejection after organ transplantation including liver, heart and lung[4)5)6)]. But little is known about nitric oxide content of bronchoalveolar lavage during infection or acute rejection after lung transplantation.

The present study was aimed to investigate whether or not nitric oxide content of bronchoalveoloar lavage is elevated during infection or acute rejection after lung allotransplantation.

Materials and Methods

Male mongrel dogs of similar weight(20-25kg) were used as donors and recipients. Animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources and the "Guides for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health(NIH Publication No. 86-23, revised 1985).

1. Operative procedure

After induction of anesthesia, donor lungs were flush-perfused with cold modified Euro-Collin's solution(60mL/kg) and topically cooled with cold saline. Prostaglandin E_1 was infused intravenously before perfusion. Explanted lung was transplanted to the donor dog with the standard operative technique[7]. All dogs were treated with 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). Heparin was used during and after transplantation.

2. Grouping

At the postoperative day 5, chest radiography was taken and open lung wedge biopsy was done under general anesthesia. If there was no pathologic evidence, dogs were randomly assigned into one of the three groups.

Group 1(Immunosuppression ; n=4) was maintained triple therapy as control group.

In group 2(Infection ; n=6), fiberoptic bronchoscopy was done through tracheostomy at postoperative day 5. A tip of bronchoscope was wedged into the lower lobe of transplanted lung and 10milliliters of 10^{10} colony-forming units(CFUs) of *Escherichia coli* with culture media was flushed into the bronchus. In preliminary experiments, three types of bacteria were routinely found from bronchial swabs in dogs-*E coli, B bronchoseptica and P aeruginosa.* A strain resistant to the antibiotics(gentamycin, clindamycin and cefazolin) was identified, colonized and used to induce pneumonia[8].

In group 3(Rejection ; n=15), assigned dogs were discontinued triple therapy to induce acute rejection at postoperative day 5.

3. Bronchoalveolar lavage

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 30ml of saline was flushed into the bronchus. Approximately 10ml of the fluid was aspirated through the suction channel of the bronchoscope. The bronchoscopic lavage was repeated in the same way in the transplanted lung. Bron-choscopic lavage was repeated in group 1, 2 and 3 respectively.

4. Measurement of nitric oxide

Collected samples were immediately centrifuged at 500g for 10 minutes at 4-5℃. The cell-free supernatant was decanted and aprotinin(300U/ml) and EDTA(2.5mg/ml) were added. Samples were kept in the siliconized tube at the -70℃ freezer until measurement. Assay was done within a week after sampling.

Chemiluminescence method : Nitrite/nitrate was reduced to nitric oxide by 0.1 M vanadium III in 3 M hydrochloric acid. Heating(85℃) helped rapid reduction of nitrate. Gaseous nitric oxide was removed from the liquid fluid by scrubbing with inert N_2 gas in modified purge and trap micro reaction vessel. Nitric oxide was oxidized by ozone and emitted the light in Sievers Nitric Oxide Analyzer(Model 270B, Boulder Co., CO, USA). Intensity of light was recorded on Shimadzu Chromatopac Integrator (Model CR 601, Shimadzu Corp., Japan). Output signals were calculated from the known standard curves of sodium nitrite and potassium nitrate[9][10][11].

5. Statistical analysis

All results were reported as the mean standard error of mean. Nitric oxide content of bronchoalveolar lavage in native unoperated lung was compared among three groups by one-way ANOVA test. Nitric oxide content of bronchoalveolar lavage in transplanted lung was compared among three groups by one-way ANOVA test. In each group, nitric oxide content of bronchoalveolar lavage was compared between native unoperated and transplanted lung by paired *t* test. A p value less than 0.05 was regarded as statistically significant.

Results

Nitric oxide content of bronchoalveolar lavage in native unoperated lung was comparable among three groups(group 1, 3.35±0.35 ; group 2, 4.69±1.92 ; group 3, 2.71±0.26uM/L ; $p>0.05$). Nitric oxide content of bronchoalveolar lavage in transplanted lung was also comparable three groups(group 1, 2.60±0.27 ; group 2, 3.11±0.56 ; group 3, 3.18±0.74uM/L ; $p>0.05$). Nitric oxide content of bronchoalveolar lavage was comparable between native unoperated and transplanted lung in each group($p>0.05$) (Table 1 and Fig. 1).

Table 1. Nitric oxide content of bronchoalveolar lavage (uM/L) in three groups

	Group 1 (Control)	Group 2 (Infection)	Group 3 (Rejection)
Native lung	3.35±0.35	4.69±1.92	2.71±0.26
Transplanted lung	2.60±0.27	3.11±0.56	3.18±0.74

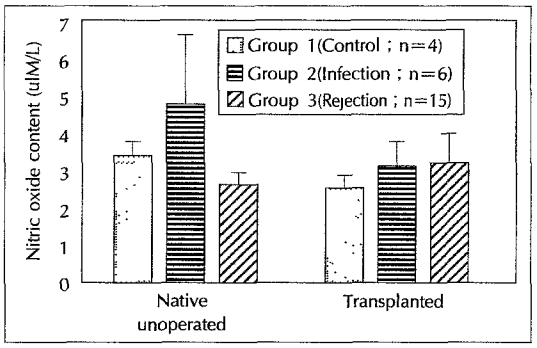

Fig. 1. Nitric oxide content of bronchoalveolar lavage(uM/L) in three groups.

Discussion

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 is infused through the bronchoscope, mix with epithelial lining fluid and its components are recovered along with it[12][13]. 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[14]. In this experiment, normalization was tried by protein content and aspirated volume respectively, but without statistical significance. So normalization was not applied in the final results of this experiment.

In 1980, Furchgott and Zawadzki reported that blood vessel with endothelium relaxed in response to

acetylcholine[15]. Moncada proved that EDRF was identical to nitric oxide by releasing of EDRF from endothelial cells and its relaxing effect on smooth miuscle[16][17].

Nitric oxide is produced by the enzyme NO synthase(NOS). Three isoforms of NOS was discovered. Two isoforms are always present and termed constitutive(cNOS). Endothelial-derived NO emanates from an endothelial cNOS(ecNOS) and neuronal cNOS(ncNOS). The third isoform is inducible NOS (iNOS), which is expressed after stimulation with cytokines, microbes or xenobiotics[18][19][20].

Nitric oxide in biological system can be measured by its physiological effects, such as the relaxation of blood vessels, activation of guanylyl cyclase, increased cGMP concentration, production of citrulline and inhibition of platelet aggregation. Also inhibitors of nitric oxide synthesis such as the L-arginine analogues or hemoglobins, have been used to estimated nitric oxide production. These indirect methods can provide incorrect information. Direct measurements including spectroscopic and electroanalytic methods are more sensitive techniques[11].

Spectroscopic methods include chemiluminescence, ultraviolet-visible spectroscopy, electron spin resonance spectroscopy and flow cytometry. Chemiluminescence method is based on the measurement of intensity of the flluorescent radiation emitted after chemical oxidation of nitric oxide by ozone. Chemiluminescence method has good detection threshold and sensitivity in determination of the total amount of nitric oxide in the system. But electrochemical method may be better used in the dynamic process which currently generates nitric oxide[9][10].

In summary, nitric oxide content of bronchoalveolar lavage was not elevated during infection or acute rejection after lung allotransplantaion.

References

1) Cooper JD, Patterson GA, Trulock EP : *Washington University and the Lung Transplant Group. Results of 131 consecutive single and bilateral lung transplant recipients. J Thorac Cardiovasc Surg 1994 ; 107 : 460*

2) Couraud L, Baudet E, Nashef SA, et al : *Lung transplantation with bronchial revascularization. Surgical anatomy, operative technique and early results. Eur J Cardiothorac Surg 1992 ; 6 : 490*

3) Sundaresan S, Gregory DT, Aoe M, et al : *Donor lung procurement: Assessment and operative technique. Ann Thorac Surg 1993 ; 56 : 1409*

4) Tanaka S, Kamiike W, Ito T, Nozaki S, Uchikoshi F, Miyata M, et al : *Evaluation of nitric oxide during acute rejection after heart transplantation in rats. Transplantation Proceedings 1995 ; 27 : 576-577*

5) Ioannidis I, Hellinger A, Dehmlow C, Rauen U, Erhard J, Eigler FW, et al : *Evidence for increased nitric oxide production after liver transplantation in humans. Transplantation 1995 ; 59 : 1293-1297*

6) Langrehr JM, Murase N, Markus PM, Cal X, Neuhaus P, Schraut W, et al : *Nitric oxide production in host-versus-graft and graft-versus-host reaction in the rat. J Clin Invest 1992 ; 90 : 679-683*

7) Nilson FN, McGregor CGA, Miller VM : *Pulmonary arterial reactivity following transplantation : differential effects of denervation and rejection. J Thorac Cardiovasc Surg 1992 ; 103 : 751-762*

8) Kim HK, Tazelaar HD, Odell J, Park YS, Steckelberg JM, Miller VM, ET AL : *An animal model of pulmonary infection after single lung transplantation. Transplantation Proceedings 1996 ; 28 : 1818-1819*

9) Menon NK, Pataricza J, Binder T, Bing RJ : *Reduction of biological effluents in purge and trap micro reaction vessels and detection of endothelium-derived nitric oxide(Edno) by chemiluminescence. J Mol Cell Cardiol 1991 ; 23 : 389-393*

10) Braman RS, Hendrix SA : *Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection. Anal Chem 1989 ; 61 : 2715-2718*

11) Archer S : *Measurement of nitric oxide in biological models. FASEB J 1993 ; 7 : 349-360*

12) Selvaggi SM : *Bronchoalveolar lavage in lung transplant patients. Acta Cytol 1992 ; 36 : 674-679*

13) Shennib H, Nguyen D : *Bronchoalveolar lavage in lung transplantation. Ann Thorac Surg 1991 ; 51 : 335-340*

14) Rennard SI, Basset G, Lecossier D, ODonnel KM,

Pinkston P, Martin PG, Crystal RG : *Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. J Appl Physio 1986 ; 60(2) : 532-538*

15) Furchgott RF, Zawadzki JV : *The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980 ; 288 : 373-376*

16) Palmer RMJ, Ferrige AG, Moncada S : *Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor[letter]. Nature 1987 ; 327 : 524-526*

17) Palmer RM, Ashton DS, Moncada S : *Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988 ; 333 : 664-666*

18) Michel T, Li GK, Busconi L : *Phosphorylation and subcellular translocation of endothelial nitric oxide synthase. Proc Natl Acad Sci USA 1993 ; 90 : 6252-6256*

19) Nishida K, Harrison DG, Navas JP, et al : *Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. J Clin Invest 1992 ; 90 : 2092-2096*

20) Marsden PA, Heng HHQ, Scherer SW, et al : *Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 1993 ; 268 : 17478-17488*