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Change of Plasma Nitric Oxide during Acute Rejection or Infection after Lung Allotransplantation

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= Abstract =

Objective : This study was aimed to investigate whether there is any change in plasma nitric oxide during acute rejection or infection after lung allotransplantation.

Methods : After lung allotransplantation, dogs were immunosuppressed with standardized triple therapy and divided into 3 groups : in group 1(control ; n=4), immunosuppression was maintained ; in group 2(n=7), triple therapy discontinued to induce acute rejection at the postoperative day 5 ; in group 3(n=6), infection was induced by bronchoscopic inoculation of *E. coli* at the postoperative day 5. Plasma nitric oxide was measured by chemiluminescence method prior to surgery(day 0), and at postoperative day 5 and 9. In each group, plasma nitric oxide level at day 9 was compared to that at day 0. Plasma nitric oxide levels at day 9 were compared in three groups.

Results : During acute rejection period, plasma nitric oxide concentration was found to be elevated significantly at postoperative day 9, compared to day 0(11.52 ± 2.58 vs 6.01 ± 0.88uM/L ; $p<0.05$). However, plasma nitric oxide concentration wasn't altered by the E. coli-induced infection(14.53 ± 5.19 vs 6.12 ± 0.98uM/L ; $p>0.05$). Plasma nitric oxide of day 9 weren't different in three groups($p>0.05$).

Conclusion : Plasma nitric oxide may be a good marker for acute rejection after allotransplantation, but not for infection.

KEY WORDS : Lung transplantation · Acute rejection · Infection · Nitric oxide.

Introduction

Lung transplantation is an accepted treatment for end-stage lung disease. Acute rejection and pulmonary infection are the two main causes of morbidity and mortality after transplantation. However, it is not easy to get accurate 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 acute rejection or infection after organ transplantation including heart and liver[4)5)6)]. However little is known about the plasma concentration of nitric oxide in acute rejection or infection after lung transplantation. Therefore the present study was aimed to investigate whether plasma nitric oxide is altered during acute rejection or infection during or after lung al-

lotransplantation.

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-perfuse 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 standard operative technique[7]. All dogs were treated with triple immunosuppressive therapy(cyclosporine, 10mg/kg/day ; azathioprine, 2.5mg/dg/day ; methylprednisolone acetate, 1mg/kg/day) and antibiotics(gentamycin sulfate 40mg twice a day ; clindamycin phosphate 300 mg 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 group) was maintained triple therapy.

Group 2(Rejection group) ; At postoperative day 5, assigned dogs discontinued triple therapy to induce acute rejection.

Group 3(Infection group) ; At postoperative day 5, fiberoptic bronchoscopy was done through tracheostomy. A tip of bronchoscope was wedged into the lower lobe of transplanted lung and 10mmilliliters of 10^{10} colony-forming units(CFU_s) 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].

3. Measurement of nitric oxide

Plasma nitric oxide was measured prior to surgery (day 0), and at postoperative day 5 and 9. Blood was collected in EDTA-contained vacutainer tube and immediately centrifuged at 3200g for 15 minutes at 4－5℃. The supernatant was kept in the siliconized tube at the －70℃ freezer until measurement. Assay was done within a week after sampling.

Chemiluminescence method ; Plasma nitrite/nitrate was reduced to nitric oxide by 0.1M vanadium III in 3M hydrochloric acid. Heating(85℃) helped rapid reduction of nitrate. Gaseous nitric oxide was removed from the liquid plasma 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].

4. Statistical analysis

All results are reported as the mean±standard error of mean and analyzed by the computer program SigmaStat. In each group, plasma nitric oxide at day 9 was compared to that at day 0 by student t-test. Plasma nitric oxide of day 9 in three groups were compared by one-way ANOVA test. A p value less than 0.05 was regarded as statistically significsant.

Results

In group 1(Immunosuppression), plasma nitric oxide remained unchanged through day 0, 5 and 9. In group 2(Rejection), plasma concentration of nitric oxide of day

Table 1. Plasma nitric oxide level(uM/L) at day 0, 5 and 9

	day 0	day 5	day 9
Group 1(IS ; n=4)	7.52±2.39	7.32±0.66	7.25±0.51
Group 2(RJ ; n=7)	6.01±0.88	7.83±1.02	11.52±2.58*
Group 3(IF ; n=6)	6.12±0.98	5.87±0.41	14.53±5.19

Data are presented as mean±standard error of the mean
IS ; immunosuppression, RJ ; Rejection, IF ; Infection
*Plasma nitric oxide of day 9 was elevated in rejection group compared to day 0($p<0.05$)

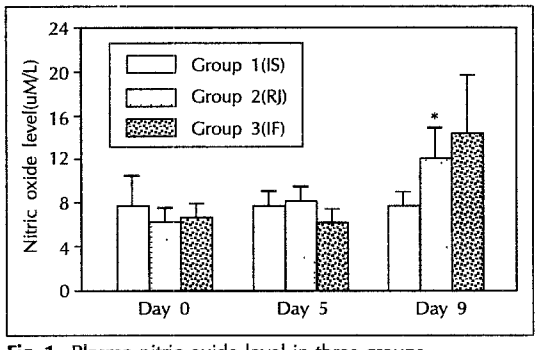

Fig. 1. Plasma nitric oxide level in three groups.
*Plasma nitric oxide level at day 9 was elevated compared to day 0 in group 2(Rejection)($p<0.05$)
(IS ; Immunosuppression, RJ ; Rejection, IF ; Infection).

9 was elevated compared to that of day 0(11.52±2.58 vs 6.01±0.88uM/L ; $p<0.05$). In group 3(Infection), pla-sma concentration of nitric oxide at day 9 was relatively elevated, but no statistical significance(14.53±5.19 vs 6.12±0.98uM/L ; $p>0.05$). There was no significant difference in plasma nitric oxide at day 9 in three groups(7.25±0.51, 11.52±2.58 and 14.53±5.19uM/L ; $p>0.05$)(Table 1 and Fig. 1).

Discussion

In 1980, Furchgott and Zawadzki reported that blood vessel with endothelium relaxed in response to acetylcholine. This EDRF was proved to be identical to nitric oxide by Moncada in 1987, by releasing of EDRF from endothelial cells and its relaxing effect on smoth miuscle.

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

During the uneventful course after human liver transplantation, plasma nitrate was reported to be elevated temporarily on 4-6 postoperative day and rapidly returned to baseline level. With rejection or infection, elevation of peak plasma nitrate level became pronounced and prolonged and showed another second peak[5]. During the acute rejection after heart transplantation in rats, nitric oxide level was increased and mRNA and protein of inducible nitric oxide synthase (iNOS) were detected in ventricular homogenates and isolated cardiac myocytes. Increases of iNOS staining in infiltrating macrophages, in microvascular endothelial cells and cardiac muscle fibers were also reported. This showed acute rejection induced iNOS in infiltrating macrophage, myocyte and endothelium in heart[12].

During the acute rejection after lung transplantation in dogs, expression of ecNOS was shown to be decreased but iNOS was induced in lung parenchyma [13]. Thus the elevation of nitric oxide level was induced by the increased expression of iNOS during the acute rejection in heart or lung transplantation.

Nitric oxide modulates vasoconstrictor mechanism, which includes inhibition of the adhesion and aggregation of leukocytes and platelets. Increased production of nitric oxide during acute rejection period might enhance the perfusion to rejecting graft and promote graft survival. But nitric oxide also had graft toxicity through peroxynitrite, which was formed from the interaction with superoxide[14][15][16].

In infection, bacteria or their immunological stimuli such as lipopolysaccharide and cytokines induce iNOS in macrophages, neutrophils and Kupffer cells of liver. Some vascular endothelium and vascular smooth muscle also expressed mRNA of iNOS. This nitric oxide is cytostatic/cytotoxic to invading bacteria at molecular target[17][18]. In group 3, infection increased the average concentration of nitric oxide from 6.12±0.98uM/L (day 0) to 14.53±5.19uM/L(day 9) and p value was close to 0.05 but failed to show the statistical significance. Theis might be merely from too small sample

size(n=6) and further study with proper sample size can confirm the result.

Nitric oxide in biological system may be measured by its physiological effects, such as the relaxation of blood vessels, activation of guanylyl cyclase, increased cGMP concentration, production of citrulline, or 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 by spectroscopic and electroanalytic methods are currently used techniques.

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 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.

Plasma nitric oxide can be a useful marker for screening the acute rejection after lung allotransplantation. For infection, further study is necessary.

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동종 폐이식술후에 합병된 거부반응 또는 감염증시 혈중 Nitric Oxide 농도의 변화

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= 국 문 초 록 =

본 연구는 동종 폐이식술후에 합병된 급성 거부반응이나 감염증시 혈중의 Nitric oxide 농도가 어떻게 변화되는지를 검사하기 위하여 계획되었다.

황견에서 일측 폐를 이식한후 세군으로 분리하였다. 제1군(면역억제제군)은 수술후 면역억제제를 투여받았다. 제2군(거부반응군)에서는 수술후 5일에 면역억제제의 투여를 중단하여, 급성 거부반응을 유도하였다. 제3군(감염증군)에서는 수술후 5일에 기관지경을 통하여, 대장균을 이식된 폐에 주입하여 감염증을 유발시켰다. 혈중 Nitric oxide 농도는 Chemiluminescence 방법으로 측정하여, 각 군에서 수술전과 수술후 9일의 혈중 농도를 각각 서로 비교하였다. 또 수술후 9일 혈중 농도를 세군에서 서로 비교하였다.

제2군(거부반응군)에서 9일의 혈중농도가 수술전의 농도에 비하여 의의있게 상승되었다(11.52±2.58 vs 6.01±0.88uM/L ; $p<0.05$). 제1군(면역억제제군)과 제3군(감염증)군에서는 의의있는 상승을 보이지 않았다. 동종 폐이식술후 혈중 Nitric oxide 농도를 측정하여 급성 거부반응을 진단하는 지표로 이용할 수 있으리라 생각된다.