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Does Plasma Nitric Oxide Concentration Correlate with the Pathological Grade of Rejection after Lung Allograft ?*

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= **Abstract** =

Objective : Experiments were designed to investigate whether there is any correlation between concentration of plasma nitric oxide and pathological severity of acute rejection after lung allograft.

Methods : After single lung allograft, 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=15), triple therapy was discontinued to induce acute rejection from postoperative day 5. Plasma concentration of nitric oxide was measured by chemiluminescence method prior to surgery and at postoperative day 9. At postoperative day 9, dogs were sacrificed and rejection was graded pathologically by a working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: lung rejection study group.

Plasma nitric oxide level of day 9 was compared to that of prior to surgery in each group. Correlation between plasma nitric oxide level and pathological grade of acute rejection at day 9 in group 2(Rejection) was examined.

Results : In group 2(Rejection), plasma nitric oxide level of day 9 was elevated significantly, compared to that of prior to surgery(12.28 ± 2.10 vs 6.46 0.57μM/L ; $p < 0.05$). But plasma nitric oxide levels of day 9 didn't significantly correlate with the pathological grades of rejection in group 2(Spearman's coefficient $r = -0.2094$; $p > 0.05$).

Conclusion : Plasma concentration of nitric oxide was elevated during acute rejection, but didn't correlate with the pathological severity of rejection.

KEY WORDS : Lung allograft · Acute rejection · Nitric oxide.

Introduction

Lung allograft is treatment option for end-stage parenchymal and vascular lung disease. As organ preservation, surgical technique and immunosuppression were improved, acute rejection had remained one of the most important impediments. To improve survival after lung allograft, diagnosis of acute rejection is significant[1)2)3)].

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For diagnosis and grading of acute rejection, histologic evaluation is useful method, which was performed by transbronchial lung biopsy[4)5)]. Plasma nitric oxide was possibly used for early detection of acute rejection because elevation was reported during acute rejection after lung allograft[6)7)].

This experiments were designed to study 1) whether plasma nitric oxide was elevated during acute rejection and 2) whether there was any correlation between plasma concentration of nitric oxide and pathological severity of rejection.

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)** was maintained triple therapy. In **group 2(Rejection)**, triple therapy was discontinued from day 5 and induced acute rejection.

3. Measurement of nitric oxide

Plasma concentration of nitric oxide was measured at prior to surgery(day 0) and postoperative day 9. Blood was collected in EDTA-contained vacutainer tube and immediately centrifuged at 3200g for 15minutes at 4 – 5℃. Supernatant was kept in the siliconized tube in the – 70℃ freezer and measured within a week.

Chemiluminescence method : Plasma 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 is 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 fluorescent light in Sievers Nitric Oxide Analyzer(Model 270B, Boulder, 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[8)9)10)].

4. Pathologic grading of acute rejection

At postoperative day 9, dogs were anesthetized and transplanted lungs were harvested. Each lobe was pefused with 10% buffered formalin via bronchus immediately after excision and stored in 10% formalin. Tissues were fixed in formalin at least 24 hours before sections were taken. Nine representative slices of tissue were taken from each dogs. Slides were prepared from paraffin embedding blocks and stained with hematoxylin and eosin.

The severity of rejection was graded from grade 0 to grade 4, by a working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection : lung rejection study group. No significant abnormality is in grade 0. Grade 1(minimal rejection) is characterized by very occasional small perivascular lymphocyte aggregates. Grade 2 (mild rejection) is reflected by more numerous perivsascular aggregates that usually are larger in size than those of minimal rejection. There may be an associated lymphocytic bronchiolitis. In grade 3(moderate rejection), both vessels and bronchioles are involved but, in addition, the lymphocytic infiltrate extends away from these structures into alveolar septae. These changes are even more marked in severe rejection(grade 4) where, in addition, alveolar damage and alveolar exudates appear[11)12)].

The highest rejection grade among nine slices was used for analysis. During grading, experienced pathologists were blinded to the origin of the tissue.

5. **Statistic Analysis**

All results were reported as the mean standard error of mean and analyzed by statistical program Microsoft Excel and GraphPad Prism. Plasma nitric oxide level of day 9 was compared to that of day 0 in each group by paired t-test. Correlation between plasma nitric oxide level and the pathological grades was analyzed by Spearmans rank correlation test. Correlation coefficient(Spearman's coefficient *r*) and P value were calculated. P value was regarded as significant when less than 0.05.

Results

In group 2(Rejection), plasma nitric oxide level of day 9 was elevated significantly, compared to that of day 0(12.28±2.10 vs 6.46 0.57μM/L ; $p<0.05$) (Table 1 and Fig. 1).

Eleven dogs of group 2 were pathological grade 2 and four dogs were grade 3 (Table 2). But plasma ni-

Table 1. Plasma nitric oxide concentration in day 0 and 9(μM/L)

	Day 0	Day 9
Immunosuppression(n=4)	7.52±2.39	7.25±0.51
Rejection(n=15)	6.46±0.57	12.28±2.10*

*In group 2(Rejection), plasma nitric oxide of day 9 was elevated significantly, compared to day 0($p<0.05$). At day 9, plasma nitric oxide of group 2(Rejection) was elevated significantly, compared to group 1(Immunosuppression) ($p<0.05$)

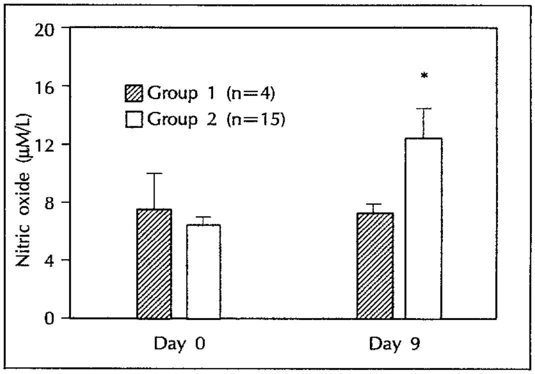

Fig. 1. Plasma nitric oxide in day 0 and 9.
*In Group 2(rejection), nitric oxide of day 9 was elevated compared to day 0($p<0.05$) ; at day 9, nitric oxide was elevated in group 2 compared to group 1(immunosuppression : $p<0.05$)

Table 2. Plasma nitric oxide and grades of rejection

No. of dogs	Plasma nitric oxide(μM/L)	Grades of rejection
1	15.38	2
2	7.94	2
3	15.91	2
4	23.36	2
5	7.07	2
6	5.04	2
7	5.92	2
8	8.72	2
9	33.91	2
10	20.20	2
11	8.87	2
12	9.56	2
13	6.94	2
14	6.21	3
15	9.14	3

Correlation coefficient(Spearman's $r=-0.2094$; $p>0.05$)

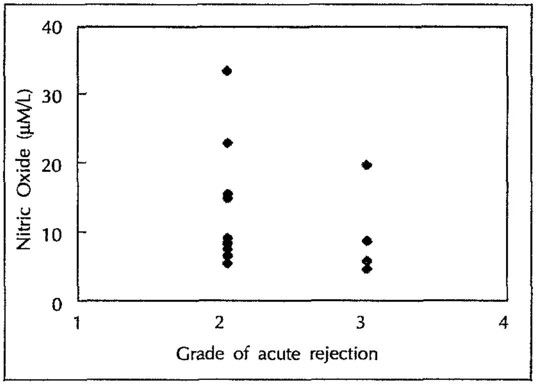

Fig. 2. Plasma nitric oxide and grades of rejection in group 2(rejection) ($r = -0.2094$; $p > 0.05$)

tric oxide levels didn't significantly correlated with the pathological grades of rejection(Spearman's coefficient $r = -0.2094$; $p > 0.05$) (Fig. 2).

Discussion

Nitric oxide is a short-lived molecule which has a various physiologic and pathophisiologic functions including endothelium-derived vasodilatation, inhibition of platelet aggregation, inhibition of leukocyte adhesion, macrophage-induced cytotoxicity and signal transduction in brain[13][14][15][16][17][18].

In organ transplantation of rat, serum $NO_2^- + NO_3^-$ level was elevated in early rejection period and further increased when the rejection became clinically apparent. Administration of immunosuppressive therapy such as cyclosporine or FK506, reduced the serum $NO_2^- + NO_3^-$ level to control level. When this therapy was discontinued and rejection recurred, the serum $NO_2^- + NO_3^-$ level was elevated again[19]. A similar observation was made with chronic rejection of small bowel graft[20]. Plasma level of nitric oxide was elevated during acute rejection after allograft of liver, small bowel and heart in rat or human[6][7]. During acute rejection after lung allograft, plasma nitric oxide level was also elevated and can be a used as a marker for acute rejection[21][22].

During acute rejection, cytokines was produced by T lymphocyte and particularly interferon gamma (IFNg) initiated macrophage nitric oxide synthesis. Expression of iNOS was increased in activated macrophages[23] This nitric oxide was cytotoxic to grafted cells by its effect on iron-containing protein function[24][25].

Paradoxically increased production of nitric oxide may have beneficial effect to grafted organ by increased perfusion. Nitric oxide induces vasodilation and inhibition of platelet aggregation and leukocyte adhesion in grafted organ.

In July 1990, the International Society of Heart Transplantation sponsored a meeting in which pathologists from seven institutions(Lung Rejection Study Group) participated in creating a working formulation for the standardization of nomenclature in the diagnosis of pulmonary rejection. This formulation was used for the evaluation of pathological severity in acute rejection[11][12].

If there is good correlation between plasma nitric oxide level and the pathological grades of acute rejection, both will be a powerful indicator for diagnosis of acute rejection in the term of sensitivity and specificity. But this study failed to show any correlation. The reason why plasma nitric oxide level didn't correlate with the grade of rejection is not clear. If plasma nitric oxide level is elevated transiently during the initial stage of rejection process, which was partially observed by unpublished author's own data, further study for continuous dynamic measurement of plasma nitric oxide may explain this result.

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황견에서 동종 폐이식후에 합병된 거부반응시 병리조직학적 소견과 혈중 Nitric Oxide농도와의 상관관계

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박 영 식

= 국 문 초 록 =

본 연구는 황견에서 동종 폐이식을 시행한 후에 합병된 거부반응시의 혈중 Nitric oxide농도와 병리조직학적 소견과의 상관관계를 보기위하여 시행되었다.

황견에서 일측 동종 폐 이식을 시행한 뒤, 면역억제제를 투여하였다. 실험견을 두군으로 분리하여 제1군(대조군 ; 황견 4마리)은 정상적으로 면역억제제를 투여하였다. 제2군(거부반응군 ; 황견 15마리)은 수술후 5일부터 면역억제제의 투여를 중단하여 급성 거부반응을 유도하였다. 혈중 Nitric oxide는 수술전과 수술후 9일에 각군에서 각각 측정하여 비교하였다. 수술후 9일에 실험견을 희생시켜 병리조직학적 검사를 시행하여 급성 거부반응의 등급을 Grade 0-4로 분류하였다. 제2군에서 수술후 9일의 혈중 Nitric oxide농도와 거부반응의 병리조직학적 등급과의 상관관계를 검사하였다.

제2군(거부반응군)에서 수술후 9일의 혈중 Nitric oxide농도가 수술전에 비하여 의의있게 증가되었다(12.28±2.10 vs 6.46±0.57μM/L ; $p<0.05$). 수술후 9일에, 제2군(거부반응군)의 Nitric oxide농도가 제1군(대조군)에 비하여 의의있게 증가되었다(12.28±2.10 vs 7.25±0.51μM/L; $p<0.05$). 그러나 제2군(거부반응군)에서 수술후 9일의 Nitric oxide농도와 거부반응의 병리조직학적 등급과의 상관관계는 유의하지 않았다(상관계수 $r=-0.2094$; $p>0.05$).