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Nitrotyrosine Formation and its Role in Various Pathological Conditions

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The formation of peroxynitrite and nitrotyrosine was examined in a variety of *in vitro* and *in vivo* animal models and its relation to cell or tissue damage was examined. In polymorphonuclear leukocyte (PMN)-induced injury to cardiac myocytes or endothelial cells, activated PMN produced peroxynitrite. Peroxynitrite appears to be responsible for the injury but it was not a major mediator of endothelial cell injury. In the experiment of ischemia-reperfusion injury of the rat brain nitrotyrosine was formed in the peri-infarct and core-of infarct regions. The degradation curve of nitrotyrosine revealed that its t^1_2 was about 2.2 hours. In the radiation-induced lung injury of rats, nitrotyrosine was also formed but it was not the sole mechanism for the injury. Levels of nitrotyrosine correlated with the severity of myocardial dysfunction in the canine model of cytokine-induced cardiac injury. Inhibition of NO generation abolished the formation of peroxynitrite and nitrotyrosine in all experiments. In conclusion; although nitrotyrosine is formed in a variety of pathological conditions where the generation of NO is increased, its presence does not always correlate with the severity of injury.

Keywords: Nitrotyrosine, Superoxide, Polymorphonuclear leukocyte, Ischemia reperfusion, Radiation injury

INTRODUCTION

Through the epoch-making[1] [2] discovery of nitric oxide (NO) as a biological signaling molecule, research in free radical-induced tissue injury has broadened to include NO-induced injury since NO is also a free radical. Various studies have showen that NOS inhibitors are capable of reducing tissue injury using animal models[3] [4], which have elucidated the cytotoxic nature of NO. However the studies reported here demonstrated that, in some pathological conditions, particularly in the case of inflammation, the mediator of cytotoxicity is not NO itself but, rather, peroxynitrite ($ONOO^-$)[5]. $ONOO^-$, formed from NO and $O_2\cdot^-$, is highly reactive with respect to protein thiol groups, tyrosine residues and phospholipids[6] [7] [8] [9], and its biological role was initially described by Beckman J et al as the culprit responsible for the nitration of tyrosine residues to form nitrotyrosine[10]. Thus in addition to lipid peroxidation reactions via superoxide ($O_2\cdot^-$) and hydroxyl radicals, nitra-

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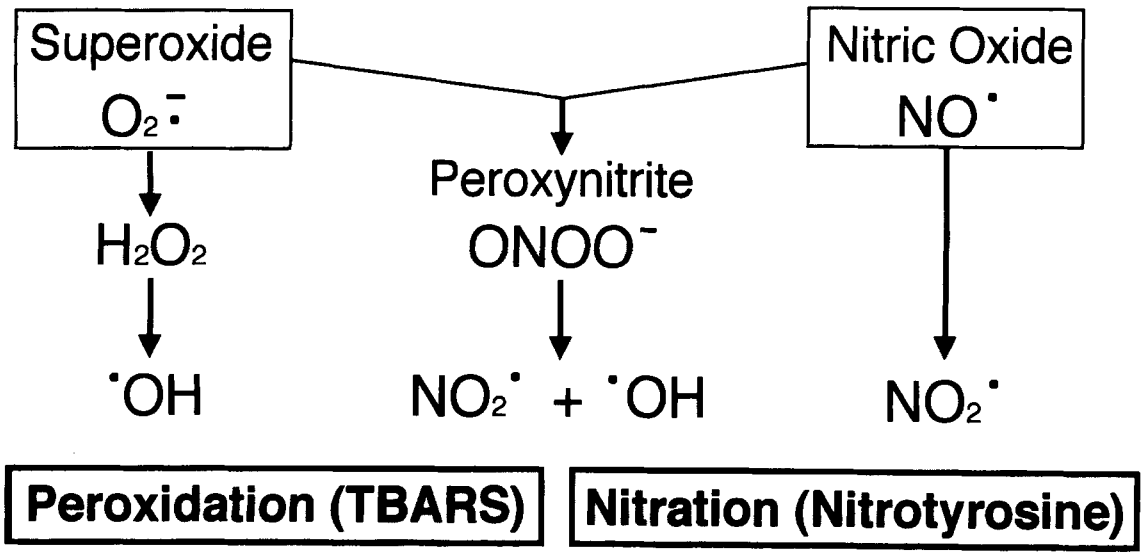


FIGURE 1 Peroxidation and Nitration Reactions by Free Radicals Hydroxyl radical (· OH) derived from superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) induces peroxidation. NO and NO-derived active molecules can induce nitration as well as peroxidation. Peroxynitrite ($ONOO^-$), a reaction product of O_2^- and NO is also able to induce nitration and peroxidation. $ONOO^-$ has high affinity to molecule with phenol residue such as tyrosine, which yields nitrotyrosine

tion reactions via NO and $ONOO^-$ can be included as free radical reactions (Fig. 1). Furthermore current findings relative to nitrotyrosine formation by my eloperoxidase dependent pathways[11] [12] in addition to $ONOO^-$ pathway shed light on the pathological roles of nitration reaction. The existence of nitrotyrosine in pathological conditions has been demonstrated in various lesions such as inflammation focus[13], atherosclerotic plaque[14] and ischemia-reperfused tissue[15]. However the issue of whether or not the activity of the protein is affected when its tyrosine residues are nitrated has not been clarified. In addition, the relationship between tyrosine nitration and impairment of cell or organ function has not been clearly demonstrated.

Thus the purpose of this paper is to demonstrate the formation of nitrotyrosine and its relation to cell or tissue damage in several pathological conditions. For this purpose a variety of *in vitro* and *in vivo* animal models were used including polymorphonuclear leukocyte-induced injury to cardiac myocytes or endothelial cells, ischemia-reperfusion injury of the brain, radiation-induced lung injury, and cytokine-induced myocardial dysfunction.

The animals in this study were maintained and used according to both National Institutes of Health Guidelines for Laboratory Animal Care and Animal Care Protocol of Tokai University. The protocols of all experiments were approved by the Committee of Tokai University.

(1) INJURY OF CARDIAC MYOCYTES AND ENDOTHELIAL CELLS BY POLYMORPHONUCLEAR LEUKOCYTE (PMN)[16]

We investigated the issue of whether nitrotyrosine is formed in cardiac myocytes or endothelial cells when these are cocultured with activated human PMNs. The experiment is based on the following two pieces of evidence; 1) PMN is one

of the major cytotoxic effectors in myocardial cells or endothelial cell injury including myocardial infarction or my ocarditis and 2) we previously demonstrated that human PMN is capable of producing $O_2^{\cdot -}$, NO and $ONOO^-$ as well[17] when these are activated by phorbol myristate acetate (PMA).

Method

Cardiac cells in monolayer were prepared from mouse embryo hearts as described previously[18]. Cardiac myocytes were cocultured with PMA-activated human PMN (effector-to-target ratio, 10:1). LDH release from myocytes was measured, to evaluate cell injury. Bovine aortic endothelial cells (BAEC) were isolated from aorta which were freshly obtained from a slaughterhouse as described previously[19]. Cultures from passages 6–12 were used for the study. ^{51}Cr loaded BAEC by preincubation were cocultured with PMA-activated human PMN (effector-to-target ratio, 10:1) using the same protocol with that of cardiac myocytes and injury to BACE was evaluated at intervals by a ^{51}Cr release assay. The levels of NO, $O_2^{\cdot -}$, $ONOO^-$ and nitrotyrosine were measured, and the influence of NO synthase inhibitor, $O_2^{\cdot -}$ and hydroxyl radical scavengers and other effectors was examined.

The formation of nitrotyrosine was measured by HPLC analysis. Briefly, cardiac my ocytes or BAEC which had been cocultured with activated PMN for 2 h in buffered saline with 1 mM Fe^{3+}/EDTA were hydrolyzed with 6 N HCl for 24 h at 110°C under vacuum and then centrifuged at 10000 rpm for 30 min. The supernatants were analyzed by HPLC with a C18 nucleosil column (JASCO Corp., Tokyo, Japan). The eluent was 0.5 M KH_2PO_4-H_3PO_4(pH 3.0) with 10% methanol (v/v) at a flow rate of 1 ml/min. The UV absorbance of the effluent was monitored at 274 nm[12] [20]. The peak was identified on the basis of the retention time of authentic 3-nitro-L-tyrosine or tyrosine. The results are expressed as the percentage ratio of nitrotyrosine to tyrosine.

Results and discussion

Figure 2 shows LDH release from cardiac myocytes cocultured with PMN. LDH release was expressed as a percentage of released LDH to total LDH in myocytes. The LDH release with non-activated PMN during the observation period remained very low. LDH release was increased in a time-dependent manner by the exposure to PMA-stimulated PMN, to 21.4 % and 38.6 % at 3 and 4 hours, respectively. By the treatments of SOD and L-NMMA, a NOS inhibitor, respectively ($P<0.001$ vs PMA) at the time point of 3 hours. LDH releases were reduced to 14.3 % and 9.4 %

Fig. 3 shows, the ^{51}Cr release from BAEC cocultured with PMN. For the loading of ^{51}Cr, BAEC were preincubated with $NA_2{}^{51}CrO_4$ (5μCi/well) for 20 hours and the ^{51}Cr release was expressed as a percentage of released ^{51}Cr to total ^{51}Cr in BAEC. ^{51}Cr release remained at a low level (< 4%) throughout the 4 hours observation period with non-activated PMN. In the case of BAEC cocultured with activated PMN, ^{51}Cr release was significantly increased (14.6 ± 2.2%, at 2 hours, $p<0.05$ and 42.6±2.7% at 4 hours, $p<0.01$). In the presence of SOD (100 U/ml), the release was reduced significantly at all time points (4.6 ± 1.2%, $p<0.05$; 11.4 ± 1.8%, $p<0.01$; 23.6 ± 2.1%, $p<0.01$ at 2, 3 and 4 hours, respectively). In contrast, L-NMMA (0.1 mM), an NOS inhibitor[21], potentiated the ^{51}Cr release at all time points (30.6 ± 3.8%, $p<0.01$; 45.3 ± 3.1%, $p<0.01$; 53.8 ± 2.8%, $p<0.05$ at 2, 3 and 4 hours, respectively). This L-NMMA-potentiated ^{51}Cr release was completely blocked by pretreatment of BAEC with a monoclonal antibody directed against the adhesion molecule CD18 (4.1 ± 1.4%, $p<0.05$; 3.3 ± 1.1%, $p<0.01$; 8.6 ± 2.8%, $p<0.01$ vs L-NMMA at 2, 3 and 4 h, respectively), which reduced the release to about the level seen with

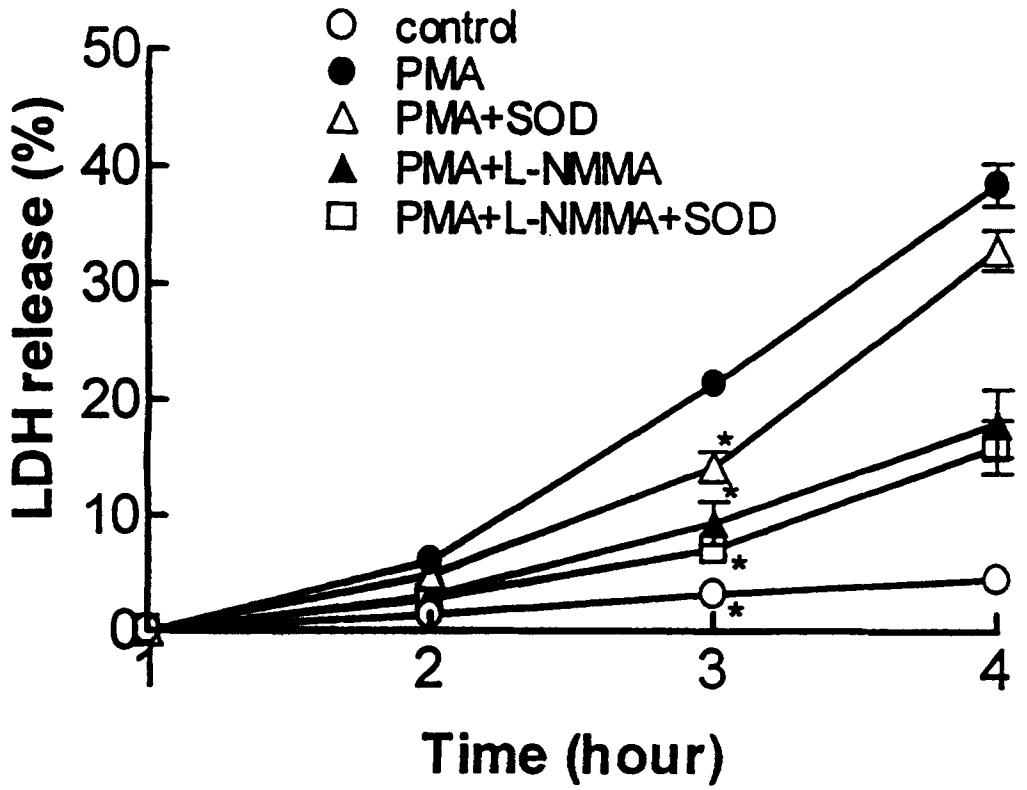


FIGURE 2 Effects of SOD and/or L-NMMA on LDH Release from Cardiac Myocytes Cocultured with PMN. Data are presented as mean ± S.E. (n=7–13 for each time point in each group). ○, control; ●, PMA; △, PMA+SOD; ▲, PMA+L-NMMA; □, PMN+L-NMMA+SOD; * $p<0.05$, compared with the control

non-activated PMN. The anti-CD18 mAb pretreatment also reduced the level of ^{51}Cr release to about the same level in the absence of L-NMMA.

Fig. 4 shows the formation of nitrotyrosine in cardiac myocytes (A) and in BAEC (B) cocultured with PMN. Nitrotyrosine formations in cardiac myocytes or BAEC were measured at the 2 hour time point (Fig. 4, A and B). Nitrotyrosine level in myocytes remained very low when cocultured with non-activated PMNs but reached high levels when cocultured with activated PMNs (0.39 ± 0.12 %). In the presence of SOD or L-NMMA it was attenuated to 0.32 ± 0.07 % and 0.10 ± 0.08 %, respectively. Contrary to cardiac myocytes in the BAEC experiments, nitrotyrosine formation was low in BAEC alone (0.10 ± 0.10%), and remained at the same low level both in the case of BAEC cocultured with non-activated PMA (0.125 ± 0.05 %) or with PMA-activated PMN (0.125 ± 0.08%).

The finding of significant nitrotyrosine formation and the attenuation of injury by the NOS inhibitor, as well as by SOD in cardiac myocytes suggests that $ONOO^-$ is involved in PMN-induced cardiac myocyte injury. However, in the BAEC experiment the NOS inhibitor potentiated BAEC injury, and nitrotyrosine was not formed at detectable levels. This indicates that $ONOO^-$ did not contribute substantially to BAEC injury. This was the opposite of our initial hypothesis, namely that $ONOO^-$ is the major cytotoxic species in the BAEC and PMN coculture systems, since BAEC themselves can gener-

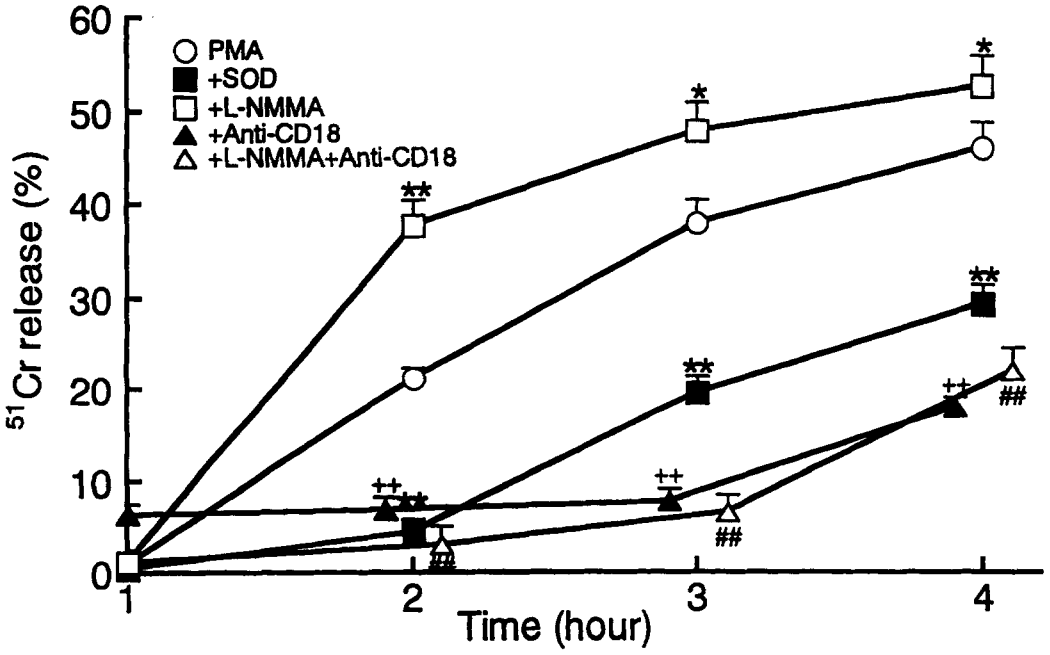


FIGURE 3 Effects of SOD, L-NMMA, or Anti-CD18 mAb on ^{51}Cr Release from BACE Cocultured with PMN[16]. Data are presented as mean±S.E. (n=7–13 for each time point in each group). ○, PMA; ■, +SOD; □, +L-NMMA; ▲, +Anti-CD_{18}; △, +L-NMMA+Anti-CD_{18}; # $p<0.05$, # # $p<0.01$ compared with the nonactivated PMN (◊). * $p<0.05$, * * $p<0.01$ compared with the PMN+PMA group (○). + $p<0.05$, + + $p<0.01$ compared with the L-NMMA group (□)

ate NO, and may result in a greater production of $ONOO^-$ than in the myocytes system. To explain the lack of nitrotyrosine formation in BAEC, despite the fact that PMN produces $ONOO^-$, two mechanisms can be considered. First, the intracellular concentration of tyrosine residues may be lower in BAEC than in cardiac myocytes. Secondly, $ONOO^-$ may be scavenged more efficiently in BAEC than in cardiac myocytes. The first possibility can be ruled out, since the addition of synthesized $ONOO^-$ to BAEC resulted in the formation of nitrotyrosine (1.70 ± 0.33%). The amount of nitrotyrosine in BAEC was even higher than that of the cardiac myocytes exposed to the same concentration of $ONOO^-$(data not shown). The second explanation is more likely since Szabo and Salzman showed that uric acid is a scavenger of $ONOO^-$ [22] and that uric acid-synthesizing enzymes such as xanthine dehydrogenase and oxidase are present at high concentrations in endothelial cells.

The aggravation of BAEC injury by NOS inhibitors can be explained by the anti-adhesion effect of NO[23,24] PMN-mediated endothelial cell injury is highly adhesion-dependent and NO is a well-known endogenous inhibitor of PMN adherence. This is supported by the results obtained with anti-CD18 mAb, an anti-adhesion molecule mAb: ^{51}Cr release was completely inhibited by anti-CD18 mAb, and in its presence, addition of NOS inhibitors did not aggravate the injury to a measurable extent (Fig. 3).

Overall, the data presented herein suggest that, although $ONOO^-$ is generated by activated PMN and appears to responsible for cardiac myocyte injury, it is not a major mediator of BAEC injury.

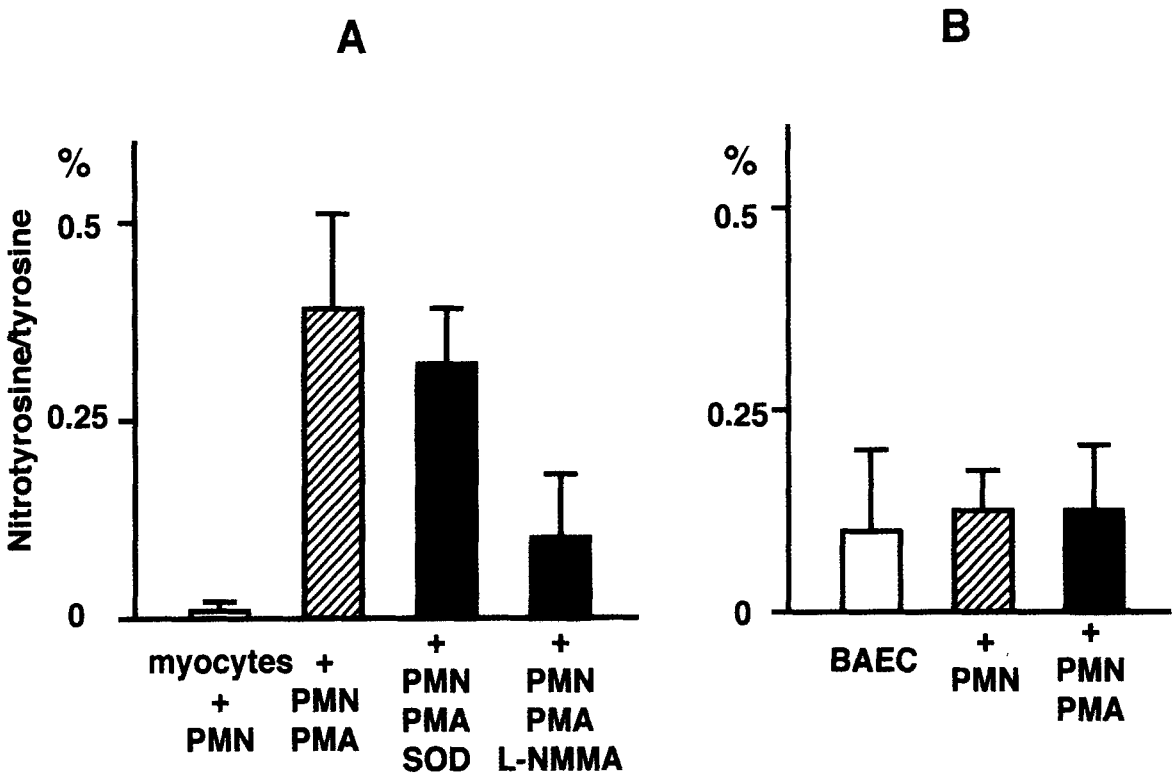


FIGURE 4 PMN-Induced Tyrosine Nitration in Cardiac Myocytes (A) and in BAEC (B)[16]. Tyrosine nitration was monitored by measuring cellular concentrations of tyrosine and nitrotyrosine and is expressed as the ratio of NO2-tyrosine/tyrosine. Data are expressed as mean±S.E. of four experiments. $^{**}P<0.01$ compared with other groups

(2) NITROTYROSINE FORMATION AND ITS DEGRADATION RATE ON FOCAL BRAIN ISCHEMIA-REPERFUSION INJURY[25] [26]

In brain ischemia or ischemia followed by reperfusion, NO generation from neuronal NOS has been shown to increase and to play an important role in brain injury [27–29]. Thus in this study the primary purpose was to examine the issue of whether $ONOO^-$ is formed in brain subjected to focal ischemia/reperfusion injury using rats. The secondary purpose was to clarify the dynamics of nitrotyrosine formation and elimination by examining the half-life of nitrotyrosine. This was achieved via sequential measurements of nitrotyrosine in the brain after blocking the further formation of nitrotyrosine with L-NMMA in experimental condition where the rise of nitrotyrosine was already demonstrated.

Method and discussion

Male Sprague-Dawley rats, weighing approximately 300 to 350 g were anesthetized with halothane. Transient focal cerebral ischemia was induced by occluding the right middle cerebral artery for 2 hours and reopening it with a permanent ligation of the right common carotid artery. For the primary purpose, animals were divided into five groups: group 1 (n=6), 2-hour ischemia in which the rats were killed at 2 hours after the occlusion; group 2 (n=7), 2-hour is chemia/3-hour reperfusion in which the rats were killed at 3

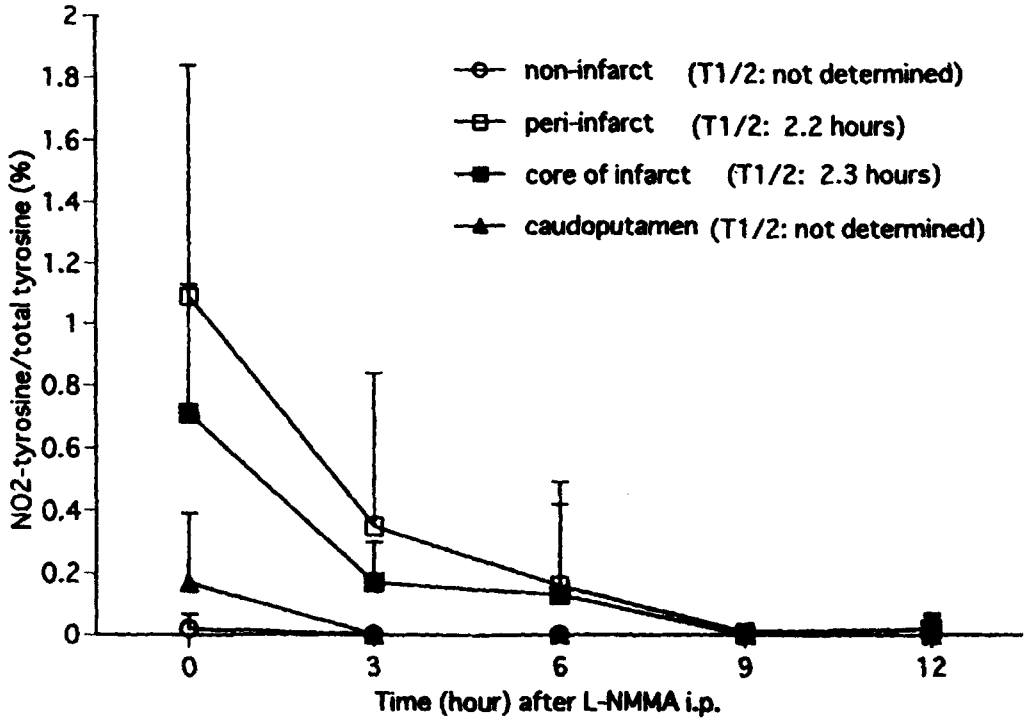


FIGURE 5 The Degradation of Accumulated Nitrotyrosine in the Brain[25]

hours after the release of the left MCA; group 3 (n=6), 2-hour ischemia/3-hour reperfusion with administration of L-NMMA, (50 mg/kg intrap eritoneally) at 30 minutes before occlusion; group 4 (n=6), and group 5 (n=6), sham-operated. Regional Cerebral blood flow (CBF) was measured during ischemia and reperfusion using different sets of rats. For the secondary purpose, L-NMMA was administered at 24 h after the start of reperfusion to halt the further production of nitrotyrosine. Nitrotyrosine was then measured at 0, 3, 6, 9 and 12 hours (n=5 in each group) to construct decay curves of nitrotyrosine.

Result and discussion

Percentage of nitrotyrosine total tyrosine in group 1 were 0% in the case of the noninfarct region, 0.42 ± 0.135. in the periinfarct region, and 0.29 ± 0.10% in the core-of-infarct region. The periinfarct and core-of-infarct regions showed significantly higher nitrotyrosine levels than the noninfarct region ($P<0.05$), but there was no significant difference between the periinfarct and core-of-infarct regions. In group 2, the levels of nitrotyrosine in the periinfarct region were 0.89% ± 0.22% which was significantly higher than that in the core-of-infarct region (0.35% ± 0.09%). Further, nitrotyrosine in the periinfarct region was significantly increased in group 2 compared with group 1.

Fig 5 demonstrates the decrease in nitrotyrosine formed in the four regions at 24 hours of reperfusion as has already been observed when sufficient amounts of nitrotyrosine are present to examine its decay rate.

The nitrotyrosine levels In all four regions declined with time when 50 mg/kg of L-NMMA was injected at 24 hours at the start of reperfusion to halt its further production; the limit of

detection was reached within 3 hours in the noninfarct region and caudoputamen within 9 hours in the periinfarct and the core-of-infarct regions. The degradation curves for nitrotyrosine in the periinfarct and the core-of-infarct regions were fitted by a nonlinear regression method ($y=2.91 \times e^{(-x/4.03)}$ and $y=2.91 \times e^{(-x/6.77)}$, and the $T_{1/2}$ values of nitrotyrosine were obtained as 2.2 and 2.3 hours, respectively. The $T_{1/2}$ values of nitrotyrosine in the noninfarct region and caudoputamen could not be determined because the values were too low to be fitted reliably to a nonlinear regression equation.

In this model we were again able to observe the formation of nitrotyrosine and the inhibition of its generation by a NOS inhibitor. The degradation rate of already formed nitrotyrosine was then examined. Although the formation rate of nitrotyrosine has already been determined (Ischiropoulos et al., 1992[30] [31]), the degradation rate of nitrotyrosine has not been evaluated. Based on the finding that the L-NMMA at a dose of 50 mg/kg completely inhibited nitrotyrosine formation in rat brain during ischemia-reperfusion[25], we measured the decay rate of nitrotyrosine after the administration of L-NMMA. The $T_{2/1}$ value of nitrotyrosine in both the periinfarct and and core-of-infarct regions was approximately 2 hours. Although the mechanisms of the decrease in nitrotyrosine were not examined in this study, a novel protein called "denitrase" may have played a major role. The presence of an activity that modifies nitrotyrosine-containing BSA and other nitrated proteins was detected in rat spleen and lung extracts[32–34]. Since the activity of denitrase was labile to heat and trypsin treatment, and increased with endotoxin treatment, it appears to be an inducible enzyme. The present information on the rate of degradation nitrotyrosine is helpful in analyzing the pathophysiology and devising therapeutic strategies so that the injured region can be rescued, although actual the values may differ in different tissue or organs which continue to be a target of future study.

(3) RADIATION-INDUCED ACUTE LUNG INJURY[35]

Irradiation is one of the established therapies for chest malignancies, but the risk of radiation pneumonitis and subsequent induction of fibrosis limits the dose of irradiation or may even contraindicate its application. The currently accepted mechanism of the injury is that irradiation stimulates alveolar macrophages or epithelial cells to produce various cytokines. One of an injurious pathway following cytokine production is proposed as an active oxygen-dependent one caused by the overproduction of sup eroxide ($O_2^{\cdot -}$) and nitric oxide (NO)[36–39]. However the responsible isoform of NOS and the detailed reaction of the active oxygen species remain to be clarified. In particular, the involvement of $ONOO^-$ or nitration reactions has not been examined yet. Thus we performed the following experiment using rats to investigate 1) which isoforms of NOS is responsible in irradiated lung injury, 2) whether nitrotyrosine is formed in the irradiated lung, and 3) if so, whether nitrotyrosine formation is responsible for the injury.

Experiment

Wistar rats aged 9–10 weeks were use (n=44). The left hemithorax of rats was irradiated with an absolute dose of 20 Gy in one fraction. The right hemithorax and other organs were shielded during irradiation and the right lung served as a control. Two weeks after the irradiation, the degree of lung injury was evaluated by histology, and Nuclear Magnetic Resonance (NMR) measurement of the lung. Protein concentration, LDH activity, and NO_2^-/NO_3^- in bronchoalveolar lavage fluid (BALF) were also measured as indices of the injury. The expression of NOS isoforms, and the formation of nitrotyrosine and $O_2^{\cdot -}$ production from BAL cells were examined. Nitrotyrosine in the tissue was detected biochemically by HPLC after homogenizing and hydrolyzing the tissue in 0.1% phenol

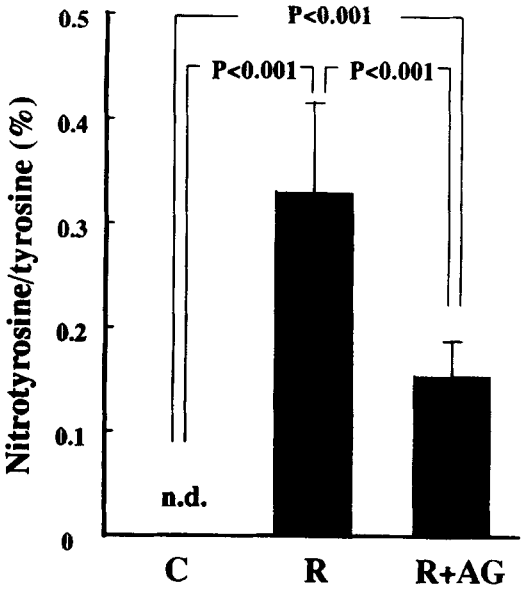


FIGURE 6 Nitrotyrosine Formation in the Lung. Values are mean±SD. C: control group (n=4), R; irradiated group (n=4) and R+AG: aminoguanidine-treated irradiated group(n=3), AG: aminoguanidine control group. Each group: n=4

containing 6N HCl using the same method as was used for the case of brain ischemia and by immuno-histochemical staining using a nitrotyrosine antibody. The experiment was performed in the following 4 groups: 1) control group, 2) radiation group, 3) radiation plus aminoguanidine, an iNOS specific NOS inhibitor group, and 4) an aminoguanidine group.

Results

At 2 weeks, the expression of iNOS mRNA was induced and endothelial NOS (eNOS) mRNA was markedly increased in the irradiated lung (data not shown). NO_2^- /NO_3^- in the BALF was increased (data not shown). Nitrotyrosine was detected both biochemically (Fig. 6) and immunohistochemically in the radiation group but nitrotyrosine staining was not observed in the control group (Fig. 7). Nitrotyrosine positive regions tended to coincide with sites of lung tissue injury, and were located in the vicinity of airways, alveolar epithelia and alveolar macrophages. In the radiation + aminoguanidine group, nitro tyrosine staining was not as marked as that in the radiation group. $O_2\cdot^-$ production from BAL cells was also significantly increased in the radiation group. All indices of acute lung injury were markedly improved as indicated by decreased protein concentration and LDH activity in BALF in the aminoguanidine treatment as shown in Fig 8. Histological changes relative to injury were almost negligible in the radiation + aminoguanidine group. The degree of attenuation in the injury by aminoguanidine was much higher compared with that of the decrease in the nitrotyrosine formation, since a nearly complete protection against injury was observed. This suggests that tyrosine nitration is not the only mechanism ending to injury and that the contribution of an $O_2\cdot^-$-mediated reaction is likely based on the persistent increase in $O_2\cdot^-$ production from BAL cells, as the result of aminoguanidine treatment.

(4) MYOCARDIAL NITROTYROSINE LEVEL IN DOGS[40] [26]

It has been concluded that inflammatory cytokines have been implicated to play an important role in the pathogenesis of inflammatory cardiovascular diseases, including acute my ocarditis. The purpose of this study was to elucidate the mechanisms of the cytokine-induced myocardial dysfunction in vivo. To achieve this, we developed a new canine model in which microspheres with IL-1β were selectively injected into the left main coronary artery to produce IL-1β-induced myocardial dysfunction[40] [26].

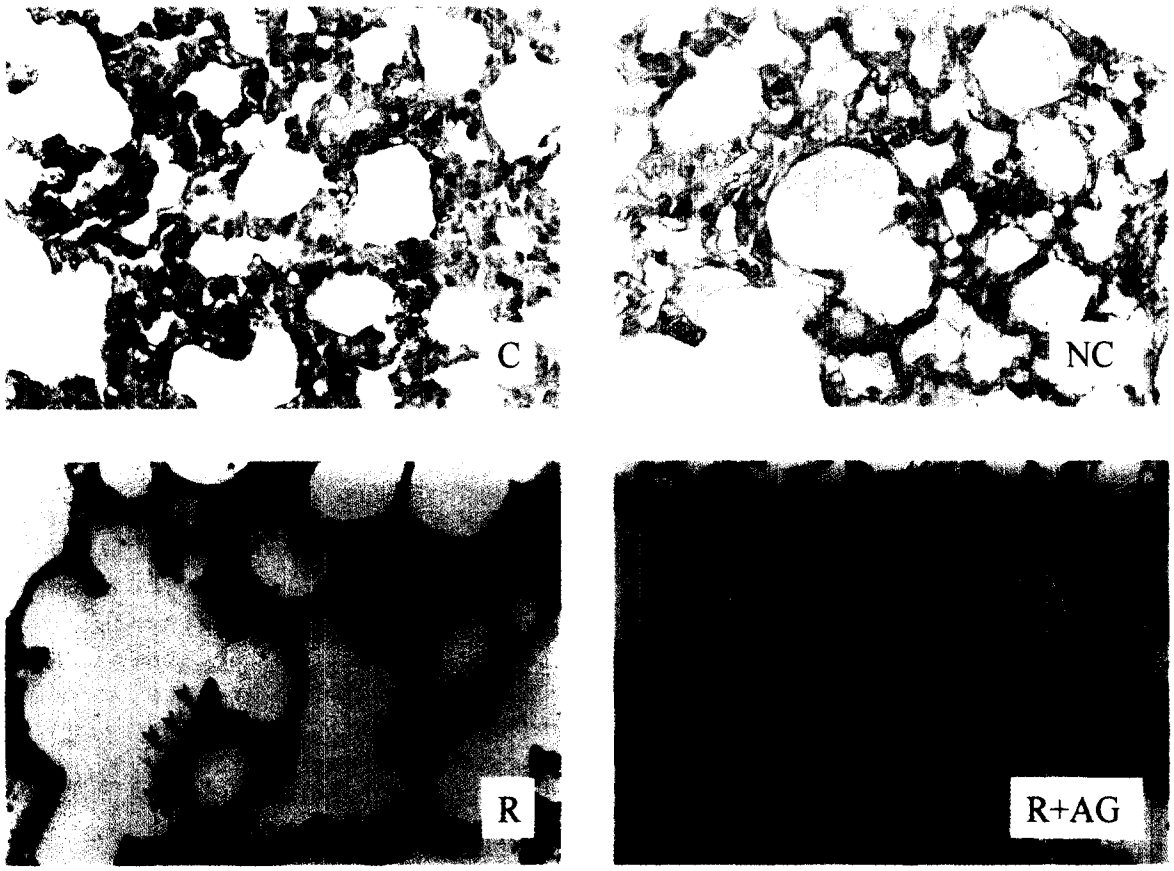
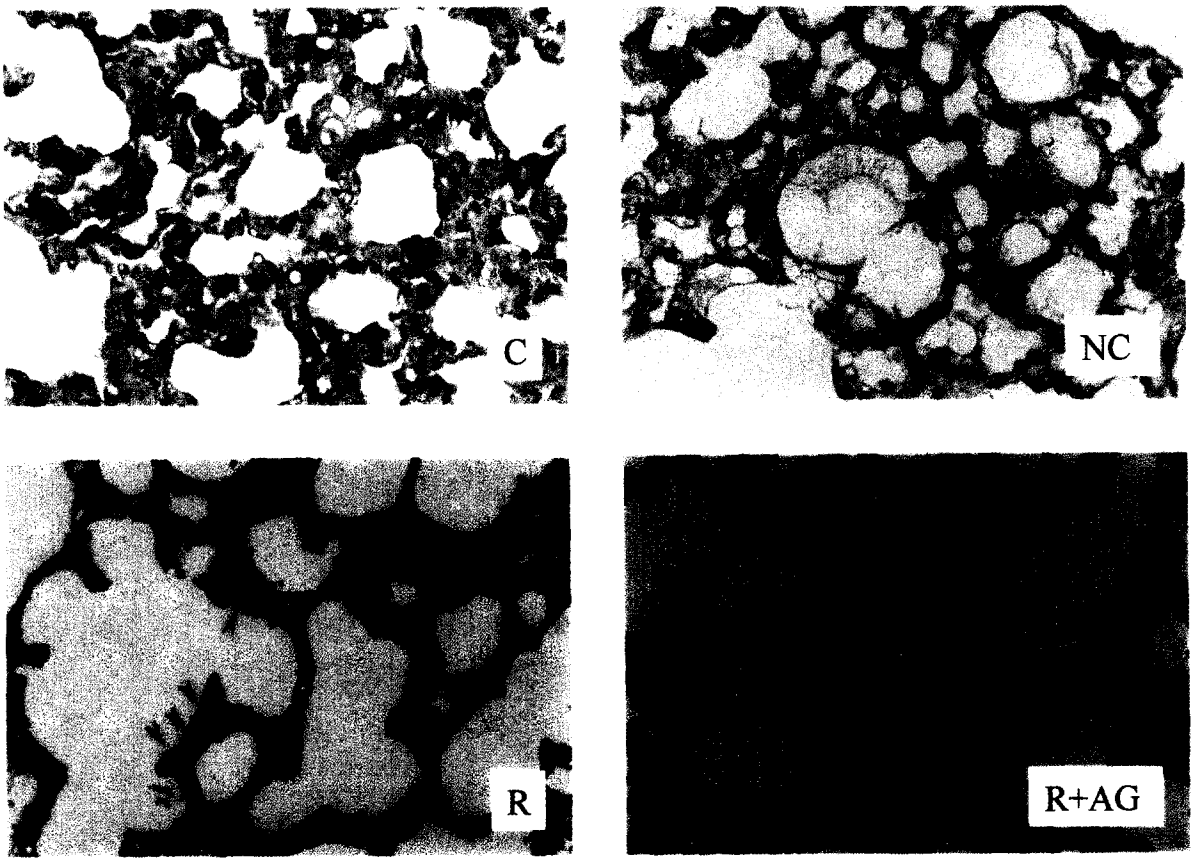


FIGURE 7 Immunohistochemical Detection of Nitrotyrosine[25]. Nitrotyrosine formation was demonstrated visualized as a brown color with the peroxidase-labeled second antibody by using the diaminobenzidene-peroxidase reaction. C; control, NC; negative control without the primary antibody. R; Radiation group, R+AG; Radiation + aminoguanidine, Arrows; alveolar epithelium and macrophages are positively stained for nitrotyrosine (see Color Plate I at the back of this issue)

Experiment

Adult mongrel dogs weighing 9–18 kg were sedated with ketamine hydrochloride and anesthetized with sodium pentobarbital (25 mg/kg intravenously) under mechanical ventilation. IL-1β-bound microspheres (MS) (15μm in diameter. 10^6 MS/kg) were injected into the main left coronary artery via the catheter inserted from the right carotid artery. Heart rate, systemic blood pressure and left ventricular end-diastolic pressure (LVEDP) were continuously measured for 7 days. The LV ejection fraction was evaluated by echo-cardiography at the indicated days. On day 2 or 7 animals were then sacrificed to obtain the myocardium. Nitrotyrosine formation in the myocardium was evaluated using the same HPLC method. Animals were divided into 4 groups: control (n=10), IL-1β group, human IL-1βconjugated MS was injected, IL-1β+DEX group, dexamethasone (3 mg/kg) was iv injected 1 hour before MS injection and continuously infused at a dose of 0.5 mg/kg/h until day 2, IL-1β+AG group; aminoguanidine was intravenously administered at a dose of 100 mg/kg before MS injection and continuously infused thereafter at a dose of 10 mg/kg/h until day 2.



Color Plate I (See page 780, Figure 7) Immunohistochemical Detection of Nitrotyrosine[25]. Nitrotyrosine formation was demonstrated visualized as a brown color with the peroxidase-labeled second antibody by using the diaminobenzidene-peroxidase reaction. C; control, NC; negative control without the primary antibody. R; Radiation group, R+AG; Radiation + aminoguanidine, Arrows; alveolar epithelium and macrophages are positively stained for nitrotyrosine

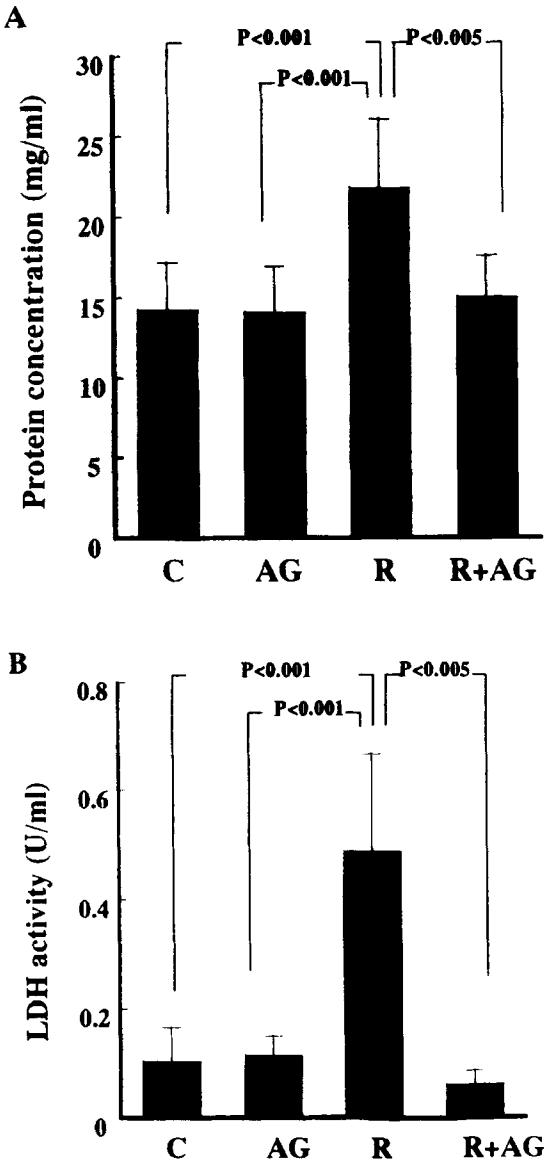


FIGURE 8 Protein Concentration (A) and LDH Activity (B) in BALF[25]. Values are mean±SD. Protein concentration was measured by modified Lowry method, and LDH activity was measured using an enzyme assay kit. Abbreviations are the same as in Fig. A. n. d. means not detected. Each group n=4

Result and discussion

Myocardial nitrotyrosine concentrations were significantly higher in the IL-1β group than in the control group on both day 2 and day 7. The treatment with dexamethasone or aminoguanidine prevented the IL-1β-induced tyrosine nitration determined on day 7. Their LV ejection fractions were compared, with respect to nitrotyrosine concentrations on day 7 (Fig. 9). A significant negative linear relationship between myocardial nitrotyrosine concentrations and LV ejection fraction was found suggesting a possible association between nitrotyrosine formation and cardiac dysfunction. NO significant increase in the myocardial nitrotyrosine concentrations from 2 to day 7 were detected, which suggests that during this period either no significant amount of nitrotyrosine was formed or, at the least, no significant increase in myocardial nitrotyrosine occurred. In this study, dexamethasone (which nonspecifically inhibits the synthesis of proteins including iNOS)[41]and aminoguanidine both prevented the IL-1β-induced sustained myocardial dysfunction and nitrotyrosine formation. These results indicate that NO, produced by iNOS, plays an important role in the pathogenesis of the cytokine-induced myocardial dysfunction and further, NO-derived $ONOO^-$ is responsible for the dysfunction, at least in part.

Summary

Extensive research work on NO and its reaction products over the last 10 years has led to a rapid change in the general concepts relating to free radical biochemistry. In parallel with these new concepts, the importance of free radical-induced redox modulation in cellular signal transduction systems has been recognized recently. These include, nitration, nitrosation and nitration-mediated and redox-mediated modulations. The finding of the presence of nitrotyrosine in 5 different pathological conditions indicates the direction of future research in this field.

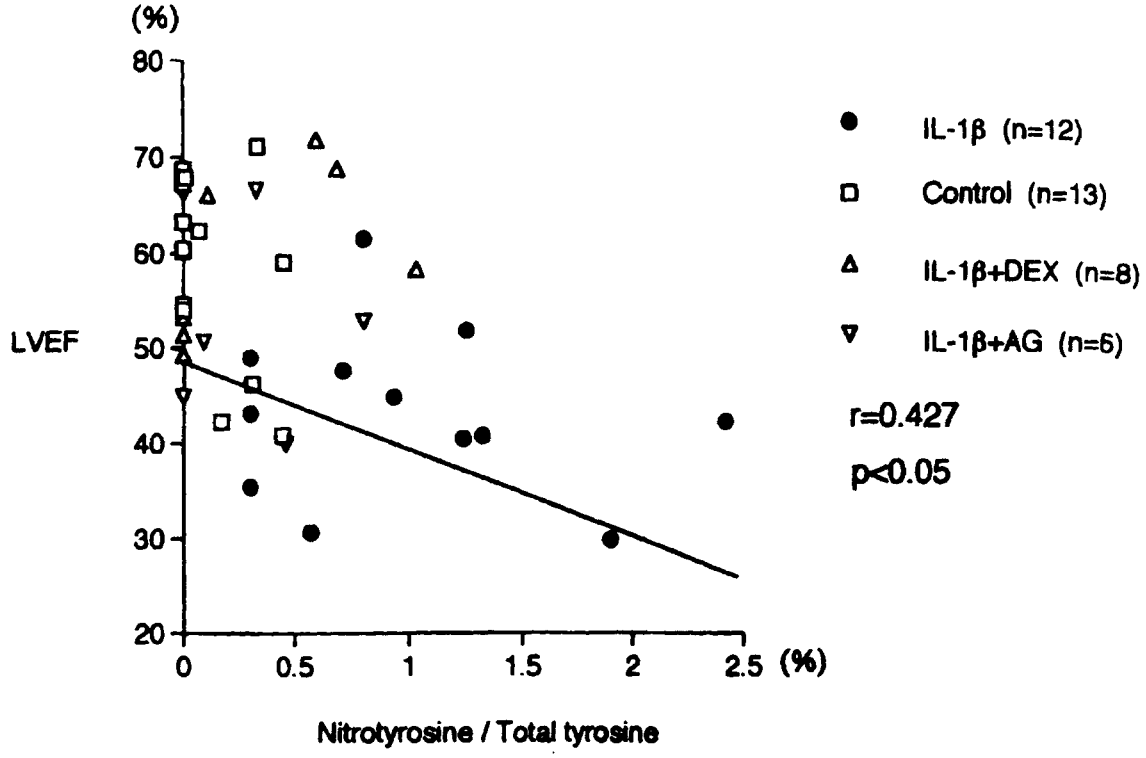


FIGURE 9 The relationship between Myocardial Nitrotyrosine Levels and LV Ejection Fraction[40]. An inverse linear relationship exists between myocardial nitrotyrosine levels and LV ejection fraction. Abbreviations are indicated in the text

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