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An unexpected interaction between N^G-nitro-L-arginine methyl ester and L-arginine in α-naphthylthiourea-induced pulmonary oedema in rats

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Abstract

This study was designed to investigate the possible participation of the L-arginine-nitric oxide (NO) pathway in the lung oedema induced by α-naphthylthiourea, which is a well-known noxious chemical agent in the lung. Lung oedema was assessed by measuring fluid accumulation in the pleural cavity and the lung weight/body weight ratio following α-naphthylthiourea injection. Administration of N^G-nitro-L-arginine methyl ester, a NO synthase inhibitor, prior to α-naphthylthiourea, produced a significant inhibition of pleural effusion and lung weight/body weight ratio in a dose-dependent manner. L-Arginine, but not D-arginine, when used higher doses (above 300 mg/kg) prior to α-naphthylthiourea injection caused a significant inhibition of pleural effusion without altering lung weight/body weight ratio. Lower doses of L-arginine (below 100 mg/kg) did not elicit an inhibitory effect against α-naphthylthiourea-induced pulmonary damage. However, lower doses of L-arginine greatly potentiated the inhibitory effect of N^G-nitro-L-arginine-methyl ester against α-naphthylthiourea-induced lung oedema when used in combination. The interesting aspect of this study is the inhibition by N^G-nitro-L-arginine methyl ester, a NO synthase inhibitor, and L-arginine, an endogenous donor of NO, of the lung oedema induced by α-naphthylthiourea. The possible role of the L-arginine-NO pathway in lung oedema induced by α-naphthylthiourea and the possible underlying mechanisms are discussed.

Keywords: α-Naphthylthiourea; Nitric oxide (NO); L-Arginine; D-Arginine; N^G-Nitro-L-arginine methyl ester; Pulmonary oedema

1. Introduction

α-naphthylthiourea is a chemical agent largely used to produce lung injury by causing prominent endothelial damage in the pulmonary vascular bed (Cunningham and Hurley, 1972; Meyrick et al., 1972). Such damage has been shown to be partially mediated through arachidonic acid metabolites (Pankhania and Bakhle, 1982; Ercan et al., 1993). Besides arachidonic acid metabolites, it has been speculated that some other vasoactive substances originating from the pulmonary vascular bed and airways may also contribute to the pulmonary oedema induced by α-naphthylthiourea. We have recently presented evidence indicating a lack of participation of endothelin peptides in this pathological event (Sipahi et al., 1996). The L-arginine-NO pathway may be considered another endothelium-derived system contributing to the lung oedema induced by α-naphthylthiourea.

NO, an endothelium-derived free radical, is synthetized from L-arginine by three isoforms of NO synthase (for review, see Gross and Wolin, 1995). Two of these isoforms, Ca^{2+}-dependent type I NO synthase and type III NO synthase, located in neuronal cells and endothelium respectively, produce relatively small quantities of NO and are constitutive in nature (Förstermann et al., 1993), Ca^{2+}-independent type II NO synthase, the inducible form causes the production of NO in high quantities in many cell types including macrophages (Marletta et al., 1988; Stuehr et al., 1989; Kilbourn et al., 1990), neutrophils (McCall et al.,

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1991; Kolls et al., 1994) and vascular smooth muscle (Busse and Mülsch, 1990; Gross and Levi, 1992). Recently, it has been shown that large quantities of NO may contribute to carrageenan-induced pleurisy in rats (Tracey et al., 1995).

The basic aim of this study was to investigate the involvement of the L-arginine-NO pathway in α-naphthylthiourea-induced pulmonary oedema in rats.

2. Materials and methods

2.1. Assessment of pulmonary oedema

Experiments were carried out on albino rats of either sex weighing 160–220 g obtained from the animal house of the Medical Faculty, Gazi University. They were housed under standard laboratory conditions with a 12 h light/12 h dark cycle and allowed free access to food and water. The procedures and protocols of the study were in accord with our institutional guideline, which is similar to 'Guide for the Care and Use of Laboratory Animals (US National Institute of Health, revised 1985)'.

During the experimental procedure, the animals were placed in separate cages and kept at room temperature (22°C). α-Naphthylthiourea (suspended in olive oil 4 mg/kg) was injected intraperitoneally (i.p.) at the dose of 10 mg/kg. The control group received the same volume of olive oil. Four hours later, the animals were anaesthetized with urethane (1.5 g/kg s.c.) and were bled by cutting the carotid artery. The thorax was opened and pleural effusion was carefully collected by suction and measured volumetrically. Care was also taken to eliminate blood contamination with pleural effusion. The lungs were removed, dissected from surrounding tissues and weighed with an analytical balance. The volume of pleural effusion (ml), the lung weight/body weight and pleural effusion/body weight ratios were calculated and considered as an index of pulmonary oedema.

2.2. Experimental protocol

The animals were divided into 9 groups. The first group of animals received only olive oil and the second group α-naphthylthiourea. Both groups were kept as controls. The third, fourth and fifth groups were injected with

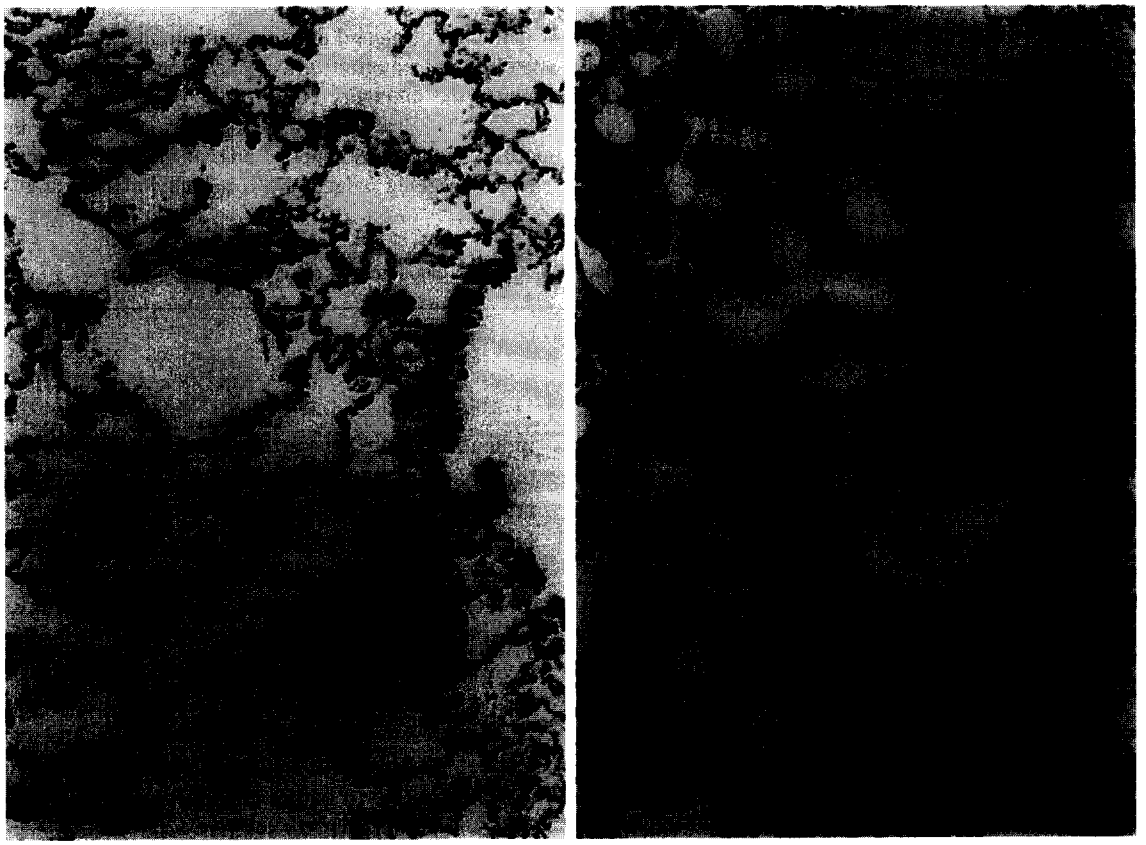

Fig. 1. (A) Normal histological appearance of olive oil-treated rat lungs. Section was taken from the middle lobe of right lung. Haematoxylin-eosin (H-E) stain ×200. (B) Prominent perivascular (indicated with upper and lower arrows), peribronchial oedema (middle arrow), thickening of alveolar septa and eosinophilic oedema fluid deposition after ANTU treatment (H-E × 400). Section was taken from the same place.

N^G-nitro-L-arginine methyl ester, an inhibitor of NO synthase (Moncada et al., 1988), at doses of 10, 20 and 40 mg/kg (i.p.) 15 min before α-naphthylthiourea injection. The sixth and seventh groups were injected with L-arginine (50 and 300 mg/kg s.c.) and the eighth group received L-arginine 50–75 mg/kg s.c. with N^G-nitro-L-arginine methyl ester 20 mg/kg i.p. simultaneously. The last group was injected with D-arginine s.c. prior to α-naphthylthiourea injection. All these treatments were made 15–30 min before α-naphthylthiourea injection.

2.3. Histological examination

For histopathological examination, the lungs were immersed in 10% formalin and allowed to fix for 2–3 days. All lobes of each lung were examined. 10 μm cross-sections were processed for standard haematoxylin and eosin staining (Sheehan and Hrapchak, 1973). These sections were then examined via a light microscope and photographed.

2.4. Drugs

The following drugs were used in this study: α-naphthylthiourea (Interchim) was suspended in olive oil (4 mg/kg); N^G-nitro-L-arginine methyl ester, L-arginine and D-arginine were purchased from Sigma and were dissolved in saline just before use.

2.5. Statistical analysis of results

Results were expressed as means ± S.E.M. Comparisons among multiple groups were evaluated non-parametrically by the Kruskal-Wallis methods (Gibbons, 1976). $P < 0.05$ was considered significant.

3. Results

3.1. Effect of α-naphthylthiourea on pulmonary vasculature

A significant lung oedema was observed 4 h after i.p. injection of α-naphthylthiourea at the dose of 10 mg/kg as indicated by an increase in lung weight/body weight ratio and pleural effusion when compared with olive oil-injected rats. Lung weight/body weight ratio was calculated as $103.6 \pm 4.4 \times 10^{-4}$ for α-naphthylthiourea-treated rats while it was found to be $58.2 \pm 2.8 \times 10^{-4}$ for olive oil-injected rats ($P < 0.001$). Although pleural effusion

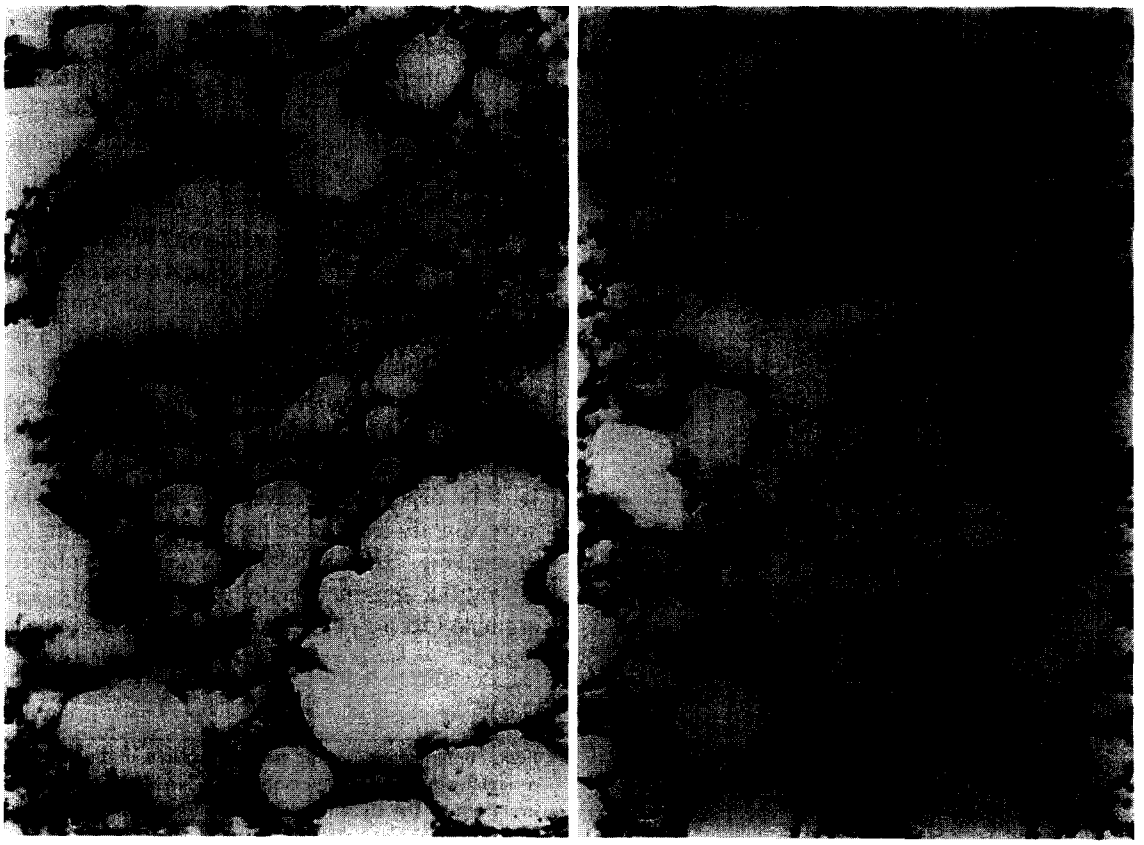

Fig. 2. (A) Reduced perivascular oedema and fluid deposition in some alveoli (indicated by both arrows) after pretreatment with L-NAME (20 mg/kg) of an α-naphthylthiourea-injected rat. Section was taken from the lower lobe of left lung (H-E × 400). (B) Section from the same place indicates that perivascular oedema is still visible (arrow) without intra alveolar fluid in L-NAME (40 mg/kg) pretreated ANTU-injected rats (H-E × 400).

was measured as 2.9 ± 0.3 ml in α-naphthylthiourea-treated rats, no detectable pleural effusion was observed in vehicle-injected rats (Fig. 4). On microscopic examination α-naphthylthiourea-treated rats were shown to have severe lung injury associated with perivascular, peribronchial, alveolar septal oedema, loss or destruction of interstitial cellular elements and deposition of eosinophilic oedema fluid in alveoli (Fig. 1B), while no change was observed in olive oil-treated rats (Fig. 1A).

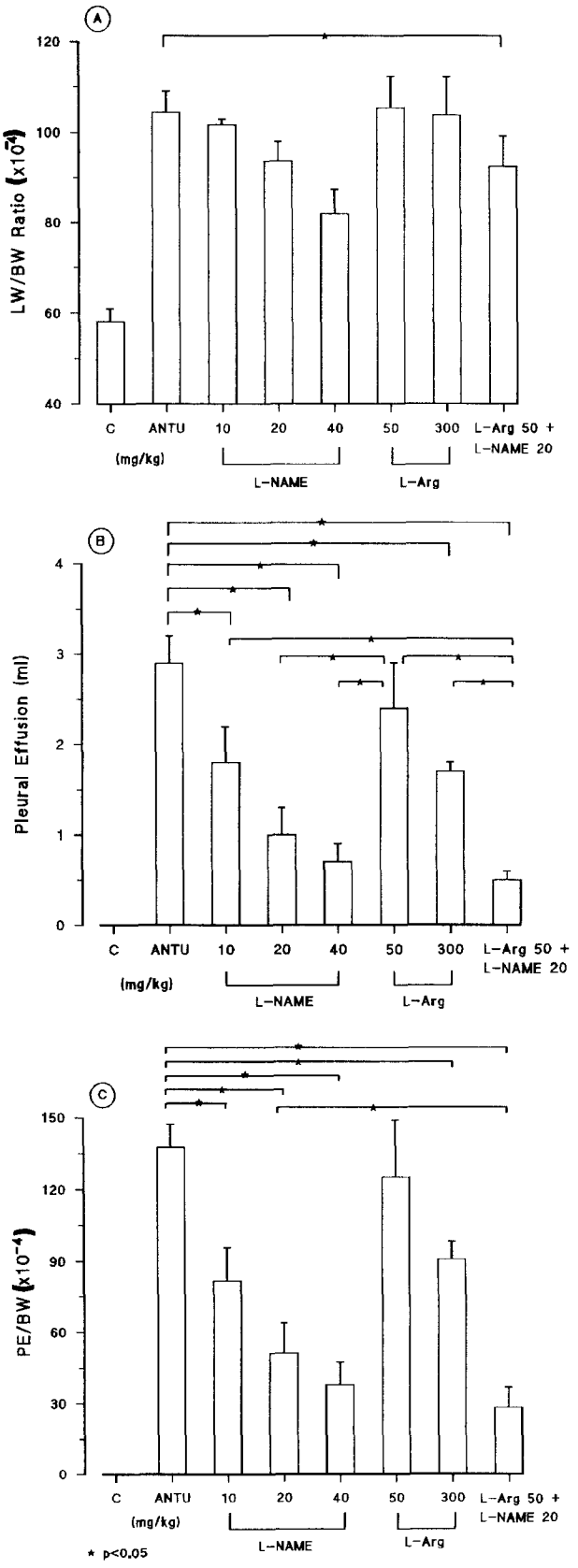

Fig. 3. Figure represents the calculated results of lung oedema induced by α-naphthylthiourea and alterations by L-NAME and L-arginine (L-Arg), as evaluated by the changes of lung weight/body weight (LW/BW) ratio (A), pleural effusion (PE) (B) and pleural effusion/body weight (PE/BW) ratio (C). Each column (except columns for olive oil control (C) ($n = 15$) and α-naphthylthiourea ($n = 22$)) shows the mean value of 10 experiments, vertical bars on the columns represent S.E.M.

3.2. The effect of N^G-nitro-L-arginine methyl ester on α-naphthylthiourea-induced oedema

Administration of N^G-nitro-L-arginine methyl ester caused an inhibition of pulmonary oedema and pleural effusion in a dose-dependent manner (Fig. 4). A reduction was observed in the destruction of interstitial cellular elements and alveolar septal oedema but still there was a slight deposition of oedema fluid in some alveoli in 20 mg/kg N^G-nitro-L-arginine methyl ester-treated rats (Fig. 2A). Although the reduction in oedema was more prominent in 40 mg/kg N^G-nitro-L-arginine methyl ester-treated rats, there was still some perivascular oedema observed microscopically (Fig. 2B).

3.3. Effects of L-arginine, D-arginine and combination with N^G-nitro-L-arginine methyl ester

Although L-arginine at the dose of 50 mg/kg did not change the increased lung weight/body weight ratio and pleural effusion induced by α-naphthylthiourea, it significantly potentiated the inhibitory effect of N^G-nitro-L-arginine methyl ester (20 mg/kg) on pulmonary oedema (Fig. 4). L-Arginine alone at a higher dose (300 mg/kg) caused a significant inhibition of the α-naphthylthiourea-induced increased pleural effusion (1.7 ± 0.1 ml), compared with α-naphthylthiourea-treated controls (2.9 ± 0.3 ml) ($P < 0.01$). Pleural effusion/body weight ratio was also significantly decreased (Fig. 4). L-arginine at a dose of 100 mg/kg did not significantly alter lung weight/body weight ratio and pleural effusion but caused a potentiation of the inhibitory effect of N^G-nitro-L-arginine methyl ester (data are not shown). However, no change was observed in either parameter following D-arginine (300 mg/kg) pretreatment (Table 1). Although there was prominent alveolar fluid deposition, perivascular and peribronchial oedema were slightly reduced in the L-arginine (50 mg/kg)-treated group (Fig. 3A). Similar changes were observed in the N^G-nitro-L-arginine methyl ester (20 mg/kg) plus L-arginine (50 mg/kg)- and N^G-nitro-L-arginine methyl es-

Table 1
Effect of D-arginine (300 mg/kg s.c.) on α-naphthylthiourea-induced lung oedema

	Lung weight/body weight ($\times 10^{-4}$)	Pleural effusion (ml)
α-Naphthylthiourea	124.1 ± 14.6	2.6 ± 0.6
	(166-81.4)[r]	(1.5–3.5)[r]
	NS	NS
α-Naphthylthiourea + D-arginine	116.3 ± 9.7	2.95 ± 0.55
	(103.5–136.8)[r]	(2.2–4.0)[r]

D-Arginine was injected 15 min before i.p. injection of α-naphthylthiourea (10 mg/kg). Measurements were made 4 h later after α-naphthylthiourea injection. [r]Range, NS = not significantly different. Values are given as means ± S.E.M. of 5 rats.

ter (40 mg/kg)-treated rats (Fig. 3B). Another interesting histological finding was the potentiation of the infiltration of eosinophilic leukocytes both in the L-arginine (50 mg/kg) and N^G-nitro-L-arginine methyl ester (20 mg/kg) plus L-arginine (50 mg/kg)-treated groups (Fig. 3A,B).

4. Discussion

Lung oedema observed 4 h after α-naphthylthiourea injection was indicated by the increase in lung weight/body weight ratio and pleural effusion when compared with olive oil-injected controls. Pretreatment with N^G-nitro-L-arginine methyl ester, a NO synthase inhibitor (Moncada et al., 1988), caused a significant decrease in both lung weight/body weight ratio and pleural effusion induced by α-naphthylthiourea. A protective effect on cellular elements, observed as a reduction in perivascular and alveolar septal oedema by N^G-nitro-L-arginine methyl ester, was also observed by microscopic examination. These results indicate that pulmonary oedema induced by α-naphthylthiourea partly depends on the increase in NO production in the pulmonary vascular bed. Feddersen et al. (1986) have reported that the lung endothelial injury induced by either α-naphthylthiourea or hyperoxia does not change the vascular responses to acetylcholine, which depend on NO release, in rats. Additionally, the vasoconstrictor effects of angiotensin II and noradrenaline were found to be reduced after damage of the vascular endothelium following α-naphthylthiourea treatment in rats (Mc-

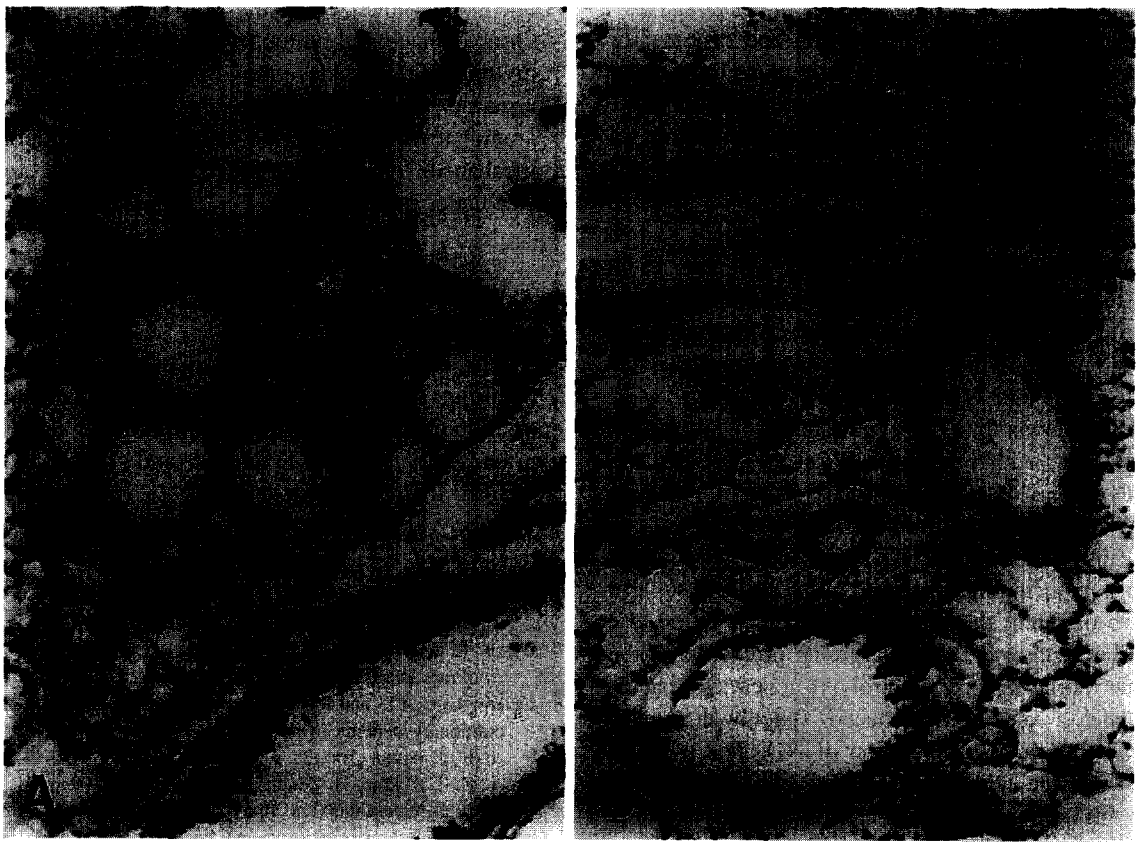

Fig. 4. (A) Section taken from the middle lobe of the right lung showing the histopathological changes following L-arginine (50 mg/kg) pretreatment of α-naphthylthiourea-injected rats. Despite a reduction in oedema, intra alveolar fluid deposition (arrow) persists (H-Ex400). (B) Sections from the same place indicating similar changes in the groups pretreated with L-NAME (20 mg/kg) + L-arginine (50 mg/kg) and L-NAME (40 mg/kg). The reduction of oedema is more prominent except perivascular oedema (arrow) (H-Ex400).

Cormick et al., 1986; Ercan et al., 1993). Acute lung injury has also been linked to the presence of oxygen radicals released from activated phagocytic cells (Jorens et al., 1993). NO is known to be a relatively stable free-radical gas, having a propensity to undergo electron transfer reactions or addition reactions with molecules having unpaired electrons (for review, see Gross and Wolin, 1995). We therefore assumed that NO acts as a free-radical cytotoxic molecule for the lung injury induced by α-naphthylthiourea. The precursor of NO, L-arginine (Moncada et al., 1991) alone, at the dose of 50 mg/kg, did not affect the vascular damage or the formation of pulmonary oedema induced by α-naphthylthiourea. An unexpected finding of the present study was the potentiation by L-arginine of the protective effect of N^G-nitro-L-arginine methyl ester against α-naphthylthiourea-induced pulmonary oedema. This potentiation was evident in all measured parameters and microscopic examinations. These results are not in accordance with the findings of Mulligan et al. (1991), who reported that protection by N^G-nitro-L-arginine methyl ester of immune complex-induced lung or skin injury is reversed by L-arginine. These authors have also reported the increasing effect of L-arginine in this inflammation model. This discrepancy might be either due to the ability of the applied noxious stimuli to produce lung oedema in rats or to the non-specific effect of this amino acid. Hence, L-arginine alone at a higher dose (300 mg/kg) caused a significant inhibition of pleural effusion and oedema formation induced by α-naphthylthiourea. D-Arginine, however, at the same dose did not alter the lung weight/body weight ratio, pleural effusion or microscopic findings induced by α-naphthylthiourea, indicating a stereospecific property of L-arginine.

NO acts as a modulator for oxidative reactions and for the generation of cytotoxic oxygen species (Kanner et al., 1991). It might react with superoxide anion to produce peroxynitrite and hydroxyl radicals, but it also acts as a protector from cytotoxic and reactive oxygen species (for review, see Gross and Wolin, 1995).

It is well known that the synthesis of NO occurs in endothelial cells, with L-arginine as an endogenous substrate (Rees et al., 1989). The protective effect of L-arginine against α-naphthylthiourea-induced lung oedema could be due to its antioxidant effect, as suggested by Xiong et al. (1994) from their findings observed in the cascade superfusion system of rabbit thoracic aorta. Recently Gumusel et al. (1996) have observed that L-arginine protects endothelium of rat aorta against electrolysis-generated reactive oxygen species and H_2O_2-induced functional damage which is not due to the generation of NO. The D-enantiomer of L-arginine, glycine and some other amino acids have also found to be effective protectors in the latter experimental model. Gumusel et al. (1996) have concluded that increased synthesis of NO as well as oxidation of L-arginine with concomitant disproportionate levels of reactive oxygen species could be responsible for the protective effect against reactive oxygen species-induced loss of the endothelial response to acetylcholine in rat aorta.

Xue et al. (1994) have shown that chronic hypoxia down-regulates endothelial constitutive NO synthase but up-regulates smooth muscle inducible NO synthase, which can be inhibited by L-arginine analogues in rats. Based on this observation, an alternative explanation was made for the mechanism of α-naphthylthiourea-induced oedema and interaction with NO. It can be thought that α-naphthylthiourea causes a brief hypoxia by increasing pulmonary oedema and consequently up-regulates inducible NO synthase. L-Arginine reduces pleural effusion and oedema formation by inhibiting inducible NO synthase. However, a non-specific action of L-arginine should be taken into consideration. Jun and Wennmalm (1994) have described that N^G-nitro-L-arginine methyl ester does not affect the hypotensive effect of L-arginine, indicating that the hypotension induced by this amino acid does not fully depend on the augmented formation of NO.

Hence, not only the potentiation of the protective effect of N^G-nitro-L-arginine methyl ester by L-arginine in α-naphthylthiourea-induced lung damage but also the significant increase in the filtration of eosinophilic leukocytes, which can not be reversed by N^G-nitro-L-arginine methyl ester, supports the non-specific effect of the amino acid.

In conclusion, the results of the present study indicate that NO synthetized in the lung may be involved in the tissue damage and pulmonary oedema induced by α-naphthylthiourea. Besides the NO-producing effect of L-arginine, a non-specific pharmacological property of this amino acid must also be taken into consideration. The mechanism of such non-specific effects of L-arginine are still under investigation.

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References

Busse, R. and A. Mülsch, 1990, Induction of nitric oxide synthase b cytokines in vascular smooth muscle cells, FEBS Lett. 275, 90.

Cunningham, A.L. and J.V. Hurley, 1972, Alphanaphthylthiourea induced pulmonary oedema in rat: a topographical and electron microscope study, J. Pathol. 106, 25.

Ercan, Z.S., S. Eren, H. Zengil and R.K. Türker, 1993, Possible involvement of eicosanoids in alpha naphthylthiourea-induced pulmonary oedema and alteration of angiotensin-converting enzyme activity, Pharmacology 46, 274.

Feddersen, C.O., I.F. McMurtry, P. Henson and N.F. Voelkel, 1986, Acetylcholine induced pulmonary vasodilation in lung vascular injury, Am. Rev. Respir. Dis. 133, 197.

Förstermann, U., M. Nakane, W.R. Tracey and J.S. Pollock, 1993, Isoforms of nitric oxide synthase: Functions in the cardiovascular system, Eur. Heart J. 14, 10.

Gibbons, J.D., 1976, Non parametric methods for quantitative analysis, International Series in Decision Processes, Vol. 23 (Halt. Rinehart and Winstons, New York, NY) p. 175.

Gross, S.S. and R. Levi, 1992, Tetrahydrobiopterin synthesis. An absolute reguirement for cytokine-induced nitric oxide generation by vascular smooth muscle, J. Biol. Chem. 267, 25722.

Gross, S.S. and M.S. Wolin, 1995, Nitric oxide; Pathophysiological Mechanisms, Ann. Rev. Physiol. 57, 737.

Gumusel, B., B.C. Tel, R. Demirdamar and I.Ş. Erdemli, 1996, Reactive oxygen species-induced impairment of Endothelin-dependent relaxation in rat aortic rings: protection by L-arginine, Eur. J. Pharmacol. 306, 107.

Jorens, P.G., P.A. Vermeire and A.G. Herman, 1993, L-Arginine-dependent nitric oxide synthase: a new metabolic pathway in the lung and airways, Eur. Respir. J. 6, 258.

Jun, T. and A. Wennmalm, 1994, L-Arginine-induced hypotension in the rat: evidence that NO synthesis is not involved, Acta Physiol. Scand. 152, 385.

Kanner, J., S. Harel and R. Granit, 1991, Nitric oxide as an antioxydant, Arch. Biochem. Biophys. 289, 130.

Kilbourn, R.G., A. Jubran, S.S. Gross, O.W. Griffith, R. Levi, J. Adams and R.F. Lodato, 1990, Reversal of endotoxin-mediated shock by N^G-methyl-L-arginine, an inhibitor of nitric oxide synthesis, Biochem. Biophys. Res. Commun. 172, 1132.

Kolls, J., J. Xie, R. Le Blanc, T. Malinski, S. Nelson, W. Summer and S.S. Greenberg, 1994, Rapid induction of messenger RNA for nitric oxide synthase II in rat neutrophils in vivo by endotoxin and its suppression by prednisolone, Proc. Soc. Exp. Biol. Med. 205, 220.

Marletta, M.A., P.S. Yoon, R. Iyengar, C.D. Leaf and J.S. Wishnok, 1988, Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate, Biochemistry 27, 8706.

McCall, T.B., R.M.J. Palmer and S. Moncada, 1991, Induction of nitric oxide synthase in rat peritoneal neutrophils and its inhibition by dexamethasone, Eur. J. Immunol. 21, 2523.

McCormick, J.R., M. Moore, R. Chrzanowski and W. Cieplinski, 1986, The effect of alpha-naphthylthiourea (ANTU)-induced acute injury on lung binding of antibody to angiotensin converting enzyme (ACE), Adv. Exp. Med. Biol. 198, 503.

Meyrick, B., J. Miller and L. Reid, 1972, Pulmonary oedema induced by ANTU or by high or low oxygen concentration in rat. An electron microscopic study, Br. J. Exp. Pathol. 53, 347.

Moncada, S., M.W. Radomski and R.M.J. Palmer, 1988, Endothelium-derived relaxing factor. Biochem. Pharmacol. 37, 2495.

Moncada, S., R.M.J. Palmer and E.A. Higgs, 1991, Nitric oxide: physiology, pathophysiology and pharmacology, Pharmacol. Rev. 43, 109.

Mulligan, M.S., J.M. Hevel, M.A. Marletta and P.A. Ward, 1991, Tissue injury caused by deposition of immune complexes is L-arginine-dependent, Proc. Natl. Acad. Sci. USA 88, 6338.

Pankhania, J.J. and Y.S. Bakhle, 1982, Effect of pulmonary oedema by alphanaphtylthiourea on synthesis of cyclooxygenase products in isolated rat lungs, Prostaglandins 30, 37.

Rees, D.D., R.M.J. Podmer, H.F. Hodson and S. Moncada, 1989, A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation, Br. J. Pharmacol. 96, 418.

Sheehan, D.C. and B.B. Hrapchak, 1973, Theory and Practice of Histotechnology (C.V., St. Louis, MO) p. 18.

Sipahi (Yılmaz), E., U. Hodoğlugil, Z.S. Ercan and R.K. Türker, 1996, Acute effect of endothelin-1 on lung oedema induced by alpha-naphthylthiourea (ANTU), Pharmacol. Res. (in press).

Stuehr, D.J., S.S. Gross, I. Sakuma, R. Levi and C.F. Nathan, 1989, Activated murine macrophages secrete a metabolite of arginine with the bioactivity of endothelium-derived relaxing factor and the chemical reactivity of nitric oxide, J. Exp. Med. 169, 1011.

Tracey, W.R., M. Nakane, J. Kuk, G. Budzik, V. Klinghofer, R. Harris and G. Carter, 1995, The nitric oxide synthase inhibitor, L-N^G-monomethyl arginine, reduces carrageenan-induced pleurisy in the rat, J. Pharmacol. Exp. Ther. 273, 1295.

Xiong, Y., Y.J. Li and H.W. Deng, 1994, Protection of L-arginine against oxygen free radicals-injured rabbit aortic endothelium, Acta Pharmacol. Sin. 15, 119.

Xue, C., A. Rengasamy, T.D. LeCras, P.A. Koberna, G.C. Dailey and R.A. Johs, 1994, Distribution of NOS in normoxic vs. hypoxic rat lung upregulation of NOS by chronic hypoxia, Am. J. Physiol. 267, L667.