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Inhibition of Inducible Nitric Oxide Synthase Attenuates Interleukin-1β Induced Vascular Hyporeactivity in the Rabbit

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Abstract

Inhibition of nitric oxide (NO) synthesis has been indicated to improve vasopressor-responsiveness and to increase blood pressure in most septic models. However, numerous adverse effects of non-selective NO synthase (NOS) inhibition have been reported, and the effect of NOS inhibition on vascular responsiveness to vasodilators has not been well studied. Using an isometric tension measurement system of vascular rings, we evaluated the effects of an inducible NOS (iNOS) inhibitor, L-canavanine (L-CAN) and a non-selective NOS inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) on interleukin-1β (IL-1β)-induced vascular hyporeactivity in the four different rabbit arteries.

Pretreatment of IL-1β inhibited phenylephrine (Phe)-induced vascular constriction in the carotid artery (CA, 49% of control), pulmonary artery (PA, 66%), femoral artery (FA, 71%) and in the renal artery (RA, 83%). A combination of NOS inhibitors attenuated the vascular hyporeactivity to Phe in all arteries. Pretreatment of IL-1β also inhibited acetylcholine (Ach)-induced vascular relaxation in FA, RA and CA. In PA, the rings were inversely constricted after Ach administration. The combination of IL-1β with L-CAN, but not with L-NAME, attenuated the Ach-induced vasorelaxation to the control level in all arteries. These data suggest that the selective inhibition of iNOS attenuates the direct endothelial damage induced by IL-1β *in vitro*. (J Nippon Med Sch 2002; 69: 149–153)

Key words: nitric oxide, interluekin-1β, vascular reactivity, L-canavanine

Introduction

Septic shock is characterized by a systemic inflammatory response syndrome, hypotension with vasopressor-resistant systemic vasodilation, and the development of multiple organ failure and dysfunction. Sepsis leads to the elaboration of a self-amplifying cascade of pro- and anti-inflammatory cytokines and mediators, including a number of vasoactive substances, such as nitric oxide (NO), endothelins, platelet activating factor, and leukotrienes[1].

NO appears to be an important mediator of impaired vascular responsiveness to vasoconstrictor agents in sepsis[2–4]. Inhibition of NO synthesis improves vasopressor-responsiveness and increases blood pressure in most septic animal models and in humans[5–7]; however, animal studies have revealed numerous adverse effects of non-selective NO synthase (NOS) inhibition[3,8–11]. As shown in our previous study[12], a selective inducible NOS (iNOS) inhibitor, L-canavanine (L-CAN), attenuates vascular hypore-

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activity without hemodynamic instability in a rat septic shock model, suggesting that selective iNOS inhibition favorably affects hemodynamics and improves short-term survival when compared with either standard or non-selective NOS inhibitor support.

Although many previous reports focused on the effects of NOS inhibitors on vasopressor responsiveness, the effects of NOS inhibitors on vasodilator responsiveness and a comparison among the different kinds of vasculatures have not been extensively studied. In this study, we investigated the direct effects of iNOS and non-selective NOS on interluekin-1β (IL-1β)-induced vascular hyporeactivity using a rabbit vascular ring model, and also evaluated those effects on four different arteries.

Materials and Methods

With the approval of the Animal Experimental Ethical Review Committee, Nippon Medical School, twenty-three adult male Japanese White rabbits weighing between 2.5 and 3.0 kg were used. The animals were anesthetized with intravenous pentobarbital sodium (40 mg/kg) and pancuronium bromide (1～2 mg). They were intubated and mechanically ventilated with oxygen. Then 10 mg/kg of heparin sodium was given intravenously before the removal of arteries. The femoral (FA), renal (RA), carotid (CA), and pulmonary arteries (PA) were removed and placed in modified Krebs-Henseleit bicarbonate (KHB) solution (composition (mM): NaCl 119, KCl 4.75, $CaCl_2$ 2.54, $MgSO_4$ 1.19, KH_2PO_4 1.19, $NaHCO_3$ 25, and glucose 11; pH was adjusted to 7.4). Each artery was dissected free of fat and connective tissue and cut into 3 mm length rings, taking special care not to damage the endothelial cell layers or stretch the vessels. Using an isometric tension measurement system (Easymagunus, UFER co. Ltd., Osaka), each ring was mounted between two stainless wires in an organ bath containing 5 ml of KHB solution. The KHB solution was gassed continuously with a mixture of 95% O_2 and 5% CO_2, and maintained at 37℃. Ring tension was recorded isometrically with a force-displacement transducer (TB-611 T, Nihon Kohden). The transducer was connected to a computer to change the electrical signal into contracting and relaxing tensions, and the data were saved to disk (MacLab Chart/s, Macintosh 7200).

Effect of IL-1β on vascular reactivity

The rings were stretched stepwise to adjust the resting tension to 2 g except for PA, which were stretched 1 g, and was equilibrated for 60 min by changing the bath fluids every 15 min. After attaining equilibrium, the rings were exposed to phenylephrine (Phe 10^{-6}M) to induce contraction, which was taken as the 100%level for each ring throughout the experiment. From previous studies and preliminary studies on the response of Phe, the initial tensions of each ring mentioned above were determined. Five min after Phe administration, at which time maximal contraction was achieved, the rings were exposed to acetylcholine (Ach 10^{-6}M). Relaxation caused by Ach was measured by the percent change in tension and then sodium nitroprusside (SNP 10^{-6}M) was added. After the solution was washed and equilibrated for 60 min, the rings were exposed to human recombinant IL-1β (25 ng/ml). Four hours after the administration of IL-1β, the solution was washed and equilibrated for 60 min, and the same experimental procedure was repeated.

Effect of L-NAME and L-CAN on IL-1β-induced vascular hyporeactivity

Arterial rings removed in the same way as described above were cut into two pairs of rings; one pair was exposed to NG-nitro-L-arginine methyl ester (L-NAME 10^{-4}M) with IL-1β, and the other was exposed to L-canavanine (L-CAN 10^{-3}M) with IL-1β. Four hours after the administration of the drugs, the same experiment was repeated. A previous study used endotoxin-treated rats that received either L-NAME (10 mg/kg/h) or L-CAN (100 mg/kg/h) to compare muscle intracellular pH and intracellular bioenergic patterns *in vitro*[13]. Another study showed that high concentrations of L-CAN (2×10^{-3}M) were able to inhibit the activity of constitutive NOS (cNOS) *in vitro*[14].From those experiments and our previous *in vivo* experiment, the doses of L-CAN and L-NAME used were 10^{-3}M and 10^{-4}M, respectively.

Data analysis

All data are expressed as mean ± standard deviation of the mean. Statistical evaluation between the groups was performed with one-way factorial analysis of variance (ANOVA) using Scheffe's test to perform multiple comparisons. A p value of less than 0.05 was considered statistically significant.

Results

Phenylephrine-induced contraction (Fig. 1)

Fig. 1 shows the changes in Phe-induced contraction after treatment of four arteries. Four hours after the administration of IL-1β, the Phe-induced contractions decreased in CA (49% of control), PA (66%), FA (71%) and in RA (83%). Additional NOS inhibitors (L-NAME, L-CAN) attenuated the vascular hyporeactivity to Phe in FA, CA and PA.

Ach and SNP-induced relaxation (Table 1)

In FA, RA and CA, the relaxation after administration of Ach was significantly inhibited by pretreatment of IL-1β. In PA, the rings were inversely constricted after Ach administration. There were no significant differences in vasorelaxation between the rings with IL-1β pretreatment, or with the combination of IL-1β and L-NAME in any of the arteries. On the other hand, the combination with L-CAN attenuated the vasorelaxation to the control level in all arteries.

The relaxation after the addition of SNP showed no significant differences among the control and treatment groups in any of the arteries.

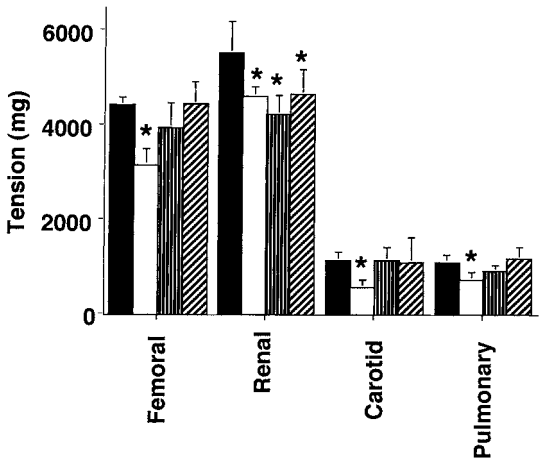

Fig. 1 Phenylephrine-induced contraction in femoral, renal, carotid and pulmonary arteries after treatment with IL-1β and the effect of NOS inhibitors
■ control group: without IL-β or NOS inhibitors before the addition of phenylephrine, □ IL-β group: addition of IL-1 β alone before the addition of phenylephrine, ▥ L-NAME group: addition of IL-1β and L-NAME before the addition of phenylephrine, ▨ L-CAN group: addition of IL-1β and L-CAN before the addition of phenylephrine.
*$p<0.05$ compared with the control group.

Discussion

In septic shock, bacterial lipopolysaccharide (LPS) activates the induction of a calcium-independent nitric oxide and stimulates macrophages, monocytes, endothelial cells to synthesize IL-1β[15]. Thus, IL-1β has been implicated as a mediator of systemic response to infection. IL-1β drives a cascade of mediators, which results in the loss of vascular tone and hypotension primarily through an increase in NO[2, 4, 16]. Increased production of NO has been implicated as a mediator during septic shock and septic syndrome. NO is synthesized by two distinct NOS: cNOS and iNOS. LPS, IL-1β and tumor necrosis factor-α induces a pronounced expression of iNOS m-RNA in rat aortic smooth muscle cells[17]. Excessive production of iNOS results in an increase in NO, which may be a major contributing factor to vasodilatation during septic shock[5].

L-arginine reversibly inhibits both forms of NOS, and has restored LPS-induced hypotension *in vivo*[2, 4–6]. Also the NOS inhibitors, *N* G-monomethyl-L-arginine (L-NMMA) and L-NAME have been reported to increase systemic vascular resistance and blood pressure in patients with septic shock[7]. Therefore, inhibition of NOS may represent a novel therapeutic approach towards managing hypotension in septic shock. However, L-NMMA produces a fall in cardiac output, which might worsen tissue perfusion[8], increase renal vascular resistance and decrease renal blood flow[3, 9, 10]. Furthermore, administration of L-NMMA to endotoxemic rats[6] and rabbits[11] induces acute hypotension and death. These data suggest that non-selective NOS inhibitors inhibit

Table 1 Effects of L-NAME and L-CAN on Ach and SNP-induced vascular relaxation

	treatment	n	Ach (% of contraction)	SNP (% of contraction)
femoral artery	control	8	48.3 + 15.6	32.4 + 12.8
	IL-1β	8	63.2 + 12.3 *	36.2 + 10.2
	L-NAME	12	66.8 + 6.7 *	31.6 + 9.5
	L-CAN	12	54.5 + 13.7	27.2 + 8.9
renal artery	control	9	42.5 + 5.1	3.1 + 1.5
	IL-1β	9	68.2 + 6.0 *	7.8 + 3.4
	L-NAME	11	70.7 + 8.0 *	4.9 + 2.7
	L-CAN	11	43.6 + 4.6	4.2 + 1.6
carotid artery	control	8	40.9 + 7.4	4.3 + 2.3
	IL-1β	8	77.1 + 22.0 *	7.9 + 3.9
	L-NAME	11	74.4 + 12.6 *	5.4 + 3.6
	L-CAN	11	48.3 + 12.9	4.0 + 2.5
pulmonary artery	control	9	47.2 + 9.6	13.3 + 4.9
	IL-1β	9	132.9 + 26.5 *	15.8 + 11.9
	L-NAME	10	126.9 + 8.7 *	7.0 + 3.4
	L-CAN	10	47.6 + 15.5	9.0 + 6.4

control: without IL-β or NOS inhibitors before the addition of Phe
IL-β : addition of IL-1β alone before the addition of Phe
L-NAME: addition of IL-1β and L-NAME before the addition of Phe
L-CAN: addition of IL-1β and L-CAN before the addition of Phe
* $p < 0.05$ compared with the control group.
IL-1β inhibited vascular relaxation after administration of Ach, and the combination with L-CAN attenuated the vasorelaxation to the control level in all arteries.

not only iNOS, but also cNOS and physiological production of NO, which is not appropriate for the treatment of septic shock.

A selective blocker of iNOS, L-CAN, attenuates the effects of LPS on hemodynamics, and improves short term survival during rodent endotoxemia[18]. L-NAME, an inhibitor of cNOS and iNOS, enhances liver damage and tends to accelerate the time of death, but L-CAN significantly reduces 7 day mortality and has no deleterious effects in terms of organ damage[19]. In contrast to nitroarginine, L-CAN largely reverses LPS-induced vascular hyporeactivity in a dose-dependent manner in the rat aorta without any inhibition of vasodilator responses to Ach[20]. Treatment with L-CAN reduces endotoxin-induced electron microscopic changes in the kidneys and lungs. Although *in vitro* selectivity is relatively modest compared with L-NAME, L-CAN does not modify blood pressure, carotid blood flow, or carotid vascular resistance in normal rats[21].

In this study, the IL-β pretreatment rings were constricted after Ach administration in PA, while the rings were dilated in FA, RA and CA. This suggests that the endothelium in PA was damaged more severely than in other arteries, and that Ach could stimulate receptors on vascular smooth muscle cells and caused vasoconstriction. An abnormal vascular response to Ach may represent a defect in the vasodilator function in the endothelium[22]. L-CAN attenuated vascular hyporeactivity even in serious disturbances of the endothelium, although L-NAME did not attenuate hyporeactivity. Previous studies reported that L-CAN only attenuated endotoxin-induced vascular hyporeactivity *in vivo*. In this study, L-CAN, a selective iNOS inhibitor, improved the endothelium disturbance of vasorelaxation *in vitro*, suggesting that inhibitors of iNOS may be beneficial in endotoxin-induced shock. Although the mechanism by which L-CAN protected against IL-β-induced endothelial damage *in vitro* cannot be deduced directly from our results, there are two possible explanations. First, decreasing NO production may reduce oxidative stress by slowing the formation of peroxynitrite, a highly reactive species formed from the reaction of NO with superoxide radicals[23]. Secondly, decreasing NO production may reduce endothelial damage

by preventing a NO-mediated block of high-energy phosphate generation at the cellular level[24]. A previous study revealed that L-CAN enhanced ATP concentration in various organs during rat endotoxic shock[18].

In conclusion, IL-1β inhibits the response of rabbit arteries, especially the pulmonary artery, to vasodilators as well as vasoconstrictor agents. L-CAN, but not L-NAME, attenuates the response to Ach to the control level in all the arteries. These data suggest that the selective inhibition of iNOS attenuates not only *in vivo* tissue perfusion, but also direct endothelial damage induced by IL-1β *in vitro*.

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