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Short communication

Effects of aminoguanidine on systemic inflammatory response syndrome induced by platelet activating factor and by lipopolysaccharide in rats

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Abstract

We investigated the effects of aminoguanidine, a relatively selective inhibitor of inducible nitric oxide (NO) synthase, on the systemic inflammatory response syndrome induced by platelet activating factor (PAF) and by lipopolysaccharide in rats, with emphasis on NO production in vivo. Aminoguanidine treatment improved survival rates after lipopolysaccharide challenge, whereas it aggravated the lethality caused by PAF. Lipopolysaccharide induced a marked increase in the concentrations of nitrate and nitrite in plasma compared with vehicle administration, and the increase was prevented by aminoguanidine. In contrast, PAF challenge with or without aminoguanidine did not affect the concentrations of nitrate and nitrite in plasma compared with vehicle administration. These results suggest that NO derived from inducible NO synthase is not a major participant in the systemic inflammatory response syndrome induced by PAF. Aminoguanidine is not likely to provide beneficial effects in conditions where PAF is produced and the concentrations of nitrate and nitrite in plasma are not significantly increased. © 1997 Elsevier Science B.V.

Keywords: Aminoguanidine; N^G-Nitro-L-arginine; PAF (platelet-activating factor); Lipopolysaccharide; Systemic inflammatory response syndrome; Nitric oxide (NO); (Rat)

1. Introduction

Bacterial endotoxin (lipopolysaccharide), cytokines and platelet activating factor (PAF) are implicated in the pathogenesis of the systemic inflammatory response syndrome including circulatory shock, acute lung injury and multiple organ failure induced by sepsis and other inflammatory responses (Members of American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee, 1992; Yoshikawa et al., 1994, 1997). These mediators induce the expression of inducible nitric oxide (NO) synthase isoform in many cell types (Thiemermann, 1994). The expression of inducible NO synthase and the production of a large amount of NO may contribute to the pathophysiology of the systemic inflammatory response syndrome and the subsequent lethality. Although non-isoform-specific NO synthase inhibitors are of some benefit in this syndrome via the restoration of blood pressure (Kilbourn et al., 1990), the concomitant inhibition of NO synthase in the endothelium may increase the incidence of organ ischemia, microvascular thrombosis and mortality (Harbrecht et al., 1992; Hutcheson et al., 1990; Shultz and Ralj, 1992; Wright et al., 1992). Recently, aminoguanidine, a relatively selective inhibitor of inducible NO synthase, has been reported to attenuate the delayed circulatory failure caused by lipopolysaccharide in rats and improve survival in a murine model of endotoxemia (Wu et al., 1995). However, little attention has been focused on the effects of relatively selective inhibitors of inducible NO synthase on the PAF-induced systemic inflammatory response syndrome and the subsequent lethality, or on the relation between NO production in vivo after PAF or lipopolysaccharide challenge and the therapeutic effects of NO synthase inhibitors.

We investigated the effects of aminoguanidine and N^G-nitro-L-arginine, a relatively selective inhibitor of endothelial NO synthase, on the lethality induced by PAF or by lipopolysaccharide in rats, with emphasis on the associ-

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ation between concentrations of nitrate and nitrite in plasma and the therapeutic effects.

2. Materials and methods

2.1. Chemicals

Platelet activating factor (PAF: 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine) and aminoguanidine were purchased from Sigma (St. Louis, MO, USA). Lipopolysaccharide from *Escherichia coli* 055:B5 was obtained from Difco Laboratories (Detroit, MI, USA). N^G-Nitro-L-arginine was purchased from Peptide Institute (Osaka, Japan).

2.2. Animals

Male Wistar rats weighing 180–220 g were obtained from Keari (Osaka, Japan). The animals were housed in our animal quarters prior to the experiments, and were maintained at 18–24°C with a 12 h light/dark cycle. They were fed a standard diet (Oriental Yeast, Tokyo, Japan) and water ad libitum. Maintenance of animals and experimental procedures were carried out in accordance with the NIH guidelines for the use of experimental animals. The experiments were approved by the Kyoto Prefectural University of Medicine, Animal Care Committee (Kyoto, Japan). The animals were anesthetized with urethane (1000 mg/kg, i.p.).

2.3. Study protocol

Animals were divided into nine experimental groups as follows: Group 1: vehicle (i.v.) + vehicle (i.v.); Group 2: N^G-nitro-L-arginine (10 mg/kg, i.v.) + vehicle (i.v.); Group 3: aminoguanidine (10 mg/kg, i.v.) + vehicle (i.v.); Group 4: vehicle (i.v.) + lipopolysaccharide (10 mg/kg, i.v.); Group 5: N^G-nitro-L-arginine (10 mg/kg, i.v.) + lipopolysaccharide (10 mg/kg, i.v.); Group 6: aminoguanidine (10 mg/kg, i.v.) + lipopolysaccharide (10 mg/kg, i.v.); Group 7: vehicle (i.v.) + PAF (2 μg/kg, i.v.); Group 8: N^G-nitro-L-arginine (10 mg/kg, i.v.) + PAF (2 μg/kg, i.v.); Group 9: aminoguanidine (10 mg/kg, i.v.) + PAF (2 μg/kg, i.v.).

The first intravenous administration of vehicle, N^G-nitro-L-arginine or aminoguanidine was given 5 min prior to the second intravenous injection of vehicle, lipopolysaccharide or PAF in each group. Survival was monitored at 3, 6, 12, 24 h after the second injection. In a separate series of experiments, the animals were anesthetized with diethyl ether 24 h after the injection, and blood samples were collected. Plasma was prepared and frozen at −80°C until assayed for nitrate and nitrite.

2.4. Nitrate and nitrite assay

Concentrations of nitrate and nitrite were determined with a spectrophotometric method based on the Griess reaction (Schmidt et al., 1988). Briefly, 80 μl diluted samples were mixed with nitrate reductase and enzyme cofactor mixture at room temperature for 3 h, and then mixed with 100 μl Griess, consisting of 1% sulfanylamide, 0.1% naphthylethylenediamide dihydrochloride, and 2.5% H_3PO_4, and incubated for 10 min at room temperature. The absorbance of the reaction product was read at 540 nm.

2.5. Statistics

Data are the means ± S.E.M. Differences in nitrate and nitrite concentrations among groups of rats were evaluated using an analysis of variance (Statview; Abacus Concepts, Berkeley, CA, USA). If differences among groups were significant ($P < 0.05$), Scheffe's F-test was used to distinguish between pairs of groups.

3. Results

All animals were alive in the absence of PAF or lipopolysaccharide challenge. As shown in Table 1, N^G-

Table 1
Effects of N^G-nitro-L-arginine and aminoguanidine on survival rates after challenge with lipopolysaccharide or platelet activating factor (PAF)

Group	Survival rate (%)			
	3 h	6 h	12 h	24 h
Vehicle plus lipopolysaccharide	91.7	91.7	83.3	83.3
N^G-Nitro-L-arginine plus lipopolysaccharide	83.3	66.7	41.7	41.7
Aminoguanidine plus lipopolysaccharide	100	100	100	100
Vehicle plus PAF	85.7	85.7	85.7	85.7
N^G-Nitro-L-arginine plus PAF	78.6	28.6	28.6	28.6
Aminoguanidine plus PAF	84.6	76.9	76.9	76.9

The first intravenous administration of vehicle, N^G-nitro-L-arginine (10 mg/kg) or aminoguanidine (10 mg/kg) was given 5 min prior to the second intravenous injection of vehicle, lipopolysaccharide (10 mg/kg) or PAF (2 μg/kg) in each group of rats ($n = 12–14$). Survival was monitored at 3, 6, 12 and 24 h after the second injection.

Table 2
Concentrations of nitrate and nitrite in plasma

Group	(*n*)	Nitrate and nitrite (μM)
Vehicle plus vehicle	(6)	17.2 ± 1.34
N^G-Nitro-L-arginine plus vehicle	(6)	14.9 ± 0.43
Aminoguanidine plus vehicle	(6)	17.6 ± 1.32
Vehicle plus lipopolysaccharide	(5)	123.0 ± 10.0 [a]
N^G-Nitro-L-arginine plus lipopolysaccharide	(1)	104.4 ± 0
Aminoguanidine plus lipopolysaccharide	(6)	62.0 ± 3.76 [b]
Vehicle plus PAF	(6)	16.8 ± 1.66
N^G-Nitro-L-arginine plus PAF	(4)	18.5 ± 5.55
Aminoguanidine plus PAF	(6)	18.8 ± 3.88

The first intravenous administration of vehicle, N^G-nitro-L-arginine (10 mg/kg) or aminoguanidine (10 mg/kg) was given 5 min prior to the second intravenous injection of vehicle, lipopolysaccharide (10 mg/kg) or PAF (2 μg/kg) in each group of rats. Concentrations of nitrate and nitrite were determined with a spectrophotometric method based on the Griess reaction 24 h after the second injection.
[a] $P < 0.0001$ vs. the vehicle plus vehicle group.
[b] $P < 0.0001$ vs. the vehicle plus lipopolysaccharide group.

nitro-L-arginine treatment markedly exacerbated the mortality caused by lipopolysaccharide and by PAF throughout the experimental period. Aminoguanidine treatment dramatically improved survival rates after lipopolysaccharide challenge, whereas it decreased survival rates after PAF challenge.

To estimate the total NO production in vivo, we measured the sum of both nitrate and nitrite in plasma. As shown in Table 2, neither N^G-nitro-L-arginine nor aminoguanidine treatment prior to vehicle administration changed the concentrations of nitrate and nitrite in plasma 24 h after the injection. Lipopolysaccharide administration induced a 7-fold increase in concentrations of nitrate and nitrite as compared with vehicle administration ($P < 0.0001$, vehicle plus lipopolysaccharide vs. vehicle plus vehicle). This increase was prevented by aminoguanidine treatment ($P < 0.0001$, vehicle plus lipopolysaccharide vs. aminoguanidine plus lipopolysaccharide). In contrast, PAF administration in the presence or absence of treatment with each NO synthase inhibitor did not affect the concentrations of nitrate and nitrite in plasma as compared with vehicle administration with or without each NO synthase inhibitor.

4. Discussion

We have previously reported that either PAF or lipopolysaccharide challenge induces a systemic inflammatory response syndrome, including hypotension, acute lung injury and multiple organ failure, in rats (Takahashi, 1993; Yoshikawa et al., 1994, 1997). In the present study, we demonstrated that the effects of aminoguanidine on mortality and on NO production in vivo were different in these two experimental models of systemic inflammatory response syndrome induced by PAF or by lipopolysaccharide.

Since N^G-nitro-L-arginine aggravated the mortality caused by PAF and by lipopolysaccharide, NO derived from endothelial NO synthase is suggested to be protective against the systemic inflammatory response syndrome induced by PAF and by lipopolysaccharide. It is suggested that a large amount of NO derived from inducible NO synthase plays an important role in lipopolysaccharide-induced systemic inflammatory response syndrome, since aminoguanidine improved lipopolysaccharide-caused mortality, and since concentrations of nitrate and nitrite in plasma, which is an index of the total NO production in vivo, dramatically increased 24 h after lipopolysaccharide challenge. Furthermore, the protective effect of aminoguanidine on lipopolysaccharide-caused mortality was concomitant with a significant decrease in the concentrations of nitrate and nitrite. These results are mostly consistent with those in a recent report (Tracey et al., 1995).

A striking finding of the present study was that aminoguanidine did not improve the PAF-caused mortality but aggravated it. It has been reported that PAF contributes to the induction of inducible NO synthase by lipopolysaccharide in the rat lung in vivo and in murine macrophages in vitro (Szabo et al., 1993). PAF causes a very low level of inducible NO synthase expression on its own and enhances the induction of inducible NO synthase by lipopolysaccharide in cultured rat Kupffer cells (Mustafa et al., 1996), whereas PAF alone does not induce nitrite production in cultured murine macrophages (Szabo et al., 1993). Intravenous injection of PAF into rats causes induction of NO synthase activity in lung homogenates (Szabo et al., 1993). In our study, however, the concentrations of nitrate and nitrite in plasma did not increase significantly 6 h (data not shown) and 24 h after the PAF challenge, when multiple organ failure was already manifested (Yoshikawa et al., 1997). The present results imply that NO generated from inducible NO synthase is not a major participant in the pathophysiology of the PAF-induced systemic inflammatory response syndrome and the subsequent lethality,

and that treatment with aminoguanidine is not a good intervention for the systemic inflammatory response syndrome induced by PAF. Aminoguanidine is not likely to have beneficial effects in conditions where PAF is produced and the concentrations of nitrate and nitrite in plasma are not significantly increased.

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