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Detrimental Effects of N^{ω}-nitro-L-arginine Methyl Ester (L-NAME) in Experimental *Escherichia coli* Sepsis in the Newborn Piglet

The role of nitric oxide during neonatal sepsis is complex. We tested the hypothesis that nonselective inhibition of nitric oxide synthase with N^{ω}-nitro-L-arginine methyl ester (L-NAME) is detrimental during the early phase of experimental sepsis in the newborn piglet. Newborn piglets were divided into four groups: 6 in the control group, 6 in the L-NAME control group, 12 in the sepsis group (SG), and 11 in the sepsis with L-NAME group (NS). Sepsis was induced by intravenous injection of 10^8 colony forming units of *Escherichia coli*. L-NAME 10 mg/kg was given intravenously 60 min before the induction of sepsis. The survival rate of piglets after 4 hr was 27% in NS, while it was 100% in other groups. Systemic hypotension, observed in both SG and NS, were more profound in NS. Leukopenia was observed in both SG and NS. Thrombocytopenia, prolongation of prothrombin time and activated partial thromboplastin time, and increase in thrombin-antithrombin complexes were observed only in NS. Decreased PaO_2/FiO_2 ratio, arterial pH and base excess, and increased blood lactate levels observed in both SG and NS, but were more profound in NS. These findings suggest that nonselective inhibition of nitric oxide synthase with L-NAME is detrimental during the early phase of experimental neonatal sepsis.

Key Words : *Sepsis; Nitric Oxide; Animals, Newborn; Escherichia coli; NG-Nitroarginine Methyl Ester*

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INTRODUCTION

Sepsis occurs most frequently during the neonatal period, and is associated with a poor prognosis despite continuous improvements in intensive care medicine and antibiotic therapy (1, 2). The causes of poor prognosis during sepsis might be attributable to multiple organ dysfunction syndrome, including septic shock, disseminated intravascular coagulation (DIC), and acute respiratory distress syndrome, caused by activation of acute inflammatory responses in response to systemic infection (3).

Although nitric oxide (NO) has been implicated to play a key role in mediating the acute pathophysiological processes during sepsis (4), the use of NO synthase (NOS) inhibitors in sepsis has yielded both beneficial and detrimental effects. Treatment with NOS inhibitor increased blood pressure and systemic vascular response (5-9), protected against DIC (10), and improved survival rate (9) in both animal and clinical studies of sepsis. However, detrimental hemodynamic effects (11-14), increased mortality (15-18), and aggravation of DIC (19) have also been reported with NOS inhibition in both neonatal and adult models of experimental sepsis. Therefore, the available data on the role of NO and the efficacy of NOS inhibitors as a novel adjuvant therapy in sepsis are still controversial, and further study will be necessary to clarify this.

This study was done to evaluate the role of NO and the efficacy of NOS inhibitors as a novel adjuvant therapy in neonatal sepsis. We tested the hypothesis that nonselective inhibition of NOS with N^{ω}-nitro-L-arginine methyl ester (L-NAME) is detrimental during the early phase of experimental sepsis in the newborn piglet. In the present study, we used the newborn piglet as an animal model of neonatal sepsis because the piglet is comparable in growth spurt, energy metabolism, and vascular anatomy to human newborns at birth (20, 21). It is also suitable in size for continuous hemodynamic monitoring during the experiment. *Escherichia coli* was used to induce sepsis because it is the most common Gram-negative pathogen of neonatal sepsis (1, 2).

MATERIALS AND METHOES

Animal Preparation

The experimental protocols described herein were reviewed and approved by the Institutional Animal Care and Use Committee of the Samsung Biomedical Research Center, Seoul, Korea. This study also followed the institutional and National Institutes of Health guidelines for laboratory animal care.

Newborn piglets less than 3 days old were anesthetized with sodium thiopental (5 mg/kg intravenously), paralyzed with pancuronium (1 mg/kg intravenously), tracheotoimzed,

and artificially ventilated with mechanical ventilator (Sechrist Infant Ventilator, IV-100B, Sechrist Industries Co., Anaheim, CA, U.S.A.). Femoral arteries and veins were cannulated for blood pressure monitoring and blood sampling, and for medication and fluid infusion, respectively. Electrocardiogram (ECG), oxygen saturation, and blood pressure were continuously monitored using Hewlett Packard neonatal monitoring system (Hewlett Packard Model M1276A, Hewlett Packard Co., MA, U.S.A.). Throughout the experiment, the piglet was placed under the servo-controlled warmer (Airshields Inc., Hatboro, PA, U.S.A.) and rectal temperature was maintained at between 38.0 and 39.0℃.

Experimental Protocol

After surgery and the period of stabilization, 35 newborn piglets were divided randomly into four groups: 6 in the control group (CG), 6 in the L-NAME control group (NG), 12 in the sepsis group (SG), and 11 in the sepsis with L-NAME group (NS). Sepsis was induced by intravenous injection of 10^8 colony forming units of *E. coli* in 100 μL of saline. L-NAME 10 mg/kg was given intravenously 60 min prior to the induction of sepsis. Continuous monitoring of ECG, systemic blood pressure, and oxygen saturation was done during the experiment. Arterial blood gas analyses, concentrations of glucose, and lactate in the blood were measured at baseline, and at every 1 hr for 4 hr after bacterial inoculation. Complete blood count (CBC), prothrombin time (PT), activated partial thromboplastin time (aPTT) and thrombin-antithrombin complexes were measured at baseline and at the end of the experiment. Arterial blood gases were measured using a blood gas analyzer (Ciba-Corning Diagnostics Corp., Medfield, MA, U.S.A.) and the concentrations of glucose and lactate were measured using an YSI model 2300 dual analyzer (Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.).

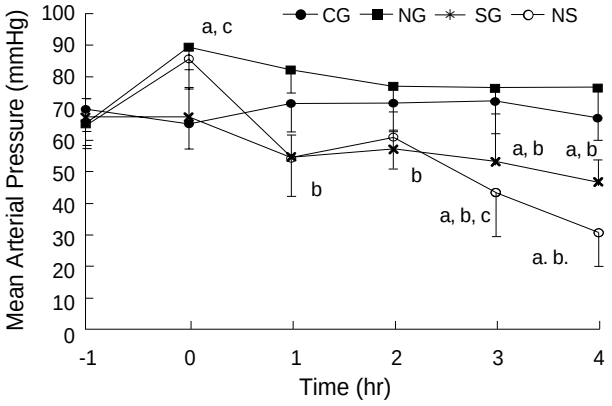

Fig. 1. Time course of changes in mean arterial pressure in newborn piglets in each experimental group. 'CG' means control group. 'NG' means L-NAME control group. 'SG' means sepsis group. 'NS' means sepsis with L-NAME group. Data are expressed as mean ±standard deviation. [a]: $p<0.05$ compared to CG, [b]: $p<0.05$ compared to NG, [c]: $p<0.05$ compared to SG.

Statistical Analysis

Data were analyzed by unpaired t test for inter-group comparisons. To detect significant changes over time within each group, data were compared using repeated measures analysis of variance with Bonferroni correction. Statistical analysis described above was done using SAS software program ver. 6.04. A p-value of <0.05 was considered significant. Data were given as mean±standard deviation.

RESULTS

Survival

All piglets in the SG (n=12), CG (n=6), and NG (n=6) were found alive at 4 hr after induction of sepsis. In the NS (n=11), only 3 piglets (27%) were found alive until the end of the experiment.

Hemodynamic Changes

The CG showed no significant changes in mean arterial pressure (MAP) during the experiment (Fig. 1). In both NG and NS, MAP increased transiently but significantly, compared to CG and SG at 1 hr, and returned to baseline values in NG at 2 hr after L-NAME injection. MAP decreased progressively in both SG and NS, and became significantly different from corresponding values in CG and NG after 3 hr. This systemic hypotension was found more profound in NS.

Physiologic Variables

No significant differences in physiologic values such as heart rate, arterial pH, PaO_2, $PaCO_2$, base excess, and blood lactate

Table 1. Physiological data at 4 hr of experiment in each group of newborn piglets

	CG (n=6)	NG (n=6)	SG (n=12)	NS (n=11)
Heart rate (/min)	201±32	199±47	186±41	235±49
Arterial base excess (mEq/L)	0.2±2.0	0.4±2.9	-5.5±3.5[a]	-22.4±7.5[a,b,c]
Arterial pH	7.41±0.11	7.35±0.08	7.28±0.14[a]	6.82±0.26[a,b,c]
$PaCO_2$ (mmHg)	41±17	47±12	45±11	66±23[a,b,c]
PaO_2/FiO_2 ratio	260±33	238±37	210±74	139±62[a,b,c]
Blood lactate (mmol/L)	1.4±0.6	1.0±0.4	2.9±5.2	9.5±6.1[a,b,c]

CG: Control group, NG: L-NAME control group, SG: Sepsis group, NS: Sepsis with L-NAME group.Values given present mean±standard deviation. [a]:$p<0.05$ compared to CG, [b]:$p<0.05$ compared to NG, [c]:$p<0.05$ compared to SG.

Table 2. Hematologic data of the newborn piglets in each experimental group

Group	CG (n=6)	NG (n=6)	NG (n=6)	NS (n=11)
White blood cell (/μL)	9,040±1,017	9,980±3,300	2,500±1,570[a,b]	1,900±2,820[a,b]
Platelet (×1,000/μL)	242±83	302±77	223±91	108±30[a,b,c]
PT (sec)	11.5±1.6	11.7±2.1	12.6±2.1	23.4±9.9[a,b,c]
aPTT (sec)	10.6±2.1	11.7±2.2	11.5±3.1	39.3±10.5[a,b,c]
TAT (ng/mL)	5.7±3.5	2.8±1.2	13.4±10.6	49.5±12.9[a,b,c]

CG: Control group, NG: L-NAME control group, SG: Sepsis group, NS: Sepsis with L-NAME group, PT: Prothrombin time, aPTT: activated partial thromboplastin time, TAT: Thrombin-antithrombin complexes. Values given present mean±standard deviation. [a]: $p<0.05$ compared to CG, [b]: $p<0.05$ compared to NG, [c]: $p<0.05$ compared to SG.

levels were observed in CG and NG throughout the experiment (Table 1). Arterial pH and base excess were significantly reduced in both SG and NS, and these abnormalities were more profound in NS. Increased $PaCO_2$ and blood lactate levels, and decreased PaO_2/FiO_2 ratio were observed only in NS.

Hematologic Data

In both CG and NG, no significant abnormalities in hematologic values were observed during the experiment (Table 2). Leukopenia was observed in both SG and NS until the end of experiment. Thrombocytopenia, prolongation of PT and aPTT, and increase in thrombin-antithrombin complexes were observed only in NS.

DISCUSSION

The role of NO during sepsis is generally assessed by the effect of NOS inhibitors (5-19). In the present study, pretreatment with L-NAME increased mortality, aggravated systemic hypotension, leukopenia, and induced disseminated intravascular coagulation during the early phase of experimental *E. coli* sepsis in the newborn piglet. These findings suggest that NO plays a protective role by attenuating the sepsis-induced multiple organ dysfunction syndrome, caused by acute inflammatory responses, and that inhibition of NOS might therefore be detrimental at least during the early phase of neonatal sepsis.

The beneficial effects of NOS inhibitors such as improved survival rate (9), protection against DIC (10) and increase in blood pressure (5-9), and brain injury observed in other animal and clinical studies of sepsis have not been observed in the present study. However, we have also observed the beneficial effects of NOS inhibition in our previous study of experimental neonatal bacterial meningitis (21). Significant attenuation of the acute inflammatory responses and prevention of brain damage were observed with L-NAME pretreatment during the early phase of experimental *E. coli* meningitis in the newborn piglet. These findings implicate that the friend or foe simple dichotomy could not account for the complex role of NO in bacterial sepsis and meningitis.

As the animal model, type and dosage of NOS inhibitors, etiologic pathogen, and stages of the disease were same in our present study of sepsis and in our previous study of meningitis (21); the reasons for this discrepancy between these two different compartments of infection remain to be explained. NO can exert both beneficial and deleterious effects in different tissues and under different conditions. Three isoforms of NOS have been described: endothelial NOS (eNOS), neuronal NOS, and inducible or immunological NOS. Among the three isoforms of NOS, NO produced by vascular endothelial cells (eNOS) has been known to have anti-inflammatory effects such as limiting platelet aggregation (22), leukocyte adherence to endothelial cells (23), and microvascular permeability (24). Therefore, blockade of these beneficial effects of NO with NOS inhibitors would be responsible for the detrimental effects observed in the present study of experimental neonatal sepsis. It is also noteworthy in this regard that a number of studies have demonstrated that the nonselective inhibition of NOS activity aggravates organ function (11-14, 19) and survival rate (15-18) in various animal models of sepsis or endotoxemia.

In our previous study of meningitis (21), secondary bacterial invasion from central nervous system to the bloodstream, which would result in secondary septicemia, has not been observed in the meningitis with L-NAME group, and the blood brain barrier might also be relatively intact during the early phase of meningitis. Thus, the activation of vascular eNOS might have been minimal, and the detrimental effects with NOS inhibition have not been observed during our previous study of meningitis. Further studies using selective inhibitors of NOS will be necessary to substantiate this hypothesis.

Our data of transient but significant increase in blood pressure in both NG and NS immediately after L-NAME pretreatment supports the assumption that NO is a potent endogenous vasodilator (4). In this study, however, L-NAME aggravated septic shock and increased mortality. These detrimental effects might be attributable to pulmonary hypertension and reduced cardiac output due to severe vasoconstriction with NOS inhibition (11-14). More profound decrease in arterial pH and base excess, and the increase in blood lactate level observed in NS compared to SG suggests compromised microcirculatory blood flow and oxygen delivery to the tissues due to active vasoconstriction with NOS inhibition during neonatal sepsis. Taken together, these findings suggest that NO may

play an important role in maintaining organ blood flow and tissue oxygenation during the early phase of neonatal sepsis.

Our data of leukopenia, thrombocytopenia, and induction of DIC in NS suggest that nonspecific inhibition of NOS might be detrimental by blocking the anti-inflammatory effects of eNOS (19, 22-24). Besides aggravating pulmonary hypertension and physiologic shunting (11-14), increased leukocyte and platelet infiltration into the lung tissue with NOS inhibition might also contribute to decreased PaO_2/FiO_2 ratio observed in NS during this study (19).

In summary, inhibition of NOS with L-NAME is detrimental as evidenced by increasing mortality, aggravating systemic hypotension, leukopenia, and inducing disseminated intravascular coagulation during the early phase of experimental *E. coli* sepsis in the newborn piglet. These findings suggest that some of the NO produced during neonatal sepsis is beneficial, and inhibition of NOS as adjuvant therapy must be approached with great caution.

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