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PNEUMOTOXICITY OF LIPOPOLYSACCHARIDE IN NITRIC OXIDE — DEFICIENT RATS IS LIMITED BY A THROMBOXANE SYNTHASE INHIBITOR

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Both nitric oxide and arachidonic acid metabolites have been implicated in pathogenesis of septic shock. We have recently described a model of endotoxin-induced acute lung injury in rats in which nitric oxide synthase is inhibited. The possible interplay between nitric oxide and eicosanoids (thromboxane A_2 , prostacyclin) in this model have been presently studied. Animals were randomly assigned to four experimental groups which received the following treatment. 1. Lipopolysaccharide (LPS) infusion only, $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during 10 min (LPS group). 2. N^{ω} -Nitro-L-Arginine $10 \text{ mg} \cdot \text{kg}^{-1}$ (L-NNA, nitric oxide synthase inhibitor) pretreatment followed by LPS infusion (L-NNA+LPS group). 3. L-NNA and camonagrel $25 \text{ mg} \cdot \text{kg}^{-1}$ (CAM, thromboxane synthase inhibitor) pretreatment followed by LPS infusion (L-NNA+CAM+LPS group). 4. L-NNA and iloprost $0.3 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (ILO, stable analog of prostacyclin) pretreatment followed by LPS infusion (L-NNA+ILO+LPS group). LPS infusion resulted in a biphasic response in mean arterial blood pressure. A transient but deep fall in arterial blood pressure was followed by a long-lasting hypotension that led to death after 278 ± 49 min. L-NNA+LPS rats died within 22 ± 5 min among the symptoms of systemic hypotension and acute lung injury. In L-NNA+CAM+LPS group a significant attenuation of early phase of hypotension occurred and survival time was comparable with that of the LPS group (298 ± 68 min). In rats of the L-NNA+ILO+LPS group survival time increased insignificantly to 48 ± 41 min. It is concluded that immediate deleterious effects of lipopolysaccharide in NO-deficient rats are at least partially mediated by thromboxane A_2 while prostacyclin cannot replace NO in its pneumoprotective action.

Key words: *lung injury, nitric oxide, thromboxane, prostacyclin, lipopolysaccharide*

INTRODUCTION

Sepsis and septic shock are the most severe clinical manifestations of bacterial infections. In spite of major progress both in understanding their pathophysiology and in critical care of severely ill patients not much improvement of mortality rate (1) was observed and the morbidity rate is still

increasing (2). A large number of endogeneous mediators have been implicated in pathologic sequence leading from bacteremia to multiple organ dysfunction syndrome (MODS) or even their failure (MOFS). Free oxygen radicals (3), eicosanoids (4), cytokines (5) or activated coagulation factors (6) are just a few examples. In fact these substances, if overproduced may be even more toxic to the host than bacterial products by themselves. A long hunt for a magic bullet which could prevent all the deleterious effects of sepsis seems now to be over, unfortunately, with no single offender to be named. Numerous clinical trials failed to show significant benefit of various antagonists or inhibitors of synthesis of the above endogenous agents (7—9), although animal studies were often promising (10, 11). We become therefore more and more aware that complex interactions between various mediators may be much more important for the final outcome than even most severe insults caused by a single mediator. One of such mediators implicated in the endotoxin-mediated hypotension (12, 13) and hyporesponsiveness to vasoconstrictors (14) is nitric oxide. Inhibition of its production has been, however, claimed beneficial (15) or detrimental (16) by various authors depending on the animal species, type of shock and dose and specificity of nitric oxide synthase inhibitor used. In general it is believed that nitric oxide derived from constitutive, endothelial nitric oxide synthase (NOS-III) is beneficial whereas inducible NOS (NOS-II) found in various tissues (e.g. in macrophages, endothelial or smooth muscle cells) after prolonged exposure to the products of bacterial cell wall or to cytokines is detrimental. We have recently described a model of endotoxic shock in rats in which pretreatment with non-selective NOS inhibitor proved to be detrimental causing acutely lethal lung injury (17). Presently, using this model we have investigated a possible interaction between nitric oxide and two metabolites of arachidonic acid with opposite actions: prostacyclin and thromboxane A₂.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (180—250 grams) were maintained at $22 \pm 1^\circ\text{C}$ with a 12/12 hour light/dark cycle and were allowed water and standard rat chow *ad libitum*.

Reagents

Lipopolysaccharide (LPS, *Escherichia coli* serotype O127:B8) and N^o-Nitro-L-Arginine (L-NNA) were purchased from Sigma. Camonagrel (CAM) was a gift from Ferrer Int., Spain and Iloprost (ILO) was purchased from Schering AG, Germany.

LPS was dissolved in saline and administered as an intravenous (i.v.) infusion ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 10 min, total dose $20 \text{ mg} \cdot \text{kg}^{-1}$). L-NNA ($10 \text{ mg} \cdot \text{kg}^{-1}$) was dissolved in 0.1 mol/L phosphate buffer $\text{pH} = 7.4$ and injected as an i.v. bolus. CAM ($25 \text{ mg} \cdot \text{kg}^{-1}$) was dissolved in saline and administered i.v. as a bolus. ILO ($0.3 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was dissolved in saline and infused i.v. over 30 min.

Surgical procedure

Under thiopentone anesthesia (Thiopental, Vuab $120 \text{ mg} \cdot \text{kg}^{-1}$, i.p.) rats were intubated intratracheally and ventilated with room air (Ugo Basile, 7025 rodent ventilator, tidal volume 10 mg/kg , at 50 breaths/minute). Polyurethane catheters were placed in the left jugular and left femoral veins for drug administration. Another catheter was inserted in the right carotid artery and connected to a pressure transducer for continuous recording of mean arterial blood pressure (MAP).

Experimental protocol

Twenty four rats were included in the study. They were assigned randomly into four groups ($n = 6$ each) which were treated as follows: 1) LPS group- LPS infusion alone, 2) L-NNA+LPS group- injection of L-NNA 45 min prior to infusion of LPS, 3) L-NNA+CAM+LPS group- camonagrel was injected 30 min after L-NNA and 15 min before LPS, 4) L-NNA+ILO+LPS group- iloprost infusion started 30 min after L-NNA administration and 15 min before LPS infusion and lasted 30 min, therefore it was stopped 5 min after the end of LPS infusion.

Statistics

Mean arterial blood pressure (MAP, mmHg) and survival time (minutes) were expressed as arithmetic means \pm standard deviations. Differences between studied groups and reference group (i.e. LPS group) were evaluated by two-tailed Student's t-test.

RESULTS

The results are presented in *Fig. 1* and *Fig. 2*.

Effects of LPS on mean arterial blood pressure (MAP) and survival time (LPS group)

After a 15 min period of stabilisation MAP in the LPS group was $124 \pm 17 \text{ mmHg}$. At the end of LPS infusion (early hypotension) it fell to $57 \pm 13 \text{ mmHg}$ (i.e. by $54 \pm 8\%$), at 45 min after LPS administration it was partially restored to $83 \pm 18 \text{ mmHg}$ and at 180 min it was $65 \pm 17 \text{ mmHg}$. Survival time in this group was $278 \pm 49 \text{ min}$.

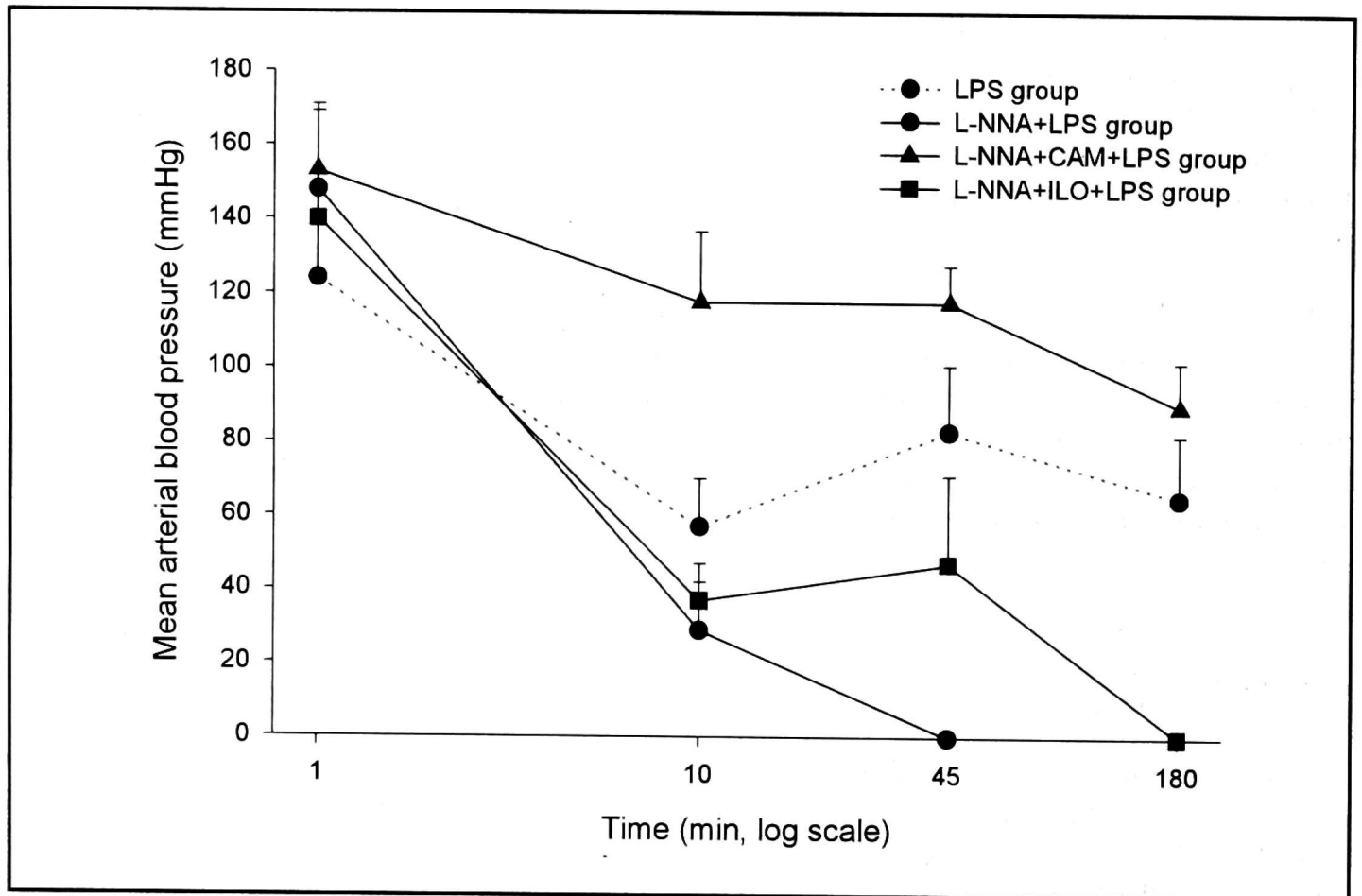


Fig. 1. Mean arterial blood pressure (MAP) studied 10, 45 and 180 min since the start of lipopolysaccharide (LPS) infusion. In the LPS group transient deep hypotension at 10 min was followed by partial recovery at 45 min and a subsequent second phase of hypotension at 180 min. Pretreatment with N^G -Nitro-L-Arginine (L-NNA) caused higher initial MAP in three other groups (L-NNA + LPS, L-NNA + ILO + LPS, L-NNA + CAM + LPS). In L-NNA + LPS group a profound hypotension occurred at 10 min followed soon by death of all animals. In L-NNA + ILO + LPS group after a similar hypotensive phase at 10 min there was observed a tendency to recovery at 45 min in three surviving rats, which all the same died before 180 min. All six rats of the L-NNA + CAM + LPS group survived up to 180 min and hypotensive responses in comparison to the L-NNA + LPS group and even to the LPS group were attenuated at all time intervals.

Effects of the pretreatment with L-NNA (L-NNA + LPS group)

In the L-NNA + LPS group pretreatment with L-NNA raised MAP within 15 min from the initial 126 ± 17 to 148 ± 23 mmHg. Administration of LPS resulted in a significantly greater early hypotension to 29 ± 13 mmHg (i.e. by $78 \pm 10\%$, $P < 0.01$ versus LPS group). All rats died in the symptoms of acute lung injury within 22 ± 5 min ($P < 0.001$).

Effects of the pretreatment with L-NNA and CAM (L-NNA + CAM + LPS group)

In the L-NNA + CAM + LPS group administration of L-NNA raised MAP from 129 ± 12 to 153 ± 16 mmHg. Subsequent administration of CAM did not

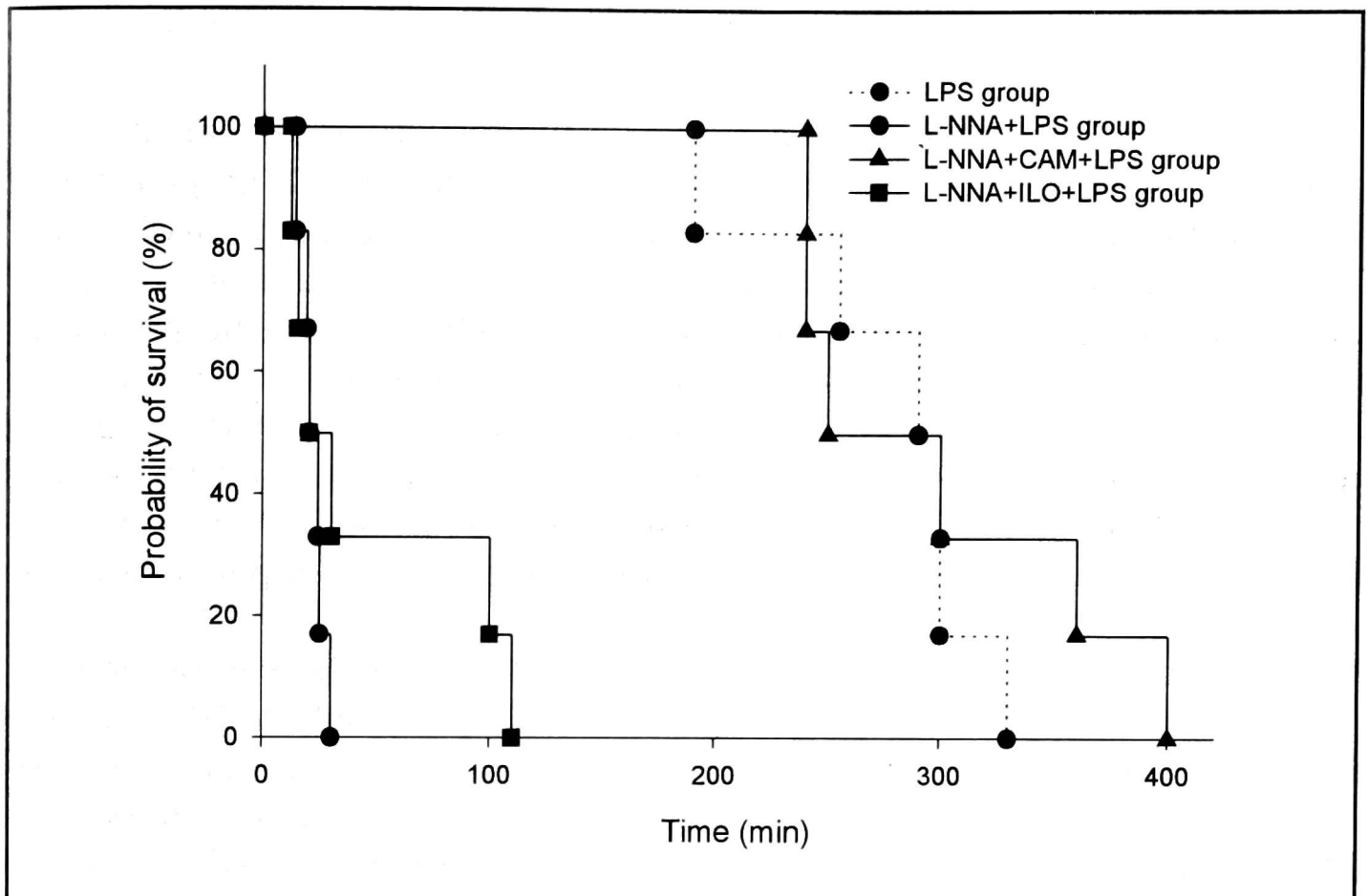


Fig. 2. Probability of survival (ordinate) of each of six rats within four studied groups (abscissa—survival time in min). The lethal shock was induced in all groups by an infusion of LPS $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during 10 min (LPS group). In three groups animals were pretreated with N^{ω} -Nitro-L-Arginine (L-NNA + LPS, L-NNA + CAM + LPS, L-NNA + ILO + LPS groups) while in two latter groups pretreatment with Camonagrel or Iloprost were also implemented, respectively.

change MAP while early hypotension after LPS was attenuated to $118 \pm 19 \text{ mmHg}$ (i.e. fall by $24 \pm 9\%$ only, $P < 0.01$ versus L-NNA + LPS group). 45 min after LPS MAP was still $118 \pm 10 \text{ mmHg}$ while at 180 min after LPS administration it fell to $90 \pm 12 \text{ mmHg}$. Survival time was restored to values comparable to the LPS group ($298 \pm 68 \text{ min}$).

Effects of the pretreatment with L-NNA and ILO (L-NNA + ILO + LPS group)

In the L-NNA + ILO + LPS group administration of L-NNA elevated MAP from $115 \pm 9 \text{ mmHg}$ to $140 \pm 12 \text{ mmHg}$. Infusion of ILO resulted in a small drop of MAP to $133 \pm 12 \text{ mmHg}$. Administration of LPS resulted in a profound early hypotension to $37 \pm 10 \text{ mmHg}$ (i.e. by $72 \pm 8\%$, non-significant versus L-NNA + LPS group). 45 min after LPS infusion only three out of six animals were alive and their MAP was $47 \pm 24 \text{ mmHg}$. The survival time could not be significantly elongated in this group and eventually all animals died with the same symptoms in $48 \pm 41 \text{ min}$.

DISCUSSION

Understanding of interaction between endogenous mediators of sepsis seems to be crucial for better management of septic patients as no panacea to treat all the symptoms seems to be on the way. We concentrated on the interaction between nitric oxide and two metabolites of arachidonic acid, thromboxane and prostacyclin, with physiologically antagonistic properties (18). Our recently described model allows for discrimination between beneficial and deleterious effects of NOS inhibition during sepsis. At early stages of endotoxaemia NO, most probably made by NOS-III protects rat lungs from acute toxicity of LPS. After few hours excessive production of NO by NOS-II is likely to be responsible, along with other mediators, for septic shock and MOFS.

Thromboxane A₂ has since long been implicated in the pathogenesis of sepsis (19). Its importance was evidenced in many animal models. Porcine model of sepsis described by Slotman *et al.* (20) revealed correlation of increased thromboxane levels with impairment of many important hemodynamic parameters: cardiac index, pulmonary vascular resistance, stroke volume, left ventricular stroke work and arterial oxygen partial pressure. Relative specificity of thromboxane antagonists for pulmonary vasculature in sepsis is another interesting issue. Kuhl *et al.* (21) demonstrated that thromboxane antagonism would ameliorate pulmonary hypertension and lung compliance reduction while increased airway resistance remained unchanged. Redl *et al.* (22) evidenced prevention of a decrease of right ventricle ejection fraction in thromboxane inhibited endotoxaemic sheep. Work of Schutzer *et al.* (23) shows that dezmegelel, another thromboxane synthase inhibitor, attenuated pulmonary response to bacteriemia while systemic circulation was not protected. Interestingly, the same study demonstrated a significantly greater improvement of systemic parameters after dextran infusion in the dezmegelel treated animals as compared to controls. This shows how minor modifications of experimental protocol can largely affect the outcome. It is not surprising that Cook *et al.* (24) noted thromboxane-dependent protection in early endotoxic shock and lack of protection in a cecal ligation and puncture model of shock while Jesmok *et al.* (25) and Furman *et al.* (26) observed no decrease of mortality in other models of shock.

Our data fit into scenario of a concomitant release by endotoxin of thromboxane and nitric oxide. These two exert opposite actions on vascular patency and functioning of platelets. In endotoxic shock early surge of thromboxane has been evidenced many times (27, 28), while a prompt synthesis of nitric oxide is more elusive. It may well be so because of instability of NO, however cultured endothelial cells (29) and isolated rat heart (30) seem to release instantly NO in response to LPS. Recently in collaboration with

Dr. T. Malinski (17) we have also shown that LPS given intravenously to Sprague-Dawley rats releases within seconds NO to the lung parenchyma, as evidenced by a porphyrinic electrode. Therefore it is likely that an increase in synthesis of endogenous NO is required to combat TXA₂, and inhibition of NOS-III leaves the pneumotoxic action of TXA₂ unopposed. The use of iloprost, a prostacyclin receptor mimetic was justified as follows. Firstly it is well known that prostacyclin opposes many thromboxane effects, and secondly thromboxane inhibition by camonagrel may simply divert arachidonic acid metabolism from thromboxane towards endogenous prostacyclin (31) and then an extra supply with exogenous prostacyclin is expected to be beneficial. A collaboration between NO and PGI₂ is known to occur, e.g. endotoxin-evoked decrease of red blood cell deformability was recently shown to be attenuated by both of them (32). The demonstrated ineffectiveness of iloprost in our model may reflect different timing for the release of TXA₂ and PGI₂. Camporesi *et al.* (33) demonstrated initial rise of TXB₂ followed by rise of 6-keto-PGF_{1α} in a baboon model of sepsis. Fink *et al.* (34) showed that these relationships would depend highly on the experimental setting which is used: in a slow-onset model of cecal ligation and puncture 6-keto-PGF_{1α} level increase is not accompanied by a similar rise in thromboxane. Our data go in line with those of Svartholm *et al.* (35) who demonstrated that TXA₂ receptor antagonists but not iloprost had prevented endotoxin-evoked pulmonary hypertension. Partial effectiveness of cyclooxygenase inhibitors in preventing pulmonary complications in endotoxaemia in sheep (36) also speaks against considering the role of the ratio between PGI₂ and TXA₂ as indicative for the deleterious action of LPS in shock. It is rather an interplay between pneumotoxic TXA₂ and pneumoprotective NO which is responsible for the final outcome of action of LPS in the lung. NO cannot be replaced by PGI₂.

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