Effects of nitric oxide synthase inhibition combined with nitric oxide inhalation in a porcine model of endotoxin shock

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1 The present investigation compares the effects of intravenous infusion of the NO synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) with that of an inhalation with NO gas in a porcine model of endotoxin (lipopolysaccharide, LPS) shock. In addition, the effects of the combination of these two treatments were also investigated.

2 Male pigs were anaesthetized and instrumented for the measurement of haemodynamic parameters. Blood samples were withdrawn at different time intervals for determination of blood gases, pH, and plasma levels of nitrite/nitrate and tumour necrosis factor.

3 Endotoxin infusion (15 µg kg⁻¹ h⁻¹ for 3 h) caused a progressive fall in mean arterial blood pressure (MABP) and cardiac output (CO) and a biphasic increase in mean pulmonary arterial pressure (MPAP) and pulmonary vascular resistance (PVR). A continuous infusion of L-NMMA (0.1 mg kg⁻¹ min⁻¹) significantly attenuated the fall in MABP, but did not affect MPAP, CO and PVR. NO-inhalation (50 p.p.m.) did not affect MABP, but significantly blunted the biphasic increase in MPAP and PVR and significantly delayed the fall in CO. The combination of L-NMMA infusion (0.1 mg kg⁻¹ min⁻¹) with NO-inhalation (50 p.p.m.) completely prevented the fall in MABP, significantly improved CO, and attenuated the biphasic increase in MPAP and PVR.

4 Endotoxin also caused a decline in PaO₂ and a rise of PaCO₂. Infusion of L-NMMA neither affected the fall in PaO₂ nor the increase in PaCO₂. In contrast, inhalation with NO gas alone as well as the combined administration of L-NMMA infusion and NO-inhalation completely prevented the fall in PaO₂ and significantly protected against the increase in PaCO₂.

5 Infusion of endotoxin for 180 min resulted in a mortality of 58%, which was not affected by L-NMMA (63%). In contrast, treatment of LPS-animals with either NO-inhalation alone or NO-inhalation plus L-NMMA completely prevented mortality.

6 This investigation demonstrates that treatment with NO-inhalation, in order to prevent the dramatic increase in MPAP, PVR and the alterations in peripheral blood gases combined with systemic L-NMMA to improve systemic MABP and thus organ perfusion, may be a new therapeutic regimen in the treatment of septic shock.

Keywords: Nitric oxide; nitric oxide inhalation; nitric oxide synthase; circulatory shock; sepsis; endotoxaemia

Introduction

Endotoxin is the initiator of the sepsis syndrome (Bone, 1991) which is characterized by hypotension, a diminished response to vasoconstrictors and myocardial dysfunction (Suffredini *et al.*, 1989; Parillo *et al.*, 1990). In contrast to the systemic vasodilatation, pulmonary hypertension often occurs in the acute phase of endotoxaemia. This may lead to right-sided heart failure, pulmonary oedema, hypoxia and death (Zapol *et al.*, 1977; Demling *et al.*, 1981).

Endotoxin leads to the induction of a calcium-independent nitric oxide (NO) synthase (NOS) (Szabo *et al.*, 1993; Julou-Schaeffer *et al.*, 1991) resulting in an overproduction of the vasodilator. This excessive formation of NO contributes importantly to the severe hypotension seen in endotoxin shock (Thiernemann & Vane, 1990; see Thiernemann, 1994 for review). NO synthase inhibition by N^G-monomethyl-L-arginine (L-NMMA) ameliorates the hypotension elicited by endotoxin in various animal models of circulatory shock (Rees *et al.*, 1989; Thiernemann & Vane, 1990; Cobb *et al.*, 1992) as well as in human septic shock (Petros *et al.*, 1991). However, the administration of NOS inhibitors, which are not selective for the inducible isoforms of NOS, also causes a concomitant inhibition of the constitutive NOS in the endothelium and may increase the incidence of organ

ischaemia, microvascular thrombosis and mortality (Hutchinson *et al.*, 1990; Harbrecht *et al.*, 1992; Shultz & Raji, 1992; Wright *et al.*, 1992). Moreover, the non-selective NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) causes detrimental haemodynamic effects in pigs with septic shock (Robertson *et al.*, 1994).

Inhalation with NO gas, on the other hand, reduces the endotoxin-induced pulmonary hypertension without affecting systemic vascular resistance in pigs with septic shock (Weitzberg *et al.*, 1993).

Therefore, the present investigation compares the effects of an intravenous infusion with the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) with that of an inhalation with NO gas in a porcine model of endotoxin shock. In addition, the effects of a combination of systemic administration of L-NMMA with inhalation of NO gas was also investigated.

Methods

Haemodynamic measurements

Thirtyone male pigs weighing 18.0 to 22.0 kg were used. The animals were fasted overnight, but had free access to water. The pigs were premedicated (ketaminchloride 25 mg kg⁻¹,

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i.m.) and then anaesthetized with pentobarbitone sodium (20 mg kg^{-1} , i.v. bolus, followed by an i.v. infusion of $15 \text{ mg kg}^{-1} \text{ h}^{-1}$). All animals were intubated, and connected to a respirator for positive pressure ventilation with a mixture of room air and 60% oxygen (tidal volume: 400–450 ml breath^{-1} ; respiration rate: 10–15 breaths min^{-1}). Arterial blood gases were monitored at regular time intervals. The femoral artery, carotid artery and jugular vein were cannulated for the measurement of the haemodynamic parameters. Haemodynamic data were recorded continuously on a multi-channel polygraph recorder. The mean arterial blood pressure (MABP) was measured with a catheter-tip manometer (Typ PC 350 Millar Instruments, Houston, Texas, U.S.A.) placed in the femoral artery. Left ventricular pressure (LVP) was recorded with a tip-catheter passed retrogradely from the carotid artery into the left ventricle. Heart rate (HR) was calculated from the LVP waveform and the left ventricular end-diastolic pressure (LVEDP) was measured on a high sensitivity scale. Myocardial contractility (dP/dt_{max}) was measured as the rate of rise of LVP. Mean pulmonary artery pressure (MPAP) was measured by means of a thermodilution catheter (Statham 5F, SP 5105) positioned in the pulmonary artery via the jugular vein. Cardiac output (CO) was determined by the thermodilution method and calculated as the mean of three subsequent measurements (CO computer, Statham SP 1435, Gould Statham Instruments, CA, U.S.A.). In addition, venous catheters were placed in the left and right external thoracic veins for administration of compounds and LPS. During the course of the experiment, 500 ml of Ringer solution was infused. The experiment started after a 1 h stabilization period.

Nitrite and nitrate determination

Nitrite (NO_2^-) was measured by means of the Griess reaction. The determination of nitrate (NO_3^-) was realized via reduction with a high-pressure cadmium column according to Green *et al.* (1982). The system allows quantitative reduction of NO_3^- and automatically eliminates interference from other compounds normally present in the plasma.

Determination of tumor necrosis factor

Plasma levels of tumour necrosis factor ($\text{TNF}\alpha$) were determined by means of a $\text{TNF}\alpha$ -bioassay which is based on the porcine kidney cell line PK (15) as recently described by Bertoni *et al.* (1992). $\text{TNF}\alpha$ activity was determined by measuring the cytostatic effect of $\text{TNF}\alpha$ on the PK (15) cell line. A standard curve of cytostasis was obtained by using human recombinant $\text{TNF}\alpha$ (Biomar, Marburg) yielding progressive cytostasis ranging from 10–1000 u ml^{-1} of $\text{TNF}\alpha$. The bioactivity of $\text{TNF}\alpha$ in the experimental samples was determined and compared against the standard curve.

Experimental protocol

Twentyfive animals received a continuous infusion of *Escherichia coli* lipopolysaccharide (LPS, $15 \mu\text{g kg}^{-1} \text{ min}^{-1}$ for 180 min, Serotype 0111:B04, Sigma) and were randomly divided into four groups. A group of 7 pigs (LPS) received LPS only and served as endotoxin controls. In another group of 6 animals, L-NMMA was given as an infusion ($0.1 \text{ mg kg}^{-1} \text{ min}^{-1}$) starting 30 min prior to LPS and continued throughout the duration of the experiment. Another 6 animals were ventilated with a mixture of room air and oxygen (60%) containing NO gas (50 p.p.m.). Inhalation with NO gas was started 30 min prior to LPS infusion and continued until the end of the experiment. The concentration of the NO-gas (Messer Griesheim, Germany) was adjusted via a volumetrically calibrated flowmeter. In a further group of 6 animals, a combination of an intravenous infusion of L-NMMA ($0.1 \text{ mg kg}^{-1} \text{ min}^{-1}$) and NO-inhalation (50 p.p.m.)

was administered. Another 6 animals received saline alone and served as sham-operated controls.

In all groups, haemodynamic parameters were measured and recorded continuously and samples of arterial blood were taken before and at 15, 30, 45, 60, 90, 120, 150 and 180 min after starting the infusion of LPS and used for the determination of NO_2^- , NO_3^- and $\text{TNF}\alpha$, arterial oxygen tension (PaO_2), arterial carbon dioxide tension (PaCO_2), pH as well as leukocyte and platelet counts. At the end of the experiment, all animals were killed by an overdose of sodium pentobarbitone.

Materials

Lipopolysaccharide (*E. coli* serotype 0111:B04), N^G -monomethyl-L-arginine (L-NMMA) were obtained from Sigma Chemical Co. The NO-gas inhalation setup was supplied by Messer Griesheim (Hoechst, Wuppertal).

Statistical analysis

All results in figures and tables are expressed as mean $\pm$ s.e.mean of n determinations. Statistical analysis of the results was performed by one-way analysis of variance (ANOVA) followed, if appropriate, by a Bonferroni test for multiple comparisons.

Results

Cardiovascular response

Baseline values for MABP, MPAP, CO and PVR (Figure 1a–d) were not significantly different between any of the groups studied. In sham-operated controls, no change in either haemodynamics or blood gas parameters were detected during the 180 min experimental period. Endotoxin infusion ($15 \mu\text{g kg}^{-1} \text{ min}^{-1}$) caused a progressive fall in MABP from $111 \pm 3 \text{ mmHg}$ (time 0) to $40 \pm 2 \text{ mmHg}$ (180 min) and in CO from $3800 \pm 280 \text{ ml min}^{-1}$ (time 0) to $1865 \pm 180 \text{ ml min}^{-1}$ (time 180 min). This severe hypotension in LPS-controls was associated with a rise in HR from $101 \pm 6 \text{ beats min}^{-1}$ (time 0) to $137 \pm 11 \text{ beats min}^{-1}$ (time 180 min) (data not shown). MPAP and PVR markedly increased within the first 60 min of endotoxin infusion, after which time the values slightly declined and then slowly rose again for the rest of the experiment (Figure 1b,d). Continuous infusion of L-NMMA partially and significantly attenuated the fall in MABP caused by LPS-infusion (Figure 1a), but had no effect on MPAP, CO and PVR (Figure 1b–d). Treatment of LPS-pigs with NO-inhalation alone significantly attenuated the acute rise in MPAP and PVR caused by infusion of LPS (Figure 1b,d) and completely abolished the secondary delayed increases in MPAP and PVR seen in LPS-controls between 90 and 180 min. NO-inhalation also significantly delayed the fall in CO (Figure 1c), but did not significantly affect the fall in MABP (Figure 1a). The combination of infusion with NO-inhalation completely prevented the LPS-induced fall in MABP, significantly improved CO, attenuated the initial rise in MPAP and PVR (0–60 min) and completely abolished the delayed increase (90–180 min) of both these parameters (Figure 1a–d).

Blood gases and pH

Basal values for PaO_2 , PaCO_2 and pH were not different among any of the groups studied (Figure 2a,b). Infusion of endotoxin resulted in a decline in PaO_2 and a rise in PaCO_2 to 74.2 ± 16 and $62.5 \pm 6.6 \text{ mmHg}$ at 180 min, respectively (Figure 2a,b). Infusion of L-NMMA in LPS-treated animals neither affected the fall in PaO_2 , nor the increase in PaCO_2 (Figure 2a,b). In contrast, both inhalation with NO gas alone and the combined administration of L-NMMA infusion and

Figure 1 Mean arterial blood pressure (MABP; a), mean pulmonary artery pressure (MPAP; b), cardiac output (CO; c) and pulmonary vascular resistance (PVR; d) during endotoxin infusion in anaesthetized pigs. Groups of animals received either lipopolysaccharide (LPS) alone (O, $15 \mu\text{g kg}^{-1} \text{min}^{-1}$, $n = 7$) or LPS and NO-inhalation (●, 50 p.p.m., $n = 6$), LPS and N^G-monomethyl-L-arginine (L-NMMA, ◆, $0.1 \text{ mg kg}^{-1} \text{min}^{-1}$, $n = 6$) or an infusion of L-NMMA combined with NO-inhalation (■, concentrations as above, $n = 6$). Data are expressed as means $\pm$ s.e.mean of n observations. * $P < 0.05$ represent significant differences when compared to LPS at the same timepoint.

Figure 2 Arterial partial pressure of oxygen (PaO₂; a), arterial partial pressure of CO₂ (PaCO₂; b), arterial pH (pH; c) and plasma nitrite level (NO₂⁻; d) during endotoxin infusion. Groups of animals received either lipopolysaccharide (LPS) alone (O, $15 \mu\text{g kg}^{-1} \text{min}^{-1}$, $n = 7$) or LPS and NO-inhalation (●, 50 p.p.m., $n = 6$), LPS and N^G-monomethyl-L-arginine (L-NMMA, ◆, $0.1 \text{ mg kg}^{-1} \text{min}^{-1}$, $n = 6$) or an infusion of L-NMMA combined with NO-inhalation (■, concentrations as above, $n = 6$). Data are expressed as means $\pm$ s.e.mean of n observations. * $P < 0.05$ represent significant differences when compared to LPS at the same timepoint.

Figure 3 Effect of the different treatments on survival rate in anaesthetized pigs. Groups of animals received either only lipopolysaccharide (LPS, $n = 7$) or LPS and NO-inhalation (50 p.p.m., $n = 6$), LPS and L-NMMA, ($0.1 \text{ mg kg}^{-1} \text{ min}^{-1}$, $n = 6$) or an infusion of L-NMMA combined with NO-inhalation (concentrations as above, $n = 6$).

NO-inhalation completely prevented the fall in PaO_2 and partially, but significantly, protected against the increase in PaCO_2 (Figure 2a,b).

In pigs treated with LPS-infusion, the arterial pH fell from 7.5 ± 0.02 (time 0) to 7.27 ± 0.01 at 180 min (Figure 2c). Treatment with L-NMMA did not affect this acidosis, while both NO-inhalation and the combined treatment with L-NMMA infusion plus NO-inhalation partially prevented the fall in arterial pH (Figure 2c).

White blood cell and platelet counts

Infusion of LPS caused a significant fall in white blood cell and platelet counts from $13000 \pm 1000 \mu\text{l}^{-1}$ and $638000 \pm 39000 \mu\text{l}^{-1}$ (time 0) to $3000 \pm 320 \mu\text{l}^{-1}$ and $238000 \pm 16000 \mu\text{l}^{-1}$ (time 180 min), respectively. Neither the infusion of L-NMMA, nor the inhalation of NO or the combination treatment affected the neutropenia or thrombocytopenia caused by LPS (data not shown).

Plasma levels of nitrite, nitrate and $\text{TNF}\alpha$

In LPS-animals, baseline plasma levels of NO_2^- and NO_3^- were $2.0 \pm 0.15 \text{ nmol ml}^{-1}$ and $54.5 \pm 7.3 \text{ nmol ml}^{-1}$, respectively. NO_2^- increased significantly in the first 30 min after LPS and reached a maximum between 60 and 120 min after commencing the LPS-infusion (Figure 2d). In contrast, infusion of LPS did not affect the plasma levels of NO_3^- (data not shown). L-NMMA infusion, NO-inhalation and the combination treatment significantly inhibited the LPS-induced increase in plasma NO_2^- levels (Figure 2d).

LPS infusion resulted in a transient increase in plasma $\text{TNF}\alpha$ levels with peak $\text{TNF}\alpha$ levels being detected at 90 min after starting the LPS-infusion. None of the different treatments caused a significant attenuation of the rise in plasma $\text{TNF}\alpha$ -levels afforded by LPS.

Mortality

The number of pigs in each group which died during the course of the experiment is presented in Figure 3. In control animals, endotoxaemia for 180 min was associated with a mortality of 58%. This high mortality associated with endotoxaemia was not affected by L-NMMA treatment. In contrast, none of the LPS-animals treated either with NO inhalation alone or with NO-inhalation plus L-NMMA died during the course of the experiment.

Discussion

Using a porcine model of septic shock, this study demonstrates that (i) neither the systemic administration of the NOS inhibitor L-NMMA, nor the inhalation of NO-gas alone prevented all of the haemodynamic changes caused by endotoxaemia, (ii) the combined treatment of NO-gas inhalation together with an intravenous infusion of L-NMMA abolished all of the haemodynamic alterations afforded by LPS; and (iii) the improved survival seen either with NO-gas inhalation alone or with the combined treatment is due to an attenuation of the severe increase in PVR afforded by endotoxin in this species.

Infusion of low-dose endotoxin in the anaesthetized pig resulted in a rapid and sustained fall in MABP, a fall in PaO_2 , an increase in PaCO_2 , acidosis as well as an increase in the plasma levels of $\text{TNF}\alpha$. Endotoxaemia also resulted in a rapid and sustained increase in plasma NO_2^- (breakdown product of NO) levels, which were significantly higher than in sham-operated controls between 30 and 180 min after the start of the endotoxin infusion. This increase in plasma NO_2^- is due to an enhanced formation of NO arising from both activation of the constitutive NOS (early phase) as well as the induction of NOS (delayed phase) (Szabo *et al.*, 1993). The finding, however, that endotoxaemia was not associated with a significant rise in plasma nitrate levels is not entirely surprising, for NO_2^- is the predominant metabolite of NO (Ignarro *et al.*, 1993). This finding also suggests that plasma NO_2^- levels are a more sensitive indicator than nitrate levels for measuring the kinetics of NO formation *in vivo*. All of the above-mentioned haemodynamic and biochemical alterations also occur in patients with septic shock (Parillo *et al.*, 1990; Bone, 1991).

Interestingly, endotoxaemia in the anaesthetized pig was also associated with a significant fall in CO which, in man, is only seen in the late, hypodynamic phase of septic shock (Parillo *et al.*, 1990). It is well known that even brief periods of endotoxaemia in the pig are associated with a substantial, biphasic rise in MPAP and PVR (Weitzberg *et al.*, 1993), which in turn accounts for the observed fall in CO. This biphasic rise in MPAP and PVR is due to the formation of the vasoconstrictor thromboxane A_2 (first phase) (Hardie & Olson, 1987; Leeper-Woodford *et al.*, 1981; Weitzberg *et al.*, 1993), as well as the development of a pulmonary oedema (second phase) (Demling *et al.*, 1981). These pathophysiological alterations in MPAP and PVR during the acute phase of endotoxaemia are peculiar to this species and have not been described in man (Parillo *et al.*, 1990).

Intravenous administration of L-NMMA largely attenuated the rise in plasma NO_2^- and partially attenuated the severe hypotension afforded by endotoxaemia in the pig. Similar beneficial haemodynamic effects of L-NMMA have been observed in a variety of animal models of endotoxaemia (see Thiemeermann, 1994 for review) as well as in patients with septic shock (Petros *et al.*, 1994). Systemic administration of L-NMMA, however, did not affect the rise in MPAP and PVR, the fall in CO, the rise in plasma $\text{TNF}\alpha$ or the changes in arterial blood gases brought about by endotoxaemia. In contrast, systemic administration of L-NMMA to patients with septic shock significantly enhanced MPAP and PVR, and also resulted in a fall in CO from elevated (hyperdynamic phase of septic shock) towards normal levels (Petros *et al.*, 1994). A fall in CO below normal levels has, however, been reported in septic dogs treated with the NOS inhibitor NO_2Arg (Kilbourn *et al.*, 1990; Klabunde & Ritger, 1991). In contrast to L-NMMA however, L-NAME is a more potent inhibitor of the constitutive NOS present in the vascular endothelium (Moore *et al.*, 1990) and, hence, causes a much more pronounced coronary vasoconstriction. The fact that L-NAME is a more potent inhibitor of endothelial NOS than L-NMMA may also account for the finding that L-NAME (Robertson *et al.*, 1994), but not L-NMMA (this study), caused a significant rise in MPAP and PVR in pigs with

septic shock. Inhibition of endothelial NOS by non-selective NOS inhibitors such as L-NMMA may result in excessive vasoconstriction and also enhanced adhesion of platelets and polymorphonuclear cells to the endothelium (see Thiemermann, 1994 for review). We demonstrate here, however, that infusion of L-NMMA, at doses which attenuated the fall in MABP caused by endotoxaemia in the pig, did not exacerbate the endotoxin-induced fall in platelet or polymorphonuclear cell counts. Similarly, in patients with septic shock, L-NMMA enhances the peripheral vascular resistance without exacerbating the thrombocytopenia or neutropenia associated with sepsis (Petros *et al.*, 1994).

Treatment of septic pigs with NO-gas inhalation alone attenuated the rise in MPAP and PVR afforded by endotoxin. Moreover, NO-gas inhalation also attenuated the LPS-induced fall in CO. This finding further supports our hypothesis that the fall in CO associated with endotoxaemia in the pig is secondary to a rise in MPAP and PVR and, hence, due to a reduction in left ventricular filling pressure. In addition, inhalation with NO-gas also attenuated the alterations in gas exchange (fall in PaO_2 and rise in PaCO_2) as well as the resulting acidosis afforded by endotoxaemia in pigs. Inhalation with NO-gas leads to a selective vasodilatation of blood vessels adjacent to ventilated areas, for NO is rapidly inactivated by haemoglobin and, hence, has no systemic haemodynamic effects (Weitzberg *et al.*, 1993). This selective vasodilatation of ventilated areas leads to a shift in blood flow from non-ventilated to ventilated areas of the lung (positive steal phenomenon), which in turn causes an improved ventilation to perfusion matching (Sprague *et al.*, 1992; Weitzberg *et al.*, 1993). Thus, NO-inhalation therapy improves the efficacy of the Euler-Liljestrand reflex. A similar redistribution of blood flow from non-ventilated to ventilated areas of the lung was also observed in patients with ARDS receiving NO-inhalation therapy (Rossaint *et al.*, 1993). Whether the beneficial effects of NO-gas inhalation therapy on gas exchange are primarily due to the above-mentioned effects on pulmonary haemodynamics or, in part, due to inhibition of platelet aggregation (Radomski *et al.*, 1987) or neutrophil adhesion (Kubes, 1991) in the pulmonary circulation is unclear.

Surprisingly, our study demonstrates that inhalation of

NO-gas also attenuated the rise in plasma NO_2^- afforded by LPS. Although the mechanism of this effect is unclear, it is conceivable that inhalation with NO-gas inhibits the formation of NO , and hence NO_2^- , by alveolar macrophages and polymorphonuclear leucocytes. The finding that NO inhalation alone did not prevent the rise in plasma NO_2^- between 120 and 180 min may well be due to the fact that inhalation with NO-gas for several hours may itself result in an accumulation of NO_2^- in the plasma (Forstell *et al.*, 1991).

NO-gas inhalation therapy, but not infusion of L-NMMA prevented the high mortality caused by endotoxaemia in the pig. This finding strongly suggests that the cause of death is related to the LPS-induced, pronounced rise in MPAP and PVR as well as severe alterations in gas exchange, but not due to the observed severe hypotension, for (i) NO-gas inhalation, but not L-NMMA, abolished these alterations in pulmonary haemodynamics and gas exchange; and (ii) L-NMMA, but not NO-gas inhalation, partially prevented the fall in MABP caused by LPS.

Interestingly, treatment of pigs with NO-gas inhalation together with infusion of L-NMMA abolished all of the LPS-induced alterations in gas exchange, haemodynamics and mortality. Most importantly, the combined treatment abolished the hypotension afforded by LPS, while L-NMMA alone only partially prevented this hypotension. Thus, the severe hypotension observed in this model of endotoxaemia in the pig is due to a fall in peripheral vascular resistance (which is prevented by L-NMMA) and a fall in CO which occurs secondary to pulmonary vasoconstriction (which is prevented by NO-gas inhalation).

Thus, this study demonstrates that the combined treatment with NO-gas inhalation and systemic administration of L-NMMA is superior to either treatment alone in preventing the LPS-induced alterations in gas exchange, haemodynamics and mortality in anaesthetized pigs. Whether this combined treatment is also superior to the use of non-selective NOS inhibitors alone in the treatment of patients with septic shock remains to be investigated. We speculate that NO-gas inhalation therapy will, at least, attenuate the rise in PVR afforded by non-selective NOS inhibitors, such as L-NMMA, in patients with septic shock (Petros *et al.*, 1994).

References

- BERTONI, G., KUHNERT, P., PETERHANS, E. & PAULI, U. (1993). Improved bioassay for the detection of porcine tumor necrosis factor using a homologous cell line: PK(15). *J. Immunol. Methods*, **93**, 267–271.
- BONE, R.C. (1991). Sepsis, the sepsis syndrome, multi-organ failure: a plea for comparable definitions. *Ann. Intern. Med.*, **114**, 332–333.
- COBB, J.P., NATANSON, C. & KOEV, C.A. (1992). The haemodynamic effect of N^G -methyl-L-arginine in endotoxaemic compared to normal canines. *Crit. Care Med.*, **20**, S61–S65.
- DEMLING, R.H., SMITH, M., GUNTHER, G., FLYNN, J.T. & GEE, M.H. (1981). Pulmonary injury and prostaglandin production during endotoxaemia in conscious sheep. *Am. J. Physiol.*, **240**, H383–386.
- FORSTELL, C., FRATACCI, M.D., WAIN, J.C., JONES, R. & ZAPOL, W.M. (1991). Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation*, **83**, 2038–2047.
- GREEN, L.C., WAGNER, D.A., GLOGOWSKI, J., SKIPPER, P.L., WISHNOK, J.S. & TANNENBAUM, S.R. (1982). Analysis of nitrate, nitrite and $[15\text{N}]$ nitrate in biological fluids. *Anal. Biochem.*, **126**, 131–138.
- HARBRECHT, B.G., BILLIAR, T.R., STADLER, J., DEMETRIS, A.J., OCHOA, J., CURREN, R.D. & SIMMONS, R.L. (1992). Inhibition of nitric oxide synthesis during endotoxaemia promotes intrahepatic thrombosis and an oxygen radical-mediated hepatic injury. *J. Leukocyte Biol.*, **52**, 390–394.
- HARDIE, E.M. & OLSON, N.C. (1987). Prostaglandin and thromboxane levels during endotoxin-induced respiratory failure in pigs. *Prostaglandins Leukot. Med.*, **28**, 255–265.
- HUTCHESON, I.R., WHITTLE, B.J.R. & BOUGHTON-SMITH, N.K. (1990). Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. *Br. J. Pharmacol.*, **101**, 815–820.
- IGNARRO, L.J., FUKUTO, J.M., GRISCAVAGNE, J.M., ROGERS, N.E. & BRYNS, R.E. (1993). Oxidation of nitric oxide in aqueous solution to nitrite but not nitrate: comparison with enzymatically formed nitric oxide from L-arginine. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 8103–8107.
- JULOU-SCHAEFFER, G., GRAY, G.A., FLEMMING, G.I., SCHOTT, C.C., PARATT, J.R. & STOCLET, J.S. (1991). Activation of the L-arginine/nitric oxide pathway is involved in vascular hyporeactivity induced by endotoxin. *J. Cardiovasc. Pharmacol.*, **17**, S207–S212.
- KILBOURN, R.G., JUBRAN, A., GROSS, S.S., GRIFFITH, O.W., LEVI, R., ADAMS, J. & LODATO, R.F. (1990). Reversal of endotoxin-mediated shock by N^G -methyl-L-arginine, an inhibitor of nitric oxide synthesis. *Biochem. Biophys. Res. Commun.*, **172**, 1132–1138.
- KLABUNDE, R.E. & RITGER, R.C. (1991). NG-Monomethyl-L-arginine (NMA) restores arterial blood pressure but reduces cardiac output in a canine model of endotoxic shock. *Biochem. Res. Commun.*, **178**, 1135–1140.
- KUBES, P., SUZUKI, M. & GRANGER, D.N. (1991). Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 4651–4655.

- LEEPER-WOODFORD, S.K., CAREY, P.D., BYRNE, K., FISHER, B.J., BLOCHER, C., SUGERMAN, H.J. & FOWLER III, A.A. (1991). Ibuprofen attenuates plasma tumor necrosis factor activity during sepsis-induced acute lung injury. *J. Appl. Physiol.*, **71**, 915–923.
- MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.W., EVANS, R.A. & GIBSON, A. (1990). N^G-nitroarginine (L-NOARG), a novel L-arginine reversible inhibitor of endothelium-dependent vasodilation *in vitro*. *Br. J. Pharmacol.*, **99**, 408–412.
- PARILLO, M.M., ASHTON, D.S., NATANSON, C., SUFFREDINI, A.F., DANNER, R.L., CUNNION, R.E. & OGNIBENE, F.P. (1990). Septic shock in humans: advances in the understanding of pathogenesis, cardiovascular dysfunction and therapy. *Ann. Intern. Med.*, **113**, 227–242.
- PETROS, A., BENNETT, D. & VALLANCE, P. (1991). Effect of nitric oxide inhibitors on hypotension in patients with septic shock. *Lancet*, **338**, 1557–1558.
- PETROS, A., LAMB, G., LEONE, A., MONCADA, S., BENNETT, D. & VALLANCE, P. (1994). Effects of a nitric oxide synthase inhibitor in humans with septic shock. *Cardiovasc. Res.*, **28**, 34–39.
- RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987). The antiaggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br. J. Pharmacol.*, **92**, 639–646.
- REES, D.D., PALMER, R.M.J. & MONCADA, S. (1989). Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 3375–3378.
- ROBERTSON, F.M., OFFNER, P.J., CICERI, D.P., BECKER, W.K. & PRUIT, B.A. (1994). Detrimental haemodynamic effects of nitric oxide synthase inhibition in septic shock. *Arch. Surg.*, **129**, 149–156.
- ROSSAINT, R., PISON, U., GERLACH, H. & FALKE, K.J. (1993). Inhaled nitric oxide: its effects on pulmonary circulation and airway smooth muscle cells. *Eur. Heart Journal*, **14**, (Suppl. I), 133–140.
- SHULTZ, P.J. & RAIJ, L. (1992). Endogenously synthesized nitric oxide prevents endotoxin-induced glomerular thrombosis. *J. Clin. Invest.*, **90**, 1718–1725.
- SPRAGUE, R.S., THIEMERMANN, C. & VANE, J.R. (1992). Endogenous endothelium-derived relaxing factor opposes hypoxic pulmonary vasoconstriction and supports blood flow to hypoxic alveoli in anaesthetized rabbits. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 8711–8715.
- SUFFREDINI, A.F., FROMM, R.E., PARKER, M.M., BRENNER, M., KOVACS, J.A., WELEY, R.A. & PARILLO, J.E. (1989). The cardiovascular response of normal humans to the administration of endotoxin. *New Engl. J. Med.*, **321**, 280–287.
- SZABO, C., MITCHELL, J.A., THIEMERMANN, C. & VANE, J.R. (1993). Nitric oxide-induced hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxin shock. *Br. J. Pharmacol.*, **108**, 786–792.
- THIEMERMANN, C. (1994). The role of the arginine:nitric oxide pathway in circulatory shock. *Adv. Pharmacol.*, **28**, 45–79.
- THIEMERMANN, C. & VANE, J.R. (1990). Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharide in the rat *in vivo*. *Eur. J. Pharmacol.*, **182**, 591–595.
- WEITZBERG, E. (1993). Circulatory responses to endothelin-1 and nitric oxide with special references to endotoxin shock and nitric oxide inhalation. *Acta Physiol. Scand.*, **148**, (Suppl. 611), 1–72.
- WEITZBERG, E., RUDEHILL, A. & LUNDBERG, J.M. (1993). Nitric oxide inhalation attenuates pulmonary hypertension and improves gas exchange in endotoxin shock. *Eur. J. Pharmacol.*, **233**, 85–94.
- WRIGHT, C.E., REES, D.D. & MONCADA, S. (1992). Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc. Res.*, **26**, 48–57.
- ZAPOL, W.M. & SNIDER, M.T. (1977). Pulmonary hypertension in severe acute respiratory failure. *New Engl. J. Med.*, **296**, 476–480.

(Received June 29, 1994
Revised September 14, 1994
Accepted September 28, 1994)