Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 475 (2003) 61–67
www.elsevier.com/locate/ejphar

Comparative effects of dexamethasone and L-canavanine in experimental septic shock

Arnaud Mansart[a], Pierre-Edouard Bollaert[b,*], Bruno Levy[b], Marie-Bénédicte Nicolas[c], Jean-Pierre Mallié[a]

[a] *Laboratoire de Néphrologie Expérimentale (UPRES-JE 2165), Faculté de Médecine, 54 505 Vandoeuvre les Nancy, France*
[b] *Service de Réanimation Médicale, Hôpital Central, 54035 Nancy, France*
[c] *Laboratoire de Biochimie, Hôpital Central, 54 035 Nancy, France*

Received 20 February 2003; received in revised form 20 June 2003; accepted 27 June 2003

Abstract

Glucocorticoids can reverse hemodynamic disturbances and dependence on catecholamines in septic shock. The relevant beneficial mechanisms of steroids in septic shock are unknown, although inducible nitric oxide synthase could account for them. The aim of this study was to compare the effects of dexamethasone, a glucocorticoid and L-canavanine, a selective inhibitor of inducible nitric oxide synthase, in a rodent model of sepsis. Mean arterial pressure was restored by dexamethasone and L-canavanine administration at 24 h, no longer at 30 h. Dexamethasone but not L-canavanine improved aortic blood flow at 24 and 30 h. Although both dexamethasone and L-canavanine administration significantly reduced nitrite/nitrate production, and improved survival, steroids did better for survival. In conclusion, dexamethasone and L-canavanine displayed similar vasopressor effects. In addition, steroids improved blood flow suggesting that steroid-induced hemodynamic improvement in sepsis is not solely due to inhibition of inducible nitric oxide synthase.

Keywords: Sepsis; Steroid; Vasopressor; Hemodynamic; Nitric oxide (NO)

1. Introduction

Septic shock remains a major therapeutic concern in intensive care units, with high mortality rates (Brun-Buisson et al., 1995; Angus et al., 2001). This clinical syndrome, which associates sepsis with hypotension, generally presents hemodynamic pattern with a dramatic fall in systemic vascular resistance and generalized blood flow maldistribution (Parrillo, 1990). In addition to the treatment of the underlying infectious disease, volume expansion and vasoconstrictive drugs are used to control cardiovascular failure. However, vascular refractoriness to volume loading and pressor agents is frequently observed.

Overproduction of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) has been recognized as a major mechanism of the cardiovascular failure characterizing septic shock, leading to the proposal that inhibition of NO synthesis might be a useful adjunct to septic shock therapy (Thiemermann and Vane, 1990). Several L-arginine-related compounds, acting as competitive NOS inhibitors, were thus shown to restore vascular reactivity and to reverse hypotension in experimental (Thiemermann and Vane, 1990) as well as human septic shock (Petros et al., 1994), although this was associated in some studies with a number of detrimental consequences (Statman et al., 1994; Wright et al., 1992), including increased mortality (Grover et al., 1999a). It has been claimed that constitutive NOS (I NOS and III NOS) inhibition was responsible for their deleterious effects in septic shock and that selective inhibition of iNOS might be more suitable (Szabo, 1995; Wright et al., 1992). Indeed, beneficial effects and improved survival have been reported in animal models of septic shock after administration of selective inhibitors of iNOS (Aranow et al., 1996; Szabo et al., 1994). Results of previous studies of mortality in septic iNOS-deficient mice have been mixed, with either improved survival (Wei et al., 1995; Hollenberg et al., 2000) or no difference (MacMicking et al., 1995; Laubach et al., 1995).

Although their use has been proposed for many years in sepsis and septic shock with controversial results (Bone et al., 1987; The Veterans Administration, 1987), glucocorti-

* Corresponding author. Tel.: +33-383-85-14-94; fax: +33-383-85-26-22.
E-mail address: pe.bollaert@chu-nancy.fr (P.-E. Bollaert).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)02035-1

coids have recently been consistently demonstrated to improve hemodynamics in septic shock. In humans, they were found to increase the vasopressor response to adrenergic agents (Bellissant and Annane, 2000), thus reducing the time under vasopressor support (Bollaert et al., 1998; Briegel et al., 1999). In addition, mortality was reduced in a recent study (Annane et al., 2002). The exact mechanisms by which glucocorticoids may act in septic shock are unknown. It is however noteworthy that, among several inhibitor effects on proinflammatory cascade, they are potent inhibitors of iNOS expression (Radomski et al., 1990). Currently, no data are available to understand why inhibitors of iNOS and glucocorticoids could have different effects on survival while both correcting peripheral vascular failure.

These preliminary results prompted us to compare hemodynamic, metabolic patterns and survival effects of dexamethasone (a glucocorticoid) and L-canavanine (a selective inhibitor of iNOS) in a hypokinetic model of septic shock created by cecal ligation and puncture (CLP) in rats.

2. Animals and methods

All procedures were in accordance with the French laws on animal experimentation. The protocol complies with the European Community guidelines for the use of experimental animals.

2.1. Animal model

Adult male Wistar rats (Dépré; St. Doulchard, France) weighing from 240 to 280 g were used in the study. They were housed in cages with constant temperature (22 °C) and exposed to a 12-h light–dark cycle for at least 1 week before use in experimental protocols. All animals were fasted overnight with free access to water.

Animals were randomized to one of the following groups: (a) a sham group where animals underwent laparotomy with cecum manipulation but without ligation or perforation ($n=28$); (b) a sepsis group where animals underwent CLP ($n=28$); (c) a dexamethasone group where 1 mg/kg dexamethasone (Qualimed, Puteaux, France) was administered intraperitoneally 22 h after CLP ($n=28$); (d) a L-canavanine group where 100 mg/kg L-canavanine (Sigma Aldrich, St. Quentin Fallavier, France) was administered intraperitoneally 22 h after CLP ($n=28$). In order to test whether the combination could display additive effects on hemodynamics, a dexamethasone + L-canavanine group where 1 mg/kg dexamethasone and 100 mg/kg L-canavanine were administered intraperitoneally 22 h after CLP ($n=5$) was also investigated.

All groups, except dexamethasone + L-canavanine group, were randomly allocated in three subgroups: one subgroup for hemodynamic and metabolic results 24 h after surgery ($n=9$), the second one for results 30 h after surgery ($n=9$) and the third one ($n=10$) for survival analysis. The dexamethasone + L-canavanine group was only used for hemodynamic results 24 and 30 h after surgery.

2.2. Cecal ligation and perforation model

Sepsis was induced by CLP as described by Wichterman et al. (1980) with minor modifications. Briefly, rats were anesthetized with ketamine (150 mg/kg, intraperitoneally, additional doses were given when necessary) and a 3–4-cm abdominal incision was made to expose the cecum. It was ligated and punctured once with a 21-gauge needle and a small amount of feces extruded. The bowel was returned to the abdomen and abdominal cavity was closed in two layers. For sham rats, a laparotomy was performed and the cecum was manipulated but neither ligated nor punctured. All these animals were resuscitated with 5 ml/100 g body weight of normal saline subcutaneously at the completion of surgery. They were fasted with only free access to water. They were carefully observed after surgery.

2.3. In vivo hemodynamic measurements

Twenty-four or 30 h after CLP or sham procedure, baseline arterial pressure (mean, systolic and diastolic), heart rate and abdominal aortic blood flow were recorded using the following procedure. Animals were anesthetized with sodium thiopental (60 mg/kg body weight intraperitoneally) and additional doses were given when necessary (indicated by presence of interdigital reflexes). A tracheostomy was performed by using PE-160 tubing, and the animals were ventilated using a rodent ventilator with a tidal volume of 1 ml of room air per 100 g. Temperature was monitored by a rectal probe. The left carotid artery was cannulated (PE 50 tubing) and carotid arterial blood pressure continuously monitored using a disposable pressure transducer (5265014 Viggo-Spectramed; Bilthoven, The Netherlands) and an amplifier-recorder system (Sirecust 302A; Siemens, Berlin, Germany). The upper abdominal aorta was carefully dissected and a perivascular (1RB) probe (Transonic Systems, Ithaca, NY, USA) was placed around this. The abdominal aortic blood flow was measured with a Transonic small animal flow meter (T206, Transonic Systems). The preparation lasted about 20 min.

2.4. Biochemical measurements

Blood was withdrawn in each subgroup after hemodynamic measurements. Samples were obtained 24 and 30 h after initiating the model from the left carotid artery.

Lactate determination. Arterial blood samples were collected and then deproteinized by adding 500 μl of perchloric acid (1 M) to 500 μl of whole blood. Lactate was measured by enzymatic–colorimetric method adapted to a Wako

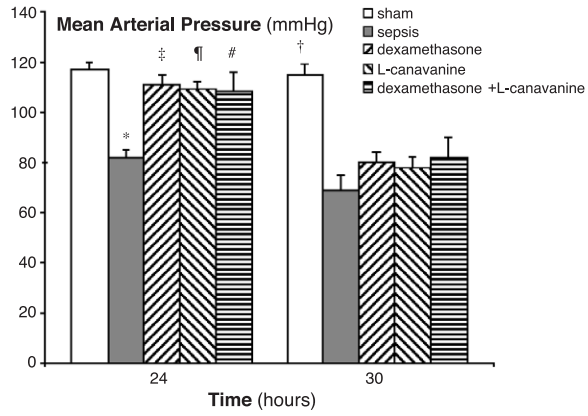

Fig. 1. Mean arterial pressure 24 (T_{24}) and 30 h (T_{30}) after cecal ligation and puncture or sham procedure. Vertical bars indicate ± S.E.M. ($n=9$ for each group; except dexamethasone + L-canavanine group, $n=5$). *$P<0.05$ vs. sham T_{24}, dexamethasone T_{24}, L-canavanine T_{24} and dexamethasone + L-canavanine T_{24}. $^{\dagger}P<0.05$ vs. sepsis T_{30}, dexamethasone T_{30}, L-canavanine T_{30} and dexamethasone + L-canavanine T_{30}. $^{\ddagger}P<0.05$ vs. dexamethasone T_{30}. $^{\P}P<0.05$ vs. L-canavanine T_{30}. $^{\#}P<0.05$ vs. dexamethasone + L-canavanine T_{30}.

automatic analyser (Biochem Systems, Rungis, France). Normal value range is <2 mM.

Serum nitrite/nitrate concentrations. Samples (1 ml of whole blood) were deproteinized before analysis by using sulfosalicylic acid, centrifuged and added to a buffer containing 5% NH_4Cl and 5% NaOH. Samples were injected into a column filled with copper-plated cadmium filings to reduce nitrate to nitrite. The column effluent was mixed with Griess' reagent. Nitrite concentration was determined by measuring the absorbance at 546 nm and compared with a standard solution of sodium nitrate (Green et al., 1982). Normal values were <40 μM.

Plasma epinephrine and norepinephrine levels were measured (2 ml of whole blood) using high performance liquid chromatography–electrochemical detection.

After blood collection, all animals were sacrificed by an overdose of sodium thiopental intravenously.

2.5. Survival

Animals were housed in cages after surgical procedure, with free access to water and food 24 h after. See " cecal ligation and perforation model " for CLP and sham procedure. After first injection (dexamethasone 1 mg/kg or L-canavanine 100 mg/kg), made intraperitoneally at 22 h, same injection was done every 24 h until death.

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. A one-way analysis of variance (ANOVA) was performed to compare groups at one time. Repeated measure ANOVA was used to compare repeated measurements across time between groups. When significant, between groups comparisons were performed using *t*-tests with the Bonferroni correction for multiple comparisons. Survival curves were compared using the log rank test. A $P<0.05$ was considered significant.

3. Results

3.1. Confirmation of the septic model

A few hours after surgical manipulation, CLP-operated rats exhibited signs of sepsis, including piloerection, exudates around the eyes and nose and decreased spontaneous movement. Sham-operated rats were active in their cages. Examination of the peritoneal cavity of septic rats demonstrated copious amounts of purulent peritoneal fluid and the ligated portion of the cecum was grossly dilated and gray-black in color. Examination of the abdominal cavity of sham rats showed no noticeable odor, minimal peritoneal fluid and the bowel was pink in color.

3.2. Hemodynamic measurements

There were significant decreases in mean arterial pressure (Fig. 1) and aortic blood flow (Fig. 2) in sepsis group as compared to sham group at 24 and 30 h. Mean arterial pressure was completely restored in steroid and L-canavanine-treated animals at 24 h but no longer at 30 h. Compared to sepsis group, aortic blood flow was higher in dexamethasone group whereas no significant change was observed in L-canavanine group. Heart rate (Fig. 3) did not differ among groups, except for sepsis group at 30 h, which was decreased. The combination of dexamethasone and L-canavanine did not display any significant

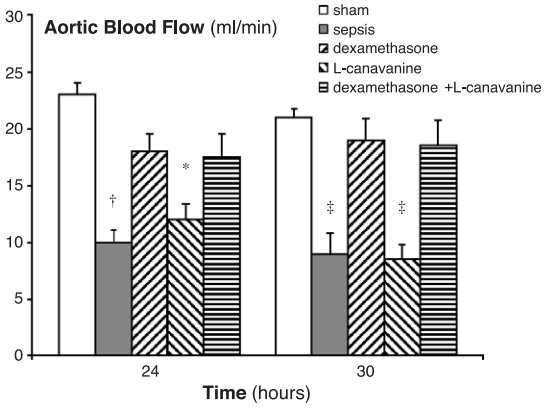

Fig. 2. Aortic blood flow 24 (T_{24}) and 30 h (T_{30}) after cecal ligation and puncture or sham procedure. Vertical bars indicate ± S.E.M. ($n=9$ for each group; except dexamethasone + L-canavanine group, $n=5$). *$P<0.05$ vs. sham T_{24}. $^{\dagger}P<0.05$ vs. sham T_{24}, dexamethasone T_{24} and dexamethasone + L-canavanine T_{24}. $^{\ddagger}P<0.05$ vs. sham T_{30}, dexamethasone T_{30} and dexamethasone + L-canavanine T_{30}.

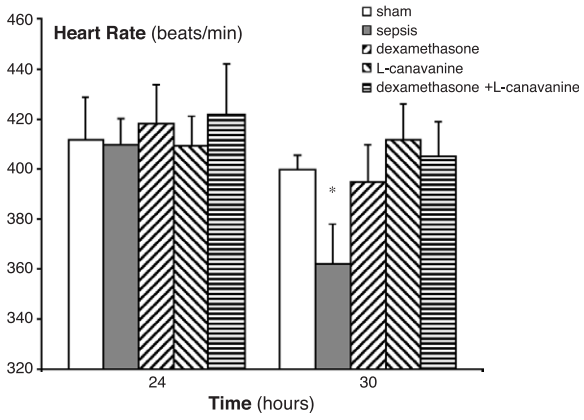

Fig. 3. Heart rate 24 (T_{24}) and 30 h (T_{30}) after cecal ligation and puncture or sham procedure. Vertical bars indicate ± S.E.M. ($n=9$ for each group; except dexamethasone + L-canavanine group, $n=5$). *$P<0.05$ vs. sepsis T_{24}, sham T_{30}, dexamethasone T_{30}, L-canavanine T_{30} and dexamethasone + L-canavanine T_{30}.

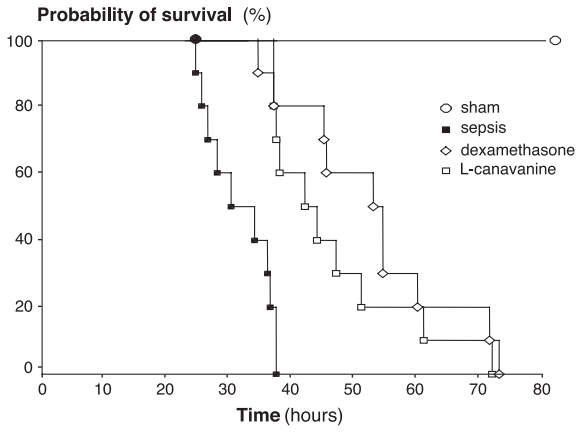

Fig. 4. Survival curves obtained in sham, sepsis, dexamethasone and L-canavanine groups ($n=10$ for each group).

additive effects on mean arterial pressure, aortic blood flow or heart rate.

3.3. Biochemical measurements

Twenty-four and thirty hours after CLP or sham procedure, lactate, norepinephrine, epinephrine and nitrite/nitrate concentrations are shown in Table 1.

3.4. Survival

Dexamethasone and L-canavanine administration improved survival time (52.8 ± 4.1 and 46.6 ± 3.7 h, respectively, vs. 31.6 ± 1.6 h for sepsis group). Time-related survival was better for dexamethasone-treated animals within 50 h ($P=0.05$), but no longer after (Fig. 4).

4. Discussion

In this experimental model of hypotensive, hypokinetic sepsis, both L-canavanine and dexamethasone improved survival. For similar restoration of arterial pressure and nitrite/nitrate inhibition, dexamethasone significantly improved blood flow, whereas L-canavanine did not. In addition, L-canavanine administration increased lactate and catecholamines levels, whereas, in part, dexamethasone administration reduced them, and improved survival rather better than L-canavanine.

Treatment with L-canavanine largely prevented hypotension at 24 h, while attenuating the rise in plasma nitrite/nitrate

Table 1
Lactate, norepinephrine, epinephrine and nitrite/nitrate concentrations 24 (T_{24}) and 30 h (T_{30}) after cecal ligation and puncture or sham procedure

	Lactate (mM)	Norepinephrine (μg/l)	Epinephrine (μg/l)	Nitrite/nitrate (μM)
Sham T_{24}	1.1 ± 0.06[a]	0.36 ± 0.05[a]	0.18 ± 0.01[b]	28.1 ± 2.3
Sepsis T_{24}	3.5 ± 0.5	0.65 ± 0.06	0.87 ± 0.16	56.2 ± 8.1[c]
Dexamethasone T_{24}	1.8 ± 0.4[d]	0.58 ± 0.11	0.39 ± 0.14[a]	18.4 ± 5.7
L-Canavanine T_{24}	4.6 ± 0.8	1.32 ± 0.3[e]	3.75 ± 1.4[f]	33.7 ± 6.1
Sham T_{30}	1.2 ± 0.2[g]	0.23 ± 0.01[g]	0.23 ± 0.1[b]	20 ± 2.8
Sepsis T_{30}	4.9 ± 0.6	1.09 ± 0.18	2.1 ± 0.4	42.6 ± 5[h]
Dexamethasone T_{30}	4.7 ± 0.6	0.77 ± 0.15	1.86 ± 0.5[i]	20.4 ± 5.8
L-Canavanine T_{30}	6.4 ± 0.5[j]	1.37 ± 0.27[e]	6.02 ± 1.2f	31.4 ± 7.1

Values are mean ± S.E.M. $n=9$ animals for each group.
[a] $P<0.05$ vs. sepsis T_{24}, L-canavanine T_{24}.
[b] $P<0.05$ vs. sepsis rats, L-canavanine rats.
[c] $P<0.05$ vs. sham T_{24}, dexamethasone T_{24}, L-canavanine T_{24}.
[d] $P<0.05$ vs. sepsis T_{24}, L-canavanine T_{24}, dexamethasone T_{30}.
[e] $P<0.05$ vs. dexamethasone rats, sepsis T_{24}.
[f] $P<0.05$ vs. sepsis rats.
[g] $P<0.05$ vs. sepsis T_{30}, dexamethasone T_{30}, L-canavanine T_{30}.
[h] $P<0.05$ vs. sham T_{30}, dexamethasone T_{30}.
[i] $P<0.05$ vs. sham T_{30}, L-canavanine T_{30}.
[j] $P<0.05$ vs. sepsis T_{30}, dexamethasone T_{30}.

(stable oxidation product of NO in blood) (Kelm et al., 1992). In previous experiments, evidence was provided that these effects are related to the selective inhibition of iNOS (Liaudet et al., 1996), in agreement with the properties of this compound demonstrated in vitro (Knowles et al., 1990; Umans and Samsel, 1992). However, in the present study, L-canavanine administration did not change aortic blood flow, whereas other experiments have shown an increase of cardiac output (Fishman et al., 1997; Liaudet et al., 1996) during sepsis. Regarding blood flow the discrepancy with the present hypokinetic findings in this study and the hyperkinetic findings in other studies (Fishman et al., 1997; Liaudet et al., 1996) could be related to the model used. Inducible NO synthesis has been implicated as a potential mediator of the myocardial dysfunction (Brady et al., 1992; Ullrich et al., 2000). Modest improvement in myocardial function cannot be ruled out in the L-canavanine-treated animals, since increase in afterload could have overridden it.

Glucocorticoids are also potent inhibitors of iNOS (Radomski et al., 1990; Simmons et al., 1996). Indeed, as in L-canavanine-treated animals, nitrite/nitrate concentrations were markedly reduced in steroid-treated animals and similar findings have been observed in septic shock patients treated with hydrocortisone in a randomized cross-over study (Keh et al., 2003). In addition, pressor effects of dexamethasone and L-canavanine were strictly similar and their combination did not have additive effects. Taken together, these findings suggest that the vasoconstrictive effects of dexamethasone were related to inhibition of iNOS. However, the improvement in blood flow we observed with steroid-treated animals suggests a specific mechanism of steroids on myocardial performance. The decrease in catecholamine levels may be subsequent to improved response of myocardial tissue to adrenergic agonists. An hypothesis to explain these findings could be a specific action of glucocorticoids on adrenergic receptors desensitization, a time-dependent event related to high circulating catecholamines concentrations (Vatner et al., 1989). In sepsis, the high catecholamine concentrations which are observed whatever the model (Jones and Romano, 1984; Tang and Liu, 1996) could theoretically account for a desensitization of both β and α adrenergic receptors (Silverman et al., 1993; Tang and Liu, 1996; Tang et al., 1998; Wakabayashi et al., 1989). However, in an identical model of septic shock, Mansart et al. (2003) have suggested that the overall number and the binding affinity of β-adrenergic receptors are maintained in sepsis at 24 h. Furthermore, the similar increase in cAMP in isoproterenol-stimulated animals provided evidence of normal receptor coupling to adenylyl cyclase. Thus, these findings suggest that myocardial dysfunction is probably not related in a significant way to receptor abnormalities at 24 h. This is consistent with more distal mechanisms of myocardial dysfunction at the subcellular level. They include abnormalities of signal transduction, free radicals action, dysfunction of Ca^{2+}-contractile protein (Tavernier et al., 2001a,b). Dexamethasone could more specifically act on one or several of these targets. Studies have shown a regulation by steroids of phosphorylation of specific protein common to several targets organs (Liu and Greengard, 1976), free radicals inhibition (Marumo et al., 1998) that could explain the improvement of aortic blood flow, observed in this study.

Finally, while correction of hypotension was clearly followed by survival improvement in both groups, increase in blood flow in the dexamethasone-treated animals was associated with decrease in lactate and catecholamine levels and a trend to further survival improvement. Taken together, these findings suggest that in this severely hypokinetic septic model, blood flow improvement was able to reduce tissue hypoxia. Lactate and survival profiles support this hypothesis. In addition, the decrease in catecholamine levels in steroid-treated animals might be a reflect of improved circulatory conditions.

Could these findings be relevant to steroid or NOS inhibitors use in human sepsis? Compared with bolus endotoxin-infused animal models, the present fluid resuscitated CLP model is closer to human sepsis. In addition, the doses of bolus endotoxin used in rodents to produce frank hypotension could induce specific effects not usually observed in human sepsis (Piper et al., 1996). The time course of mortality in the present experiments is not very different from human sepsis. Two differences should however limit the interpretation of our findings to humans. First, animals did not receive antibiotics that could have led to suboptimal management despite the fact that antibiotics are of less clinical value than surgical treatment of peritonitis. Second, animals remained hypokinetic that is less frequent than hyperkinetic profiles in humans. In clinical practice, hydrocortisone has been demonstrated to reduce the vasopressor needs, while maintaining or slightly improving arterial pressure and maintaining or slightly reducing oxygen transport in hyperdynamic patients (Bollaert et al., 1998; Briegel et al., 1999). Similar findings in similar conditions have been observed with low doses of N^G-methyl-L-arginine; higher doses were associated with more marked reduction in oxygen transport secondary to increased afterload (Grover et al., 1999b). However, no data are available in subgroups with hypokinetic sepsis. Although speculative, the positive effect of steroids on myocardial function could better maintain oxygen transport in front of an increased afterload, thus preserving tissue oxygenation. Specific studies on this point are needed to definitely validate this concept in human sepsis.

Acknowledgements

Supported, in part, by grants from Ministère de l'Education Nationale et de la Recherche (UPRES 2165) and from Perrier-Vittel France.

We thank C. Montemont and E. Vauthier for expert technical assistance. We also thank Dr. M.A. Gelot, chemistry laboratory, for biochemical measurements.

References

Angus, D.C., Linde-Zwirble, W.T., Lidicker, J., Clermont, G., Carcillo, J., Pinsky, M.R., 2001. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit. Care Med. 29, 1472–1474.

Annane, D., Sebille, V., Charpentier, C., Bollaert, P.E., Francois, B., Korach, J.M., Capellier, G., Cohen, Y., Azoulay, E., Troche, G., Chaumet-Riffaut, P., Bellissant, E., 2002. The effect of a seven-day treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. JAMA 288, 862–871.

Aranow, J., Zhuang, J., Wang, H., Larkin, V., Smith, M., Fink, M.P., 1996. A selective inhibitor of inducible nitric oxide synthase prolongs survival in a rat model of bacterial peritonitis. Comparison with two nonselective strategies. Shock 5, 116–121.

Bellissant, E., Annane, D., 2000. Effect of hydrocortisone on phenylephrine—mean arterial pressure dose–response relationship in septic shock. Clin. Pharmacol. Ther. 68, 293–303.

Bollaert, P.E., Charpentier, C., Levy, B., Debouverie, M., Audibert, G., Larcan, A., 1998. Reversal of late septic shock with supraphysiologic doses of hydrocortisone. Crit. Care Med. 26, 645–650.

Bone, R.C., Fisher, C.J., Clemmer, T.P., Slotman, G.J., Metz, C.A., Balk, R.A., The Methylprednisolone Severe Sepsis Study Group, 1987. A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. N. Engl. J. Med. 317, 653–658.

Brady, A.J.B., Poole-Wilson, P.A., Harding, S.E., Warren, J.B., 1992. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxaemia. Am. J. Physiol. 263, H1963–H1966.

Briegel, J., Forst, H., Haller, M., Schelling, G., Kilger, E., Kuprat, G., Hemmer, B., Hummel, T., Lenhart, A., Heyduck, M., Stoll, C., Peter, K., 1999. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomised, double-blind single-center study. Crit. Care Med. 27, 723–732.

Brun-Buisson, C., Doyon, F., Carlet, J., Dellamonica, P., Gouin, F., Lepoutre, A., Mercier, J.C., Offenstadt, G., Regnier, B., 1995. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU Group for Severe Sepsis. JAMA 274, 968–974.

Fishman, D., Liaudet, L., Lazor, R., Feihl, F., Perret, C., 1997. L-Canavanine, an inhibitor of inducible nitric oxide synthase, improves venous return in endotemic rats. Crit. Care Med. 25, 469–475.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.C., Tannenbaum, F.R., 1982. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal. Biochem. 126, 131–138.

Grover, R., Lopez, A., Lorente, J., Steingrub, J., Bakker, J., Wilatts, S., McLuckie, A., Takala, J., for the International 546C88 Septic Shock Study Group, 1999a. Multi-center, randomized, placebo-controlled, double blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock (abstract). Crit. Care Med. 27 (Suppl. 1), A33.

Grover, R., Zachardelli, D., Colice, G., Guntupalli, K., Watson, D., Vincent, J.L., 1999b. An open-label dose escalation study of the nitric oxide synthase inhibitor, N^G-methyl-L-arginine hydrochloride (546C88), in patients with septic shock. Crit. Care Med. 27, 913–922.

Hollenberg, S.M., Broussard, M., Osman, J., Parrillo, J.E., 2000. Increased microvascular reactivity and improved mortality in septic mice lacking inducible nitric oxide synthase. Circ. Res. 86, 774–778.

Jones, S.B., Romano, F.D., 1984. Plasma catecholamines in the conscious rat during endotoxicosis. Circ. Shock 14, 189–201.

Keh, D., Boehnke, T., Weber-Cartens, S., Schulz, C., Ahlers, O., Bercker, S., Volk, H.D., Doecke, W.D., Falke, K.J., Gerlach, H., 2003. Immunologic and hemodynamic effects of "low-dose" hydrocortisone in septic shock: a double-blind, randomized, placebo-controlled, crossover study. Am. J. Respir. Crit. Care Med. 167, 512–520.

Kelm, M., Feelisch, M., Grube, R., Motz, W., Strauer, B.E., 1992. Metabolism of endothelium-derived nitric oxide in human blood. In: Moncada, M.A., Marletta, M.A., Hibbs, J.B., Higgs, E.A. (Eds.), The Biology of Nitric Oxide. Portland Press Proceedings, London, pp. 453–463. Part 1.

Knowles, R.G., Merrett, M., Salter, M., Moncada, S., 1990. Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in the rat. Biochem. J. 270, 833–836.

Laubach, V.E., Shesely, E.G., Smithies, O., Sherman, P.A., 1995. Mice lacking inducible nitric oxide synthase are not resistant to lipopolysaccharide-induced death. Proc. Natl. Acad. Sci. U. S. A. 92, 10688–10692.

Liaudet, L., Feihl, F., Rosselet, A., Markert, M., Perret, C., 1996. Beneficial effects of L-canavanine, a selective inhibitor of inductible nitric oxide synthase, during rodent endotoxemia. Clin. Sci. 90, 369–377.

Liu, A.Y., Greengard, P., 1976. Regulation by steroid hormones of phosphorylation of specific protein common to several target organs. Proc. Natl. Acad. Sci. U. S. A. 73, 568–572.

MacMicking, J.D., Nathan, C., Hom, G., Chartrain, N., Fletcher, D.S., Trumbauer, M., Stevens, K., Xie, Q.W., Sokol, K., Hutchinson, N., Chen, H., Mudgett, J.S., 1995. Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. Cell 81, 641–650.

Mansart, A., Bollaert, P.E., Seguin, C., Levy, B., Longrois, D., Mallié, J.P., 2003. Hemodynamic effects of early versus late glucocorticoid administration in experimental septic shock. Shock 19, 38–44.

Marumo, T., Schini-Kerth, V.B., Brandes, R.P., Busse, R., 1998. Glucocorticoids inhibit superoxide anion production and p22 phox mRNA expression in human aortic smooth muscle cells. Hypertension 32, 1083–1088.

Parrillo, J.E., 1990. Cardiovascular pattern during septic shock. Ann. Intern. Med. 113, 227–231.

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.

Piper, R.D., Cook, D.J., Bone, R.C., Sibbald, W.J., 1996. Introducing critical appraisal to studies of animal models investigating novel therapies in sepsis. Crit. Care Med. 24, 2059–2070.

Radomski, M.W., Palmer, M.R.J., Moncada, S., 1990. Glucocorticoids inhibit the expression of an inducible but not the constitutive, nitric oxide synthase in vascular endothelial cells. Proc. Natl. Acad. Sci. U. S. A. 87, 10043–10047.

Silverman, H.J., Penaranda, R., Orens, J.B., Lee, N.H., 1993. Impaired beta-adrenergic receptor stimulation of cyclic adenosine monophosphate in human septic shock: association with myocardial hyporesponsiveness to catecholamines. Crit. Care Med. 21, 31–39.

Simmons, W.W., Ungureanu-Longrois, D., Smith, G.K., Smith, T.W., Kelly, R.A., 1996. Glucocorticoids regulate inducible nitric oxide synthase by inhibiting tetrahydrobiopterin synthesis and L-arginine transport. J. Biol. Chem. 271, 23928–23937.

Statman, R., Cheng, W., Cunningham, N., Henderson, J.L., Damiani, P., Siconolfi, A., Rogers, D., Horovitz, J.H., 1994. Nitric oxide inhibition in the treatment of the sepsis syndrome is detrimental to tissue oxygenation. J. Surg. Res. 57, 93–98.

Szabo, C., 1995. Alterations in nitric oxide production in various forms of circulatory shock. New Horiz. 3, 2–32.

Szabo, C., Southan, G.J., Thiemermann, C., 1994. Beneficial effects and improved survival in rodent models of septic shock with *S*-methyl-isothiourea sulfate, a potent and selective inhibitor of inducible nitric oxide synthase. Proc. Natl. Acad. Sci. U. S. A. 91, 12472–12476.

Tang, C., Liu, M.S., 1996. Initial externalization followed by internalization of beta-adrenergic receptors in rat heart during sepsis. Am. J. Physiol. 270, R254–R263.

Tang, C., Yang, J., Wu, L.L., Dong, L.W., Liu, M.S., 1998. Phosphorylation of b-adrenergic receptor leads to its redistribution in rat heart during sepsis. Am. J. Physiol. 274, R1078–R1086.

Tavernier, B., Li, J.M., El-Omar, M.M., Lanone, S., Yang, Z.K., Trayer, I.P., Mebazaa, A., Shah, A.M., 2001a. Cardiac contractile impairment associated with increased phosphorylation of troponin I in endotoxemic rats. FASEB J. 15, 294–296.

Tavernier, B., Mebazaa, A., Mateo, P., Sys, S., Ventura-Clapier, R., Veksler, V., 2001b. Phosphorylation-dependent alteration in myofilament Ca2+ sensitivity but normal mitochondrial function in septic heart. Am. J. Respir. Crit. Care Med. 163, 362–367.

The Veterans Administration Systemic Sepsis Cooperative Study Group, 1987. Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. N. Engl. J. Med. 317, 659–665.

Thiemermann, C., Vane, J., 1990. Inhibition of nitric oxide synthesis reduces the hypotension induced by bacterial lipopolysaccharides in the rat in vivo. Eur. J. Pharmacol. 182, 591–595.

Ullrich, R., Scherre-Crosbie, M., Bloch, K.D., Ichinose, F., Nakajima, H., Picard, M.H., Zapol, W.M., Quezado, Z.M., 2000. Congenital deficiency of nitric oxide synthase 2 protects against endotoxin-induced myocardial dysfunction in mice. Circulation 102, 1440–1446.

Umans, J.G., Samsel, R.W., 1992. L-Canavanine selectively augments contraction in aortas from endotoxemic rats. Eur. J. Pharmacol. 210, 343–346.

Vatner, D.E., Vatner, S.F., Nejima, J., Uemura, N., Susanni, E.E., Hintze, T.H., Homcy, C.J., 1989. Chronic norepinephrine elicits desensitization by uncoupling the b-receptor. J. Clin. Invest. 84, 1741–1748.

Wakabayashi, I., Hatake, K., Kakishita, E., Hishida, S., Nagai, K., 1989. Desensitization of alpha 1 adrenergic receptor mediated smooth muscle contraction in aorta from endotoxic rats. Life Sci. 45, 509–515.

Wei, X.Q., Charles, I.G., Smith, A., Ure, J., Feng, G.J., Huang, F.P., Xu, W., Muller, W., Moncada, S., Liew, F.Y., 1995. Altered immune responses in mice lacking inducible nitric oxide synthase. Nature 375, 408–411.

Wichterman, K.A., Baue, A.E., Chaudry, I.H., 1980. Sepsis and septic shock—a review of laboratory models and a proposal. J. Surg. Res. 29, 189–201.

Wright, C.E., Rees, D.D., Moncada, S., 1992. Protective and pathological roles of nitric oxide in endotoxin shock. Cardiovasc. Res. 26, 48–57.