Tirilazad mesylate (U-74006F) inhibits effects of endotoxin in dogs

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Zhang, Haibo, Herbert Spapen, Panayotis Manikis, Peter Rogiers, Gaëtane Metz, Wim A. Buurman, and Jean-Louis Vincent. Tirilazad mesylate (U-74006F) inhibits effects of endotoxin in dogs. *Am. J. Physiol.* 268 (*Heart Circ. Physiol.* 37): H1847–H1855, 1995.—The present study explored the effects of a potent antioxidant, the 21-aminosteroid U-74006F, on the systemic and regional hemodynamics and the oxygen extraction capabilities during endotoxic shock. Twenty-four anesthetized dogs were randomized into three groups. Group 1 ($n = 8$) served as control. Group 2 ($n = 8$) and group 3 ($n = 8$) received 2 mg/kg iv of *Escherichia coli* endotoxin, followed 30 min later by saline infusion. Group 3 was given U-74006F as an intravenous bolus of 80 $\mu\text{g/kg}$ followed by an infusion of 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, and group 2 received an equivalent volume of vehicle. Tamponade was induced 30 min later to study the oxygen extraction capabilities of the animals. Compared with the endotoxin-alone group, the U-74006F-treated dogs maintained higher mean arterial pressure, cardiac index, stroke volume index, and left ventricular stroke work index and lower pulmonary vascular resistance. They also showed a higher fractional blood flow to mesenteric and renal beds. Endotoxin administration increased whole body critical oxygen delivery ($\text{DO}_{2\text{crit}}$) from 7.7 ± 2.4 to $12.0 \pm 1.9 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ($P < 0.05$), but U-74006F decreased $\text{DO}_{2\text{crit}}$ to $7.8 \pm 2.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ($P < 0.05$ vs. endotoxin alone). Endotoxin decreased critical oxygen extraction ratio ($\text{O}_2\text{ER}_{\text{crit}}$) from 75.0 ± 12.7 to $44.3 \pm 8.7\%$ ($P < 0.05$), but U-74006F increased $\text{O}_2\text{ER}_{\text{crit}}$ to $64.1 \pm 11.2\%$ ($P < 0.05$ vs. endotoxin alone). U-74006F also decreased endotoxin-induced elevation of mesenteric and renal $\text{DO}_{2\text{crit}}$ and markedly increased regional $\text{O}_2\text{ER}_{\text{crit}}$. Systemic and regional blood lactate concentrations were lower in the U-74006F-treated animals. U-74006F also largely attenuated the endotoxin-induced release of tumor necrosis factor and plasma nitrite. We conclude that during endotoxic shock, U-74006F can enhance myocardial function, increase mesenteric and renal blood flow, and improve whole body and regional oxygen extraction capabilities.

oxygen delivery; oxygen extraction; cardiac function; regional blood flow; lazaroid; tumor necrosis factor; nitric oxide; nitrite; lactate

SEPTIC SHOCK is a distributive form of shock, associated with peripheral vasodilation, myocardial depression, and defective oxygen extraction capabilities. The high frequency and the often devastating consequences of septic shock make it a major cause of morbidity and mortality in critically ill patients.

The pathogenesis of septic shock involves the release of a wide array of mediators, among which tumor necrosis factor (TNF) is believed to play a key role

because it can cause significant tissue damage either directly or indirectly by the stimulation of other mediators (5). Among these, oxygen free radicals seem to be important, since they can react with polyunsaturated fatty acids to produce peroxidized lipids, which degenerate proteins and inactivate sulfhydryl enzymes in the membrane (15), thereby changing membrane structure and permeability and eventually causing cell or organ dysfunction (3). Oxygen free radicals have been incriminated in the vascular alterations as well as the myocardial depression associated with septic shock (6, 13). They can also increase the production of TNF by further activating leukocytes and macrophages (14, 22, 26).

Antioxidants can exert protective effects in septic shock by a reduction of the oxidant damage and the attenuation of TNF production (20, 27, 35). With the replenishment of intracellular glutathione, *N*-acetyl-L-cysteine, an antioxidant reacting with hydroxyl radical and hydrogen peroxide, has been recently demonstrated to decrease critical oxygen delivery ($\text{DO}_{2\text{crit}}$) and increase critical oxygen extraction ratio ($\text{O}_2\text{ER}_{\text{crit}}$) during endotoxic shock when blood flow is reduced by cardiac tamponade (35). *N*-acetyl-L-cysteine also markedly reduces pulmonary hypertension and enhances myocardial performance. At least some of these effects can be mediated by attenuating TNF production (35), although *N*-acetyl-L-cysteine also has important vasodilating effects in the microvasculature.

The 21-aminosteroid, U-74006F [21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16 α -methylpregna-1,4,9(11)-triene-3,20-dione, monomethanesulfonate], has been shown to scavenge the lipid peroxyl and phenoxy radicals (7). In various animal models of ischemia and reperfusion, U-74006F has been reported to attenuate the accumulation of neutrophils (30), to protect endothelial structure (12), to diminish myocardial injury (9), to maintain a higher arterial pressure, and to increase survival (2). Importantly, U-74006F has been also shown to suppress eicosanoid and TNF production (30) and to prevent lactic acidosis (28) and to increase survival rate (27) following endotoxemia in animals.

We hypothesized that U-74006F may exert beneficial effects on hemodynamics and organ perfusion and in particular improve tissue oxygen extraction capabilities in severe sepsis. We thus explored the effects of U-74006F on the relationship between oxygen uptake ($\dot{V}\text{O}_2$) and oxygen delivery (DO_2) at systemic and regional levels during endotoxic shock in the dog. We used a previously described model (35, 36) in which endotoxin administra-

tion is followed by fluid resuscitation to obtain a hyperdynamic shock reproducing the pattern observed in clinical septic shock. Cardiac tamponade is then induced to decrease cardiac output and thereby to study the tissue oxygen extraction capabilities in these conditions.

MATERIALS AND METHODS

Surgical Preparation

This study was approved by the Institutional Review Board of Animal Research, and care and handling of the animals were in accord with National Institutes of Health guidelines. Twenty-four mongrel dogs weighing 30.7 ± 5.6 kg were anesthetized with 30 mg/kg iv of pentobarbital sodium, followed by a constant 4 mg·kg⁻¹·h⁻¹ infusion (infusion pump Infusomat II; B. Braun, Melsungen, Germany). The trachea was intubated with a no. 9 cuffed endotracheal tube, and each animal was ventilated with room air (Servo ventilator 900B; Siemens-Elema, Solna, Sweden). Controlled ventilation was facilitated with pancuronium bromide (0.15 mg/kg initially, with supplementation at 0.075 mg·kg⁻¹·h⁻¹ thereafter). Respiratory frequency was set at 12 breaths/min, and tidal volume adapted to obtain an initial end-tidal carbon dioxide tension (47210A Capnometer; Hewlett-Packard, Waltham, MA) between 28 and 38 mmHg, and the ventilatory conditions were not changed during the studies. The left forepaw vein was catheterized for intravenous administration of pentobarbital and the right forepaw vein for intravenous fluid infusion and U-7406F administration. The right femoral artery was catheterized for monitoring of arterial blood pressure and withdrawal of arterial blood samples. Through the right external jugular vein, a balloon-tipped pulmonary artery catheter (model 93A-131-7F; Baxter Healthcare, Irvine, CA) was placed under guidance of pressure waves (monitor Sirecust 302A; Siemens, Erlangen, Germany). A left thoracotomy was performed between the fourth and the fifth intercostal space to introduce a 16-gauge polyethylene catheter (Intracath; Deseret Medical, Sandy, UT) with multiple side holes around the tip segment into the pericardial space via a 2- to 3-mm incision in the anterior pericardium. The catheter was secured with silk purse-string sutures and sealed with medical glue. The pericardial cavity was drained with replacement of 30 ml of sterile saline heated at 37°C before sealing, and the thoracic cavity was then carefully closed in three layers, and a chest tube (Trocath catheter A75, 28 Ch-40 cm; Argyle, Tullamore, Ireland) was placed through the seventh intercostal space to allow gentle evacuation of the chest. The pericardial catheter was used to inject saline into the pericardial sac and to measure intrapericardial pressure. A splenectomy was performed after maximal splenic contraction to 1 ml of epinephrine (1 mg/ml) through a midline laparotomy to prevent autotransfusion of erythrocytes during hypotension. Ultrasonic flow probes were placed around the superior mesenteric (4–6 mm) and left renal (3–4 mm) arteries close to their origin from the abdominal aorta, respectively, for simultaneous measurement of blood flow of the two vessels. A 16-gauge, 20-cm intravenous catheter (Intramedicut, Sherwood; Argyle) was inserted into the main superior mesenteric vein through a distal tributary of the superior mesenteric vein of the ileum. A 20-gauge, 32-mm intravenous catheter (Surflo intravenous catheter; Terumo Europe, Leuven, Belgium) was positioned in the left renal vein and secured with a purse-string suture. The abdominal incision was then closed. A flow probe was placed around the left femoral (4–6 mm) artery and an 18-gauge, 8-cm catheter (Leader cath; Vygon, Ecouen, France) was inserted into the left femoral vein. The probes were connected to a previously calibrated blood flowmeter (model T208; Transonic Systems,

Ithaca, NY). After surgical preparation, the animal was allowed to stabilize for at least 30 min.

Measurements and Calculations

Pressures from femoral arterial, pulmonary arterial, and intrapericardial lines were monitored continuously using pressure transducers (series 966020-01; Baxter Healthcare) with amplifiers (Hellige Servomed, Freiburg, Germany) and a pen recorder (model 2600S; Gould Instruments, Cleveland, OH). All pressures were determined at end expiration. Cardiac index (l·min⁻¹·kg⁻¹) was measured by the thermodilution technique (COM-2, Baxter Healthcare), using three to five 5-ml bolus injections of cold 5% dextrose in ice water at end inspiration.

Exhaled gases were directed through a mixing chamber for sampling of expired oxygen fraction (P. K. Morgan, Chatham, Kent, UK). Expired minute volume was measured with a spirometer (Haloscale Wright Respirometer; Edrington, UK) over a 2-min period. Arterial, mixed venous, superior mesenteric, left renal, and left femoral blood samples were simultaneously withdrawn for immediate determination of blood gases (ABL-2; Radiometer, Copenhagen, Denmark) and oxygen saturation (OSM-3 hemoximeter calibrated for dog blood; Radiometer). Hematocrit was determined by the capillary method (Hettich Haematokrit, Tuttlingen, Germany). Systemic $\dot{V}O_2$ was measured from the expired gases as previously described (35). Systemic $\dot{D}O_2$ was calculated as the product of arterial oxygen content and cardiac index. Mesenteric and renal $\dot{V}O_2$ values were calculated as the product of superior mesenteric or left renal blood flow and the regional arteriovenous oxygen content difference, and normalized to the weight of the whole small intestine and the left kidney, respectively. Femoral $\dot{V}O_2$ was calculated as the product of left femoral blood flow and the regional arteriovenous oxygen content difference and was reported in milliliters per minute, since we did not measure the weight of the limb. O_2ER_{crit} was derived from the ratio of $\dot{V}O_2$ to $\dot{D}O_2$ at $\dot{D}O_{2crit}$.

Whole body and regional blood lactate concentrations (sampled from femoral artery and superior mesenteric, left renal, and left femoral veins) were determined by a glucose-lactate analyzer (2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH). TNF levels were measured using ligand immunoassay (W. Buurman); the limit of detection of the enzyme-linked immunosorbent assay was 10 pg/ml.

Nitric oxide (NO) release was determined spectrophotometrically by measuring the accumulation of both nitrite and nitrate (the latter is reduced to nitrite) in plasma. Nitrate was stoichiometrically reduced to nitrite by incubation of sample (100 μ l plasma) for 2 h at 37°C, in the presence of 0.1 U/ml nitrate reductase (NAD[P]H: nitrate oxidoreductase, EC 1.6.6.2; *Aspergillus* species; Sigma, St. Louis, MO), 120 μ M NADPH, and 5 μ M FAD (Sigma) in a final volume of 103 μ l. After nitrate had been reduced to nitrite, NADPH, which interfered with the subsequent nitrite determination, was oxidized with 10 U/ml L-lactic dehydrogenase (EC 1.1.1.27; type XI; from rabbit muscle; Sigma) and 10 mM sodium pyruvate for 30 min at 37°C in a final volume of 114 μ l. Sodium nitrate was used as a standard. Nitrite concentration in plasma was assayed by a standard Griess reaction (11). Briefly, 100 μ l of plasma was incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, 2.5% H₃PO₄) at room temperature for 10 min. The absorbance of the chromophore formed was determined at 540 nm using a microtiter plate reader (Molecular Devices, Menlo Park, CA). Sodium nitrite was used as a standard with control baseline plasma as a blank.

Fig. 1. Schematic representation of experimental protocols. Endo, *Escherichia coli* endotoxin; PAOP, pulmonary artery occlusion pressure.

Experimental Protocol

The pericardial cavity was emptied using a 5-ml syringe to ensure a slightly negative intrapericardial pressure before control measurements (baseline). The animals were randomized into three groups (Fig. 1). Group 1 ($n = 8$, control) served as control, receiving normal saline to keep pulmonary artery occlusion pressure at baseline levels. Group 2 ($n = 8$, endotoxin) and group 3 ($n = 8$, U-74006F) received a slow bolus of 2 mg/kg of *Escherichia coli* endotoxin (055:B5, control no. 3120-10-7; Difco, Detroit, MI) over 2 min through the pulmonary artery catheter. A second set of measurements was obtained 30 min after endotoxin administration. Normal saline infusion was titrated thereafter to restore pulmonary artery occlusion pressure to baseline. Group 3 was then given tirilazad mesylate (U-74006F, 1.5 mg/ml, Cas No. 110101-67-2, Upjohn, Kalamazoo, MI) as an intravenous bolus of 80 μ g/kg followed by a continuous infusion of 10 μ g $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ until the end of the study. Group 2 received an equivalent volume of vehicle over an identical time course. The U-74006F solution and the vehicle were stored at 4°C before the experiment. Thirty minutes later, a third set of measurements (U-74006F) was determined. Tamponade was then induced by repeated bolus injections of normal saline heated to 37°C into the pericardium. The amount of saline injected was 30 ml for the first two injections, then 10 ml until $\dot{V}O_2$ started to fall from baseline levels, and finally 2–5 ml until mean arterial pressure fell by 80% from baseline. The experiment was then ended. After each injection, a time interval of 20 min was permitted to reach a steady state, characterized by a stable expired oxygen fraction, end-tidal carbon dioxide tension, arterial pressure, and heart rate before the next measurements were obtained. Throughout the study, the core temperature of the animals was kept constant by a heating blanket and operating lamps.

Statistical Methodology

The $\dot{D}O_{2crit}$ was determined in each animal by dual-line regression from a plot of systemic and regional $\dot{V}O_2$ vs. $\dot{D}O_2$, respectively. For each plot, linear regression by best fit was used to calculate straight lines for the oxygen supply depen-

dency and independency (35). The point of intersection of their regression lines defined the $\dot{D}O_{2crit}$ and the corresponding critical $\dot{V}O_2$. $O_{2ERcrit}$ was calculated as the ratio of $\dot{V}O_2$ to $\dot{D}O_2$ at $\dot{D}O_{2crit}$. A two-way analysis of variance (for intrapericardial pressure and group) followed by a Dunnett's test was used for statistical analysis. A P value < 0.05 was considered statistically significant. All values are expressed as means $\pm$ SD.

RESULTS

Control Conditions

The progressive increase in intrapericardial pressure resulted in a reduction in arterial pressure, cardiac index, stroke index, and left ventricular stroke work index. Systemic vascular resistance increased initially but fell when intrapericardial pressure reached higher levels (Fig. 2). The degree of reduction in regional blood flow was similar in the mesenteric, renal, and femoral beds. In the three regions, fractional blood flow was initially well maintained and markedly fell at high levels of intrapericardial pressure (Fig. 3). There was a parallel increase in systemic and regional blood lactate concentrations (Table 1). Table 2 shows the systemic and regional oxygen-derived variables. Table 3 shows the systemic and regional $\dot{D}O_{2crit}$ calculated from whole body $\dot{V}O_2$ and regional $\dot{V}O_2$, respectively.

Endotoxic Conditions

Effects of endotoxin alone. Endotoxin administration resulted in sharp decreases in mean arterial pressure, cardiac filling pressures, cardiac index, stroke index, left ventricular stroke work index, and systemic vascular resistance and increases in pulmonary vascular resistance and systemic and regional blood lactate levels (Table 1, Fig. 2). The degree of reduction in blood flow was similar in the mesenteric, renal, and femoral beds (Fig. 3).

Fig. 2. Relationship between mean arterial pressure (MAP), cardiac index (CI), stroke volume index (SVI), left ventricular stroke work index (LVSWI), systemic vascular resistance (SVR), and pulmonary vascular resistance (PVR) and intrapericardial pressure (IPP) in the 3 groups of animals. * $P < 0.05$ vs. control ($\circ$); † $P < 0.05$ vs. endotoxin (Δ); § $P < 0.05$ vs. U-74006F ($\square$); ‡ $P < 0.05$ vs. both endotoxin and U-74006F groups. Endo, 30-min postendotoxin.

After initial fluid resuscitation, mean arterial pressure and systemic vascular resistance remained low, but cardiac index and stroke index increased, reflecting a hyperdynamic state (Fig. 2). Absolute blood flow in the mesenteric and femoral beds was higher in the endotoxin than in the control animals, but renal blood flow was not significantly influenced. However, fractional blood flow to mesenteric and renal vasculatures was significantly decreased in the endotoxin dogs compared with the controls (Fig. 3). Systemic and regional blood lactate concentrations were higher in the endotoxin than in the control animals (Table 1).

Endotoxin animals had higher $\dot{V}O_{2crit}$ in whole body, mesenteric, and femoral vasculatures than the control animals. They also had significantly lower O_2ER_{crit} in the whole body and the three regional beds (Table 2). The relation between regional $\dot{V}O_2$ and systemic $\dot{V}O_2$ indicated that oxygen supply dependency in the mesenteric and femoral beds started to occur earlier in the endotoxin than in the control animals (Table 3).

Effects of U-74006F. U-74006F administration following endotoxin significantly increased mean arterial pressure, cardiac index, stroke index, and left ventricular stroke work index. U-74006F did not influence systemic vascular resistance but prevented the increase in pulmonary vascular resistance associated with the increase in intrapericardial pressure (Fig. 2). U-74006F increased the absolute blood flow to all three regions studied and the fractional blood flow to mesenteric and renal beds at high intrapericardial pressure levels (Fig. 2). Whole body and regional blood lactate concentrations remained lower in the U-74006F-treated than in the other animals (Table 1).

U-74006F significantly reduced the endotoxin-induced increase in whole body, mesenteric, and renal $\dot{V}O_{2crit}$ and restored the corresponding O_2ER_{crit} close to the levels observed in the control conditions. Changes in the femoral vasculature, although parallel, did not reach statistical significance (Table 2).

U-74006F-treated animals tolerated larger intrapericardial volume and higher intrapericardial pressure so that the survival time was longer in these animals than in endotoxin animals receiving only vehicle (Table 4).

The endotoxin-induced increase in TNF and nitrite levels were inhibited by U-74006F (Fig. 4).

DISCUSSION

In previous studies (7, 9, 31), the 21-aminosteroid U-74006F has been shown to exert strongly protective effects in models of ischemia-reperfusion by inhibiting lipid peroxidation. The present study showed that U-74006F administration 30 min after intravenous endotoxin injection markedly attenuated the hemodynamic and the inflammatory effects of endotoxin in the dog.

A central aspect of our study was to investigate the effects of U-74006F on the tissue oxygen extraction capabilities. Healthy, anesthetized dogs show an O_2ER_{crit} between 60 and 75% when $\dot{V}O_2$ falls below a $\dot{V}O_{2crit}$ of 7–9 ml·kg⁻¹·min⁻¹ (25, 36). In the presence of endotoxin, the O_2ER_{crit} reaches only 47–56% when $\dot{V}O_2$ diminishes to a $\dot{V}O_{2crit}$ of 11–13 ml·kg⁻¹·min⁻¹ (25, 35, 36). In the present study also the endotoxin animals

Fig. 3. Changes in absolute (A) and fractional (B) regional blood flow (%) during the incremental IPP in the 3 groups of animals. See Fig. 2 legend for explanation of symbols.

receiving the vehicle had an O_2ER_{crit} of only 44% and a $\dot{D}O_{2crit}$ of $12 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. However, U-74006F administration significantly increased O_2ER_{crit} to 64% and reduced $\dot{D}O_{2crit}$ to $7.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, a value similar to the control levels (25, 36).

The U-74006F-treated dogs maintained a higher mean arterial pressure than the dogs receiving only the vehicle. U-74006F administration did not influence systemic vascular resistance but increased stroke index and cardiac index. Thus a higher left ventricular stroke work index in the presence of similar cardiac filling pressures indicated that U-74006F improved cardiac function. Myocardial protective effects of U-74006F have also been reported in ischemia and reperfusion in rabbits or in traumatic shock in rats (2, 9), where the substance diminished creatine phosphokinase release, improved peak positive and negative rates of pressure development, and reduced diastolic pressure (9). In rats, U-74006F improves myocardial contractility by inhibiting hydrogen peroxide-mediated alkane generation causing myocardial injury (29). Our observations are the first to indicate that U-74006F can improve cardiac function following endotoxin administration.

Pulmonary vascular resistance was lower in the U-74006F-treated than in the control animals. In the two endotoxic groups pulmonary artery pressures were

similar and cardiac index was significantly higher in the U-74006F-treated than in the endotoxin-alone animals, indicating that U-74006F decreased the pulmonary vascular tone. These may be at least in part attributed directly or indirectly to the antioxidant effects of U-74006F. Oxygen free radicals have been implicated in the pathogenesis of the pulmonary hypertension induced by endotoxin or TNF (1, 19). In particular, the TNF-induced decrease in glutathione can increase the sensitivity of pulmonary vascular endothelial cells to hydrogen peroxide (17). In addition, U-74006F may maintain higher levels of prostacyclin (24) and thereby inhibit the effects of thromboxane, which is a key player in the endotoxin-induced pulmonary hypertension (4). The present model is not associated with severe respiratory impairment. However, Tanigaki and co-workers (33) recently reported that U-78518F, another 21-aminosteroid, could protect the guinea pig against endotoxin-induced lung injury as reflected by decreased edema formation and lung protein fluxes.

The protective cardiovascular effects of the U-74006F-treated animals following endotoxin administration were associated with less cellular alterations, as suggested by lower systemic and regional blood lactate concentrations than in the endotoxin-alone group, particularly at higher intrapericardial pressure levels. Another study reported

Table 1. Heart rate, cardiac filling pressures, and whole body and regional blood lactate concentrations

	Baseline	Endotoxin	U-74006F	Intrapericardial Pressure, mmHg						
				0	2	4	6	8	10	12
HR, beats/min										
Control	153 ± 27	152 ± 24	154 ± 23	153 ± 27	146 ± 25	155 ± 28	157 ± 24	153 ± 27	145 ± 3	51 ± 12
Endotoxin	134 ± 28	153 ± 21	152 ± 21	151 ± 40	151 ± 12	141 ± 20	149 ± 15	150 ± 13	129 ± 13	130 ± 8*
U-74006F	143 ± 21	167 ± 28	163 ± 29	158 ± 26	169 ± 23	170 ± 44	158 ± 32	144 ± 23	145 ± 25	147 ± 42*
MPAP, mmHg										
Control	16.4 ± 3.4	16.3 ± 4.0	16.7 ± 3.6	16.5 ± 3.4	16.7 ± 4.1	16.8 ± 4.3	16.1 ± 2.9	15.1 ± 2.5	17.0 ± 2.8	11.7 ± 3.1
Endotoxin	14.4 ± 2.2	13.3 ± 3.0	16.1 ± 4.2	14.8 ± 3.5	16.3 ± 4.2	19.8 ± 0.8	16.2 ± 2.8	15.0 ± 3.9	16.7 ± 3.1	16.2 ± 1.7*
U-74006F	15.5 ± 3.8	14.2 ± 3.2	20.5 ± 2.6*†	17.2 ± 4.1	18.7 ± 3.2	19.9 ± 0.7	19.0 ± 3.4	17.6 ± 2.4	17.2 ± 3.2	18.4 ± 3.4*
PAOP, mmHg										
Control	5.0 ± 1.4	5.8 ± 2.7	5.6 ± 2.5	5.6 ± 2.2	5.8 ± 2.9	7.3 ± 3.0	8.5 ± 2.1	9.9 ± 1.8	12.5 ± 3.5	12.0 ± 3.8
Endotoxin	6.5 ± 2.3	4.1 ± 2.0	6.6 ± 1.9	5.8 ± 2.3	7.0 ± 2.4	8.5 ± 1.0	9.3 ± 1.2	10.0 ± 2.9	11.5 ± 0.7	13.0 ± 1.2
U-74006F	5.8 ± 2.9	3.6 ± 1.6*	6.7 ± 1.2	6.5 ± 2.8	7.0 ± 2.7	9.0 ± 1.2	10.5 ± 0.7	10.8 ± 1.3	12.5 ± 0.6	14.0 ± 1.6
RAP, mmHg										
Control	3.5 ± 1.5	4.6 ± 2.2	5.7 ± 2.5	5.5 ± 1.5	5.8 ± 2.5	6.3 ± 2.7	8.1 ± 2.5	9.1 ± 2.5	13.5 ± 2.1	15.0 ± 2.7
Endotoxin	4.8 ± 2.0	2.6 ± 1.8	4.6 ± 2.1	4.0 ± 2.1	5.5 ± 0.7	6.3 ± 0.6	9.0 ± 1.0	10.0 ± 2.8	11.0 ± 2.9	12.8 ± 1.6
U-74006F	4.9 ± 2.6	3.6 ± 1.8	5.6 ± 1.7	6.1 ± 2.7	6.0 ± 3.1	7.5 ± 1.3	10.0 ± 3.1	9.5 ± 0.7	12.3 ± 1.8	14.1 ± 1.6
Whole body lactate, mM										
Control	2.2 ± 0.8	2.3 ± 0.8	2.4 ± 0.7	2.3 ± 0.8	2.6 ± 0.9	2.3 ± 0.9	3.2 ± 1.5	4.3 ± 1.9	4.4 ± 1.8	5.5 ± 2.1
Endotoxin	2.2 ± 0.6	4.5 ± 1.1*	3.5 ± 1.2*	3.4 ± 1.4*	2.7 ± 1.7	3.0 ± 1.4	4.2 ± 0.9	4.3 ± 1.8	5.1 ± 2.4	6.1 ± 2.3
U-74006F	2.3 ± 0.8	4.1 ± 1.1*	3.1 ± 1.0*	3.2 ± 1.2	1.9 ± 0.9	2.9 ± 1.2	2.9 ± 1.8†	2.7 ± 1.2*†	4.0 ± 0.9	3.7 ± 1.5*†
Mesenteric lactate, mM										
Control	2.3 ± 1.0	2.5 ± 0.8	2.6 ± 0.8	2.4 ± 1.0	2.8 ± 0.9	2.8 ± 1.1	3.6 ± 1.5	4.6 ± 1.7	4.4 ± 1.9	5.4 ± 2.3
Endotoxin	2.2 ± 0.6	4.8 ± 1.3*	3.6 ± 1.3	3.5 ± 1.5	2.9 ± 1.8	3.1 ± 1.6	4.4 ± 0.9	4.4 ± 1.9	5.4 ± 2.3	6.1 ± 2.1
U-74006F	2.3 ± 0.6	4.4 ± 1.4*	3.1 ± 1.1	3.2 ± 1.3	2.0 ± 1.1	3.0 ± 1.2	3.0 ± 1.1†	2.9 ± 1.4*†	4.2 ± 0.8	3.6 ± 1.6*†
Renal lactate, mM										
Control	2.3 ± 0.7	2.3 ± 0.7	2.4 ± 0.8	2.3 ± 0.7	2.6 ± 0.8	2.4 ± 1.0	3.3 ± 1.7	4.1 ± 2.1	3.9 ± 2.1	5.0 ± 1.9
Endotoxin	2.1 ± 0.7	4.3 ± 1.0*	3.3 ± 1.3	3.2 ± 1.3	2.5 ± 1.6	2.7 ± 1.2	4.0 ± 1.0	3.9 ± 2.0	4.6 ± 1.9	7.4 ± 1.9*
U-74006F	2.0 ± 0.6	3.8 ± 1.1*	2.8 ± 1.0	2.9 ± 1.2	1.8 ± 1.1	2.8 ± 1.2	2.7 ± 1.2†	2.5 ± 1.1*†	3.6 ± 0.9	3.4 ± 0.4*†
Femoral lactate, mM										
Control	2.3 ± 0.8	2.4 ± 0.9	2.5 ± 0.9	2.3 ± 0.8	2.6 ± 1.1	2.6 ± 0.9	3.4 ± 1.5	4.0 ± 1.9	4.3 ± 2.1	4.3 ± 2.4
Endotoxin	2.5 ± 1.0	4.2 ± 1.0*	3.4 ± 1.1	3.4 ± 1.2	2.7 ± 1.4	2.9 ± 1.0	4.0 ± 0.9	3.8 ± 1.6	4.6 ± 2.1	5.7 ± 2.1*
U-74006F	2.1 ± 0.9	3.8 ± 0.9*	3.2 ± 1.0	3.0 ± 1.1	2.0 ± 1.0	2.9 ± 1.2	2.7 ± 1.1†	2.6 ± 1.2*†	3.9 ± 1.0	3.7 ± 1.6†

Values are means ± SD. HR, heart rate; MPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; RAP, right atrial pressure. **P* < 0.05 vs. control; †*P* < 0.05 vs. endotoxin.

Table 2. Values at critical oxygen delivery

	Control	Endotoxin	U-74006F
Whole body			
VO ₂ , ml·kg ⁻¹ ·min ⁻¹	5.6 ± 1.2	5.2 ± 0.9	4.8 ± 0.7
DO ₂ , ml·kg ⁻¹ ·min ⁻¹	7.7 ± 2.4	12.0 ± 1.9*	7.8 ± 2.0†
O ₂ ER, %	75.0 ± 12.7	44.3 ± 8.7*	64.1 ± 11.2†
Mesenteric vasculature			
VO ₂ , ml·100 g tissue ⁻¹ ·min ⁻¹	3.2 ± 0.6	3.0 ± 0.3	2.9 ± 0.4
DO ₂ , ml·100 g tissue ⁻¹ ·min ⁻¹	4.3 ± 0.6	9.1 ± 2.9*	5.2 ± 1.3†
O ₂ ER, %	72.3 ± 6.4	35.3 ± 13.6*	61.0 ± 10.0†
Renal vasculature			
VO ₂ , ml·100 g tissue ⁻¹ ·min ⁻¹	6.4 ± 4.0	4.9 ± 2.6	4.8 ± 2.4
DO ₂ , ml·100 g tissue ⁻¹ ·min ⁻¹	12.4 ± 4.7	15.0 ± 3.3	10.7 ± 4.5†
O ₂ ER, %	53.9 ± 25.9	33.0 ± 13.1*	47.6 ± 14.4†
Femoral vasculature			
VO ₂ , ml/min	3.6 ± 0.7	4.0 ± 0.6	3.0 ± 1.0
DO ₂ , ml/min	4.7 ± 1.0	8.6 ± 2.1*	6.3 ± 2.6
O ₂ ER, %	77.4 ± 10.7	48.0 ± 7.9*	52.4 ± 16.1*

Values are means ± SD. VO₂, oxygen uptake; DO₂, oxygen delivery; O₂ER, oxygen extraction ratio. **P* < 0.05 vs. control; †*P* < 0.05 vs. endotoxin.

that U-74006F prevented lactic acidosis and also decreased the severity of hypoglycemia following endotoxin administration in neonatal calves (28).

Endotoxin administration caused immediate decrease in blood flow in the three regional beds studied. Fluid resuscitation following endotoxin enhanced blood flow in the mesenteric and the femoral beds but not in the renal vasculature. The distribution of cardiac output was initially maintained near baseline levels in the three regions but significantly decreased in the mesenteric

Table 3. Mean critical systemic oxygen delivery calculated from whole body VO₂ or regional VO₂

	Control	Endotoxin	U-74006F
Whole body	7.7 ± 2.4	12.0 ± 1.9*	7.8 ± 2.0
Mesenteric bed	10.3 ± 3.4†	15.9 ± 4.6*†	15.4 ± 3.8*†
Renal bed	9.2 ± 2.7	11.0 ± 2.3	6.6 ± 3.7‡
Femoral bed	7.6 ± 2.3	11.6 ± 2.2*	9.0 ± 3.8

Values (in ml·kg⁻¹·min⁻¹) are means ± SD. **P* < 0.05 vs. control; †*P* < 0.05 vs. whole body; ‡*P* < 0.05 vs. endotoxin.

Table 4. Survival time, intrapericardial pressure, and volume injected into the pericardium

	Survival Time, min	Intrapericardial Pressure, mmHg	Pericardial Volume, ml
Control	218 ± 16	11 ± 2	167 ± 28
Endotoxin	195 ± 21*†	10 ± 3†	150 ± 46†
U-74006F	245 ± 23*	16 ± 3*	200 ± 68

Values are means ± SD. * $P < 0.05$ vs. control; † $P < 0.05$ vs. U-74006F.

and the renal vasculatures at high intrapericardial pressure. Our observations are consistent with previous studies demonstrating that endotoxin administration decreases the absolute blood flow and the fractional distribution of blood flow to splanchnic and renal beds but influences much less the blood flow to the skeletal muscle (16, 34). In the present study, U-74006F administration increased fractional blood flow to mesenteric and renal vasculatures but did not significantly influence femoral blood flow, indicating that U-74006F exerts beneficial effects on the distribution of blood flow in endotoxic shock.

Furthermore, U-74006F significantly influenced individual organ oxygen extraction capabilities during endotoxic shock. We explored the magnitude of changes in O_2ER_{crit} in mesenteric, renal, and femoral beds, and

observed that endotoxin significantly increased $\dot{D}O_{2crit}$ and decreased O_2ER_{crit} in mesenteric and femoral vasculatures. Previous observations on a model of hemorrhage also reported that endotoxin impaired oxygen extraction capabilities in the mesenteric bed (25) and in skeletal muscle (8). Additionally, we found that endotoxin significantly altered the renal oxygen extraction capabilities, as reflected by a lower renal O_2ER_{crit} and a somewhat higher renal $\dot{D}O_{2crit}$. The administration of U-74006F largely attenuated the endotoxin-induced alterations in oxygen extraction capabilities in mesenteric and renal beds but not in femoral vasculature.

U-74006F exerts these protective effects after endotoxemia by several intertwined mechanisms. The strong antioxidant properties of U-74006F (7, 20) and its anti-inflammatory effects on neutrophils (30) must have played a crucial role. Endotoxin is known to activate polymorphonuclear leukocytes, resulting in the release of oxygen free radicals involved in the microbial killing but also in the alterations in permeability and cellular injury (23). In addition, U-74006F has also been suggested to exert a membrane-stabilizing function preserving endothelium integrity (12, 31). In models of hemorrhage and reperfusion in rats, administration of U-74006F was shown to preserve hepatic endothelial structural integrity (12) or to preserve proximal tubular morphology in the kidneys (31). Salem et al. (29) recently found that U-74006F administration significantly decreases lactate dehydrogenase efflux, an index of cell dysfunction and death, during exposure to hydrogen peroxide in rats. In our study, the decreased inflammatory response was reflected by a large inhibition of TNF release in the U-74006F-treated group. Semrad et al. (30) also demonstrated recently that U-74006F significantly inhibited serum TNF production when administered either 1 h before or 1 h after the initiation of an endotoxin infusion in neonatal calves. This attenuating effect on TNF production can also be related to the antioxidant properties of U-74006F, since oxygen free radicals can increase the production of TNF (26). More specifically, spin trap techniques can decrease TNF levels and protect mice from endotoxic shock (26). Other mediators may also be involved. In particular, U-74006F can decrease the production of leukotrienes (30), which may increase TNF release following endotoxin (10).

We also determined systemic plasma nitrite levels, which were markedly increased 3 h after endotoxin administration. This time delay is consistent with the expression of the inducible form of NO synthase in various cells following endotoxin administration (22, 32). Although determinations of blood nitrite are not specific for NO production, the increase in nitrite levels in the vehicle-treated but not in the U-74006F-treated group strongly suggested that U-74006F inhibited NO production. In contrast to the effects of specific NO inhibitors (21), this effect was not associated with any vasoconstriction, indicating that this attenuating effect of U-74006F on nitrate metabolism was part of its anti-inflammatory effects. The anti-inflammatory effects of U-74006F can also account for the improvement in cardiac function observed in the present study, since

Fig. 4. Time course of plasma tumor necrosis factor (TNF) and nitrite concentrations of the 2 endotoxic groups. Open bars, endotoxin alone; solid bars, endotoxin + U-74006F. * $P < 0.05$ between the groups.

TNF and oxygen free radicals including NO have been incriminated in the sepsis-induced myocardial depression (6, 13, 29).

These protective effects in the U-74006F-treated animals were translated into a prolonged survival time. The treated animals tolerated larger intrapericardial volume and thus higher intrapericardial pressure than the endotoxin-alone animals receiving only the vehicle. In another study in rats, Powell et al. (27) demonstrated that the administration of U-74006F at the time of or 4 h following cecal ligation and puncture markedly improved survival rate from 3 to 22% at 72 h.

Because 21-aminosteroids were derived from methylprednisolone, it may be possible that some mechanisms of actions are similar to that of glucocorticoids (30). Although 21-aminosteroids do not have any significant glucocorticoid or mineralocorticoid activity, they can share with corticosteroids the property to stabilize membranes and to inhibit the release of eicosanoids (7, 30). A major difference in activity is that corticosteroids have been shown to be protective primarily when they were administered before endotoxin (18), whereas U-74006F was protective when administered 30 min after endotoxin in the present study.

In conclusion, U-74006F administration significantly enhanced myocardial function, increased mesenteric and renal blood flow, and improved systemic and regional oxygen extraction capabilities during endotoxic shock in dogs. These protective effects were attributed to its strong antioxidant and anti-inflammatory actions, as reflected by attenuated TNF and nitrite production. Importantly, these protective effects were observed when U-74006F was administered 30 min following endotoxin, suggesting a potential therapeutic role of this new agent.

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