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

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Department of Intensive Care, Erasme University Hospital, Laboratory of Immunology, Faculty of Medicine, Free University of Brussels, B-1070 Brussels, Belgium; and Department of Surgery (Immunology Laboratory), Rijksuniversiteit Limburg, 6200 MD Maastricht, The Netherlands

Zhang, Haibo, Herbert Spalen, 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 endotoxemia. Twenty-four anesthetized dogs were randomized into three groups. Group 1 (n = 8) was 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 µg/kg followed by an infusion of 10 µg·kg⁻¹·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 (DO₂crit) from 7.7 ± 2.4 to 12.0 ± 1.9 ml·kg⁻¹·min⁻¹ (P < 0.05), but U-74006F decreased DO₂crit to 7.8 ± 2.0 ml·kg⁻¹·min⁻¹ (P < 0.05 vs. endotoxin alone). Endotoxin decreased critical oxygen extraction ratio (O₂ERcrit) from 75.0 ± 12.7 to 44.3 ± 8.7% (P < 0.05), but U-74006F increased O₂ERcrit to 64.1 ± 11.2% (P < 0.05 vs. endotoxin alone). U-74006F also decreased endotoxin-induced elevation of mesenteric and renal DO₂ crit and markedly increased regional O₂ERcrit. 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.

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-acetylcysteine, an antioxidant reacting with hydroxyl radical and hydrogen peroxide, has been recently demonstrated to decrease critical oxygen delivery (DO₂crit) and increase critical oxygen extraction ratio (O₂ERcrit) during endotoxic shock when blood flow is reduced by cardiac tamponade (35). N-acetylcysteine 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-acetylcysteine also has important vasodilating effects in the microvasculature.

The 21-aminosteroid, U-74006F [21-{4-(2,6-dimethyl-1-pyrroolidinyl)-4-pyrimidinyl}-1-piperazinyl]-16a-methylpregna-1,4,9(11)-trien-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 also been shown to suppress eicosanoid and TNF production (30) and to prevent lactic acidosis (25) 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 (VO₂) and oxygen delivery (DO₂) at systemic and regional levels during endotoxic shock in the dog. We used a previously described model (35, 36) in which endotoxin administra-

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 (7).
tion is followed by fluid resuscitation to obtain a hyper-
dynamic 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 male Sprague-Dawley rats weighing 30.7 ± 5.6 kg were anesthetized
with 30 mg/kg iv of pentobarbital sodium, followed by a con-
stant 4 mg·kg⁻¹·h⁻¹ infusion (infusion pump Infusomat
II; B. Braun, Melsungen, Germany). The trachea was intu-
bated with a no. 9 cuffed endotraechal 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 supple-
mentation at 0.075 mg·kg⁻¹·h⁻¹ thereafter). Respiratory fre-
quency 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 catheter-
ized for intravenous administration of pentobarbital and the
right forepaw vein for intravenous fluid infusion and U-74006F
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-
121-7F; Baxter Healthcare, Irvine, CA) was placed under guid-
ance of pressure waves (monitor Sirecust 302A; Siemens,
Erlangen, Germany). A left thoracotomy was performed be-
tween 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 pericar-
dial 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
(Trocar catheter A75, 28 Ch-40 cm; Argyle, Tullamore, Ire-
land) 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
erthrocytes 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 cath-
eter (Inramedicut, 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 inci-
sion was then closed. A flow probe was placed around the left
demoral (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 al-
lowed to stabilize for at least 30 min.

Measurements and Calculations

Pressures from femoral arterial, pulmonary arterial, and
intrapericardial lines were monitored continuously using pres-
sure transducers (series 966620-01; 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
(1·min⁻¹·kg⁻¹) was measured by the thermodilution tech-
ique (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; Edmonton, UK)
over a 2 min period. Arterial, mixed venous, superior mesen-
teric, left renal, and left femoral blood samples were simulta-
neously withdrawn for immediate determination of blood
gases (ABL-2; Radiometer, Copenhagen, Denmark) and oxy-
gen saturation (OSM-3 hemoximeter calibrated for dog blood;
Radiometer). Hematocrit was determined by the capillary
method (Hettich Haematokrit, Tutlingen, Germany). Sys-
temic VO₂ was measured from the expired gases as previously
described (35). Systemic DO₂ was calculated as the product of
arterial oxygen content and cardiac index. Mesenteric and
renal VO₂ values were calculated as the product of superior
mesenteric or left renal blood flow and the regional arterio-
venous oxygen content difference, and normalized to the weight
of the whole small intestine and the left kidney, respectively.
Femoral VO₂ 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. O₂ERmax was derived
from the ratio of VO₂ to DO₂ at DO₂max.

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 lig-
ding immunoassay (W. Buurman); the limit of detection of
the enzyme-linked immunosorbent assay was 10 pg/ml.

Nitric oxide (NO) release was determined spectrophotometri-
cally 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 µl plasma) for 2 h at 37°C, in the presence of 0.1 U/ml
nitrate reductase (NADPH: nitrate oxidoreductase, EC
1.6.6.2; Aspergillus species; Sigma, St. Louis, MO), 120 µM
NADPH, and 5 µM FAD (Sigma) in a final volume of 103 µl.
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 µl. Sodium
nitrate was used as a standard. Nitrite concentration in
plasma was assayed by a standard Griess reaction (11).
Briefly, 100 µl of plasma was incubated with an equal volume of Griess
reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)ethylenedi-
amine dihydrochloride, 2.5% H₃PO₄) at room temperature for
10 min. The absorbance of the chromophore formed was
determined at 540 nm using a microtitrator plate reader (Mole-
ular Devices, Menlo Park, CA). Sodium nitrite was used as a
standard with control baseline plasma as a blank.
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, U74006F) received an initial bolus of 2 mg/kg of Escherichia coli endotoxin (055:B5, control no. 3120–10; 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 tiralaz in a bolus of 80 μg/kg, followed by a continuous infusion of 10 μg·kg⁻¹·min⁻¹ until the end of the study. Group 2 received an equivalent volume of vehicle over an identical time course. The U74006F solution and the vehicle were stored at 4°C before the experiment. Thirty minutes later, a third set of measurements (U74006F) 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 VO₂ 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 DO₂crit was determined in each animal by dual-line regression from a plot of systemic and regional VO₂ vs. DO₂, respectively. For each plot, linear regression by best fit was used to calculate straight lines for the oxygen supply depend-
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 endotoxic 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 endotoxic dogs compared with the controls (Fig. 3). Systemic and regional blood lactate concentrations were higher in the endotoxic than in the control animals (Table 1).

Endotoxic animals had higher $D_{O_2\text{crit}}$ in whole body, mesenteric, and femoral vasculatures than the control animals. They also had significantly lower $O_2ER_{\text{crit}}$ in the whole body and the three regional beds (Table 2). The relation between regional $V_O_2$ and systemic $D_{O_2}$ indicated that oxygen supply dependency in the mesenteric and femoral beds started to occur earlier in the endotoxic 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 $D_{O_2\text{crit}}$ and restored the corresponding $O_2ER_{\text{crit}}$ 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 endotoxic 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 90 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 $O_2ER_{\text{crit}}$ between 60 and 75% when $D_{O_2}$ falls below a $D_{O_2\text{crit}}$ of 7–9 ml·kg$^{-1}$·min$^{-1}$ (25, 36). In the presence of endotoxin, the $O_2ER_{\text{crit}}$ reaches only 47–56% when $D_{O_2}$ diminishes to a $D_{O_2\text{crit}}$ of 11–13 ml·kg$^{-1}$·min$^{-1}$ (25, 35, 36). In the present study also the endotoxic animals...
receiving the vehicle had an $O_2ER_{crit}$ of only 44% and a $D_O_{crit}$ of 12 ml·kg$^{-1}$·min$^{-1}$. However, U-74006F administration significantly increased $O_2ER_{crit}$ to 64% and reduced $D_O_{crit}$ to 7.8 ml·kg$^{-1}$·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

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Endotoxin</th>
<th>U-74006F</th>
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<tr>
<td><strong>HR, beats/min</strong></td>
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<tr>
<td>Control</td>
<td>153 ± 27</td>
<td>152 ± 24</td>
<td>154 ± 23</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>154 ± 28</td>
<td>153 ± 21</td>
<td>152 ± 21</td>
</tr>
<tr>
<td>U-74006F</td>
<td>143 ± 21</td>
<td>167 ± 28</td>
<td>163 ± 29</td>
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<td><strong>MAP, mmHg</strong></td>
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<tr>
<td>Control</td>
<td>164 ± 3.4</td>
<td>163 ± 4.0</td>
<td>167 ± 3.6</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>144 ± 2.2</td>
<td>133 ± 3.0</td>
<td>161 ± 4.2</td>
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<tr>
<td>U-74006F</td>
<td>155 ± 3.8</td>
<td>142 ± 3.2</td>
<td>20.5 ± 26*</td>
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<td><strong>PAOP, mmHg</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>5.0 ± 1.4</td>
<td>5.8 ± 2.7</td>
<td>5.6 ± 2.5</td>
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<tr>
<td>Endotoxin</td>
<td>5.5 ± 2.3</td>
<td>4.1 ± 2.0</td>
<td>6.6 ± 1.9</td>
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<tr>
<td>U-74006F</td>
<td>5.8 ± 2.9</td>
<td>3.6 ± 1.6*</td>
<td>6.7 ± 1.2</td>
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<tr>
<td><strong>Whole body lactate, mM</strong></td>
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<tr>
<td>Control</td>
<td>2.2 ± 0.8</td>
<td>2.3 ± 0.8</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>2.2 ± 0.6</td>
<td>4.5 ± 1.1*</td>
<td>3.5 ± 1.2*</td>
</tr>
<tr>
<td>U-74006F</td>
<td>2.3 ± 0.8</td>
<td>4.1 ± 1.1*</td>
<td>3.1 ± 1.0*</td>
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<tr>
<td><strong>Mesenteric lactate, mM</strong></td>
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<tr>
<td>Control</td>
<td>2.3 ± 1.0</td>
<td>2.5 ± 0.8</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>2.2 ± 0.6</td>
<td>4.8 ± 1.3*</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td>U-74006F</td>
<td>2.3 ± 0.6</td>
<td>4.4 ± 1.4*</td>
<td>3.1 ± 1.1</td>
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<td><strong>Renal lactate, mM</strong></td>
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<tr>
<td>Control</td>
<td>2.3 ± 0.7</td>
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<td>2.4 ± 0.8</td>
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<tr>
<td>Endotoxin</td>
<td>2.1 ± 0.7</td>
<td>4.3 ± 1.9*</td>
<td>3.3 ± 1.3</td>
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<tr>
<td>U-74006F</td>
<td>2.0 ± 0.6</td>
<td>3.8 ± 1.1*</td>
<td>2.8 ± 1.0</td>
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</table>

Values are means ± SD. HR, heart rate; MAP, 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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Endotoxin</th>
<th>U-74006F</th>
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<tr>
<td></td>
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<tr>
<td><strong>Whole body</strong></td>
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<tr>
<td>VO₂, ml·kg⁻¹·min⁻¹</td>
<td>5.6 ± 1.2</td>
<td>5.2 ± 0.9</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td>DO₂, ml·kg⁻¹·min⁻¹</td>
<td>7.7 ± 2.3</td>
<td>12.0 ± 1.9*</td>
<td>7.8 ± 2.0†</td>
</tr>
<tr>
<td>O₂ER, %</td>
<td>75.0 ± 12.7</td>
<td>44.3 ± 8.7*</td>
<td>64.1 ± 11.2†</td>
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<tr>
<td><strong>Mesenteric vasculature</strong></td>
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<tr>
<td>VO₂, ml·100 g tissue⁻¹·min⁻¹</td>
<td>3.2 ± 0.6</td>
<td>3.0 ± 0.3</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>DO₂, ml·100 g tissue⁻¹·min⁻¹</td>
<td>4.3 ± 0.6</td>
<td>5.1 ± 2.5*</td>
<td>5.2 ± 1.3†</td>
</tr>
<tr>
<td>O₂ER, %</td>
<td>72.3 ± 6.4</td>
<td>35.3 ± 13.6*</td>
<td>61.0 ± 10.0†</td>
</tr>
<tr>
<td><strong>Renal vasculature</strong></td>
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<tr>
<td>VO₂, ml·100 g tissue⁻¹·min⁻¹</td>
<td>6.4 ± 4.0</td>
<td>4.9 ± 2.6</td>
<td>4.8 ± 2.4</td>
</tr>
<tr>
<td>DO₂, ml·100 g tissue⁻¹·min⁻¹</td>
<td>12.4 ± 4.7</td>
<td>16.0 ± 3.3</td>
<td>10.7 ± 4.5†</td>
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<tr>
<td>O₂ER, %</td>
<td>53.9 ± 25.9</td>
<td>33.0 ± 13.1*</td>
<td>47.6 ± 14.4†</td>
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<tr>
<td><strong>Femoral vasculature</strong></td>
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<tr>
<td>VO₂, ml/min</td>
<td>3.6 ± 0.7</td>
<td>4.0 ± 0.6</td>
<td>3.0 ± 1.0</td>
</tr>
<tr>
<td>DO₂, ml/min</td>
<td>8.7 ± 1.0</td>
<td>8.6 ± 2.1*</td>
<td>6.3 ± 2.6</td>
</tr>
<tr>
<td>O₂ER, %</td>
<td>77.4 ± 10.7</td>
<td>48.0 ± 7.9*</td>
<td>52.4 ± 16.1*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Endotoxin</th>
<th>U-74006F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Whole body</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂, ml·kg⁻¹·min⁻¹</td>
<td>7.7 ± 2.4</td>
<td>12.0 ± 1.9*</td>
<td>7.8 ± 2.0</td>
</tr>
<tr>
<td>VO₂, ml·100 g tissue⁻¹·min⁻¹</td>
<td>5.6 ± 1.2</td>
<td>5.2 ± 0.9</td>
<td>4.8 ± 0.7</td>
</tr>
<tr>
<td><strong>Mesenteric bed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂, ml·kg⁻¹·min⁻¹</td>
<td>10.3 ± 3.4†</td>
<td>15.9 ± 4.5*</td>
<td>15.4 ± 3.8†</td>
</tr>
<tr>
<td><strong>Renal bed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂, ml·kg⁻¹·min⁻¹</td>
<td>9.2 ± 2.7</td>
<td>11.0 ± 2.3</td>
<td>6.6 ± 3.7†</td>
</tr>
<tr>
<td><strong>Femoral bed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂, ml·kg⁻¹·min⁻¹</td>
<td>7.6 ± 2.3</td>
<td>11.6 ± 2.2*</td>
<td>9.0 ± 3.8</td>
</tr>
</tbody>
</table>

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.
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 $D_{02crit}$ 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 $D_{O2crit}$. 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. Somrad 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

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

<table>
<thead>
<tr>
<th></th>
<th>Survival Time, min</th>
<th>Intrapericardial Pressure, mmHg</th>
<th>Pericardial Volume, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>218 ± 16</td>
<td>11 ± 2</td>
<td>167 ± 28</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>195 ± 21*†</td>
<td>10 ± 2†</td>
<td>180 ± 46†</td>
</tr>
<tr>
<td>U-74006F</td>
<td>245 ± 23*</td>
<td>16 ± 3*</td>
<td>200 ± 68</td>
</tr>
</tbody>
</table>

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