British Journal of Pharmacology (1996) 118, 1223-1231

Effects of UR-12633, a new antagonist of platelet-activating factor, in rodent models of endotoxic shock

Marta Giral, Dolors Balsa, Rosa Ferrando, Manuel Merlos, Julian Garcia-Rafanell & [1]Javier Forn

Research Center, J. Uriach & Cía, S.A. Degà Bahí 59-67, 08026 Barcelona, Spain

1 The effects of the selective and potent novel platelet-activating factor (PAF) antagonist, UR-12633 (1-(3,3-diphenylpropionyl)-4-(3-pyridylcyanomethyl)piperidine) on several markers of endotoxic shock syndrome were evaluated in rats and mice.

2 UR-12633, administered 60 min after *E. coli* lipopolysaccharide (LPS), reversed the LPS-induced sustained hypotension in rats at doses of 0.01 to 1 mg kg^{-1}, i.v. The reference compound WEB-2086 (1 mg kg^{-1}) also reversed the LPS-induced hypotension. UR-12633 (1 mg kg^{-1}), administered 10 min before LPS, almost fully inhibited sustained hypotension. The immediate hypotension (within 1 min) caused by LPS was not prevented by either UR-12633 or WEB-2086.

3 Pretreatment with 10 mg kg^{-1}, i.v. of either UR-12633 or WEB-2086 inhibited the increase in disseminated intravascular coagulation markers, such as activated partial thromboplastin time (55 and 74% inhibition, respectively), and prothrombin time (22 and 72% inhibition) and prevented the decrease in plasma fibrinogen content (100 and 29% inhibition).

4 Increases in acid phosphatase (ACP) plasma activity, a marker of lysosomal activation, and in lactate dehydrogenase (LDH), a marker of tissue damage, were inhibited by pretreatment with 10 mg kg^{-1}, i.v. of either UR-12633 or WEB-2086 (100% and 69% inhibition, ACP; 62 and 48% inhibition, LDH). Hyperglycaemia (71 and 46%) and hyperlactacidaemia (92 and 56%) were also inhibited.

5 UR-12633, but not WEB-2086, inhibited the LPS-induced increase in vascular permeability in rats, as shown by prevention of haemoconcentration and, to a lesser degree, the increase in Evans blue dye extravasation.

6 In a series of nine reference compounds and UR-12633, we found a high correlation ($P<0.001$) between PAF antagonist activity, measured as the inhibition of PAF-induced rabbit platelet aggregation or PAF-induced mortality in mice and the inhibition of LPS-induced mortality.

7 In spite of the multifactorial nature of endotoxic shock, in which many mediators may be involved, the new potent PAF antagonist, UR-12633, proved effective in protecting against changes in most shock markers. These data strongly suggest a key role for PAF in the pathogenesis of endotoxic shock in rodents.

Keywords: UR-12633; WEB-2086; endotoxic shock; platelet-activating factor; disseminated intravascular coagulation; haemoconcentration; hyperglycaemia; hyperlactacidaemia

Introduction

The administration of endotoxins (lipopolysaccharides, LPS) from gram-negative bacteria to laboratory animals mimics many of the clinical features of septic shock syndrome (Parrillo, 1993). Despite the ongoing controversy over the usefulness of endotoxic shock models as valid approach to mimic clinical sepsis (McCabe *et al.*, 1981; Hurley, 1993), such models have generally been well accepted since the role of LPS in triggering pathogenic processes that lead to shock has been largely demonstrated (Morrison & Ryan, 1987; Danner *et al.*, 1991; Parrillo, 1993; Taveira da Silva *et al.*, 1993).

Platelet-activating factor (PAF) is a natural phospholipid synthesized by several different cells (basophils, macrophages, neutrophils and platelets) and tissues (heart, lung and kidney). There is strong evidence for the participation of PAF in endotoxic shock. First, LPS stimulates the generation of PAF, both *in vitro* (Rylander & Beijer, 1987) and *in vivo* (Doebber *et al.*, 1985; Whittle *et al.*, 1987; Chang *et al.*, 1987). Second, many of the pathological changes seen following PAF administration, such as hypotension, increased vascular permeability, thrombocytopenia and gastrointestinal damage, are very similar to those observed during endotoxaemia (Koltai *et al.*, 1991a, b). And third, PAF antagonists of diverse structure are able to prevent and/or reverse some of the symptoms of endotoxic shock in experimental animal models (for reviews see Toth, 1990 and De Joy *et al.*, 1993) and in human subjects (Thompson *et al.*, 1994).

Endotoxic shock involves a complex and not always well-understood cascade of events affecting most body systems and organs. Changes in haemodynamic, haematological and biochemical parameters have been extensively documented (Morrison & Ryan, 1987; Parrillo, 1993). Thus, we have selected a spectrum of tests to study changes in key markers of the pathophysiology of septic shock. This approach was chosen to determine the potential of the new PAF antagonist, UR-12633 (see Figure 1) for the treatment of endotoxic shock. As a reference compound we selected the PAF antagonist WEB-2086, which has been widely used to elucidate the role of PAF and PAF antagonists in a variety of diseases (for review, see Ikegami *et al.*, 1992). Changes in arterial blood pressure were measured, as systemic hypotension is a major haemodynamic event following LPS administration. Haematocrit values and Evans Blue dye extravasation into several tissues were considered as markers of changes in vascular permeability. Blood platelet count, activated partial thromboplastin time (APTT), prothrombin time (PT), and fibrinogen concentration were measured to monitor the development of disseminated intravascular coagulation (DIC). Plasma markers (glucose, lac-

[1] Author for correspondence.

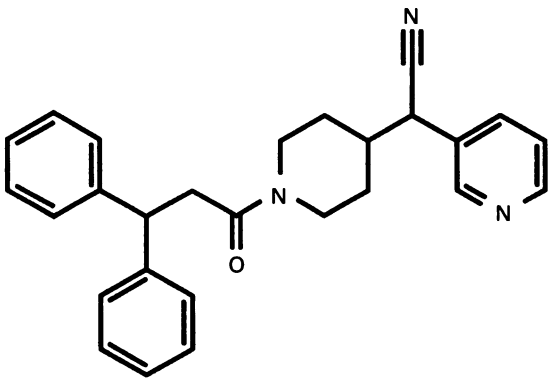

Figure 1 Structure of UR-12633.

tate, acid phosphatase and lactate dehydrogenase) were measured as indicators of altered metabolism or tissue damage. Finally, we also investigated the correlation between PAF antagonist activity *in vitro* and *in vivo* with the LPS-induced mortality in mice.

Methods

Endotoxin-induced hypotension and haematological changes in anaesthetized rats

Fasted male Sprague-Dawley rats weighing 250–275 g were anaesthetized with 50 mg kg^{-1} i.p. sodium pentobarbitone (Sigma Chemical Co., Saint Louis, MO, U.S.A.). Animals were kept under anaesthesia by continuous infusion of 20 mg kg^{-1} h^{-1} of sodium pentobarbitone. Blood pressure was recorded from the left carotid artery, with a Letica pressure transducer connected to a Letica PRS-206 pressure amplifier (Letica S.A., Barcelona, Spain). Both femoral veins were catheterized to inject LPS or test compound (dissolved in saline). LPS from *E. coli*, serotype 0111:B4 at a dose of 20 mg kg^{-1} was used. Experiments were started after blood pressure stabilization. To test their preventive effects, compounds were administered by intravenous injection 10 min before the administration of LPS. To test their curative effects, compounds were administered 60 min after LPS administration. Control animals received only the vehicle (saline). Blood pressure was monitored continuously and the percentage of pressure variation with respect to baseline was calculated. In an independent series of experiments, compounds were administered 10 min before LPS and blood (0.5 ml) was collected from the jugular vein at times 0, 5, 20, 45 and 90 min after LPS injection. Blood parameters were determined with a whole blood analyser Celloscope 1260-PC (Analys Instruments, Stockholm, Sweden).

Effects on vascular permeability in anaesthetized rats

Fasted male Sprague-Dawley rats weighing 180–220 g were used. Vascular permeability was evaluated by a modification of the Evans Blue dye extravasation method (Evans *et al.*, 1988). In a previous study (Balsa *et al.*, 1993) we administered several doses of LPS (lipopolysaccharide from *E. coli* serotype 0127:B8) and studied their effects on permeability in several organs and tissues. From these experiments we selected the dose of 25 mg kg^{-1} of LPS, as it elicited a clear and reproducible effect. The LPS was dissolved in Evans Blue (1% in saline, 1 ml kg^{-1}) and was injected into the femoral vein of pentobarbitone-anaesthetized rats. The animals were exsanguinated by cardiac puncture 1 h later. The trachea, thymus, stomach and seminal vesicle were dissected and weighed. Organs were immersed in a solution of sodium sulphate (0.5%) and acetone (30:70 v/v) at room temperature for 24 h to extract Evans Blue. Afterwards, the mixture was filtered and organs were dried at 60°C for 24 h and weighed. Evans Blue concentration in the filtrate was determined by spectrophotometry at 620 nm and the results were plotted on a standard curve (0.2–2 $\mu g\ ml^{-1}$). The Evans Blue content of each sample was expressed as $\mu g\ g^{-1}$ dry weight tissue. Animals were treated intravenously with the test compounds 5 min before administration of LPS.

Effects on coagulation and biochemical parameters in conscious rats

Fasted male rats weighing 180–220 g were used. Two hours after administration of LPS (lipopolysaccharide from *E. coli* 0127:B8, 25 mg kg^{-1}, i.v.), rats were exsanguinated and 4.5 ml of blood was mixed with 0.5 ml of 3.8% sodium citrate. Plasma was obtained by centrifugation (1000 *g*, 4°C, 15 min). Test compounds were administered i.v. 5 min before LPS. Coagulation parameters (activated partial thromboplastin time, prothrombin time and plasma fibrinogen content) were determined with commercially available tests (Pacific Hemostasis, Huntersville, NC, U.S.A.). Glucose, lactate, lactate dehydrogenase (LDH) and acid phosphatase (ACP) were determined with commercially available kits (Boehringer Mannheim, Barcelona, Spain) in an automatic chemistry analyser (Technicon RA-1000, Bayer Diagnostica, Madrid, Spain).

[^{3}H]WEB-2086 binding in rabbit platelet membranes

Rabbit platelet membranes were prepared as described by Hosford *et al.* (1990). The platelet membrane homogenate was resuspended in HEPES buffer, composition (mM): HEPES 10, pH: 7.4, NaCl 145, KCl 5, $MgCl_2.6H_2O$ 1, NaH_2PO_4 0.6, K_2HPO_4 0.4, glucose 6, 0.1% w/v, bovine serum albumin and stored at −80°C. Protein content in membrane suspensions was determined by Bradford's method (1976) using bovine serum albumin as the standard.

Binding studies were carried out as described by Ring *et al.* (1992) with slight modifications. Platelet membrane samples (0.6 mg ml^{-1}) were incubated at 25°C in HEPES buffer, containing [^{3}H]WEB-2086 (20 nM) in a final assay volume of 0.5 ml. Binding reaction was terminated at 90 min by adding 5 ml of ice-cold HEPES buffer to the incubation mixture. The [^{3}H]WEB-2086 bound to membranes was separated from the free radiolabel by rapid filtration through Whatman GF/C glass fibre filters, that had been presoaked for at least 60 min in HEPES buffer containing 1% (w/v) bovine serum albumin. Then, the filters were washed with 2 × 5 ml ice-cold buffer. Radioactivity was measured by liquid scintillation counting in 3 ml Hisafe3 (LKB, Bromma, Sweden). Non specific binding was determined in the presence of a large molar excess (10 μM) of unlabelled C_{18}-PAF. The affinity of UR-12633 and WEB-2086 for the [^{3}H]WEB-2086 binding sites was expressed as the K_i value.

PAF-induced platelet aggregation in rabbit plasma in vitro

Blood was collected into 3.8% sodium citrate (1 volume for 9 volumes of blood) by cardiac puncture from anaesthetized (sodium pentobarbitone 40 mg kg^{-1} i.p.) male New Zealand rabbits (2–2.5 kg body weight). Platelet-rich plasma (PRP) was prepared by centrifugation of blood (250 *g*, 10 min, 4°C). PRP was diluted with platelet-poor plasma obtained by further centrifugation of PRP (3000 *g*, 10 min, 4°C). The platelet count was adjusted to 3.5×10^8 cells ml^{-1}. PRP was incubated with the inhibitors for 4 min before addition of PAF. Platelet aggregation was induced by C_{18}-PAF (1.5×10^{-8} M) and measured with a dual-channel aggregometer Chrono-log 560 (Havertown, PA, U.S.A.). The activity of each inhibitor was expressed as the IC_{50} value.

PAF-induced mortality in mice

Groups of 10 fasted male Swiss mice weighing 22–26 g were used. A dose of 100 $\mu g\ kg^{-1}$ C_{18}-PAF plus 1 $mg\ kg^{-1}$ propranolol was administered through a lateral tail vein 5 min after i.v. administration of either the test compound (10 $ml\ kg^{-1}$) or saline (control group). Mortality was recorded 2 h after PAF injection. Percentage inhibition of mortality due to the treatments in comparison with the control group was calculated. The results were expressed as the ID_{50} values.

Endotoxin-induced mortality in mice

Groups of 10 fasted male Swiss mice weighing 22–26 g were used. Twenty $mg\ kg^{-1}$ of LPS (lipopolysaccharide from *E. coli* 0111:B4) was injected through a lateral tail vein 5 min after i.v. administration of the test compound. Vehicle (saline) was administered to control animals. Mortality was recorded 7 days after LPS injection. The results were expressed as the ID_{50} values.

Drugs

Propranolol and lipopolysaccharides from *E. coli* (serotypes 0111:B4 and 0127:B8) were obtained from Sigma (St. Louis, MO, U.S.A.). Evans Blue dye was purchased from Fluka (Buchs, Switzerland). C_{18}-PAF, UR-12633 (1-(3,3-diphenylpropionyl)-4-(3-pyridylcyanomethyl)piperidine) and WEB-2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]-triazolo[4,3-a][1,4]diazepine-2-yl]-1-(4-morpholinyl)-1-propanone) were synthesized in our laboratories following published procedures. Other reference drugs were also synthesized in our laboratory (UR-11353, 2-[[N-acetyl-N-[[[2-[octadecyloxy]-4-tetrahydrofuranyl]methoxy]carbonyl]amino]methyl]-1-ethylpyridinium chloride; WEB-2170, 6-(2-chlorophenyl)-8,8-dihydro-1-methyl-8-(4-morpholinylcarbonyl)-4*H*,7*H*-cyclopenta[4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine; SCH-37370, 1-acetyl-4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidine)piperidine; CV-6209, 2-[N-acetyl-N-(2-methoxy-3-octadecylcarbamoyloxypropoxy-carbonyl)aminomethyl]-1-ethylpyridinium chloride; TCV-309, 3-bromo-5-[N-phenyl-N-[2-[[2-(1,2,3,4-tetrahydro-2-isoquinolylcarbonyloxy)ethyl] carbamoyl]ethyl]carbamoyl]-1-propylpyridinium nitrate; BB-882, (S)-4-methyl-2-[methyl-[4-(2-methylimidazo[4,5-c]pyridin-1-ylmethyl)benzenesulphonyl]-amino]-pentanoic acid ethyl ester; L-659,989, *trans*-2-(3-methoxy-5-methylsulphonyl-4-propoxyphenyl)-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran; BN-52021, 3-*terc*-butylhexahydro-4,7b,11-trihydroxy-8-methyl-9*H*-1,7a(epoxymethano)-1*H*,6a*H*-cyclopenta[c]furo[2,3-b]furo[3',2':3,4]cyclopenta[1,2-d]furan-5,9,12(4*H*)-trione) or kindly provided by Merck & Co., Rahway, NJ, U.S.A. (L-659,989) and Henri Beaufour, Le Plessis Robinson, France (BN-52021). All other chemicals used were of reagent grade or of the purest commercially available grade.

Statistical analysis

All values in the figures and text are expressed as mean ± s.e. mean of n observations, where n represents the number of animals. IC_{50} and ID_{50} values and their 95% confidence intervals were calculated with Tallarida's computer program (Tallarida *et al.*, 1981). The binding data were evaluated by using LIGAND programme. Statistical evaluation of the data was performed by analysis of variance (ANOVA) using INSTAT programme. Where ANOVA showed there to be significant difference, the results were further analysed with an *a posteriori* Bonferroni test. A P value less than 0.05 was considered to be statistically significant.

Results

Inhibition of LPS-induced hypotension in anaesthetized rats

Immediately after LPS administration an acute initial phase of hypotension occurred, with mean arterial blood pressure (MABP) falling to about 50% of baseline within 1 min and holding to about 75% of baseline afterwards. About 45 min after LPS administration, a slower and gradual fall in MABP was observed. When administered 60 min after LPS, UR-12633 immediately reversed the hypotension in a dose-dependent manner, showing significant effects even at doses as low as 10 $\mu g\ kg^{-1}$, i.v. Two hours after LPS administration, the MAPB of control animals was 71% of baseline, whereas the

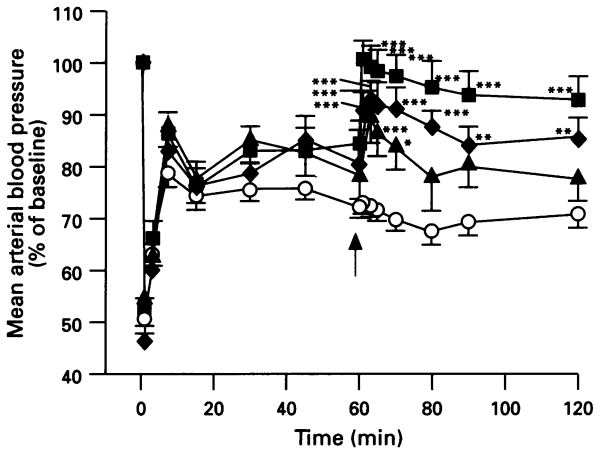

Figure 2 Reversion of lipopolysaccharide-induced hypotension in rats. PAF antagonists UR-12633 at a dose of 1 $mg\ kg^{-1}$, i.v. (■, $n=13$) or 0.01 $mg\ kg^{-1}$, i.v. (▲, $n=13$), and WEB-2086 at a dose of 1 $mg\ kg^{-1}$, i.v. (◆, $n=20$) were administered (indicated by the arrow) 60 min after the intravenous injection of 20 $mg\ kg^{-1}$ lipopolysaccharide from *E. coli* serotype 0111:B4. Control animals (○, $n=48$) received vehicle (saline, 1 $ml\ kg^{-1}$). n in parentheses indicates the number of animals used. ***$P<0.001$, **$P<0.01$, *$P<0.05$ vs. control.

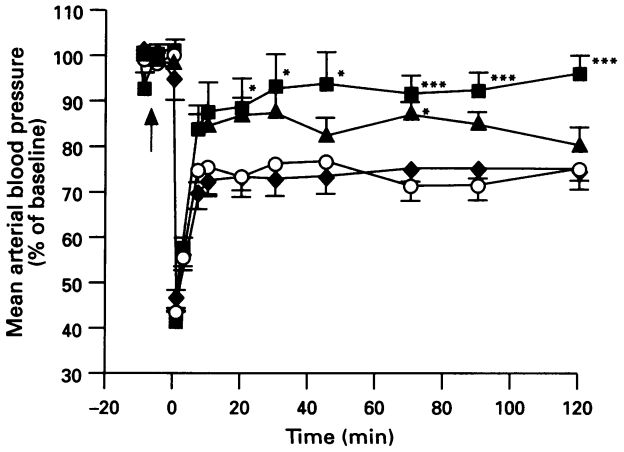

Figure 3 Prevention of lipopolysaccharide-induced hypotension in rats. PAF antagonists, UR-12633 at a dose of 1 $mg\ kg^{-1}$, i.v. (■, $n=15$) or 0.1 $mg\ kg^{-1}$, i.v. (▲, $n=10$), and WEB-2086 at a dose of 1 $mg\ kg^{-1}$, i.v. (◆, $n=9$) were administered (indicated by the arrow) 10 min before the intravenous injection of 20 $mg\ kg^{-1}$ lipopolysaccharide from *E. coli* serotype 0111:B4. Control animals (○, $n=37$) received vehicle (saline, 1 $ml\ kg^{-1}$). n in parentheses indicates the number of animals used. ***$P<0.001$, **$P<0.01$, *$P<0.05$ vs. control.

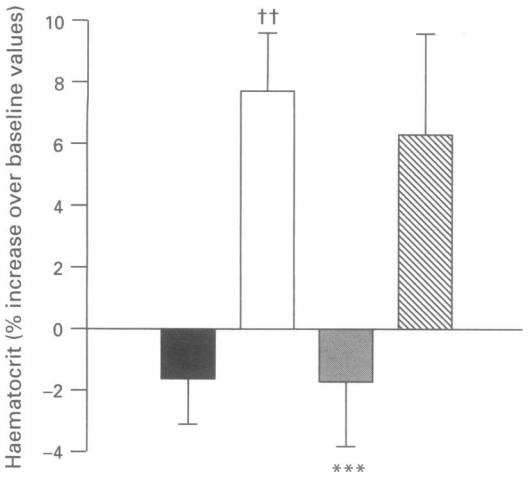

Figure 4 Inhibition of lipopolysaccharide-induced increase in haematocrit in rats. PAF antagonists were administered 10 min before the intravenous injection of 20 mg kg^{-1} lipopolysaccharide from *E. coli* serotype 0111:B4. Haematocrit was measured 90 min after lipopolysaccharide administration. Vehicle (saline)-treated animals with no lipopolysaccharide (sham, $n=12$), solid column; vehicle-treated plus lipopolysaccharide (control, $n=9$), open column; UR-12633-treated animals (1 mg kg^{-1}, i.v.) ($n=11$), cross-hatched column; WEB-2086-treated animals (1 mg kg^{-1}, i.v.) ($n=7$), hatched column. ††$P<0.01$ vs sham; ***$P<0.001$ vs control.

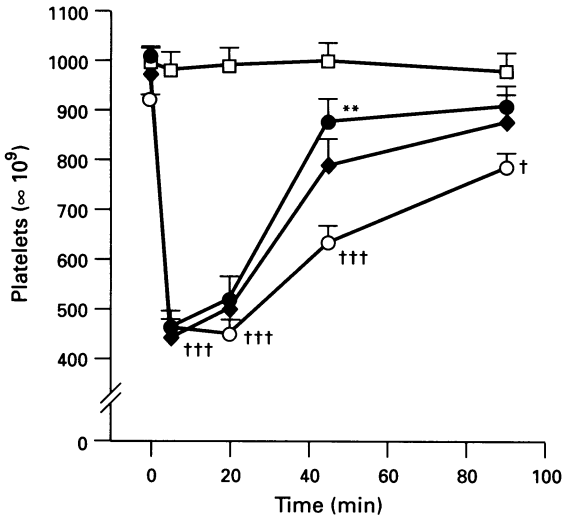

Figure 5 Inhibition of lipopolysaccharide-induced drop in platelet count in rats. PAF antagonists UR-12633 (●, $n=9$) and WEB-2086 (◆, $n=8$) were administered at a dose of 1 mg kg^{-1}, i.v. 10 min before the intravenous injection of 20 mg kg^{-1} lipopolysaccharide from *E. coli* serotype 0111:B4. The sham group (□, $n=12$) were vehicle (saline)-treated animals with no lipopolysaccharide; control group (○, $n=8$) were vehicle-treated plus lipopolysaccharide. n in parentheses indicates the number of animals used. †††$P<0.001$, †$P<0.05$ vs sham; **$P<0.01$ vs control.

MABP of animals treated with 1 mg kg^{-1}, i.v. UR-12633 or WEB-2086 was 93% and 86%, respectively (Figure 2).

The initial phase of hypotension was not inhibited by administration of either UR-12633 or WEB-2086 (1 mg kg^{-1}, i.v.) when given 10 min before LPS. However, UR-12633 completely abolished the second hypotensive phase, so that 2 h after LPS administration, UR-12633-treated rats had recovered baseline MABP. During the same period, MABP in the untreated control group fell to 75%. WEB-2086 was not able to prevent the LPS-induced hypotension (Figure 3).

Inhibition of LPS-induced changes in blood parameters in anaesthetized rats

LPS administration produced slight, transient leukopenia (79% of baseline at 5 min) which disappeared afterwards, thus preventing us from assessing the effectiveness of either UR-12633 or WEB-2086. LPS administration provoked haemoconcentration, measured by an increase in haematocrit (Figure 4). Thrombocytopenia was detected immediately after LPS administration with a drop of platelet count to about 50% of baseline at 5 min, followed by a slow recovery (80% of baseline at 90 min) (Figure 5). Pretreatment with UR-12633 (1 mg kg^{-1}, i.v.) completely prevented any increase in haematocrit and produced a faster recovery of platelet count. WEB-2086 at the same dose slightly improved both parameters, but the difference between the WEB-2086 and control groups was not statistically significant.

Inhibition of LPS-induced increase in vascular permeability in anaesthetized rats

The administration of LPS to rats elicited a significant increase in plasma extravasation in the trachea, thymus, seminal vesicle and stomach. UR-12633 (10 mg kg^{-1}, i.v.) decreased this extravasation of plasma into the tissues (100, 62, 50 and 35% inhibition in thymus, seminal vesicle, stomach, and trachea, respectively), although differences for stomach and trachea were not statistically significant. WEB-2086 failed to decrease extravasation when administered at the same dose (Figure 6).

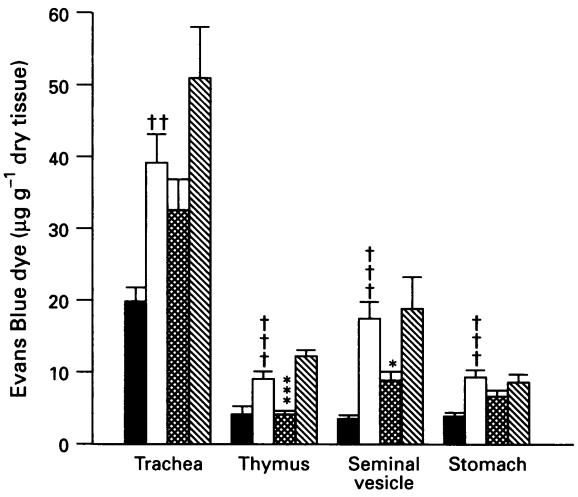

Figure 6 Inhibition of lipopolysaccharide-induced increase in vascular permeability in several tissues of the rat. PAF antagonists were administered 5 min before the intravenous injection of 25 mg kg^{-1} lipopolysaccharide from *E. coli* serotype 0127:B8 plus 10 mg kg^{-1} Evans Blue dye. Animals were killed 1 h after lipopolysaccharide administration and extravasation was measured as described in the Methods section. Vehicle (saline)-treated animals with no lipopolysaccharide (sham, $n=9$), solid columns; vehicle-treated plus lipopolysaccharide (control, $n=10$), open columns; UR-12633-treated animals (10 mg kg^{-1}, i.v.) ($n=8$), cross-hatched columns; WEB-2086-treated animals (10 mg kg^{-1}, i.v.) ($n=5$), hatched columns. †††$P<0.001$, ††$P<0.01$ vs sham; ***$P<0.001$, *$P<0.05$ vs control.

Inhibition of LPS-induced increase in APTT and PT in conscious rats

LPS produced a significant increase in APTT and PT, indicating activation of both intrinsic and extrinsic pathways of coagulation. Baseline values were 15.2±0.4 (APTT) and

13.4±0.4 s (PT). The coagulation times, measured 2 h after LPS administration, increased to 29.4±2.1 and 23.3±2.1 s, respectively. Pretreatment with either UR-12633 or WEB-2086 (10 mg kg^{-1}, i.v.) inhibited the increase in APTT by 55% and 74%, respectively, whereas WEB-2086, but not UR-12633, significantly inhibited the increase in PT, by 72% (Figure 7).

Inhibition of LPS-induced decrease of plasma fibrinogen concentration in conscious rats

A 35–50% decrease in plasma fibrinogen concentration was observed 2 h after LPS administration. Pretreatment with UR-12633 (10 mg kg^{-1}, i.v.) completely prevented the reduction in fibrinogen concentration, whereas WEB-2086 was only partially effective at the same dose (Figure 8). Data for both compounds are given in separate graphs, as experiments were performed in rat populations with different basal fibrinogen concentrations (412 vs 218 mg dl^{-1}).

Inhibition of LPS-induced changes in biochemical parameters and enzymatic activities in plasma of conscious rats

LPS administration induced large increases in both LDH and ACP plasma activities (of about 600% and 100%, respectively, 2 h after LPS). UR-12633 and WEB-2086 (10 mg kg^{-1}, i.v.) attenuated the increase in LDH (62% and 48% inhibition, respectively) and in ACP (100% and 69% inhibition, respectively) (Table 1). LPS-induced hyperglycaemia (70% increase) and hyperlactacidaemia (123% increase) were also inhibited when the rats were treated with these PAF antagonists. Hyperglycaemia was inhibited by 71% in animals treated with UR-12633 and by 46% in those treated with WEB-2086. Hyperlactacidaemia was also inhibited by both UR-12633 and WEB-2086 (by 92% and 56%, respectively) (Table 1).

Inhibition of [^{3}H]WEB-2086 binding in rabbit platelet membranes

UR-12633 potently displaces [^{3}H]WEB-2086 from its binding sites in rabbit platelet membranes with a K_i value of 0.9 (0.5–1.5, 95% confidence limits) nM. In the same conditions, WEB-2086 showed a K_i value of 10 (7–20) nM. Analysis of saturation curves suggests a competitive antagonism, since UR-12633 increased the K_D value for [^{3}H]WEB-2086 binding (e.g. 4.6±0.3 to 132±27 nM in the absence or in the presence of 0.5 nM of UR-12633, respectively) while the maximum number of receptors B_{max} remained unaltered (403±6 vs 333±40 fmol mg^{-1} protein in the absence or in the presence of 0.5 nM of UR-12633, not significant).

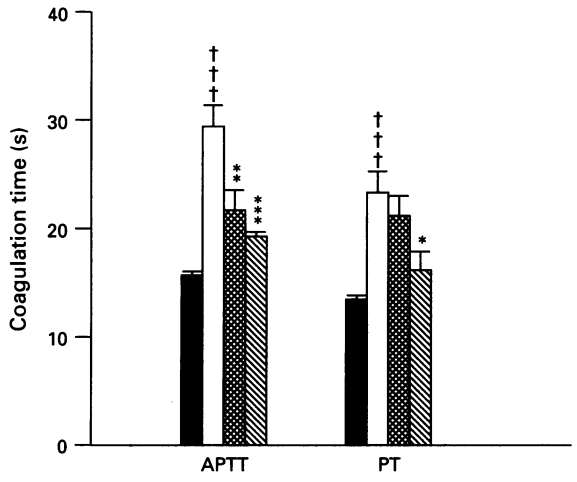

Figure 7 Inhibition of lipopolysaccharide-induced increase in APTT (activated partial thromboplastin time) and PT (prothrombin time) in rats. PAF antagonists were administered 5 min before the intravenous injection of 25 mg kg^{-1} lipopolysaccharide from *E. coli* serotype 0127:B8. Animals were exsanguinated 2 h after lipopolysaccharide administration. Coagulation times were measured with standard kits as described in the Methods section. Vehicle (saline)-treated animals with no lipopolysaccharide (sham, $n=13$), solid columns; vehicle-treated plus lipopolysaccharide (control, $n=19$), open columns; UR-12633-treated animals (10 mg kg^{-1}, i.v.) ($n=8$), cross-hatched columns; WEB-2086-treated animals (10 mg kg^{-1}, i.v.) ($n=13$), hatched columns. †††$P<0.001$ vs sham; ***$P<0.001$, **$P<0.01$, *$P<0.05$ vs control.

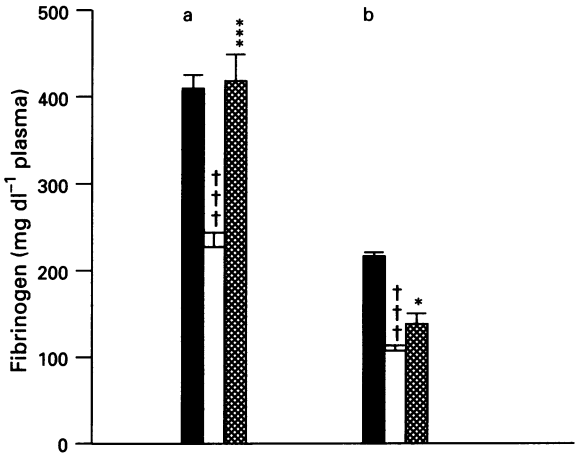

Figure 8 Inhibition of lipopolysaccharide-induced decrease in plasma fibrinogen concentration of rats. PAF antagonists were administered 5 min before the intravenous injection of 25 mg kg^{-1} lipopolysaccharide from *E. coli* serotype 0127:B8. Animals were exsanguinated 2 h after lipopolysaccharide administration. Fibrinogen concentration was measured with standard kit as described in the Methods section. Vehicle (saline)-treated animals with no lipopolysaccharide (sham, $n=10$), solid columns; vehicle-treated plus lipopolysaccharide (control, $n=10$), open column; (a) UR-12633-treated animals (10 mg kg^{-1}, i.v.) ($n=12$), cross-hatched or (b) WEB-2086-treated animals (10 mg kg^{-1}, i.v.) ($n=5$), cross-hatched column. †††$P<0.001$ vs sham; ***$P<0.001$, *$P<0.05$ vs control.

Table 1 Effect of PAF antagonists on biochemical and enzymatic parameters in rats treated with lipopolysaccharide (LPS)

	Lactate dehydrogenase (*u* l^{-1})		*Acid phosphatase* (*u* l^{-1})		*Glucose* (*mg* dl^{-1})		*Lactate* (*mg* dl^{-1})	
Vehicle–LPS	155±9	($n=43$)	32.6±0.9	($n=47$)	90.5±5.3	($n=54$)	203±12	($n=26$)
Vehicle+LPS	1081±90	($n=37$)†††	60.7±3.6	($n=44$)†††	154.3±6.1	($n=55$)†††	452±41	($n=36$)†††
UR-12633+LPS	503±93	($n=15$)***	30.8±1.3	($n=15$)***	108.8±8.1	($n=15$)***	222±37	($n=12$)***
WEB-2086+LPS	641±69	($n=15$)***	41.4±2.3	($n=15$)***	124.8±9.0	($n=18$)*	312±43	($n=17$)*

Test compounds (10 mg kg^{-1}) or saline were administered intravenously 5 min before LPS (lipopolysaccharide from *E.coli* serotype 0127:B8, 25 mg kg^{-1}, i.v.). Measurements were made 2 h after LPS administration. *n* in parentheses are the number of animals used. ††† $P<0.001$ vs. vehicle -LPS; ***$P<0.001$ vs. vehicle+LPS; *$P<0.05$ vs. vehicle+LPS.

Table 2 *In vitro* and *in vivo* PAF antagonist activity of several compounds: comparison with the activity in the model of lipopolysaccharide (LPS)-induced mortality in mice

Drug	*PAF-induced platelet aggregation*[a] (IC_{50}, μM)	*PAF-induced mortality in mice*[b] (ID_{50}, mg kg^{-1}, i.v.)	*LPS-induced mortality in mice*[c] (ID_{50}, mg kg^{-1}, i.v.)
UR-12633	0.014 (0.012-0.016)	0.010 (0.009-0.012)	0.050 (0.042-0.060)
UR-11353	0.012 (0.009-0.014)	0.023 (0.014-0.040)	0.28 (0.23-0.33)
WEB-2086	0.091 (0.071-0.12)	0.084 (0.042-0.167)	1.89 (1.39-2.56)
WEB-2170	0.016 (0.011-0.023)	0.0070 (0.0052-0.0094)	0.66 (0.54-0.80)
SCH-37370	0.84 (0.70-0.93)	1.11 (0.93-1.32)	36.7 (7.6-178)
CV-6209	0.012 (0.010-0.014)	0.022 (0.010-0.046)	0.36 (0.27-0.46)
TCV-309	0.012 (0.007-0.021)	0.0028 (0.0021-0.0038)	0.15 (0.10-0.24)
BB-882	0.0036 (0.0021-0.0063)	0.0021 (0.0010-0.0042)	0.11 (0.07-0.16)
BN-52021	0.55 (0.38-0.77)	0.10 (0.06-0.17)	13.8 (9.5-20.1)
L-659989	0.010 (0.009-0.012)	0.34 (0.10-1.17)	11.9 (5.3-26.8)

[a] Platelet-aggregation in rabbit platelet-rich plasma was obtained with 15 nM C_{18}-PAF. Inhibitors were incubated for 4 min before PAF addition. [b] Mortality was produced by the administration of 100 μg kg^{-1}, i.v. C_{18}-PAF plus 1 mg kg^{-1}, i.v. propranolol. Inhibitors were given 5 min before PAF administration. [c] Mortality was produced by the administration of 20 mg kg^{-1}, i.v. lipopolysaccharide from *E.coli* serotype 0111:B4. Inhibitors were given 5 min before LPS administration. Values in parentheses are 95% confidence limits.

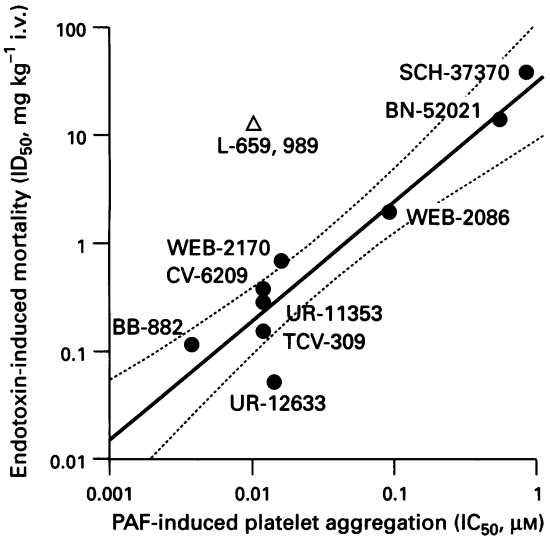

Figure 9 Correlation between the potency of PAF antagonists in the lipopolysaccharide-induced mortality test in mice and in the PAF-induced platelet aggregation test in rabbit platelet-rich plasma. Mortality was induced by the injection of 20 mg kg^{-1}, i.v. lipopolysaccharide from *E. coli* serotype 0111:B4 5 min after the administration of PAF antagonists. Platelet aggregation was provoked by the addition of 15 nM C_{18}-PAF to platelet-rich plasma prepared as described in the Methods section. Regression parameters were $\gamma = 1.109\alpha + 1.488$, $r^2 = 0.890$. Dotted lines indicate 95% confidence intervals for the regression line.

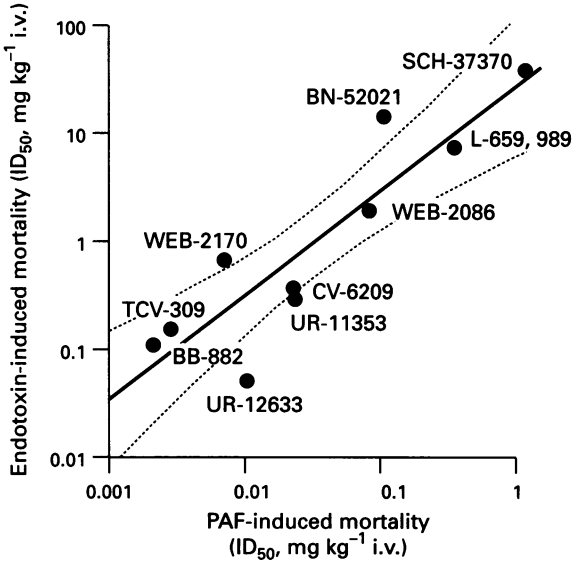

Figure 10 Correlation between the potency of PAF antagonists in the lipopolysaccharide-induced mortality test in mice and in the PAF-induced mortality test in mice. Mortality was induced by the injection of either 20 mg kg^{-1}, i.v. lipopolysaccharide from *E. coli* serotype 0111:B4 or 100 μg kg^{-1}, i.v. C_{18}-PAF plus 1 mg kg^{-1}, i.v. propranolol 5 min after the administration of the PAF antagonists. Regression parameters were $\gamma = 0.969\alpha + 1.429$, $r^2 = 0.802$. Dotted lines indicate 95% confidence intervals for the regression line.

Inhibition of PAF-induced platelet aggregation in vitro

The most potent compound was BB-882 (IC_{50} = 3.6 nM). UR-12633, L-659,989, UR-11353, WEB-2170, TCV-309 and CV-6209 showed similar potencies, with IC_{50} values of about 10^{-8} M, all of them being more potent than WEB-2086 (10^{-7} M) or BN-52021 and SCH-37370 (between 10^{-7} and 10^{-6} M) (Table 2).

Inhibition of PAF-induced mortality in mice

Intravenous administration of 100 μg kg^{-1} PAF plus 1 mg kg^{-1} propranolol produced a mortality of 70–100% in the control group (average = 90.0%, n = 1170). UR-12633 protected mice from PAF-induced death with an ID_{50} value of 10 μg kg^{-1}, i.v., being 2, 8 and more than 10 times more potent than CV-6209, WEB-2086 and L-659,989, respectively. The most active compounds in this test were BB-882 and TCV-309 (ID_{50} = 2–3 μg kg^{-1}, i.v.) (Table 2).

Inhibition of LPS-induced mortality in mice

Administration of 20 mg kg^{-1}, i.v. LPS (lipopolysaccharide from *E. coli* 0111:B4) to mice caused 80–100% mortality in the control group (average = 97.3%, n = 310). UR-12633 was the most potent inhibitor of LPS-induced death, with an ID_{50} value of 50 μg kg^{-1}, i.v. (Table 2).

Correlation between PAF-antagonist activity and the inhibition of LPS-induced mortality in mice

A good correlation was found between the inhibition of PAF-induced platelet aggregation *in vitro* and the inhibition of LPS-induced mortality in mice (r^2 = 0.890, P < 0.001) (Figure 9).

Compound L-659,989 was not included in the correlation, as it showed discrepant activities depending on the test. Thus, L-659,989 was very potent in inhibiting platelet aggregation but it was clearly less potent in other tests such as PAF-induced mortality (see Table 2) or hypotension (data not shown). Good correlation was also found between the two *in vivo* tests, the inhibition of PAF- or LPS-induced mortality in mice ($r^2=0.802$, $P<0.001$) (Figure 10).

Discussion

It is generally accepted that many biological activities brought about by LPS are not due to its direct effects, but secondary to the production and/or release of various mediators (tumour necrosis factor (TNF), interleukins, PAF and arachidonic acid metabolites) from target cells like monophages, polymorphonuclear leukocytes and endothelial cells. The question that arises is whether a selective inhibitor of one mediator, in our case of PAF, is able to ameliorate the myriad symptoms associated with LPS administration affecting many organs or systems. In an attempt to respond to that concern, we sought to demonstrate here the effect of UR-12633 on several different aspects of the pathophysiology of endotoxic shock.

UR-12633 is a novel and potent PAF antagonist, as it displaces [^{3}H]WEB-2086 from PAF receptors in rabbit platelet membranes ($K_i=0.9$ nM). UR-12633's activity profile is excellent in comparison with other well known PAF antagonists, when studied both *in vitro* (rabbit platelet aggregation) and *in vivo* (PAF-induced mortality in mice). Overall, UR-12633 is an order of magnitude more potent than WEB-2086 both *in vitro* and *in vivo*. Further studies (unpublished observations) have demonstrated good selectivity of UR-12633 for PAF-induced effects. UR-12633 (10 μM) does not show antihistamine, anticholinergic, anti-5-hydroxytryptamine, α- or β-adrenoceptor properties and does not affect cyclo-oxygenase, 5-lipoxygenase and phospholipase A_2 activities. UR-12633 (100 μM) is not a nonspecific platelet aggregation inhibitor, as it does not affect arachidonic acid- or ADP-induced platelet aggregation.

LPS caused a biphasic fall in blood pressure in rats. UR-12633 abolished the late hypotensive phase when given either after or before LPS. Neither UR-12633 nor WEB-2086, however, inhibited the immediate hypotension. It has been suggested that LPS-induced hypotension is caused by the activation of the constitutive calcium-dependent isoform of NO synthase (NOS) and the induction of the inducible, calcium-independent isoform of NOS (Szabó *et al.*, 1993a), and PAF by itself can stimulate NO synthesis through both isoforms of NOS (Szabó *et al.*, 1993b). The kinin-bradykinin system is also involved in the early stages of LPS-induced hypotension, as elevated levels of circulating kinins generated by endothelial cells (Fleming *et al.*, 1992) are found almost immediately after LPS administration (Katori *et al.*, 1989). In our experimental conditions, kinins are probably important in triggering the immediate hypotension (within 1 min); while the LPS-induced PAF production and the subsequent stimulation of NO synthesis may become relevant for the subsequent, sustained hypotension. The lack of effect of the pretreatment with WEB-2086 (1 mg kg^{-1}) compared with the reversion of the hypotension produced by the same dose of WEB-2086 administered 60 min after LPS also points to a role of PAF in the late phase of LPS-induced hypotension.

Early hypotension has also been attributed to a direct action of LPS on endothelial cells (Harlan *et al.*, 1983; Meyrick *et al.*, 1986). Incubation of cultured monolayers of bovine pulmonary artery endothelial cells with LPS causes changes in cell morphology within a few minutes and can lead to cell death within several hours (Meyrick *et al.*, 1986). The most relevant changes observed during this early stage are alterations in endothelial cell junctions. The observed enlargement of intercellular spaces produces an increase in vascular permeability, which we have observed in several rat tissues even 15 min after LPS administration, although maximal extravasation was achieved at 1 h (Balsa *et al.*, 1993). Some PAF antagonists such as CV-3988 or SRI 63-441 have been reported to decrease the LPS-induced changes in vascular permeability (Chang *et al.*, 1987). In our study of this effect, the differences in inhibitory potency in different organs observed after UR-12633 treatment and the lack of effect of WEB-2086 could be explained not only by the different potencies of these compounds but also by their different pharmacokinetic properties, such as their tissue distribution patterns. Thus, UR-12633, which is a relatively lipophilic drug with an apparent volume of distribution (V_d) of 3.2 l kg^{-1} in rats, tends to accumulate in tissues (unpublished results). WEB-2086, on the other hand, is more hydrophilic and its V_d in human subjects has been reported to be 0.3–0.5 l kg^{-1} (Brecht *et al.*, 1991).

Haemoconcentration, clinically detectable by an increase in haematocrit, is another marker of an increase in vascular permeability. In previous studies we have seen that both PAF and LPS cause an increase in haematocrit, with the maximum increase being reached earlier after administration of PAF than after administration of LPS (unpublished results). These results are in accordance with those reported by Floch and coworkers, who hypothesized that this slower effect of LPS is due to the gradual release of an endogenous mediator, and that this mediator could be PAF (Floch *et al.*, 1990). This hypothesis is supported by the observation of a time-dependent increase in plasma PAF levels after LPS injection in rats (Doebber *et al.*, 1985). Furthermore, some PAF antagonists (such as RP 59227 and SRI 63-441) are able to inhibit the haemoconcentration induced by LPS (Floch *et al.*, 1990; Lang & Bagby, 1990). In our study, pretreatment with UR-12633 did in fact completely inhibit the increase in haematocrit observed 90 min after LPS administration, a finding which further underlines the role of PAF in the increase in vascular permeability caused by LPS.

LPS administration in rats induces changes very similar to disseminated intravascular coagulation (DIC) syndrome (Kawamura *et al.*, 1993). Low circulating levels of LPS can induce activation of coagulation which is mediated by cytokines, particularly TNFα. This effect seems to be dependent on the activation of the extrinsic pathway of blood coagulation (van Deventer *et al.*, 1990). PAF by itself is able to reproduce some of the changes in coagulation parameters elicited by LPS (Imura *et al.*, 1986). PAF activates the blood coagulation system through the generation of tissue factor (Kawamura *et al.*, 1993), and the fibrinolytic system through the release of tissue-type plasminogen activator (Emeis & Kluft, 1985). The hypothesis that PAF antagonists may exert beneficial effects on DIC syndrome has been put forward by several authors (Imura *et al.*, 1986; Kawamura *et al.*, 1993). In our study, both UR-12633 and WEB-2086 attenuated the rise in APTT and the decrease of fibrinogen plasma concentrations, both of which are markers of the development of DIC. Immediate thrombocytopenia (within 5 min) was not inhibited by these PAF antagonists, while UR-12633 showed some effectiveness against the late fall in platelet count. The LPS-induced thrombocytopenia in rats is not directly mediated by PAF, because rat platelets are devoid of specific PAF receptors (Iñarrea *et al.*, 1984). Thus, PAF seems to produce thrombocytopenia in rats through TNFα production (Rabinovici *et al.*, 1993), although the observed thrombocytopenia may also be due to incorporation of platelets into thrombi during blood coagulation (Kawamura *et al.*, 1993).

After LPS administration, blood glucose concentration increases as glucose production outpaces tissue uptake. The increase in glucose plasma levels is the consequence of higher concentrations of plasma catecholamines and/or glucagon, which are released through sympathetic activation. Some authors have speculated that the sympathetic system may be activated to compensate for LPS-induced hypotension (Lang & Bagby, 1990). PAF also induces increases in plasma catecholamines and glucagon (Lang *et al.*, 1988). Pretreatment with WEB-2086 in conscious unrestrained rats that had received LPS from *S. enteritidis* blocked hypotension, but not the increase in catecholamines, thus suggesting that mecha-

misms other than hypotension are involved in the sympathetic activation that precedes hyperglycaemia and hyperlactacidaemia (Qi & Jones, 1990). In our study, UR-12633 abolished the LPS-induced increase in the blood levels of glucose and lactate, a result that provides further evidence for a role of PAF production in LPS-induced hyperglycaemia and hyperlactacidaemia.

The increase in plasma LDH activity after LPS administration, which is considered a marker of tissue damage, was partially prevented by UR-12633 or WEB-2086. The cellular origin of LDH in endotoxic shock may be multiple, as LPS provokes extensive tissue damage. Thus, partial effectiveness may arise from PAF antagonists' affinity for different tissues, in a way that is similar to the process that probably occurs in the permeability studies. Both PAF antagonists also suppressed the increase in ACP activity. Above-normal ACP activity indicates lysosomal activation and in our conditions it is mainly associated with prostatic inflammation (unpublished results).

PAF antagonists not only improve many of the symptoms induced by LPS administration, but also protect against death. We found a good linear correlation between PAF antagonist activity, both *in vitro* (platelet aggregation test) and *in vivo* (mortality test), and antagonist activity found in the LPS-induced mortality test in mice. These data point strongly to PAF's role as a key mediator in the cascade of events that lead to mortality after LPS administration. The particularly good results obtained with UR-12633 in the LPS-induced mortality test could be attributed to high compound accumulation in the target organs, whose failure leads to the animal's death.

In conclusion, the novel potent PAF antagonist UR-12633 was effective in protecting against haemodynamic, haematological and metabolic changes following LPS administration to rats. Discrepant results in different tests reported for UR-12633 and WEB-2086 in this paper and for other PAF antagonists in the literature may arise from differences in potency, in pharmacokinetics (e.g. half-life and tissue distribution) and in lack of selectivity.

The authors wish to thank Alejandro Moliner, Esther Samper and Rosa María Oliva for their excellent technical assistance, Drs Elena Carceller and Javier Bartrolí, for the synthesis of many of the PAF antagonists used and Mary Ellen Kerans for a review of the English manuscript.

References

BALSA, D., MERLOS, M., GIRAL, M., GARCIA-RAFANELL, J. & FORN, J. (1993). Tissue distribution of increased vascular permeability and gastric damage induced by endotoxin in rats: effect of PAF antagonists. *World Congress Inflammation* '93. Vienna Austria, p.84.

BRADFORD, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

BRECHT, H.M., ADAMUS, W.S., HEUER, H.O., BIRKE, F.W. & KEMPE, E.R. (1991). Pharmacodynamics, pharmacokinetics and safety profile of the new platelet-activating factor antagonist apafant in man. *Arzneim. Forsch.*, **41**, 51–59.

CHANG, S.-W., FEDDERSEN, C.O., HENSON, P.M. & VOELKEL, N.F. (1987). Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rats. *J. Clin. Invest.*, **79**, 1498–1509.

DANNER, R.L., ELIN, R.J., HOSSEINI, J.M., WESLEY, R.A., REILLY, J.M. & PARRILLO, J.E. (1991). Endotoxemia in human septic shock. *Chest*, **99**, 169–175.

DEJOY, S.O., ORONSKY, A.L., WICK, M.M. & KERWAR, S.S. (1993). Preclinical studies with platelet-activating factor antagonists in models of septic shock. *Int. J. Antimicrob. Agents*, **3**, 175–186.

DOEBBER, T.W., WU, M.S., ROBBINS, J.C., CHOY, B.M., CHANG, M.N. & SHEN, T.Y. (1985). Platelet activating factor (PAF) involvement in endotoxin-induced hypotension in rats. Studies with PAF-receptor antagonist kadsurenone. *Biochem. Biophys. Res. Commun.*, **127**, 799–808.

EMEIS, J.J. & KLUFT, C. (1985). PAF-acether-induced release of tissue-type plasminogen activator from vessel walls. *Blood*, **66**, 86–91.

EVANS, T.W., DENT, G., ROGERS, D.T., AURSUDKIJ, B., CHUNG, K.F. & BARNES, P.J. (1988). Effects of a PAF antagonist, WEB-2086, on airway microvascular leakage in the guinea pig and platelet aggregation in man. *Br. J. Pharmacol.*, **94**, 164–168.

FLEMING, I., DAMBACHER, T. & BUSSE, R. (1992). Endothelium-derived kinins account for the immediate response of endothelial cells to bacterial lipopolysaccharide. *J. Cardiovasc. Pharmacol.*, **20**, S135–S138.

FLOCH, A., MASSA, N. & CAVERO, I. (1990). Studies on the pharmacological mechanisms responsible for the hemoconcentration produced by PAF an endotoxin in rats. In *Platelet Activating Factor in Endotoxin and Immune Diseases*. ed. Handley, D.A., Saunders, R.N., Houlihan, W.J. & Tomesch, J.C. pp. 577–588, New York: Marcel Dekker.

HARLAN, J.M., HARKER, L.A., REIDY, M.A., GAJDUSEK, C.M., SCHWARTZ, S.M. & STRIKER, G.E. (1983). Lipopolysaccharide-mediated bovine endothelial cell injury *in vitro*. *Lab. Invest.*, **48**, 269–274.

HOSFORD, D.J., DOMINGO, M.T., CHABRIER, P.E. & BRAQUET, P. (1990). Ginkgolides and platelet-activating factor. *Methods Enzymol.*, **187**, 433–447.

HURLEY, J.C. (1993). Reappraisal of the role of endotoxin in the sepsis syndrome. *Lancet*, **341**, 1133–1135.

IKEGAMI, K., MEADE, C.J., HEUER, H.O. & BIRKE, F. (1992). Hetrazepine PAF antagonists. *J. Lipid Med.*, **5**, 177–182.

IMURA, Y., TERASHITA, Z. & NISHIKAWA, K. (1986). Possible role of platelet activating factor (PAF) in disseminated intravascular coagulation (DIC), evidenced by use of a PAF antagonist, CV-3988. *Life Sci.*, **39**, 111–117.

INARREA, P., GOMEZ-CAMBRONERO, J., NIETO, M. & SANCHEZ CRESPO, M. (1984). Characteristics of the binding of platelet-activating factor to platelets of different animal species. *Eur. J. Pharmacol.*, **105**, 309–315.

KATORI, M., MAJIMA, M., ODOI-ADOME, R., SUNAHARA, N. & UCHIDA, Y. (1989). Evidence for the involvement of a plasma kallikrein-kinin system in the immediate hypotension produced by endotoxin in anaesthetized rats. *Br. J. Pharmacol.*, **98**, 1383–1391.

KAWAMURA, M., TERASHITA, Z., IMURA, Y., SHINO, A. & NISHIKAWA, K. (1993). Inhibitory effect of TCV-309, a novel platelet activating factor (PAF) antagonist, on endotoxin induced disseminated intravascular coagulation in rats: possible role of PAF in tissue factor generation. *Thromb. Res.*, **70**, 281–293.

KOLTAI, M., HOSFORD, D., GUINOT, P., ESANU, A. & BRAQUET, P. (1991a). PAF, a review of its effects, antagonists and possible future implications (Part 1). *Drugs*, **42**, 9–29.

KOLTAI, M., HOSFORD, D., GUINOT, P., ESANU, A. & BRAQUET, P. (1991b). PAF, a review of its effects, antagonists and possible future implications (Part II). *Drugs*, **42**, 174–204.

LANG, C.H. & BAGBY, G.J. (1990). The role of platelet-activating factor in endotoxin-, sepsis-, and burn-induced changes in hemodynamics and metabolism. In *Platelet Activating Factor in Endotoxin and Immune Diseases*. ed. Handley, D.A., Saunders, R.N., Houlihan, W.J. & Tomesch, J.C. pp. 609–628, New York: Marcel Dekker.

LANG, C.H., DOBRESCU, C., HARGROVE, D.M., BAGBY, G.J. & SPITZER, J.J. (1988). Platelet-activating factor-induced increases in glucose kinetics. *Am. J. Physiol.*, **254**, E193–E200.

MCCABE, W.R. & OLANS, R.N. (1981). Shock in Gram-negative bacteremia: Predisposing factors, pathophysiology, and treatment. In *Current Clinical Topics in Infectious Diseases*. Vol. 2. ed. Remington, J.S., Swartz, M.N. pp. 121–150. New York: McGraw-Hill.

MEYRICK, B.O., RYAN, U.S. & BRIGHAM, K.L. (1986). Direct effects of *E. coli* endotoxin on structure and permeability of pulmonary endothelial monolayers and the endothelial layer of intimal explants. *Am. J. Pathol.*, **122,** 140–151.

MORRISON, D.C. & RYAN, J.L. (1987). Endotoxins and disease mechanisms. *Annu. Rev. Med.*, **38,** 417–432.

PARRILLO, J.E. (1993). Pathogenetic mechanisms of septic shock. *N. Engl. J. Med.*, **328,** 1471–1477.

QI, M. & JONES, S.B. (1990). Contribution of platelet activating factor to hemodynamic and sympathetic responses to bacterial endotoxin in conscious rats. *Circ. Shock*, **32,** 153–163.

RABINOVICI, R., ESSER, K.M., LYSKO, P.G., YUE, T., GRISWOLD, D.E., HILLEGASS, L.M., BUGELSKI, P.J., HALLENBECK, J.M. & FEUERSTEIN, G. (1991). Priming by platelet-activating factor of endotoxin-induced lung injury and cardiovascular shock. *Circ. Res.*, **69,** 12–25.

RING, P.C., SELDON, P.M., BARNES, P.J. & GIEMBYCZ, M.A. (1992). Pharmacological characterization of a receptor for platelet-activating factor on guinea-pig peritoneal macrophages using [^{3}H]apafant, a selective and competitive platelet-activating factor antagonist: evidence that the noncompetitive behavior of apafant in functional studies relates to slow kinetics of dissociation. *Mol. Pharmacol.*, **43,** 302–312.

RYLANDER, R. & BEIJER, L. (1987). Inhalation of endotoxin stimulates alveolar macrophage production of platelet activating factor. *Am. Rev. Respir. Dis.*, **135,** 83–86.

SZABÓ, C., MITCHELL, J.A., THIEMERMANN, C. & VANE, J.R. (1993a). Nitric oxide-mediated hyporeactivity to noradrenaline precedes the induction of nitric oxide synthase in endotoxic shock. *Br. J. Pharmacol.*, **108,** 786–792.

SZABÓ, C., WU, C.C., MITCHELL, J.A., GROSS, S.S., THIEMERMANN, C. & VANE, J.R. (1993b). Platelet-activating factor contributes to the induction of nitric oxide synthase by bacterial lipopolysaccharide. *Circ. Res.*, **73,** 991–999.

TALLARIDA, R.J. & MURRAY, R.B. (1981). Procedure 8: Graded Dose-Response. In *Manual of Pharmacologic Calculations.* pp 14–19. New York: Spriger-Verlag.

TAVEIRA DA SILVA, A.M., KAULBACH, H.C., CHUIDIAN, F.S., LAMBERT, D.R., SUFFREDINI, A.F. & DANNER, R.L. (1993). Brief report: shock and multiple-organ dysfunction after self-administration of Salmonella endotoxin. *N. Engl. J. Med.*, **328,** 1457–1460.

THOMPSON, W.A., COYLE, S., KIMBERLY, V.Z., OLDENBURG, H., TROUSDALE, R., ROGY, M., FELSEN, D., MOLDAWER, L. & LOWRY, S.F. (1994). The metabolic effects of platelet-activating factor antagonism in endotoxemic man. *Arch. Surg.*, **129,** 72–79.

TOTH, P.D. (1990). The biological effects of PAF antagonists in endotoxemia. In *Platelet Activating Factor in Endotoxin and Immune Diseases.* ed. Handley, D.A., Saunders, R.N., Houlihan, W.J. & Tomesch, J.C. pp. 589–608, New York: Marcel Dekker.

VAN DEVENTER, S.J.H., BULLER, H.R., TEN CATE, J.W., AARDEN, L.A., HACK, C.E. & STURK, A. (1990). Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolitic and complement pathways. *Blood*, **76,** 2520–2526.

WHITTLE, B.J.R., BOUGHTON-SMITH, N.K., HUTCHESON, I.E., ESPLUGUES, J.V. & WALLACE, J.L. (1987). Increased intestinal formation of PAF in endotoxin-induced damage in the rat. *Br. J. Pharmacol.*, **92,** 3–4.

(Received January 2, 1996
Revised March 4, 1996
Accepted March 11, 1996)