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The effects of bosentan, aminoguanidine and L-canavanine on mesenteric blood flow, spleen and liver in endotoxaemic mice

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Abstract

The modulatory effects of a non-selective endothelin receptor antagonist, bosentan, were investigated together with those of relatively selective inducible nitric oxide synthase inhibitors, aminoguanidine and L-canavanine, on mesenteric blood flow decrease, liver and spleen injury elicited by endotoxaemia. Swiss albino mice (20–40 g) were administered intraperitoneally bosentan (3, 10 or 30 mg kg^{-1}), aminoguanidine (15 mg kg^{-1}) or L-canavanine (20 or 100 mg kg^{-1}) 10 min before they received saline or *Escherichia coli* endotoxin (10 mg kg^{-1}). After 4 h, the mice were anaesthetized, mesenteric blood flow values were measured, spleen and liver weight/body weight ratios were determined and the organs were examined histopathologically. Endotoxin decreased mesenteric blood flow (ml min^{-1}, saline: 3.0 ± 0.2; endotoxin: 2.2 ± 0.2; $n = 10$, $P < 0.05$), increased the weight of liver (g per kg body weight, saline: 47.5 ± 2.0; endotoxin: 60.8 ± 1.9; $n = 10$, $P < 0.05$) and spleen (g per kg body weight, saline: 3.9 ± 0.5; endotoxin: 8.6 ± 0.9; $n = 10$, $P < 0.01$) while it inflicted significant histopathological injury to both organs. Bosentan was ineffective at 3 mg kg^{-1} but at 10 and 30 mg kg^{-1} doses, it abolished all the deleterious effects of endotoxin without exception. Aminoguanidine blocked most of the effects of endotoxin except those on spleen. In contrast, L-canavanine blocked only the endotoxin-induced increase in liver weight but itself increased spleen weight and failed to block any other effects of endotoxin. Thus, it can be speculated that the beneficial effects of aminoguanidine are produced largely by mechanisms other than selective inducible nitric oxide synthase inhibition since L-canavanine was not fully effective. The beneficial effects of endothelin inhibition by using bosentan in endotoxaemia can be further exploited for the understanding and the therapy of sepsis-related syndromes.

Keywords: Bosentan; Aminoguanidine; L-canavanine; Endothelin; Nitric oxide (NO); Sepsis

1. Introduction

The release of lipopolysaccharides from the outer membrane of the bacterial cell wall is responsible for the pathological events including hypotension, microvascular injury, disseminated intravascular coagulation and diminished blood flow to vital organs that lead to multiple organ failure during gram-negative sepsis (Parrillo, 1989). Since the cardiovascular system is the main determinant of the outcome of septic shock (MacLean, 1972), endothelium serves as an important target and a modulator for the effects of endotoxin because of its close contact with circulating blood and its proximity to the underlying vascular smooth muscle. Despite some opposing views (MacMicking et al., 1995; Lefer, 1998), endothelium-derived substances such as nitric oxide (Kilbourn, 1998) and endothelin (Pittet et al., 1991) are regarded as key mediators in the systemic inflammatory response syndrome that leads to fatal multiple organ dysfunction (Bone, 1990).

Nitric oxide, produced in copious amounts by inducible nitric oxide synthase (Schulz et al., 1992) contributes significantly to the deleterious effects of endotoxin (Moncada and Higgs, 1995) such as hypotension (Thiemermann and Vane, 1990), vascular hyporesponsiveness to vasoconstrictors (Julou Schaeffer et al., 1990) and vasodilators (Guc et al., 1990) during sepsis. When the inducible enzyme was blocked selectively by using L-canavanine (Iyengar et al., 1987), the mice challenged with

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a lethal dose of endotoxin were reported to show significant improvements in haemodynamic and metabolic parameters, as well as increased survival (Liaudet et al., 1996). On the other hand, non-selective nitric oxide synthase inhibition with N^G-substituted L-arginine analogues (e.g., N^G-nitro-L-arginine methyl ester: L-NAME) was shown to exacerbate endotoxin-induced organ ischaemia and mortality due to concomitant inhibition of the constitutive nitric oxide synthase (Hutcheson et al., 1990). Thus, the inhibition of the inducible enzyme by its relatively selective inhibitors (for a review see Thiemermann, 1998) such as aminoguanidine (Misko et al., 1993) has been compared to the only partial beneficial effects of L-NAME in experimental endotoxaemia (Wu et al., 1996). Since aminoguanidine has many other pharmacological properties including the inhibition of (i) histamine metabolism (Bieganski et al., 1983), (ii) polyamine catabolism (Seiler et al., 1985), (iii) the formation of advanced glycolysation end-products and (iv) catalase or iron-/copper-containing enzymes (Ou and Wolff, 1993) as well as (v) endotoxin-induced bacterial translocation through the gut (Kavuklu et al., 1998), it is difficult to interpret the underlying mechanism exactly. In addition to the fact that nitric oxide has both beneficial and deleterious effects in biological systems (Lipton et al., 1993) depending on the redox potential of the milieu (Butler et al., 1995), the failure of nitric oxide overproduction to fully explain the hyporeactivity of the mesenteric vascular bed in endotoxin-treated rats (Mitolo-Chieppa et al., 1996) supports the hypothesis that no single agent can yet be implicated as the mediator of endotoxin-induced organ injury (Parratt, 1997) that is frequently encountered during sepsis (Ghosh et al., 1993).

The case for a role of endothelin(s) in endotoxaemia is similar. Endothelin release is stimulated by endotoxin (Sugiura et al., 1989) and increased in the circulation of sepsis patients (Weitzberg et al., 1991), suggesting that it may help maintain the blood pressure and organ perfusion which are beneficial during the initial phases of septic shock (Vemulapalli et al., 1991), while excessive rises in the plasma levels of endothelin for longer periods evoke profound vasoconstriction in the splanchnic vascular bed, which is indeed a harmful effect (Ruetten and Thiemermann, 1996). Recently, bosentan, a non-specific endothelin ET_A and endothelin ET_B receptor antagonist with no intrinsic agonist activity, became available (Clozel et al., 1994). Using a porcine model of endotoxic shock, bosentan was shown to improve cardiovascular performance by increasing the blood flow to splanchnic and intestinal vascular beds (Weitzberg et al., 1996), indicating a significant deleterious role for endothelin during endotoxaemia.

Therefore, we attempted to investigate the effects of the inducible nitric oxide inhibitors, aminoguanidine and L-canavanine, together with those of the endothelin receptor blocker, bosentan, on the endotoxin-induced decrease in mesenteric blood flow, and spleen and liver injury. A preliminary account of the current data was presented partially in abstract form at the XIIth Biannual Meeting of The Turkish Pharmacological Society held in November 1994, Antalya, Turkey.

2. Materials and methods

2.1. Animals

Swiss albino mice (25–35 g) were obtained from the Laboratory Animal Husbandry Facility of Department of Pharmacology, Hacettepe University Faculty of Medicine, and were housed under environmentally controlled conditions at 21 ± 2°C and 30–70% relative humidity with 12 h dark/12 h light illumination sequence (the lights were on between 0700–1900 h) with access ad libitum to tap water (drinking bottle) and standard pellet dairy chow (Murat Yem Sanayii, Ankara, Turkey). Guiding Principles in the Care and Use of Laboratory Animals together with The Recommendations from the Declaration of Helsinki were strictly adhered to during the execution of all the procedures described within this manuscript.

2.2. General procedures

Endotoxin derived from *Escherichia coli* (O55:B5; 10 mg kg^{-1}, i.p.) or an equivalent volume (1 ml kg^{-1}, i.p.) of non-pyrogenic sterile saline (NaCl 0.9%, w/v, dissolved in pyrogen-free distilled water) was given by intraperitoneal injection to animals 4 h before they underwent surgery. The timing of endotoxin administration was chosen on the basis of previous studies showing that inducible nitric oxide synthase activity has reached its maximum within 2–6 h after endotoxin administration (Schulz et al., 1992; Gardiner et al., 1995).

During the planning of the experiments in the present study the doses of the drugs were chosen on the basis of the information in the literature. Aminoguanidine (15 mg kg^{-1}, i.p.), L-canavanine (20 or 100 mg kg^{-1}, i.p.), bosentan (30 mg kg^{-1}, i.p.) or their solvent saline (1 ml kg^{-1}, i.p.) was given 20 min before endotoxin injection. The initial results revealed that bosentan (30 mg kg^{-1}, i.p.) virtually abolished all the effects of endotoxin, thus further experiments were performed with two more additional doses of bosentan (i.e., 3 and 10 mg kg^{-1}, i.p.) in order to complete a three-point dose–response profile. All drugs were prepared daily, dissolved in non-pyrogenic sterile saline and warmed to body temperature (approximately 37°C) before injection. Drug solutions were kept in dark containers until injection in order to protect them from light-induced decomposition.

2.3. Surgical procedure

The mice were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and placed on a heat-insulated

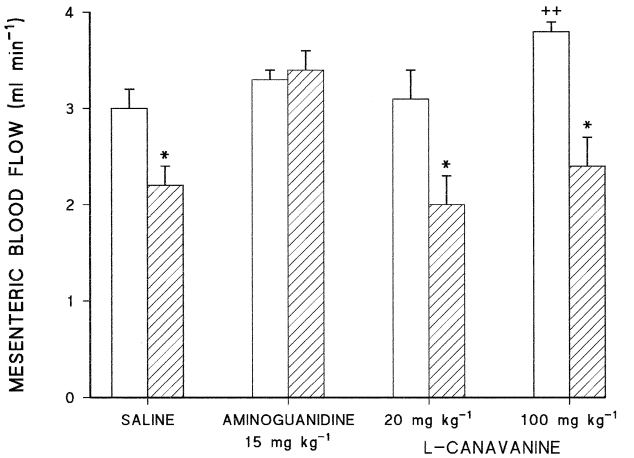

Fig. 1. Mesenteric blood flow (ml min^{-1}) after endotoxaemia for 240 min in saline (1 ml kg^{-1}, open columns) or endotoxin (10 mg kg^{-1}, hatched columns)-treated mice which also received aminoguanidine (15 mg kg^{-1}), L-canavanine (20 or 100 mg kg^{-1}) or their solvent (saline, 1 ml kg^{-1}). Vertical bars indicate the standard error of the arithmetic mean of the number of data points for that column. $n = 5–10$ for each data point. (*) Indicates significant difference ($P < 0.05$) from its adjacent column. (++) Indicates significant difference ($P < 0.01$) from the control (saline–saline).

cork-sheet-covered operating table. The animals were allowed to breathe room air spontaneously. Body temperature of the mice was stabilized at 37.0 ± 0.1°C by a rectal thermistor probe-controlled incandescent lamp placed 25 cm above the animals. The abdomen was opened by a

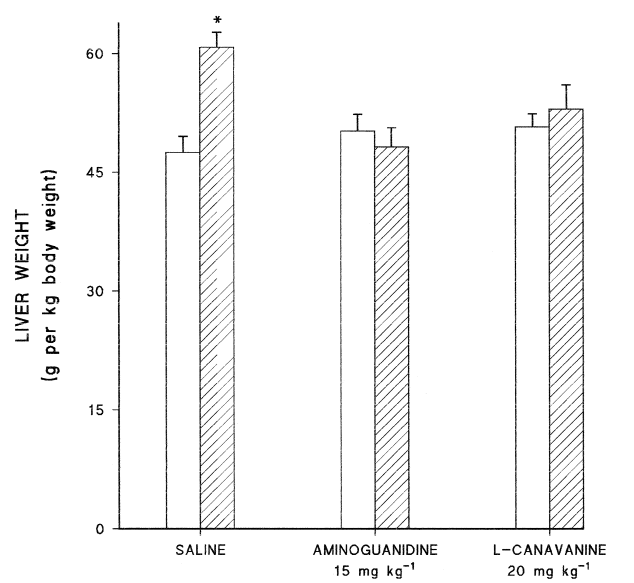

Fig. 2. Liver weight (g per kg body weight) at the end of endotoxaemia for 240 min in saline (1 ml kg^{-1}, open columns) or endotoxin (10 mg kg^{-1}, hatched columns)-treated mice which also received aminoguanidine (15 mg kg^{-1}), L-canavanine (20 mg kg^{-1}) or their solvent (saline, 1 ml kg^{-1}). Vertical bars indicate the standard error of the arithmetic mean of the number of data points for that column. $n = 5–12$ for each data point. (*) Indicates significant difference ($P < 0.05$) from its adjacent column.

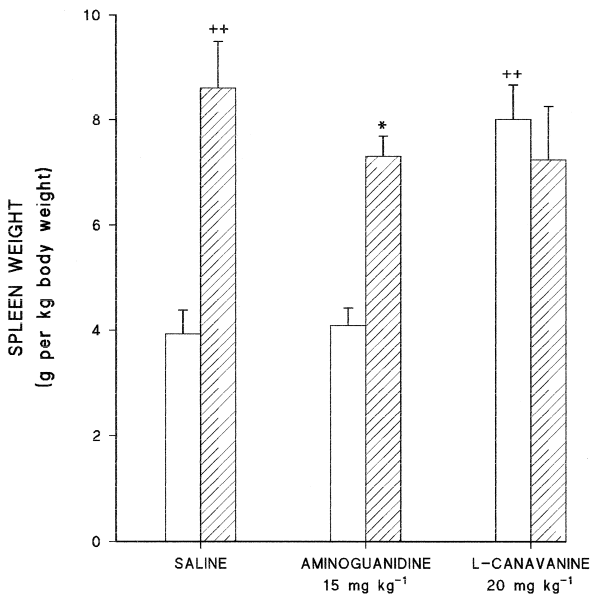

Fig. 3. Spleen weight (g per kg body weight) at the end of endotoxaemia for 240 min in saline (1 ml kg^{-1}, open columns) or endotoxin (10 mg kg^{-1}, solid columns)-treated mice which also received aminoguanidine (15 mg kg^{-1}), L-canavanine (20 mg kg^{-1}) or their solvent (saline, 1 ml kg^{-1}). Vertical bars indicate the standard error of the arithmetic mean of the number of data points for that column. $n = 5–12$ for each data point. (*) Indicates significant difference ($P < 0.05$) from its adjacent column. (++) Indicates significant difference ($P < 0.01$) from the control (saline–saline).

midline incision and a perivascular ultrasonic Doppler-flow probe (1 RB, Transonic, Ithaca, USA) coupled to a Small Animal Flowmeter System (T106, Transonic, Ithaca, USA) was placed around the common mesenteric artery. The absolute blood flow values measured in ml min^{-1} by the

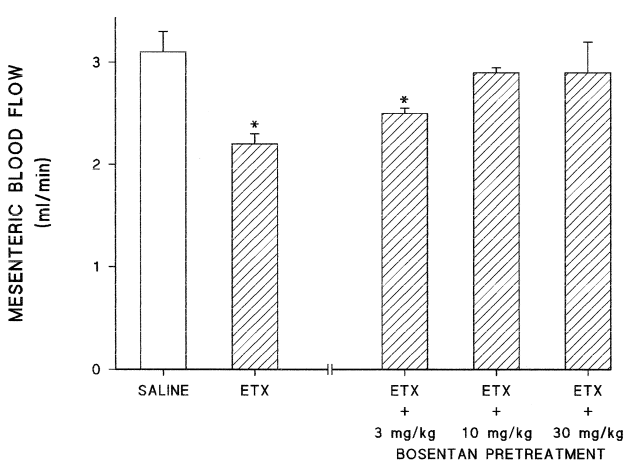

Fig. 4. Mesenteric blood flow (ml min^{-1}) after endotoxaemia for 240 min in saline (1 ml kg^{-1}, open column) or endotoxin (10 mg kg^{-1}, hatched column)-treated mice which also received bosentan (3, 10 or 30 mg kg^{-1}) 20 min before endotoxin injection. Vertical bars indicate the standard error of the arithmetic mean of the number of data points for that column. $n = 6–15$ for each data point. (*) Indicates significant difference ($P < 0.05$) from the control (saline–saline). *N.B.* The first two columns from the left also appear as the first two columns of Fig. 1.

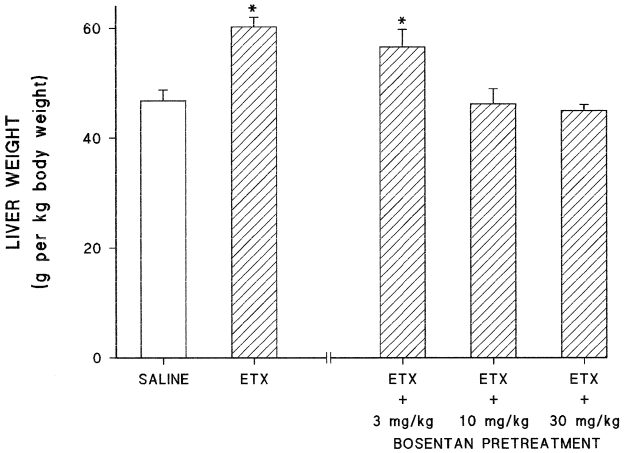

Fig. 5. Liver weight (g per kg body weight) after endotoxaemia for 240 min in saline (1 ml kg^{-1}, open column) or endotoxin (10 mg kg^{-1}, hatched column)-treated mice which also received bosentan (3, 10 or 30 mg kg^{-1}) 20 min before endotoxin injection. Vertical bars indicate the standard error of the arithmetic mean of the number of data points for that column. $n = 6–15$ for each data point. (*) Indicates significant difference ($P < 0.05$) from the control (saline–saline). *N.B.* The first two columns from the left also appear as the first two columns of Fig. 2.

system were recorded with a Harvard Oscillograph Pen-Recorder while the animals were left to stabilize for 15 min and the mesenteric blood flow values were then recorded.

2.4. Liver and organ injury

After the determination of mesenteric blood flow values, the animals were killed by exsanguination and the spleen and liver were dissected from the surrounding tissues. The organs were placed on hydrophilic paper tissue in order to eliminate the contaminating excess fluid and they were immediately transferred onto a laboratory balance for the determination of their wet tissue weights. These values were then divided by the weight of the individual mouse to obtain a spleen or liver weight normalized according to the body weight (i.e., g per kg body weight).

After weighing, liver and spleen were prepared for blind histopathological assessment. Tissues were fixed in 10% formaldehyde. Sections were prepared from paraffin blocks and stained with hematoxylin–eosin. The slides were examined under conventional light microscopy (Zeiss Axioskop, Germany).

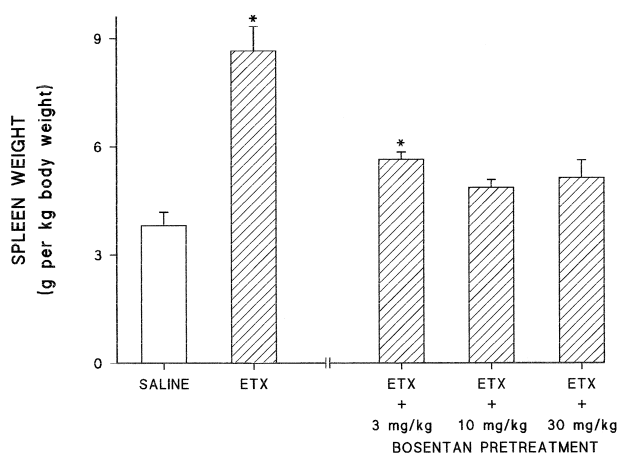

Fig. 6. Spleen weight (g per kg body weight) after endotoxaemia for 240 min in saline (1 ml kg^{-1}, open column) or endotoxin (10 mg kg^{-1}, hatched column)-treated mice which also received bosentan (3, 10 or 30 mg kg^{-1}) 20 min before endotoxin injection. Vertical bars indicate the standard error of the arithmetic mean of the number of data points for that column. $n = 6–15$ for each data point. (*) and (++) indicates significant differences ($P < 0.05$ and $P < 0.01$, respectively) from the control (saline–saline). *N.B.* The first two columns from the left also appear as the first two columns of Fig. 3.

2.5. Drugs used

Sodium chloride, hematoxylin (Merck, USA), sodium pentobarbitone (Abbott, USA), lipopolysaccharide (*E. coli*

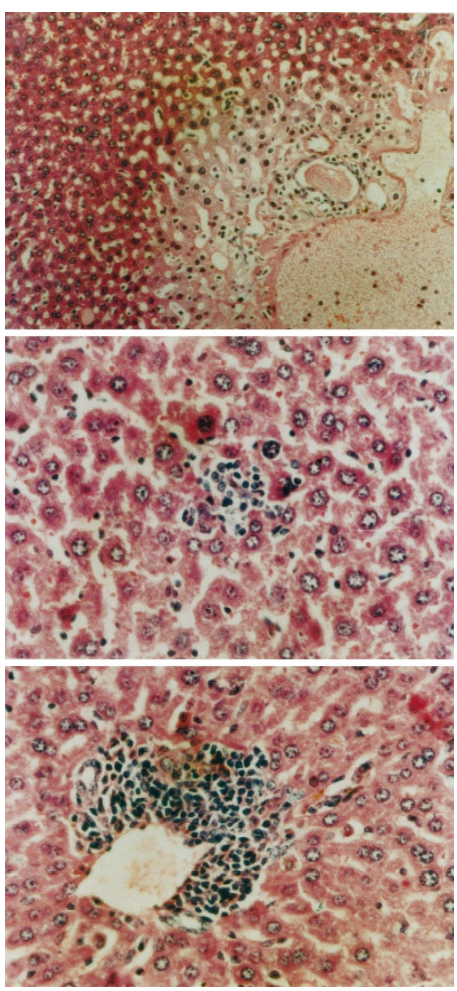

Fig. 7. Histological sections of endotoxin-treated liver exhibited hydropic degeneration around central vein (upper panel, hematoxylin and eosin staining, 230× magnification), ''spotty necrosis'' (middle panel, hematoxylin and eosin staining, 460× magnification) and infiltrate of lymphocytes around bile canaliculi (lower panel, hematoxylin and eosin staining, 460× magnification) in addition to congestion (all panels).

endotoxin, serotype O55:B5, Sigma, USA), aminoguanidine hemisulphate, L-canavanine, eosin (Sigma, USA), paraffin (Shandon, UK), formaldehyde (Carlo Elba, Italy) and bosentan (kindly provided by Dr. Martine Clozel, F. Hoffman La Roche, Basel, Switzerland).

2.6. Statistical analysis

The data are expressed as arithmetic means ± standard error of the mean (S.E.M.) of the number (n) of experiments. Differences between the means of groups were analyzed with Student's t-test and when $P < 0.05$, were considered as statistically significant.

3. Results

3.1. Aminoguanidine and L-canavanine effects on mesenteric blood flow alterations

Fig. 1 shows the mesenteric blood flow values obtained from anaesthetized mice. In endotoxin-treated animals, the mesenteric arterial blood flow was significantly lower than that in solvent (saline)-treated controls (ml min^{-1}, controls: 3.0 ± 0.2; endotoxin: 2.2 ± 0.2, $n = 10$, $P < 0.05$). When the animals were pretreated with aminoguanidine, there was no significant difference between the values obtained from endotoxin or its solvent (saline)-treated mice. However, L-canavanine failed to prevent the endotoxin-induced decrease in mesenteric blood flow at two different doses (i.e., 20 and 100 mg kg^{-1}) while it significantly increased blood flow at the higher dose. Except for the blood flow values for L-canavanine (100 mg kg^{-1})-treatment, there was no significant difference between the values obtained from saline-treated animals (Fig. 1).

3.2. Aminoguanidine and L-canavanine effects on liver and spleen weight alterations

Fig. 2 shows the effects of various treatments on liver weight. There was no significant difference between the values obtained from saline-treated animals. Endotoxin significantly increased liver weight (g per kg body weight, controls: 47.5 ± 2.0; endotoxin: 60.8 ± 1.9, $n = 10$, $P < 0.05$) while aminoguanidine or L-canavanine (20 mg kg^{-1}) pretreatment virtually abolished its effect (Fig. 2).

The effects of various treatments on spleen weight are shown in Fig. 3. Similar to liver, endotoxin significantly increased the weight of spleen (g per kg body weight, controls: 3.9 ± 0.5; endotoxin: 8.6 ± 0.9, $n = 10$, $P < 0.01$). However, in contrast to its effects in liver, aminoguanidine failed to prevent the endotoxin-induced increase in spleen weight while L-canavanine (20 mg kg^{-1}) itself caused a significant increase in spleen weight in saline-treated mice when compared to controls (L-canavanine: 8.01 ± 0.66, $n = 10$, $P < 0.01$) while it failed to block the endotoxin-induced increase (Fig. 3).

3.3. Three point dose–response profile of bosentan

In another set of experiments, bosentan failed to block the endotoxin-induced decrease in mesenteric blood flow

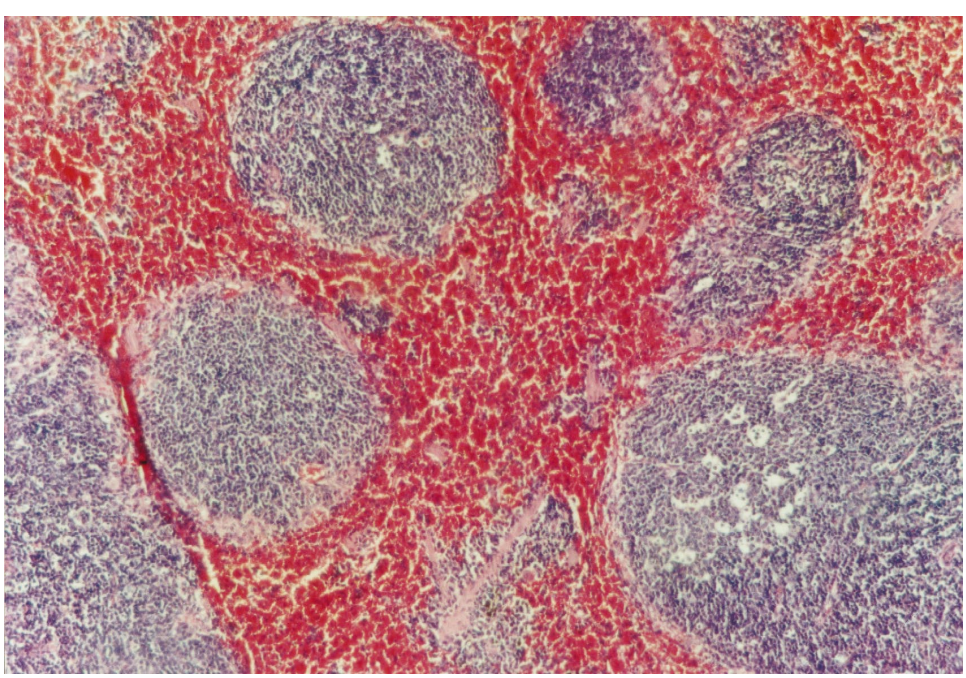

Fig. 8. Histological sections of the endotoxin-treated spleen exhibited significant congestion of the red pulp (hematoxylin and eosin staining, 230× magnification).

(Fig. 4); increase in liver (Fig. 5) and spleen (Fig. 6) weight at the 3-mg kg^{-1} dose but these effects were abolished at the 10- or 30-mg kg^{-1} doses (Figs. 4–6).

3.4. Histopathological examination of liver and spleen

Sections of the liver from endotoxin-treated animals showed profound hydropic degeneration around the central veins (Fig. 7 upper panel) and minimal parenchymal injury in the form of spotty necrosis (Fig. 7 middle panel) in addition to the presence of inflammatory infiltrates of lymphocytes, including occasional plasma cells around bile canaliculi (Fig. 7 lower panel) while the infiltrate was spilled out into the hepatic parenchyma. Also there was intense congestion in the periportal region of the liver (Fig. 4 all panels) and in the red pulp of all spleen samples (Fig. 8).

When the animals were pretreated with bosentan, both liver and spleen architecture were well preserved and the organs were completely protected from the histopathological injuries inflicted by endotoxin while bosentan itself produced no alterations of these organs in saline-treated animals. On the other hand, aminoguanidine blocked endotoxin-induced congestion in spleen but failed to modify the pathology inflicted by endotoxin in liver. In contrast to bosentan and aminoguanidine, L-canavanine itself caused cellular swelling in liver, which is regarded as the first manifestation of almost all forms of cellular injury, in addition to the necrosis of small clusters of cells and it also produced significant congestion in the spleen.

4. Discussion

This study demonstrated that endotoxaemia for 4 h produced a significant decrease in the blood flow to the mesenteric circulation, produced a significant blood congestion in liver and spleen, which was reflected as weight increase in these organs and inflicted some degree of inflammatory injury. However, all effects of endotoxin were abolished by the endothelin receptor antagonist, bosentan, while aminoguanidine, a selective inhibitor of inducible nitric oxide synthase, blocked the effects of endotoxin on blood flow and liver without modifying those on spleen. Interestingly, L-canavanine, another selective inducible nitric oxide synthase inhibitor, blocked the effects of endotoxin only in liver while it caused a significant weight increase in the spleen without blocking the endotoxin-induced decrease in mesenteric blood flow.

Our results are in agreement with those of previous studies which reported some degree of beneficial effects by endothelin or nitric oxide blockade during endotoxin-associated pathologies. Both the increase in blood flow to the splenic and intestinal vascular beds due to endothelin blockade by bosentan in a porcine model of septic shock (Weitzberg et al., 1996) and recent work on rats (Mitolo-Chieppa et al., 1996) fully support our findings that vasoconstrictor mechanisms are indeed prominent in the splanchnic area during endotoxaemia. Since maldistribution of nutritive blood flow (MacLean, 1972) and inadequate oxygen supply to the tissues, coupled to increased metabolic demands imposed by endotoxaemia are the hallmarks of septic shock (Ghosh et al., 1993), vasodilator interventions which increase regional blood flow in mesenteric circulation are expected to improve ischaemia-associated deteriorations in target tissues. Thus, endothelin, being the most potent vasoconstrictor substance known to date (Yanagisawa et al., 1988), appears to be the likely candidate for the self-perpetuating vicious circle of events resulting from intense vasoconstriction. The results of studies with the endothelin ET_B receptor antagonist, BQ 788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-L-methoxycarbonyl-tryptophanyl-D-norleucine) suggested that the type of endothelin receptor involved in endotoxin-induced injury was the endothelin ET_B receptor, which mediates the contraction of hepatic stellate (Ito) cells that control the hepatic sinusoidal blood flow. The suggestion was based on the fact that a specific endothelin ET_A receptor antagonist did not significantly modify the extent of liver dysfunction, of hepatocellular injury and hypotension caused by endotoxaemia in the rat (Ruetten and Thiemermann, 1996). Also the question of whether selective or non-selective endothelin receptor blockade is beneficial has yet to be answered because in contrast to our results, another non-selective endothelin receptor antagonist SB 209670 [(±)-(1*S*,2*R*,3*S*)-3-(2-carboxymethoxy-4-methoxyphenyl)-5-(prop-1-yloxy) indane-2-carboxylic acid] was reported to aggravate circulatory failure in the rat (Gardiner et al., 1995) an effect which may be explained by the difference(s) between the pharmacological properties of this molecule and bosentan. Interestingly, the complete attenuation of the endotoxin-induced blood flow decrease and the histopathological deterioration induced by bosentan within 4 h found in the present study are consistent with a previous report that hepatocellular dysfunction may develop within 2 h in a sepsis model of caecal ligation and puncture (Wang et al., 1995).

The significant prevention of endotoxin-induced hepatocellular injury, mesenteric blood flow decrease and increase in liver weight caused by aminoguanidine in our study can be partially explained by its relatively selective inhibitory actions on both the expression and the activity of inducible nitric oxide synthase (Thiemermann, 1998). This is in line with the concept that specific inhibition of inducible nitric oxide synthase is indeed beneficial with regard to liver dysfunction (Thiemermann et al., 1995), hepatocellular injury and multiple organ dysfunction during endotoxaemia (Wu et al., 1995) while constitutive isoforms are not (Wu et al., 1996). This is because the endogenous nitric oxide generated by constitutive nitric oxide synthase indeed plays an important role in the physiological vasodilatation which, in turn, provides pro-

tection in the intestinal microcirculation against noxious challenges such as that by endotoxin (Laszlo et al., 1994).

On the other hand, the failure of aminoguanidine to prevent the effects of endotoxin on the spleen in the present study remains to be explained, while the relevance of additional biological effects of aminoguanidine such as the inhibition of oxidative modification of low density lipoprotein and the subsequent increase in uptake by the macrophage scavenger receptor (Picard et al., 1992) must be evaluated in this context. Also, the lack of any prevention from the deleterious effects of endotoxin by another specific inhibitor of inducible nitric oxide synthase, L-canavanine, suggests that mechanisms other than excessive nitric oxide formation are responsible for endotoxin-induced organ injury. Our finding that L-canavanine itself caused cellular swelling, which is regarded as the first manifestation of a cellular injury, makes less likely any central role attributable to inducible nitric oxide synthase in endotoxin-induced organ injury. However, these assumptions should be treated with caution as both aminoguanidine (Misko et al., 1993) and L-canavanine (Iyengar et al., 1987) are highly specific but relatively weak inhibitors of inducible nitric oxide synthase (Thiemermann, 1997) when compared to the currently developed antagonists such as isothiourea derivatives. Thus, more precise conclusions could be reached by focusing on the time- and dose-dependence of inducible enzyme inhibition with its highly selective inhibitor, 1400W (Garvey et al., 1997).

Interestingly, our main suggestion that inducible nitric oxide inhibitors are relatively ineffective during endotoxaemia in comparison to endothelin receptor blockade is supported by the findings of a study by MacMicking et al. (1995). The authors reported that there was no significant difference between the knockout mice deficient in inducible nitric oxide synthase and normal-wild type mice with regard to lipopolysaccharide/*Clostridium parvum*-induced hepatic injury. They concluded that nitric oxide is not the key mediator in septic shock (MacMicking et al., 1995) while the inhibition of its production might not be the panacea most of us had hoped for (Parratt, 1997).

Thus, the main finding of the present study was that bosentan, a non-specific endothelin receptor antagonist, blocked the endotoxin-induced mesenteric blood flow decrease and attenuated the liver and spleen injury in endotoxaemic mice. Since splanchnic ischaemia contributes significantly to the high mortality rate in sepsis-related syndromes (Takala, 1996) increasing the blood flow to mesenteric circulation by endothelin blockade appears to be promising for further exploitation for the prevention of organ injury and the treatment of septic shock.

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