The endothelin receptor antagonist bosentan restores gut oxygen delivery and reverses intestinal mucosal acidosis in porcine endotoxin shock

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Abstract

Background—Endothelin-1, the most potent vasoconstrictor known, is produced in septic states and may be involved in the pathophysiology of the deteriorated splanchnic circulation seen in septic shock.

Aims—To elucidate the capability of bosentan, a non-peptide mixed endothelin receptor antagonist, to attenuate splanchnic blood flow disturbances and counteract intestinal mucosal acidosis in endotoxic shock.

Methods—In 16 anaesthetised pigs, central and regional haemodynamics were monitored by thermodilution and ultrasonic flow probes, respectively. A tonometer in the ileum was used for measurement of mucosal pH. Onset of endotoxin challenge was followed by bosentan administration (to eight pigs) two hours later.

Results—Endotoxin infusion reduced cardiac index and systemic oxygen delivery; bosentan restored these parameters. The reduced mean arterial blood pressure and renal blood flow remained unaffected by bosentan. The profound reduction in gut oxygen delivery in response to endotoxin was completely abolished by bosentan. Bosentan significantly improved the notably deteriorated intestinal mucosal pH and mucosal-arterial Pco_2 gap. The mucosal-portal vein Pco_2 gap, used to monitor the mucosa in relation to the gut as a whole (including the spleen and pancreas), was also greatly increased by endotoxaemia and significantly reversed by bosentan.

Conclusion—Bosentan completely restored the profound endotoxin induced reductions in systemic and gut oxygen delivery with a concomitant reversal of intestinal mucosal acidosis. Results suggest that endothelin is involved in the pronounced perfusion disturbances seen in the gut in endotoxic shock. Bosentan may prove useful in reducing gut ischaemia in septic shock.

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Keywords: splanchnic circulation; septic shock; tonometry; pH_i; Pco_2 gap; endothelin-1

It is a well established fact that splanchnic ischaemia is associated with multiple organ dysfunction syndrome and mortality in criti-

cally ill patients.^{1,2} However, the exact pathogenic mechanism is still unclear and has been the subject of substantial research efforts.

Barrier dysfunction of the intestinal mucosa appears to be of main pathophysiological importance. The intestinal mucosa is likely to become ischaemic in shock states.³ Under septic conditions increases in splanchnic oxygen consumption and microcirculatory disturbances may further contribute to ischaemia.⁴⁻⁶ Translocation of gut derived bacteria and endotoxin across an ischaemia induced hyper-permeable intestinal barrier has been suggested as an aetiology of multiple organ dysfunction syndrome.^{7,8} However, some studies have failed to show translocation in shock and the concept of translocation as a main promoter of multiple organ dysfunction syndrome is still under debate.⁹ Tissue damage by gut derived activated neutrophils has been suggested as an alternative aetiology.¹⁰ Mucosal acidosis assessed by gastrointestinal tonometry, proposed as an indicator of mucosal ischaemia, has in several studies been shown to be a good predictor of multiple organ dysfunction syndrome and mortality in critically ill patients.^{2,11} The results of the many studies aiming to restore gastrointestinal mucosal pH (pH_i), mainly utilising inotropic drugs, have been somewhat conflicting. Some studies have even shown reductions in pH_i despite increases in cardiac index and splanchnic oxygen delivery,¹²⁻¹⁵ suggesting inadequate blood flow distribution within the splanchnic region.

Endothelin-1 (ET-1) is an endogenous peptide with highly potent vasoconstrictive properties. It is derived mainly from the endothelium. The precursor, Big ET-1, is split by endothelin converting enzyme to form ET-1 which acts mainly on two receptors: ET_A situated on the vascular smooth muscle and ET_B situated on both vascular smooth muscle and endothelium. The vascular smooth muscle ET_A and ET_B receptors mediate contraction while ET_B receptors located on the endothelium mediate vasodilatation by release of nitric oxide and prostacyclin.¹⁶ High plasma levels of ET-1-like immunoreactivity (ET-1-LI) have been shown in various septic conditions^{17,18} and are associated with morbidity and mortality in patients with sepsis.^{19,20} As ET-1 is an extremely potent vasoactive peptide and produced in large quantities in septic states it may well be involved in the splanchnic blood flow disturbances seen in septic shock. Infusion of ET-1 in non-septic rats results in mesenteric vasoconstriction, an effect attenuated by ET receptor

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antagonism,²¹ and endothelin antiserum has previously been shown to counteract bacteraemia induced intestinal vasoconstriction.²² Furthermore, endothelin has been reported to promote endotoxin induced translocation in animal models.²³

In the present study, bosentan, a non-peptide ET_A and ET_B receptor antagonist,²⁴ was used in an attempt to attenuate splanchnic blood flow disturbances and counteract splanchnic ischemia in a porcine model of endotoxemic shock. In addition to systemic and regional haemodynamic measurements the effects of bosentan administration on the intestinal mucosa were assessed by tonometry.²⁵ The systemic haemodynamic parameters (table 1) presented in this study have been published previously.²⁶

Materials and methods

The experimental protocol for this study was approved by the ethics committee for experimental animal research at the Karolinska Institute, Stockholm, Sweden.

ANAESTHESIA AND SURGICAL PREPARATION

Sixteen landrace pigs, with mean weight of 20.6 kg (range 18.0–28.5), were fasted overnight with free access to water. An intramuscular injection of ketamine hydrochloride 20 mg/kg and atropine 25 µg/kg was given as premedication. General anaesthesia was induced by an intravenous bolus injection of pentobarbital 12 mg/kg and maintained by a continuous infusion of 3–6 mg/kg/h; incremental doses were given when needed.

Muscle paralysis was achieved by an infusion of pancuronium bromide 0.5 mg/kg/h. After tracheostomy the animals were mechanically ventilated by a Servo 900 ventilator (Siemens Elema, Sweden). The minute volume was adjusted to keep the animals normoventilated (Paco₂ 4.7–5.8 kPa). The respiratory frequency was set to 18 per minute and Fio₂ to 0.30. The settings were maintained during the experiment. The animals received a continuous infusion of saline with glucose 2.5 mg/ml at a rate of 20 ml/kg/h throughout the experiment.

The body temperature was monitored by a rectal thermistor and maintained at 38–39°C. A multiple lumen catheter was inserted into the right femoral vein and a single lumen catheter into the right femoral artery. A balloon tipped pulmonary artery catheter was positioned in the pulmonary artery by pressure guidance via the left femoral vein. The animals were placed in the semilateral position before midline laparotomy. A catheter was placed in the urinary bladder by a small cystotomy. Another catheter was introduced into the splenic vein and positioned with its tip in the portal vein; its position was verified by palpation of the catheter tip in the portal vein. Ultrasonic flow probes (Transonic Systems Inc., Ithaca, New York, USA) were placed around the right renal artery and portal vein, for continuous registration of regional blood flow. Through a small enterotomy in the ileum a tonometer (sigmoid catheter, Tonometrics Inc., Massachusetts,

USA) was inserted for measurement of mucosal PCO₂.

At the end of preparation the abdomen was closed and the animals fully turned to the left lateral position.

HAEMODYNAMIC MEASUREMENTS AND CALCULATIONS

The arterial and pulmonary artery catheters were connected to pressure transducers and heart rate (HR), mean arterial blood pressure (MAP), and central venous pressure (CVP) were continuously recorded on a polygraph (Grass 7B, Quincy, Massachusetts, USA). Cardiac index (indexed to body weight, CI) was measured by thermodilution (Edwards Lab 9520A, St Ana, California, USA) and determined as the mean of a triplicate of 10 ml of ice cold saline injections. The systemic vascular resistance index (SVRI) was calculated as MAP–CVP/CI. Blood samples were collected from the femoral artery, pulmonary artery, and portal vein catheters for analysis of blood gases on an ILS 1610 blood gas analyser (Instrumental Laboratories, USA). Systemic oxygen delivery index (Do₂i) was calculated as: haemoglobin concentration (Hb) × 0.0139 × arterial oxygen saturation (Sao₂) × CI; and global oxygen consumption index (Vo₂i) as: Hb × 0.0139 × (Sao₂ – mixed venous oxygen saturation) × CI. Regional blood flow was indexed to body weight and presented as ml/kg/min. Gut oxygen delivery index (Do₂igut) including pancreas and spleen was calculated as: portal blood flow index (Qpvi) × Sao₂ × Hb × 0.0139. Gut oxygen consumption index (Vo₂igut) including pancreas and spleen was calculated as: Hb × 0.0139 × Qpvi × (Sao₂ – portal vein oxygen saturation (Spvo₂)).

INTESTINAL TONOMETRY

The PCO₂ in the saline obtained from the tonometer was used for calculation of intestinal mucosal pH (pHi) by means of the Henderson-Hasselbalch equation assuming that the mucosal bicarbonate level equals the arterial level. (Henderson-Hasselbalch equation: pHi = 6.1 + log [HCO₃⁻] / [PCO_{2(SS)} × 0.03].) As the saline in the balloon is not fully equilibrated with the luminal PCO₂ after one hour, a correction table, provided by the manufacturer, was used to obtain PCO₂ at steady state (PCO_{2(SS)}). The mucosal-arterial PCO₂ gap was calculated as: PCO_{2(SS)} – arterial PCO₂. The mucosal-portal PCO₂ gap was calculated as: PCO_{2(SS)} – portal venous PCO₂.

BIOCHEMICAL ANALYSIS

Arterial and portal plasma levels of ET-1-LI were analysed by radioimmunoassay as described previously.²⁷ Hb concentration was measured spectrophotometrically (haemoglobin photometer, Leo, Helsingborg, Sweden).

ENDOTOXIN

Escherichia coli lipopolysaccharide (serotype 0111: B4, Sigma, St Louis, USA) was used. Prior to infusion, the endotoxin was dissolved

in saline and heated in order to dissolve any precipitate.

EXPERIMENTAL PROTOCOL

The animals were allowed one hour of rest after surgery; thereafter baseline measurements were made at one hour (T_{-1h}) and at 30 minutes ($T_{-0.5h}$) prior to onset of endotoxin challenge at T_{0h} . Endotoxin infusion was started at a rate of 2.5 $\mu\text{g/kg/h}$ and was increased stepwise until reaching 20 $\mu\text{g/kg/h}$ after 30 minutes. The infusion was discontinued after three hours (T_{3h}). At T_{2h} eight animals received a bolus injection of 10 mg/kg bosentan (F Hoffmann LaRoche Ltd, Basel, Switzerland) followed by a continuous infusion of 5 mg/kg/h maintained throughout the experiment. The other eight animals receiving only endotoxin served as a control group.

Every 30 minutes cardiac output (CO), HR, MAP, CVP, and blood flow in the portal vein and renal artery were recorded. PCO_2 in saline, obtained from the tonometer, was analysed together with samples from arterial, mixed venous, and portal blood every hour as was Hb. The experiments were terminated at T_{5h} and the animals were sacrificed by injection of a lethal dose of sodium pentobarbital.

STATISTICS

Data are presented as mean (SEM). Univariate analysis of variance for repeated measures was used for comparison between groups. In cases of significant interactions, time matched between group contrasts were used for differences between groups at T_{0h} (before endotoxaemia), T_{2h} (before intervention), and T_{5h} (at end of experiment). Changes in time were analysed between T_{0h} and T_{2h} . Data were considered significant at $p < 0.05$. Statistica 5.0 software (StatSoft Inc., Oklahoma, USA) was used for statistical calculations.

Results

No significant differences in any parameter were found between the endotoxin control group and the bosentan treated group at baseline (T_{0h}) or after two hours of endotoxin infusion, immediately before onset of bosentan intervention (T_{2h}). One animal in the control group died 2.25 hours after onset of endotoxaemia.

Table 1 Systemic haemodynamic parameters

	Time (hours)					
	0	1	2	3	4	5
Cardiac index (ml/kg/min)						
Control	111 (6)	83 (7)	74 (6)	62 (6)	63 (7)	65 (9)
Bosentan	130 (13)	88 (6)	85 (10)	110 (13)	126 (18)	131 (16)**
Mean arterial blood pressure (mm Hg)						
Control	125 (4)	85 (7)	64 (7)	59 (4)	55 (3)	59 (5)
Bosentan	141 (7)	106 (6)	73 (5)	58 (5)	59 (5)	61 (6)
Systemic vascular resistance index (mm Hg min kg/l)						
Control	1094 (56)	980 (111)	826 (106)	935 (106)	850 (77)	845 (69)
Bosentan	1111 (118)	1168 (57)	869 (104)	514 (73)	470 (69)	448 (66)**
Systemic oxygen delivery index (ml/kg/min)						
Control	18.3 (1.0)	15.1 (1.8)	12.6 (0.9)	10.8 (0.95)	10.2 (1.0)	11.5 (1.3)
Bosentan	18.9 (1.8)	15.7 (1.2)	13.5 (1.2)	16.1 (1.6)	17.6 (2.1)	18.2 (1.6)**
Systemic oxygen consumption index (ml/kg/min)						
Control	3.7 (0.2)	4.6 (0.4)	4.7 (0.5)	5.0 (0.3)	4.8 (0.3)	4.9 (0.4)
Bosentan	3.5 (0.1)	3.8 (0.2)	3.8 (0.2)	4.4 (0.4)	3.9 (0.3)	4.1 (0.4)

Data are presented as mean (SEM). ** $p < 0.01$.

SYSTEMIC HAEMODYNAMICS, OXYGEN DELIVERY, AND CONSUMPTION

Endotoxaemia resulted in a distinct reduction in CI and MAP while SVRI was unchanged apart from an early peak at $T_{0.5h}$ (table 1). Bosentan intervention resulted in a restoration of CI back to baseline levels with a significant difference between groups at T_{5h} while MAP remained unaffected. SVRI was significantly reduced in response to bosentan administration. A DO_2i decrease in parallel with CI and a slight increase in VO_2i were observed in response to endotoxaemia. Bosentan intervention reversed the reduction in DO_2i resulting in significant differences between groups at T_{5h} . VO_2i remained unaffected by bosentan treatment and no significant differences between groups were observed (table 1).

REGIONAL HAEMODYNAMICS, OXYGEN DELIVERY, AND CONSUMPTION

Qpvi and DO_2igut were notably reduced while VO_2igut was slightly but significantly increased in response to endotoxin challenge (fig 1). Bosentan completely restored Qpvi and DO_2igut with highly significant differences between groups at T_{5h} . VO_2igut was not altered by bosentan. Endotoxaemia resulted in a significant reduction in renal artery blood flow index (table 2). Bosentan administration had no influence on this parameter. SpvO_2 decreased rapidly after onset of endotoxaemia and was completely restored by bosentan treatment with a significant difference between groups at T_{5h} .

INTESTINAL TONOMETRY

While there was a small mucosal-arterial PCO_2 gap around 2 kPa, no mucosal-portal PCO_2 gap was observed at baseline. Endotoxaemia induced a significant reduction in pHi and mucosal-arterial PCO_2 gap and mucosal-portal PCO_2 gap were significantly increased (fig 2). Bosentan notably improved both pHi and mucosal-arterial PCO_2 gap resulting in significant differences between groups at T_{5h} . Mucosal-portal PCO_2 gap was also significantly reduced by bosentan administration leading to a significant difference between groups at T_{5h} .

Figure 1 Portal blood flow index (A), portal venous oxygen saturation (B), gut oxygen delivery index (C), and gut oxygen consumption index (D). Control group, $n=8$; bosentan treated group, $n=8$. Significant differences between groups at T_{0h} , T_{2h} and T_{5h} are symbolised by * $p<0.05$ and ** $p<0.001$.

ACID-BASE STATUS AND HAEMOGLOBIN

Base excess and arterial pH decreased significantly in response to endotoxin challenge while bosentan administration resulted in a discernible difference between groups at T_{5h} ; this was, however, significant only for base excess (table 2). A haemoconcentration followed induction of endotoxaemia with a peak in Hb concentration at T_{1h} . Bosentan treatment reduced Hb concentration compared with that in control

animals resulting in a significant difference between groups at T_{5h} .

ENDOTHELIN-1-LIKE IMMUNOREACTIVITY

Endotoxaemia led to a three to fourfold increase in arterial and portal vein plasma ET-1-LI levels at T_{2h} (table 2). Administration of bosentan dramatically increased both arterial and portal vein ET-1-LI plasma levels to

Table 2 Other parameters

	Time (hours)					
	0	1	2	3	4	5
Haemoglobin (g/l)						
Control	120 (5)	139 (7)	126 (8)	131 (7)	123 (6)	125 (5)
Bosentan	107 (7)	130 (6)	118 (5)	108 (4)	106 (4)	108 (6)*
pH arterial blood						
Control	7.39 (0.02)	7.29 (0.02)	7.23 (0.04)	7.17 (0.05)	7.16 (0.04)	7.19 (0.04)
Bosentan	7.39 (0.02)	7.35 (0.02)	7.23 (0.03)	7.22 (0.03)	7.25 (0.02)	7.27 (0.02)
Base excess (mmol/l)						
Control	-1.4 (1.0)	-6.3 (1.2)	-10.9 (2.2)	-13.3 (2.1)	-13.0 (2.0)	-12.5 (1.8)
Bosentan	-2.2 (1.2)	-5.3 (0.7)	-9.7 (1.4)	-9.8 (1.4)	-8.9 (1.3)	-8.7 (1.1)*
Pco₂ arterial blood (kPa)						
Control	5.1 (0.2)	5.6 (0.4)	5.2 (0.2)	5.4 (0.3)	5.7 (0.2)	5.4 (0.1)
Bosentan	5.0 (0.2)	4.9 (0.2)	5.5 (0.1)	5.8 (0.2)	5.6 (0.1)	5.3 (0.1)
Renal blood flow index (ml/kg/min)						
Control	4.9 (0.4)	3.2 (0.5)	2.7 (0.9)	2.3 (0.7)	3.1 (0.8)	3.6 (1.2)
Bosentan	5.5 (0.8)	4.6 (0.7)	3.4 (0.8)	3.5 (0.9)	3.8 (1.0)	3.7 (1.0)
Endothelin-1-like immunoreactivity in arterial plasma (pmol/l)						
Control	15.2 (3.0)	29.4 (2.8)	42.8 (3.6)	65.7 (15.2)	59.1 (12.9)	52.0 (11.9)
Bosentan	14.5 (1.2)	27.5 (2.2)	36.7 (2.5)	194 (19.0)	185 (18.9)	180 (29.9)***
Endothelin-1-like immunoreactivity in portal venous plasma (pmol/l)						
Control	17.0 (2.5)	24.5 (2.1)	47.3 (4.3)	44.9 (3.9)	60.0 (12.1)	61.1 (13.3)
Bosentan	18.3 (1.8)	30.1 (3.2)	43.1 (4.4)	207 (32.5)	163 (16.9)	184 (25.1)***

Data are presented as mean (SEM). * $p<0.05$; *** $p<0.001$.

Figure 2 Calculated pHi (A), mucosal-arterial PCO_{2gap} (B), and mucosal-portal PCO_{2gap} (C). Control group, $n=8$; bosentan treated group, $n=8$. Significant differences between groups at T_{0h} , T_{2h} and T_{5h} are symbolised by * $p<0.05$ and ** $p<0.01$.

the same extent, resulting in significant differences between groups at T_{5h} .

Discussion

The purpose of the present study was to elucidate whether the mixed ET receptor antagonist bosentan could reverse a notably deteriorated gut circulation induced by endotoxaemia. The most important findings were that bosentan completely restored portal blood flow, almost doubled gut oxygen delivery, and substantially reversed intestinal mucosal acidosis.

Endotoxin challenge induced pronounced alterations in systemic haemodynamics, causing a progressive decline in cardiac index and mean arterial blood pressure. As a consequence of the cardiac index decrease systemic oxygen delivery was reduced. Bosentan given two hours after onset of endotoxaemia notably improved cardiac performance, and as a result, systemic oxygen delivery, while mean arterial blood pressure remained unchanged, indicating systemic vasodilatation. These results are in line with previous findings where pretreatment with bosentan has been shown to improve cardiac index in porcine endotoxaemia.²⁸

In the present study endotoxin induced profound reductions in portal blood flow and gut oxygen delivery; these results are in agreement with those of other authors.²⁹ However, the results of previous studies are conflicting as endotoxin administration or sepsis may not affect or even increase gut oxygen delivery, although there are usually further increases in gut oxygen consumption. In the clinical setting, from which a number of reports of increased gut oxygen delivery in sepsis originate, patients are undoubtedly subject to volume resuscitation contributing to the hyperdynamic state and increased gut oxygen delivery.⁴⁻³⁰ Analogously, in animal studies utilising hyperdynamic, volume loaded models, maintained or increased gut oxygen delivery are common findings.³¹⁻³² In our study there was a hypodynamic response to endotoxin and one may argue that the model is inadequately resuscitated in terms of volume loading. However, in spite of the hypodynamic response, bosentan was able to restore both systemic and gut oxygen delivery. There was a significant increase in gut oxygen consumption despite the profound reduction in gut oxygen delivery in response to endotoxin administration. Furthermore, the notable improvement in gut oxygen delivery in response to bosentan treatment was not associated with an increase in oxygen consumption. These findings suggest that oxygen consumption was not supply dependent in the gut. This may seem somewhat surprising considering the profound reduction in gut oxygen delivery in response to endotoxin and the fact that gut oxygen extraction capability has been reported to be reduced due to increased gut capillary transit time heterogeneity in endotoxic shock.⁶ However, gut oxygen supply dependency has been difficult to show in several experimental studies, suggesting that gut oxygen extraction capability may be sufficient in these models despite microcirculatory disturbances.³³

The use of calculated pHi , obtained by tonometry, to assess mucosal ischaemia has been criticised as it may be affected by respiratory acid base disorders and treatment with buffering agents.³⁴⁻³⁵ Therefore, we also used the mucosal-arterial PCO_{2gap} , focusing on the difference between mucosal and arterial PCO_{2} , proposed as a more specific indicator of mucosal ischaemia by several authors.²⁵⁻³⁶ In addition, the mucosal-portal PCO_{2gap} , focusing on the difference between the intestinal

mucosa and the gut as a whole (including pancreas and spleen), was measured.

Endotoxaemia resulted in a pronounced intestinal mucosal acidosis as indicated by both calculated pHi and mucosal-arterial PCO_2 gap, findings in line with previous studies where tonometry has been shown to be a sensitive method when assessing the intestinal mucosa in experimental sepsis.^{37–39} There were no apparent differences between calculated pHi and mucosal-arterial PCO_2 gap in terms of significance between groups. This may in part be explained by the experimental situation with controlled normoventilation. The development of a mucosal-portal PCO_2 gap in response to endotoxin illustrates, as tonometry mainly reflects the mucosal layer, the susceptibility of the mucosa in relation to the gut as a whole (including the spleen and pancreas). Bosentan improved calculated pHi and decreased both mucosal-arterial PCO_2 gap and mucosal-portal PCO_2 gap. These findings suggest that endothelin may be involved in intestinal perfusion disturbances seen in endotoxaemia.

Apart from counteracting vasoconstriction bosentan may also reduce ET-1-induced capillary protein and plasma leakage, possibly mediated by increased leucocyte adhesion to the endothelium.⁴⁰ This concept is supported by the reduction in haemoconcentration in the bosentan treated group as compared with controls, indicating an increased intravascular volume in the bosentan treated group contributing to improved perfusion. Furthermore, bosentan may reduce endothelin induced tissue oedema secondary to protein leakage and thereby promote tissue oxygen transport. Tissue oedema may also be reduced by improved lymph drainage by bosentan as ET-1 has been shown to reduce lymph flow notably.⁴¹ The venoarterial response (an increase in mesenteric arterial resistance in response to an increased portal vein pressure), is abolished in endotoxaemia.⁴² As portal vein resistance is reported to increase in endotoxaemia this may contribute to intestinal oedema and splanchnic blood pooling.⁴³ Interestingly, in several studies ET-1 is reported to be a three to 10 times more potent constrictor of large veins than arteries,⁴⁴ suggesting that antagonism of ET-1 by reducing portal vein resistance may counteract intestinal oedema in endotoxaemia.

Interventions leading to an increase in cardiac output may not necessarily result in improved mucosal pH and in fact even an increased splanchnic blood flow has been shown to result in reductions in mucosal pH ,^{12–15} suggesting tissue perfusion mismatch. The present intervention aimed to counteract the action of a potent vasoactive endothelium derived peptide produced in large quantities in endotoxic shock. The increase in cardiac output and splanchnic blood flow was followed by an increased mucosal pH , suggesting that endothelin may well be involved in the microcirculatory disturbances seen in endotoxaemia. These findings are further supported by Wilson *et al* who showed, using intravital microscopy, that endothelin antiserum attenu-

ates intestinal microvascular vasoconstriction induced by bacteraemia.²²

The relation between mucosal pH and oxygenation in sepsis is complex. Antonsson and Haglund⁴⁵ showed a reduced mucosal pH despite a normal intraluminal PO_2 in a peritonitis model, whereas in haemorrhagic shock both parameters were reduced. Similar findings were made by VanderMeer *et al* who reported reductions in mucosal pH despite normal mucosal PO_2 in endotoxaemia.³¹ Revelly *et al* found a reduced mucosal pH simultaneously with an increase in mucosal blood flow in a model of porcine hyperdynamic endotoxic shock.³² These findings suggest that factors other than ischaemia may contribute to mucosal acidosis in sepsis. Among the pathophysiological factors, contributing to disturbances in oxygen utilisation, nitric oxide has been suggested.³³ This highly reactive gas, reported to be produced in large amounts in endotoxic shock,⁴⁶ has been put forward as an inhibitor of the mitochondrial electron transport chain,⁴⁷ reducing the production of ATP and thereby contributing to acidosis. Interestingly, stimulation of endothelial ET_B receptors has been shown to promote production of nitric oxide,¹⁶ suggesting that an ET receptor antagonist such as bosentan may attenuate nitric oxide formation.

Renal perfusion was not improved by bosentan, suggesting that the sustained hypoperfusion is maintained by other mechanisms and/or a different endothelin receptor distribution in the kidney, resulting in a lack of response to bosentan. In healthy volunteers, exogenous ET-1 induces a more pronounced vasoconstriction in the splanchnic than in the renal vascular bed, indicating that the splanchnic circulation may be more sensitive to ET-1.⁴⁸ These findings are somewhat inconsistent with a previous study where ET-1 antibodies were shown to improve renal function in septic rats.⁴⁹

ET-1-LI plasma levels increased substantially in response to endotoxin and the increase was notably accentuated by bosentan treatment. A rise in plasma ET-1-LI in response to bosentan administration is found in both humans and animals of different species in several studies.^{28 50 51} The levels of prepro-ET-1 mRNA and circulating Big ET-1 are unchanged in response to bosentan administration,^{28 52} indicating that bosentan has no effect on ET-1 synthesis. A rise in plasma ET-1-LI is not seen when using a selective ET_A antagonist.^{50 52} Furthermore, selective ET_B receptor antagonism almost abolishes pulmonary extraction of circulating ET-1 in dogs while ET_A receptor antagonism had no such effect.⁵³ These findings indicate that the ET_B receptor is deeply involved in the elimination of ET-1 in plasma. It is likely that the increase in circulating ET-1-LI plasma levels in response to bosentan administration is due to a decreased endothelium located ET_B receptor mediated elimination of ET-1 from plasma. There were no apparent differences between ET-1-LI levels in arterial and portal vein plasma. These results indicate that the production of ET-1 in the gut did not exceed the

elimination significantly. These findings are in line with those of Myhre *et al.*⁵⁴

In conclusion, bosentan completely restored the profound reductions in systemic and gut oxygen delivery induced by endotoxin. These changes were followed by a distinct improvement of intestinal mucosal pH. The findings suggest that endothelin is an important mediator of the notable perfusion disturbances seen in the gut during endotoxic shock. Endothelin receptor antagonism may prove useful in reducing gut ischaemia in septic shock conditions.

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- Gutierrez G, Palizas F, Doglio G, *et al.* Gastric intramucosal pH as a therapeutic index of tissue oxygenation in critically ill patients. *Lancet* 1992;339:195-9.
- Maynard N, Bihari D, Beale R, *et al.* Assessment of splanchnic oxygenation by gastric tonometry in patients with acute circulatory failure. *JAMA* 1993;270:1203-10.
- Fink MP. Gastrointestinal mucosal injury in experimental models of shock, trauma, and sepsis. *Crit Care Med* 1991;19:627-41.
- Dahn MS, Lange P, Lobdell K, *et al.* Splanchnic and total body oxygen consumption differences in septic and injured patients. *Surgery* 1987;101:69-80.
- Arvidsson D, Rasmussen I, Almqvist P, *et al.* Splanchnic oxygen consumption in septic and hemorrhagic shock. *Surgery* 1991;109:190-7.
- Humer MF, Phang PT, Friesen BP, *et al.* Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs. *J Appl Physiol* 1996;81:895-904.
- Deitch EA, Berg R, Specian R. Endotoxin promotes the translocation of bacteria from the gut. *Arch Surg* 1987;122:185-90.
- Baron P, Traber LD, Traber DL, *et al.* Gut failure and translocation following burn and sepsis. *J Surg Res* 1994;57:197-204.
- Schlichting E, Grotmol T, Kahler H, *et al.* Alterations in mucosal morphology and permeability, but no bacterial or endotoxin translocation takes place after intestinal ischemia and early reperfusion in pigs. *Shock* 1995;3:116-24.
- Biffi WL, Moore EE. Splanchnic ischaemia/reperfusion and multiple organ failure. *Br J Anaesth* 1996;77:59-70.
- Mythen MG, Webb AR. Intra-operative gut mucosal hypoperfusion is associated with increased post-operative complications and cost. *Int Care Med* 1994;20:99-104.
- Marik PE, Mohedin M. The contrasting effects of dopamine and norepinephrine on systemic and splanchnic oxygen utilization in hyperdynamic sepsis. *JAMA* 1994;272:1354-7.
- Uusaro A, Ruokonen E, Takala J. Gastric mucosal pH does not reflect changes in splanchnic blood flow after cardiac surgery. *Br J Anaesth* 1995;74:149-54.
- Kosonen P, Ruokonen E, Takala J. Assessment of splanchnic perfusion using systemic and regional blood-gastric pH gradient. *Clin Int Care* 1995;6:93.
- Parviainen I, Ruokonen E, Takala J. Dobutamine-induced dissociation between changes in splanchnic blood flow and gastric intramucosal pH after cardiac surgery. *Br J Anaesth* 1995;74:277-82.
- De-Nucci G, Thomas R, d'Orleans-Juste P. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 1988;85:9797-800.
- Lundblad R, Giercksky KE. Endothelin concentrations in experimental sepsis: profiles of big endothelin and endothelin 1-21 in lethal peritonitis in rats. *Eur J Surg* 1995;161:9-16.
- Weitzberg E, Lundberg JM, Rudehill A. Elevated plasma levels of endothelin in patients with sepsis syndrome. *Circ Shock* 1991;33:222-7.
- Pittet JF, Morel DR, Hemsn A, *et al.* Elevated plasma endothelin-1 concentrations are associated with the severity of illness in patients with sepsis. *Ann Surg* 1991;213:261-4.
- Takakuwa T, Endo S, Nakae H, *et al.* Plasma levels of TNF-, endothelin-1 and thrombomodulin in patients with sepsis. *Res Commun Chem Pathol Pharmacol* 1994;84:261-9.
- Gardiner SM, Kemp PA, March JE, *et al.* Effects of bosentan (Ro 47-0203), an ETA-, ETB-receptor antagonist, on regional haemodynamic responses to endothelins in conscious rats. *Br J Pharmacol* 1994;112:823-30.
- Wilson MA, Steeb GD, Garrison RN. Endothelins mediate intestinal hypoperfusion during bacteremia. *J Surg Res* 1993;55:168-75.
- Wang WY. Intestinal endotoxin translocation in endotoxemic rats. *Sheng Li Ko Hsueh Chin Chan (Progress in Physiological Sciences)* 1995;26:41-4.
- Clozel M. Pharmacological characterization of bosentan, a new potent orally active non-peptide endothelin receptor antagonist. *J Pharmacol Exp Ther* 1994;270:228-35.
- Groeneveld AB, Kolkman JJ. Splanchnic tonometry: a review of physiology, methodology, and clinical applications. *J Crit Care* 1994;9:198-210.
- Wanecek M, Oldner A, Rudehill A, *et al.* Cardiopulmonary dysfunction during porcine endotoxin shock is effectively counteracted by the endothelin receptor antagonist bosentan. *Shock* 1997;7:364-70.
- Hemsn A. Biochemical and functional characterization of endothelin peptides with special references to vascular effects. *Acta Physiol Scand* 1991;142 (suppl 602):5-61.
- Weitzberg E, Hemsn A, Rudehill A, *et al.* Bosentan improves cardiopulmonary vascular performance and regional blood flows in porcine endotoxin shock. *Br J Pharmacol* 1996;118:617-26.
- Fink MP, Kaups KL, Wang HL, *et al.* Maintenance of superior mesenteric arterial perfusion prevents increased intestinal mucosal permeability in endotoxic pigs. *Surgery* 1991;110:154-60.
- Ruokonen E, Takala J, Kari A, *et al.* Regional blood flow and oxygen transport in septic shock. *Crit Care Med* 1993;21:1296-303.
- VanderMeer TJ, Wang H, Fink MP. Endotoxemia causes ileal mucosal acidosis in the absence of mucosal hypoxia in a normodynamic porcine model of septic shock. *Crit Care Med* 1995;23:1217-26.
- Revelly J-P, Ayuse T, Brienza N, *et al.* Endotoxic shock alters distribution of blood flow within the intestestinal wall. *Crit Care Med* 1996;24:1345-51.
- Aranow JS, Fink MP. Determinants of intestinal barrier failure in critical illness. *Br J Anaesth* 1996;77:71-81.
- Benjamin E, Hannon EM, Oropello JM, *et al.* Effects of respiratory acidosis on gastrointestinal tonometry [abstract]. *Anesthesiology* 1992;77:A307.
- Benjamin E, Polokoff E, Oropello JM, *et al.* Sodium bicarbonate administration affects the diagnostic accuracy of gastrointestinal tonometry in acute mesenteric ischemia. *Crit Care Med* 1992;20:1181-3.
- Grdebck M, Settergren G, hquist G, *et al.* Effect of dexamine on calculated low gastric intramucosal pH following valve replacement. *Acta Anaesthesiol Scand* 1995;39:599-604.
- Montgomery A, Almqvist P, Arvidsson D, *et al.* Early detection of gastrointestinal mucosal ischemia in porcine E. coli sepsis. *Acta Chir Scand* 1990;156:613-20.
- Rasmussen I, Haglund U. Early gut ischemia in experimental fecal peritonitis. *Circ Shock* 1992;38:22-8.
- Oldner A, Gojny M, Ungerstedt U, *et al.* Splanchnic homeostasis during endotoxin challenge in the pig as assessed by microdialysis and tonometry. *Shock* 1996;6:188-93.
- Helset E. ET-1 causes accumulation of leukocytes in the pulmonary circulation. *Circ Shock* 1994;44:201-9.
- Reeder LB, Ferguson MK. Endothelin-1 synthesis and receptor-mediated activity in porcine lymph vessels. *J Surg Res* 1996;63:215-9.
- Revelly J-P, Ayuse T, Brienza N, *et al.* Dysregulation of the veno-arterial response in the superior mesenteric artery during endotoxic shock. *Crit Care Med* 1995;23:1519-27.
- Ayuse T, Brienza N, Revelly JP, *et al.* Alterations in liver hemodynamics in an intact porcine model of endotoxin shock. *Am J Physiol* 1995;37:H1106-14.
- Cocks TM, Faulkner NL, Sudhir K, *et al.* Reactivity of endothelin-1 on human and canine large veins compared with large arteries in vitro. *Eur J Pharmacol* 1989;171:17-24.
- Antonsson JB, Haglund UH. Gut intramucosal pH and intraluminal Po₂ in a porcine model of peritonitis or haemorrhage. *Gut* 1995;37:791-7.
- Thiemermann C. The role of the L-arginine: nitric oxide pathway in circulatory shock. *Adv Pharmacol* 1994;28:45-79.
- Gross SS, Wolin MS. Nitric oxide: pathophysiological mechanisms. *Ann Rev Physiol* 1995;57:737-69.
- Weitzberg E, Ahlberg G, Lundberg JM. Long-lasting vasoconstriction and efficient regional extraction of endothelin-1 in human splanchnic and renal tissues. *Biochem Biophys Res Commun* 1991;180:1298-303.
- Morise Z, Ueda M, Aiura K, *et al.* Pathophysiological role of endothelin-1 in renal function in rats with endotoxin shock. *Surgery* 1994;115:199-204.
- Hemsn A, Weitzberg E, Modin A, *et al.* Effects of Ro-47-0203 and PD 155080 on the plasma kinetics, receptor binding and vascular effects of endothelin in the pig. *Eur J Pharmacol* 1996;31:369-76.
- Kiowski W. Evidence of endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet* 1995;346:732-6.
- Lffler BM. Effect of different endothelin receptor antagonists and of the novel non-peptide antagonist Ro 46-2005 on endothelin levels in rat plasma. *FEBS Lett* 1993;333:108-10.
- Dupuis J, Gorsky CA, Fournier A. Pulmonary clearance of circulating endothelin-1 in dogs in vivo: exclusive role of ET_B receptors. *J Appl Physiol* 1996;81:1510-5.
- Myhre U, Pettersen JT, Rise C, *et al.* Endothelin-1 and endotoxemia. *J Cardiovasc Pharmacol* 1993;22(suppl 8):S291-4.