Role of Endothelin 1 in Hemorrhagic Shock-Induced Gastric Mucosal Injury in Rats

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Background/Aims: Gastric microcirculatory disturbances are involved in the pathogenesis of stress ulcers; however, vasomodulators regulating this process are not fully understood. This study was conducted to investigate the role of endothelin 1 (ET-1) in hemorrhagic shock-induced gastric mucosal damage in rats.

Methods: ET-1 contents in plasma and gastric mucosa were measured and gastric mucosal damage was evaluated during a control period, 60 minutes of ischemia, 15 minutes of reperfusion, and 30 minutes of postreperfusion. Next, effects of BQ-123, an endothelinA receptor antagonist, on the gastric mucosal damage and hemodynamics were studied.

Results: Both plasma and mucosal ET-1 significantly increased after ischemia and reperfusion compared with the control values, but only mucosal ET-1 continued to increase after reperfusion, leading to the development of gastric mucosal damage. BQ-123, administered just before reperfusion, reduced mucosal damage in the postreperfusion period dose-dependently and improved mean gastric mucosal blood flow and mucosal hemoglobin oxygen saturation during the 30-minute postreperfusion period.

Conclusions: These results suggest that endogenous ET-1 plays an important role in the pathogenesis of hemorrhagic shock-induced gastric mucosal damage through impairment of mucosal microcirculation. Further, endothelinA antagonists may have therapeutic benefits for shock-induced gastric mucosal damage.

Stress ulcers, although occurring less frequently since the development of antiulcer drugs, still result in high mortality in critically ill patients.1,2 Defects in the protective mechanism of the gastric mucosa, especially reduced gastric mucosal blood flow, are closely related to its pathogenesis.3,4 Several factors5-8 (including free radicals, neutrophil adherence, and platelet-activating factor) are reported to be involved in the mechanism of the microcirculatory disturbances in the ischemia/reperfusion model, which most nearly approximates the human stress ulcer.9

Endothelin 1 (ET-1), a potent vasoconstrictive peptide with 21-amino acid residues, regulates vascular tone in peripheral vessels.10 The vasoconstrictive action of ET-1 is mediated by endothelinA (ETa) receptors on vascular smooth muscle cells.11 Exogenous ET-1 produces a marked increase in gastric vascular tone in perfused stomach of rats and increases the susceptibility of the gastric mucosa to injury in this species.12,13 Moreover, intravascular ethanol stimulates local ET-1 release from gastric vasculature, resulting in gastric microcirculatory disturbances.14 Thus, ET-1 may have important pathophysiological roles in the stomach. Recently, ET-1 in serum was reported to be increased in patients with severe traumatic injury15 or septic shock,16 which may cause stress ulcers. However, the possible role of ET-1 in the shock states is not fully understood.

The aim of this study was to investigate the role of endogenous ET-1 in the pathogenesis of hemorrhagic shock-induced gastric mucosal damage. To achieve this goal, we evaluated the temporal relationship between changes in plasma and mucosal ET-1 contents and mucosal damage. We also examined whether BQ-123, a specific ETa receptor antagonist,17,18 improves gastric mucosal damage and hemodynamics induced by ischemia and reperfusion.

Materials and Methods
Animal Preparation and Experimental Protocols

Male Sprague-Dawley rats weighing 200–250 g were fasted for 24 hours and anesthetized with pentobarbital sodium (35 mg/kg) intraperitoneally. A polyethylene cannula (PE-50; Becton Dickinson and Co., Parsippany, NJ) was inserted into the common carotid artery to monitor systemic blood pressure and obtain blood samples. A tracheotomy was performed to ensure a free airway.

Abbreviations used in this paper: ET, endothelin; ETa, receptor; endothelinA receptor; SO2, index of gastric mucosal hemoglobin oxygen saturation.

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After a 15-minute control period, systemic blood pressure was reduced to 35–40 mm Hg by slowly withdrawing blood for a period of 15 minutes via the carotid artery into a syringe containing heparin (100 U). After 60 minutes of hypotension, blood retained in the syringe was reinfused for a period of 15 minutes and the experiment then continued for another 30 minutes. Body temperature was maintained at 36–38°C with a heating pad.

Forty rats were divided into two identical groups; one was used to assess gastric mucosal ET-1, and the other was used to assess plasma ET-1 and the area of mucosal damage. Each group was divided equally into four subgroups; samples were taken at the end of (1) the control period (basal value), (2) the ischemic period, (3) the reperfusion period, and (4) the 30-minute postreperfusion period. Plasma and gastric mucosal samples were taken from different rats because the collection of one sample may influence ET-1 content in the other.

An additional 20 rats were divided equally into four groups to assess the effect of an ET<sub>A</sub> receptor antagonist, BQ-123 (kindly provided by Tsukuba Research Institute, Banyu Pharmaceutical Co. Ltd., Tsukuba, Japan), on gastric mucosal damage; 1 mL/kg of phosphate-buffered saline (PBS) or BQ-123 (0.1, 1, or 10 mg/mL diluted with PBS) was intra-arterially injected for a period of 5 minutes just before reperfusion, and mucosal damage was evaluated 30 minutes after reperfusion.

Another 10 rats were divided into two groups to investigate the effect of BQ-123 on gastric mucosal hemodynamics; 1 mL/kg of PBS (n = 5) or BQ-123 (n = 5, 10 mg/mL) was administered just before reperfusion, and gastric blood flow and index of gastric mucosal hemoglobin oxygen saturation (ISO<sub>2</sub>) were measured continuously throughout the experiment.

BQ-123 is a synthetic analogue cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu-) based on ET<sub>A</sub> antagonist BE-18257B isolated from Streptomyces misakienisi. BQ-123 has no effect on ET-induced transient depressor action (the response to endothelin<sub>B</sub> receptor), although it blocks the pressor action of ET-1 via ET<sub>A</sub> receptors and does not affect systemic blood pressure. BQ-123 is a synthetic analogue cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu-) based on ET<sub>A</sub> antagonist BE-18257B isolated from Streptomyces misakienisi. BQ-123 has no effect on ET-induced transient depressor action (the response to endothelin<sub>B</sub> receptor), although it blocks the pressor action of ET-1 via ET<sub>A</sub> receptors and does not affect systemic blood pressure. BQ-123 was administered just before reperfusion because reperfusion is the main cause of mucosal damage in this model and because we wanted to investigate whether BQ-123 is potentially useful in prevention of shock-induced gastric mucosal lesions.

ET-1 Measurement in Gastric Mucosa and Plasma

ET-1 in the gastric mucosa and plasma was extracted and measured as previously described. Briefly, to obtain gastric mucosal samples, the forestomach was cut and the stomach gently turned inside out after an abdominal incision. The superficial mucosa of the glandular stomach was cut with small ophthalmologic scissors and immediately frozen in liquid nitrogen. Each sample was lyophilized under vacuum and weighed. Gastric mucosal and plasma samples were stored at −80°C until ET-1 extraction. ET-1 was extracted according to the method of Matsumoto et al. with minor modifications, then applied to a Sep-Pak C<sub>18</sub> cartridge (Waters, Milford, MA) and subjected to a sandwich enzyme immunoassay using a monoclonal antibody to ET-1. As mentioned above, ET-1 is also secreted by other cell types, such as vascular endothelial cells, smooth muscle cells, and platelets. However, in this study, we were interested in the ET-1 content of the gastric mucosa and plasma because ET-1 is known to be produced by gastric mucosal cells. To assess the effect of BQ-123 on gastric mucosal hemodynamics, 1 mL/kg of PBS (n = 5) or BQ-123 (n = 5, 10 mg/mL) was injected for a period of 5 minutes just before reperfusion, and mucosal damage was evaluated 30 minutes after reperfusion.

Evaluation of Gastric Mucosal Damage

The stomach was removed and opened along the greater curvature, and photographs of the stomach were taken. The extent of damage visible macroscopically, mainly hemorrhagic and erosive lesions, was determined planimetrically and expressed as a percentage of the total gastric glandular area. A sample of the corpus, 0.5-cm below the limiting ridge, was then removed. Corpus specimens contained the entire width of the anterior wall from the greater to the lesser curvature. The tissue specimens were dehydrated, embedded in paraffin, sectioned at 6-μm, stained with H&E, and examined under a light microscope. Microscopic estimation of gastric mucosal injury was made using the criteria of Whittle et al. Briefly, a 1-cm length of each histological section was assessed for epithelial cell damage (a score of 1), glandular disruption or vasocongestion in the upper mucosa (a score of 2), hemorrhage in the mid- to lower mucosa (a score of 3), and deep necrosis and ulceration (a score of 4). Each section was evaluated on a cumulative basis to give the histological index with a maximum score of 10. Histological tissue sections from all groups were coded, randomized, and examined for microscopic gastric mucosal injury. The code was not broken until all tissue sections were examined.

Evaluation of Gastric Mucosal Hemodynamics

Gastric blood flow and ISO<sub>2</sub> were simultaneously monitored at the glandular stomach using laser-Doppler flowmetry (TSI model BPM 403 flowmeter, TSI, Inc., St. Paul, MN) and reflectance spectrophotometry (TS-200; Sumitomo Electric...
Ind., Osaka, Japan), respectively. A detailed description of these methods has been presented elsewhere. Briefly, laser-Doppler flowmetry gives an estimation of tissue perfusion based on the Doppler shift of wavelength of the reflected light, which is proportional to the number and velocity of moving red cells. Experimental studies have shown a close relationship between gastric blood flow and laser-Doppler flow measured in the gastric mucosa. ISO, a marker of oxygen delivery and consumption, is determined by absorption changes at three close wavelengths (569, 577, and 586 nm) in the reflectance spectra from approximately 1 mm of mucosa. Because both parameters were continuously measured at the same point of the mucosa throughout the experiment, data were expressed as percentages of the basal values.

After the animal preparation as described above, a midline laparotomy was performed and the stomach was exposed. Through an incision in the forestomach, probes of a reflectance spectrophotometer and a flowmeter were lowered via a micro-manipulator onto the surface of the corpus mucosa. After stabilization of the optical systems, systemic blood pressure was reduced to 40 mm Hg for 1 hour. Just before reperfusion, 1 mL/kg of PBS or BQ-123 (10 mg/mL) was administered. Shed blood was then reinfused for a period of 15 minutes, and the experiment was continued for another 30 minutes. Gastric blood flow and ISO were measured continuously throughout the experiment.

Statistical Analysis

All data were expressed as means ± SEM. Comparisons between groups of parametric data were made by Student's unpaired t test or one-way analysis of variance after Fisher's least-significant difference test. Histological scores were compared using the Mann–Whitney U test and the Mantel-extension dose-response test. P < 0.05 was considered statistically significant.

Results

Plasma and Gastric Mucosal Concentrations of ET-1 and Gastric Mucosal Damage Induced by Hemorrhagic Shock

Basal values of ET-1 concentrations in plasma and gastric mucosa were 1.40 ± 0.42 pg/mL and 1.90 ± 0.29 ng/g, respectively. Plasma ET-1 increased significantly at the end of ischemia, reperfusion, and postreperfusion (2.40 ± 0.42 pg/mL, 10.42 ± 0.73 pg/mL, and 12.72 ± 1.61 pg/mL, respectively; P < 0.01) compared with the basal value. Figure 1 summarizes changes in ET-1 concentrations in the gastric mucosa and area of gastric mucosal damage. The former also significantly increased after ischemia and reperfusion; however, there was a significant difference between values in the ischemic period and those after reperfusion (P < 0.05). Namely, ET-1 in gastric mucosa (but not in plasma) continued to increase during the postreperfusion period. The latter increased significantly after reperfusion compared with damage observed in the control (P < 0.01) and ischemic periods (P < 0.05).

Effects of an ETα Receptor Antagonist

The effects of BQ-123, an ETα receptor antagonist, on gastric mucosal damage and hemodynamics after reperfusion were assessed. BQ-123 (0.1, 1, and 10 mg/kg) reduced the area of mucosal damage 30 minutes after reperfusion in a dose-dependent manner (by 47%, 68%, and 77% of the control value, respectively (Figure 2A). As shown in Figure 2B, BQ-123 also improved the histological score of mucosal damage dose dependently. Histological evaluation showed that glandular disruption
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Figure 2. Effects of BQ-123 on (A) macroscopic area and (B) histological score of gastric mucosal damage. BQ-123 (0.1, 1, or 10 mg/kg) or PBS was administered just before reperfusion, and damage 30 minutes after reperfusion was assessed macroscopically and microscopically. The column and bar represent mean and SEM of five rats per group, respectively. *P < 0.05, †P < 0.01; significantly different from the PBS-treated group using (A) Student’s unpaired t test and (B) the Mann-Whitney U test.

Table 1. Effects of BQ-123 on Mucosal Damage

<table>
<thead>
<tr>
<th>BQ-123 (mg/kg)</th>
<th>Epithelial cell damage</th>
<th>Glandular disruption†</th>
<th>Hemorrhage in the midmucosa*</th>
<th>Ulcerations or necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (n = 5)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>0.1 (n = 5)</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>1 (n = 5)</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10 (n = 5)</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

NOTE. Mucosal damage was assessed histologically after administration of BQ-123. Values are expressed as number of rats with mucosal injury from five rats per group.

*P < 0.05 compared with PBS-treated group using the Mantel extension dose-response test.

Figure 3. Time course of changes in (A) systemic blood pressure, (B) gastric blood flow, and (C) IS0 in groups with and without BQ-123. BQ-123 (10 mg/kg) or PBS was administered just before reperfusion, and these parameters were monitored continuously throughout the experiments. Gastric blood flow and IS0 were determined by laser-Doppler flowmetry and reflectance spectrophotometry, respectively. Values are expressed as means ± SEM of five rats per group. •, PBS-treated group; ■, BQ-123-treated group. *P < 0.05, †P < 0.01; significantly different from PBS-treated group using Student’s unpaired t test.

Discussion

The present study shows that ET-1 concentrations in the plasma and gastric mucosa increased significantly after ischemia and reperfusion. It has been reported that a local injection of nanogram (but not picogram) amounts of ET-1 induces gastric mucosal damage and increases gastric vascular tone in rats. In our study, the level of plasma ET-1, which might reflect the production and release from systemic endothelial cells (including those...
in gastric vasculature) would remain lower than the ET-1 concentrations that cause mucosal damage during ischemia and reperfusion. In contrast, the changes in ET-1 in the gastric mucosa, which is mainly produced by the mucosal vascular endothelial cells, would be two or three orders of magnitude greater than those in plasma and could therefore induce an increase in gastric vascular tone and gastric mucosal damage. Thus, ET-1 in the stomach may act as a local modulator of gastric vascular tone.

The increase in gastric mucosal ET-1 could be caused by stimulated production, release from damaged endothelial cells, or uptake in the stomach. Several factors related to ischemia and reperfusion stimulate ET-1 production in the endothelium, including hypoxia,

sheer stress, and neutrophils. Because ET-1 injected intravenously is mainly taken up in the lung and kidney, gastric mucosal ET-1 would be increased mainly because of increased ET-1 production rather than uptake of plasma ET-1 in the stomach.

The increase in ET-1 in the gastric mucosa was associated with the development of mucosal damage (Figure 1). The increased mucosal ET-1 seems to affect mucosal susceptibility to damage induced by hemorrhagic shock. ET-1 may act synergistically on the gastric mucosa with other causative factors induced by reperfusion, including free radicals, neutrophil adherence, and platelet activating factor. Indeed, these causative factors and ET-1 are closely related. ET-1 is a potent modulator of human neutrophils and enhances superoxide generation of stimulated human neutrophils, whereas neutrophils stimulate production of ET-1 in vitro. It is also reported that ET-1 promotes production of platelet activating factor, and a blocker of platelet activating factor inhibits gastric mucosal injury induced by ET-1. Thus, these factors are closely connected with each other, and ET-1 is a candidate for the main vasomodulator that regulates the other causative factors.

Our study shows that BQ-123, a specific ETA receptor antagonist, reduced mucosal damage in a dose-dependent manner. This result also supports the hypothesis that endogenous ET-1 is involved in the pathogenesis of hemorrhagic shock-induced gastric mucosal damage. Two ET receptors, ETA and ETB, are known; one exists on smooth muscle cells and has an important role in vasoconstriction, and the other is found on endothelial cells and is associated with production of endothelium-derived relaxing factor. Because BQ-123 is expected to act as an inhibitor of vasoconstriction induced by ET-1, it is likely that ET-1 causes mucosal damage mainly through gastric microcirculatory disturbances via ETA receptors. However, BQ-123 could not completely block the mucosal damage, possibly because it might not inhibit other actions of ET-1 such as promoting production of superoxide or platelet activating factor.

We also examined the effects of BQ-123 on gastric mucosal hemodynamics. BQ-123 significantly improved mean ISO_{2} and gastric blood flow during the 30-minute postreperfusion period. More aerobic tissues, such as the gastric mucosa, become more susceptible to injury when aberrations in blood flow deprive the tissue of oxygen. Changes in ISO_{2}, which depend on oxygen delivery and consumption, correlate as well with the degree of ulceration as do changes in gastric mucosal blood flow. These results indicate that increased ET-1 reduces tissue perfusion and subsequently augments mucosal tissue hypoxia via ETA receptors, thus leading to mucosal damage.

In conclusion, the present studies indicate that endogenous ET-1 is involved in hemorrhagic shock-induced gastric mucosal injury as a local modulator of gastric microcirculation.

References


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