Role of platelet activating factor on the fibrinolytic activation in the pathogenesis of gastric mucosal damage induced by endothelin-1

I Kurose, S Miura, D Fukumura, H Tashiro, H Imaeda, H Shiozaki, M Suematsu, H Nagata, E Sekizuka, M Tsuchiya

Abstract
We have examined the hypothesis that the release of tissue type plasminogen activator may play a prominent role in endothelin induced gastric mucosal injury. We determined tissue type plasminogen activator activity in the regional blood sample and the concentration of platelet activating factor in the gastric mucosa after the administration of endothelin-1 in a range of 50–500 pmol/kg into the left gastric artery of male Wistar rats. Endothelin-1 increased the tissue type plasminogen activator release and platelet activating factor formation, and induced subsequent gastric mucosal haemorrhagic change in a dose dependent manner. In addition CV-6209, a selective platelet activating factor blocker, attenuated the activation of regional tissue type plasminogen activator and the development of mucosal damage induced by endothelin-1. The results of this study showed that tissue type plasminogen activator activation may play an important role in the pathogenesis of endothelin induced mucosal injury of rat stomach, and suggest that the platelet activating factor may be involved in the process of regional fibrinolytic activation induced by endothelin-1.

Microcirculatory disturbances of the stomach are thought to be one of the factors contributing to the development of gastric mucosal damages.

The experimental animal. These animals were deprived of food for 24 hours before the start of experiment, but permitted of free access to drinking water. Under anaesthesia with pentobarbital sodium, a median incision was made in the abdomen and the gastric pylorus was ligated with silk thread. Endothelin induced gastric mucosal damage was carried out as described below. Two millilitres 0·1 N HCl was instilled into the gastric cavity through the forestomach with 27 gauge needle. Using 30 gauge needle, 100 μl Krebs-Ringer solution containing endothelin-1 (Peptide Laboratory, Osaka, Japan) was injected as a bolus into the left gastric artery in a range of 50–500 pmol/kg. As the control group, 100 μmol of Krebs-Ringer solution alone was injected into the left gastric artery. At various periods after the injection of endothelin-1, the stomach was removed and opened along the greater curvature. The length and width of each lesion were measured, and the area of macroscopic lesion was determined. The total area of lesions was calculated as the sum of areas of individual lesion.

To determine the tissue type plasminogen activator activity in the regional blood of rat stomach, a 200 μl blood sample was collected from the gastric vein before and after the injection of endothelin-1 which was then transferred to a glass container containing 10 μl disodium citrate dihydrate (0·13 M). The container was immediately placed in an ice bath. Plasma was separated by centrifugation for five minutes at 2000 rpm (4°C), frozen and stored at −70°C in small aliquots. Tissue type plasminogen activator activity in plasma was assayed by the specific chromogenic substrate assay, which
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Time course changes of mucosal lesions and tissue-type plasminogen activator (t-PA) activity in regional plasma of stomach induced by endothelin-1 500 pmol/kg

<table>
<thead>
<tr>
<th>Group</th>
<th>Area of mucosal lesion (mm²)</th>
<th>t-PA activity (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before injection (minutes)</td>
<td>0</td>
<td>2.5 (1.5)</td>
</tr>
<tr>
<td>Endothelin-1 (5)</td>
<td>0.1 (0.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endothelin-1 (10)</td>
<td>4.4 (3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endothelin-1 (15)</td>
<td>9.8 (4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endothelin-1 (30)</td>
<td>34.5 (10.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control (30)</td>
<td>1.0 (0.9)</td>
<td>2.8 (1.1)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD) from seven experiments.

was a modification of the method described by Ránby et al. Briefly, the activity of tissue type plasminogen activator can be measured by adding Glu-plasminogen, chromogenic plasmin substrate and fibrin (purchased from Biopool Co, Umeå, Sweden) at neutral pH. In the presence of fibrin, tissue type plasminogen activator converts plasminogen to plasmin, which subsequently cleaves the chromogenic substrate. The amount of colour developed during a fixed time is proportional to the tissue type plasminogen activator activity in the sample.

The level of platelet activating factor in the rat gastric mucosa was determined after the administration of endothelin-1 using a radio-immuno-assay method. Rats were decapitated and the stomach was removed, opened along the greater curvature. The mucosal surface was rinsed with ice cold isotonic saline solution. For separation of the mucosal layer of the granular stomach, the tissues were mounted on a glass slide, covered with another glass slide, and immersed in hexane at −60°C. The mucosal and muscular tissue layers were then separated by quickly pulling the two slides apart. Total lipids of mucosal homogenate of rat antrum were extracted by the method of Bligh and Dyer at 4°C in the presence of 50 mM glycine HCl buffer (pH 2-8) in a one phase system to inactivate platelet activating factor acetylhydrolase. The chloroform layer, which contains the purified lipid extract, was isolated and used to determine the platelet activating factor concentration, using the platelet activating factor [1H] scintillation proximity assay system purchased from Amersham International plc (UK). The assay system is based on the competition between unlabelled platelet activating factor and a fixed quantity of [1H] labelled platelet activating factor for a limited number of binding sites on a platelet activating factor specific antibody. The platelet activating factor concentration of mucosa from rat antrum was expressed as pmol/g frozen tissue.

To assess the involvement of platelet activating factor in the endothelin induced tissue type plasminogen activator activation in gastric mucosa, CV-6209 (a gift from Takeda Pharm Co, Osaka, Japan), a selective platelet activating factor blocker, was intravenously administered at a dose of 0.001–0.1 mg/kg five minutes before the injection of endothelin-1.

The statistical significance of the difference between the mean value of groups was tested by Student’s t test for unpaired values.

Results

As shown in the Table, the area of mucosal lesions was time dependently increased after the injection of endothelin-1 (500 pmol/kg). Red spots were sporadically seen on the mucosa at 10 minutes after the injection of endothelin-1. At 30 minutes after the injection, multiple haemorrhagic erosions and linear ulcers were observed grossly. The application of 0.1 N HCl caused no remarkable change in the gastric mucosa at 30 minutes (Table). Figure 1A shows the area of lesion which was calculated at 30 minutes after the injection of endothelin-1 in a range of 50–500 pmol/kg. Endothelin-1 in this range induced mucosal haemorrhagic changes in a dose dependent manner.

Before the administration of endothelin-1 (500 pmol/kg), the activity of tissue type plasminogen activator in regional blood sample of stomach was 2−5 (1.5) IU/ml. The activity of tissue type plasminogen activator was significantly raised 10 minutes after the injection of endothelin-1 and gradually increased to 8–1 (3.1) IU/ml at 30 minutes (mean (SD) n=7, p<0.001). No significant increase in tissue type plasminogen activator activity was induced, however, by the administration of 0.1N HCl alone into the gastric cavity at 30 minutes after the injection (Table). It was also shown that the local administration of endothelin-1 elicited tissue type plasminogen activator activity in a dose dependent manner at 30 minutes (Fig 1B). To see whether the area of mucosal lesion is correlated to the tissue type plasminogen activator activity, these two parameters were plotted as shown in Figure 2. The individual tissue type plasminogen activator activity and damaged area was determined 30 minutes after the administration of endothelin-1 in the various concentrations (50, 100, 250, and 500 pmol/kg), each of which consisted of seven experiments. It was clearly shown that tissue

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Figure 1: Area of gastric mucosal lesion, tissue type plasminogen activator (t-PA) activity and platelet activating factor (PAF) content after the administration of endothelin-1. The area of macroscopic mucosal lesion and the t-PA activity in the regional blood sample from rat gastric vein were determined at 30 minutes after the intravenous injection of endothelin-1 in the range of 50–500 pmol/kg. In this range endothelin-1 caused the mucosal haemorrhagic lesion in rat stomach and t-PA activation in a dose dependent manner (A and B). The PAF content of gastric mucosa was measured at five minutes after the administration of endothelin-1 in the same range. Endothelin-1 in doses greater than 250 pmol/kg significantly increased the PAF content as compared with the control value (C). Data are expressed as mean (SD) from seven experiments (A and B) or four experiments (C). *p<0.05, **p<0.01 and ***p<0.001 as compared with the control value.
type plasminogen activator activity was significantly correlated with the mucosal lesion ($r = 0.877, p < 0.001$).

To investigate one of the mechanisms by which the tissue type plasminogen activator activity in regional blood increased, the platelet activating factor content of the gastric mucosa was measured before, five, and 30 minutes after the administration of endothelin-1 (500 pmol/kg). In the gastric mucosa before the endothelin-1 injection, platelet activating factor content was 28.0 (7.1) pmol/g frozen tissue (mean (SD) n=4). Platelet activating factor content increased five minutes after the administration of endothelin-1 (64.0 (11.4) n=4, $p < 0.01$) and decreased to 44.5 (11) at 30 minutes ($p < 0.05$) (Fig 3). Endothelin-1 in doses greater than 250 pmol/kg increased the platelet activating factor content at five minutes as compared with the control value (27.0 (6.1)) (Fig 1C).

The effects of the pretreatment with CV-6209 (0.001, 0.01, or 0.1 mg/kg), a selective platelet activating factor blocker, on both the mucosal injury and the tissue type plasminogen activator activation induced by endothelin-1 500 pmol/kg were tested. Figure 4A shows the effect of CV-6209 on the endothelin induced gastric mucosal injury. The pretreatment of CV-6209 attenuated the mucosal damage at 30 minutes in a dose dependent manner. At the same time, the tissue type plasminogen activator activity induced by endothelin-1 was also attenuated by CV-6209 more than 0.01 mg/kg (Fig 4B). These results suggest the involvement of platelet activating factor in the endothelin induced fibrinolytic activation and subsequently developed mucosal haemorrhagic lesion.

Discussion

Endothelin-1 has become a major research topic since its discovery and characterisation as a potent vasoconstrictor. This potency has led to the idea that endothelin-1 might play a pathophysiological role in circulatory disorder resulting from various organs. Whittle et al described an intraarterial injection of endothelin-1 that can dose dependently produce gastric mucosal lesions. Fewer studies have been carried out on microcirculatory hemodynamic changes of the stomach induced by endothelin, however. The present authors have recently shown that endothelin-1 induces the constriction of submucosal arterioles followed by the macro- molecular permeable change in the mucosal

Figure 2: Relationship between gastric mucosal lesion and tissue type plasminogen activator (t-PA) activity induced by endothelin-1. The area of mucosal lesion and the t-PA activity were plotted (vertical: area of mucosal lesion, horizontal: t-PA activity). The individual t-PA activity and damaged area were determined as 30 minutes after the injection of endothelin-1 50, 100, 250, and 500 pmol/kg, each of which consisted of seven experiments. t-PA activity in the regional blood sample was significantly proportional to the area of lesion induced by endothelin-1 ($r = 0.877, p < 0.001$).

Figure 3: Time course change of platelet activating factor (PAF) content in the rat gastric mucosa. The PAF content before, five and 30 minutes after the administration of endothelin-1 was determined by the radioimmunoassay. The PAF content showed a significant rise at five minutes and decreased to the pretreatment level at 30 minutes after the injection. Data were obtained from four experiments and expressed as mean (SD) (pmol/g frozen tissue).

Figure 4: Effects of CV-6209 on the gastric mucosal lesion and the tissue type plasminogen activator (t-PA) activation induced by endothelin-1. The pretreatment of CV-6209 in the doses more than 0.01 mg/kg significantly attenuated the mucosal damage and increased t-PA activity induced by endothelin-1 at 30 minutes. Data are expressed as mean (SD) from seven experiments. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ as compared with the value of endothelin-1 500 pmol/kg.
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microvasculature, and causes mucosal haemorrhagic changes in the rat stomach. In these changes, it is still unknown whether endothelin can directly cause the permeability change or indirectly induce the change after arteriolar constriction. Of interest, it was recently reported that endothelin-1 and -3 can induce the release of tissue type plasminogen activator from endothelial cells. It is widely accepted that fibrin degradation products released after the action of plasmin on fibrin contribute to the development of inflammatory changes in several organs by increasing the permeability of the vasculature to protein. We previously showed that the excessive activation of tissue type plasminogen activator, mediated by microvascular endothelial cells, plays a crucial role in the development of gastric mucosal injury induced by ischaemia reperfusion in rats. The present study shows that the intraarterial injection of endothelin-1 increased tissue type plasminogen activator activity in the regional blood accompanying the mucosal injury in a time dependent manner. In addition, as shown in the Table and Figure 2, the regional tissue type plasminogen activator activity significantly increased before the development of mucosal damage and was proportional to the area of mucosal lesion at 30 minutes after the administration of endothelin-1. These results could suggest a prominent role of tissue type plasminogen activator in the pathogenesis of endothelin induced gastric mucosal damage.

Platelet activating factor is one of lipid mediators that is generated in a variety of cells upon chemical or immune stimulation. In addition to its biological activities in stimulating platelets and neutrophils, and enhancing vascular permeability, exogenous platelet activating factor has also been shown to be a potent ulcerogen, inducing extensive gastric mucosal damage and haemorrhage associated with septic shock. Sugatani et al recently determined the alteration of the platelet activating factor contents in rat gastric mucosa during water immersion stress and showed that endogenous platelet activating factor may actually be involved in the process of mucosal injury. The present study shows that endothelin-1 can increase the mucosal platelet activating factor content at five minutes after the administration in the rat stomach before the activation of regional tissue type plasminogen activator. We have shown also that CV-6209, a platelet activating factor blocker, eliminated the tissue type plasminogen activator activation and mucosal lesion induced by endothelin-1. Although the detailed mechanism is unknown, our data raise the possibility that endothelin-1 actually stimulates the production of platelet activating factor followed by the excessive release of tissue type plasminogen activator.

These results are in accordance with the previous investigation that platelet activating factor activation directly increases the release of tissue type plasminogen activator from vessel walls in the experimental model of the isolated perfused rat hindlimb bed. Taken together with the present results, platelet activating factor is likely to modulate regional fibrinolytic activity during gastric mucosal injury induced by endothelin-1.

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