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Effect of Prostaglandin E_1 on Acute Ischemia-Reperfusion of Canine Small Intestine

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Summary Ischemia-reperfusion of the small intestine associated with hemorrhage and other shock states is characterized by increased microvascular permeability and mucosal barrier dysfunction. Glycoproteins play an important role as a barrier to diffusion, and some of the functions of prostaglandin E_1 (PGE_1) are related to mucosal protein synthesis. The present experiment was conducted to clarify the effect of PGE_1 on mucosal levels of glycoproteins and ATP following acute ischemia-reperfusion of the small intestine. The canine jejunum was isolated, and the blood flow was blocked for 30 min with or without intravenous infusion of PGE_1. Mucosal levels of ATP, Na^+-K^+ ATPase activity, glucosamine, galactosamine, and cAMP decreased, and plasma endotoxin levels in portal blood increased, after reperfusion in the PGE_1-non-treated group. These changes were suppressed and mucosal levels of cAMP were increased by the administration of PGE_1. These results suggest that mucosal permeability increased and mucin synthesis decreased markedly following acute ischemia-reperfusion and that the administration of PGE_1 suppressed these changes by stimulation of ATP and cAMP synthesis. We also conclude that the administration of PGE_1 is useful to protect against acute ischemia-reperfusion injury in the small intestine.

Key Words: cAMP, glycoprotein, ischemia-reperfusion injury, Na^+-K^+ ATPase, prostaglandin E_1

Ischemia-reperfusion of the intestine is often associated with increases in vascular [1] and mucosal [2] permeability and mucosal barrier dysfunction that results in bacterial translocation to the portal system. Glycoproteins present in the

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mucosa of the small intestine, such as glucosamine and galactosamine, play an important role as a barrier to diffusion [3], and prostaglandin E_1 (PGE_1) has been implicated in the regulation of mucosal protein synthesis [4]. The present study was conducted to clarify the effect of PGE_1 on changes in the mucosal levels of glycoproteins and adenine nucleotides following acute ischemia-reperfusion of the canine small intestine.

MATERIALS AND METHODS

Experimental protocol. Mongrel dogs weighing 10 kg were fasted for 24 h, and then laparotomy was performed under general anesthesia. The small intestine was divided into four parts, and one-fourth of the intestine in the second equal part from the ligament Treitz (lower jejunum) was subjected to warm ischemia-reperfusion. The jejunum was isolated with preservation of the superior mesenteric artery and vein, and the blood flow was blocked for 30 min by clipping the superior mesenteric artery and vein with vascular forceps. An intravenous infusion of 10 μg/kg/min of PGE_1 (Ono Pharmaceutical Co., Ltd., Osaka, Japan) was continued for 1 h, beginning 15 min before the start of the vascular clipping. Blood was drawn from the portal vein into a heparinized syringe prior to ischemia and again 30 min after the start of reperfusion, centrifuged at 3,000 rpm for 15 min at 4°C, and stored at −80°C. Mucosal specimens (2 cm length) were obtained at pre-ischemia, and at 15, 30, and 60 min after the start of reperfusion by scraping with a slide glass. The mucosa was freeze-dried and stored at −80°C.

Procedures of measurement. Plasma endotoxin levels in the portal blood were measured by the *Limulus amoebocytes* lysate test (Toxicolor, Seikagaku Kogyo, Tokyo) [5]. A small segment of the freeze-dried mucosal specimen (0.5 mg) was homogenized in 1 ml of ice-cold saline, and the level of Na^+-K^+ ATPase in each homogenate was determined by Charney's method [6]. Another segment of the freeze-dried mucosa (0.5 mg) was also homogenized in 1 ml of 80% methanol, and then centrifuged at 3,000 rpm for 15 min. Phosphoric acid buffer (0.01 M, pH 6.0, 0.4 ml) was added to the supernatant, and the ATP level was measured by high-performance liquid chromatography (LC-6A, Shimadzu, Tokyo) [7]. A third segment of freeze-dried mucosa (1 mg) was heated with phosphoric acid buffer (0.1 M, pH 6.7, 0.2 ml) and hydrochloric acid (1 N, 0.2 ml) to 100°C for 1 h, and then 0.1 ml of each sample was distilled and mixed with 100 μl of phenylthiocyanic acid (0.1 N) at room temperature for 20 min. Following the addition of 1 ml 80% methanol, each sample was distilled again, and then centrifuged at 3,000 rpm for 5 min after addition of acetic acid buffer (1 M, pH 5.0, 0.4 ml). Glucosamine and galactosamine levels in the supernatant were measured by high-performance liquid chromatography (LC-6A, Shimadzu) [7]. Protein content of the pellet was determined by Lowry's method [8]. The levels of ATP, glucosamine, and galactosamine were expressed as a value per mg of protein. Another small segment of freeze-dried specimen was homogenized in 1 ml of hydrochloric acid (0.1 N) and

centrifuged at 3,000 rpm for 5 min. The cAMP level in the supernatant was determined by radioimmunoassay (cAMP Assay Kit, Yamasa, Tokyo) [9].

Statistical analysis. The findings in each group were expressed as the mean±SD. Student's *t*-test was used to determine differences between the means, and *p* values of less than 0.05 were taken as statistically significant.

RESULTS

ATP, Na$^+$-K$^+$ ATPase, and endotoxin

Mucosal levels of ATP and Na$^+$-K$^+$ ATPase activity in the control (PGE$_1$-non-treated) group had significantly decreased by 15, 30, and 60 min after the start of reperfusion. PGE$_1$ treatment prevented the decrease in ATP and Na$^+$-K$^+$ ATPase activity observed in the control group ($p<0.05$). Plasma endotoxin levels in the portal blood of the control group significantly increased 30 min after reperfusion had begun, and following PGE$_1$ treatment were significantly lower than those of the control group (Table 1).

Glucosamine, galactosamine, and cAMP

Mucosal levels of glucosamine and galactosamine in the control group

Table 1. Time course of changes in mucosal ATP levels and Na$^+$-K$^+$ ATPase activity, and in endotoxin levels in portal blood.

		Pre-ischemia	Time after start of reperfusion (min)		
			15	30	60
ATP	PGE$_1$(−)	8.1±1.5	2.0±1.3[a]	2.2±0.8[a]	2.4±1.6[a]
(μg/dry weight/mg protein)	PGE$_1$(+)	8.9±1.6	9.7±1.7[b]	8.3±2.5[b]	11.8±1.5[b]
Na$^+$-K$^+$ ATPase	PGE$_1$(−)	2.5±0.6	0.6±0.3[a]	0.5±0.3[a]	0.5±0.2[a]
(mmol Pi/ng protein/h)	PGE$_1$(+)	2.6±0.7	2.0±0.6[b]	1.8±0.5[b]	1.8±0.4[b]
Endotoxin	PGE$_1$(−)	5.3±0.7	−	14.3±3.2[a]	−
(pg/ml)	PGE$_1$(+)	5.5±0.5	−	6.7±0.9[b]	−

PGE$_1$(+), PGE$_1$ treated; PGE$_1$(−), control; −, not determined; values, mean±SD ($n=5$); [a]$p<0.05$ vs. pre-ischemia; [b]$p<0.05$ vs. PGE$_1$(−).

Table 2. Time course of changes in mucosal levels of glucosamine, galactosamine, and cAMP.

		Pre-ischemia	Time after start of reperfusion (min)		
			15	30	60
Glucosamine	PGE$_1$(−)	11.4±2.6	7.9±2.0[a]	6.4±2.4[a]	6.3±1.1[a]
(mg/dry weight/mg protein)	PGE$_1$(+)	13.9±3.3	14.0±2.4[b]	17.2±3.1[b]	14.6±2.6[b]
Galactosamine	PGE$_1$(−)	11.3±3.5	7.5±2.0[a]	7.0±2.1[a]	7.3±1.6[a]
(mg/dry weight/mg protein)	PGE$_1$(+)	12.6±2.2	12.7±2.1[b]	12.1±0.9[b]	12.3±0.5[b]
cAMP	PGE$_1$(−)	490±17	377±66[a]	387±59[a]	437±44
(pmol/dry weight)	PGE$_1$(+)	501±20	510±29[b]	577±37[ab]	637±35[ab]

PGE$_1$(+), PGE$_1$ treated; PGE$_1$(−), control; values, mean±SD ($n=5$); [a]$p<0.05$ vs. pre-ischemia; [b]$p<0.05$ vs. PGE$_1$(−).

significantly decreased after the start of reperfusion. However, the decrease in the mucosal glucosamine and galactosamine levels after reperfusion was significantly suppressed in the PGE_1 group. Mucosal levels of cAMP in the control group were also significantly decreased at 15 and 30 min after reperfusion had begun, and returned to the pre-ischemia level by 60 min. Mucosal cAMP levels in the PGE_1 group were significantly higher than those in the control group at 15, 30, and 60 min after reperfusion, and at 30 and 60 min significantly higher than the pre-ischemia levels (Table 2).

DISCUSSION

Healthy intestinal mucosa has active regeneration ability and is fairly well protected from physical, chemical, or biologic insults by the covering mucus [10]. Ischemia-reperfusion of the small intestine associated with hemorrhage and other shock states is characterized by increased microvascular permeability and mucosal barrier dysfunction. The barrier lesion might prove to be an important factor in patients recovering from shock in as much as mucosal and microvascular barrier dysfunction is closely associated with the release of toxic factors, such as endotoxin, into the circulation, leading to sepsis, and possibly, multiple organ failure [11]. The observed increase in the plasma endotoxin levels in the portal blood and decrease in the mucosal levels of Na^+-K^+ ATPase activity indicate increased mucosal and microvascular permeability. Further, mucosal glycoproteins, such as glucosamine and galactosamine, act as a barrier against enteric bacteria and oxygen-derived free radicals [12]. Mucosal levels of ATP and glycoproteins are also an index of mucin synthesis in the mucosa.

In the present study, the mechanism of injury following acute ischemia-reperfusion of the canine small intestine was clarified by evaluating changes in the plasma endotoxin levels in the portal blood and the mucosal levels of Na^+-K^+ ATPase activity. Loss of the mucosal barrier function was suggested by the changes in the mucosal levels of ATP and glycoproteins. Plasma endotoxin levels in the portal blood were significantly increased and mucosal levels of Na^+-K^+ ATPase activity were significantly decreased after 30 min of ischemia followed by reperfusion. Mucosal levels of ATP and glycoproteins were decreased after ischemia-reperfusion, suggesting that increased mucosal permeability and a marked disturbance of mucin synthesis occurred following acute ischemia-reperfusion. The mechanism that weakens the mucosal barrier and leads to microvascular dysfunction after reperfusion of ischemic tissue is poorly understood. However, reactive oxygen metabolites, vasocongestion, and leukocytes have all been implicated as potential mediators [13]. For example, administration of allopurinol, superoxide dismutase, or inhibitors of nitric oxide synthesis can protect the intestine during inflammatory conditions, such as ischemia-reperfusion [14].

Additionally, several prostaglandins were found to be cytoprotective for the stomach and intestine during inflammatory conditions. In fact, endogenously

produced prostaglandins appear to play a physiological role in protecting the mucosa [15]. The increased synthesis of prostaglandin represents a natural defense mechanism that may be necessary to maintain cellular integrity in the gastrointestinal mucosa [16]. The prostaglandins of the E series stimulate adenylate cyclase activity and increase the cellular levels of cAMP in a variety of cultured cells [17]. In the presence of insulin, PGE_1 increases cAMP levels and stimulates DNA synthesis [4]. In the present study the mucosal changes observed following ischemia-reperfusion were prevented by the administration of PGE_1 that was started prior to ischemia.

In conclusion, the present data have shown that the administration of PGE_1 had a protective effect on the mucosa following ischemia-reperfusion by stimulating ATP and cAMP synthesis. These findings indicate that the administration of PGE_1 is useful to protect against ischemia-reperfusion injury of the small intestine.

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