

Regulation of Colonic Mucosal Blood Flow by Exogenous Eicosanoids in the Rat

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We measured colonic mucosal blood flow using a reflectance spectrophotometry in Wistar rats during and after continuous injection of prostaglandins (PGs: PGE₁, PGE₂ and PGI₂), as vasodilators, and thromboxane A₂ (TXA₂) as a vasoconstrictor. Administration of PGs increased colonic mucosal blood volume and oxyhemoglobin saturation of the colonic mucosal tissue, representing a parameter of mucosal oxygenation. The dose used did not change arterial blood pressure. These results suggest that PGs regulate colonic mucosal hemodynamics and oxygenation, and may thus act as cytoprotective substances. On the other hand, TXA₂ injection diminished colonic mucosal blood flow compared with the same dose of PGI₂ injection and acted against PGI₂. Our results suggest that TXA₂ and PGI₂ regulate colonic mucosal hemodynamics.

Key words : Prostaglandins, inflammatory bowel disease, colonic vascularization, thromboxane A₂, cyclic adenosine monophosphate.

INTRODUCTION

Prostaglandins (PGs) are important substances influencing vascular reactivity, and may provide cytoprotection to the microcirculation. The cytoprotective role of PGs was initially reported in the gastric mucosa (1). Subsequent studies indicated that PGE₂, PGI₂ and dmpGE₂ increase gastric mucosal blood flow when administered at non-secretory doses (2-4). Furthermore, the cytoprotective activity of PGs has also been demonstrated in an animal model of inflammatory bowel diseases (IBD) (5, 6). It has been recognized that the vasodilatory action of PGs enhances colonic blood flow in rabbit colitis (7), and that the administration of PGE₁ in the dog increases mesenteric blood flow (8). These studies suggest that PGs play a significant cytoprotective role in both gastric and colonic mucosa. However, the relationship between colonic mucosal microcirculation and exogenous PGs is still unclear.

On the other hand, the combination of thromboxane A₂ (TXA₂) and PGI₂ has been demonstrated to regulate the cardiovascular system (9), while a reduction of 6-keto-

PGF_{1α} (PGI₂ metabolite) thromboxane B₂ (TXA₂ metabolite) ratio may alter the cytoprotective capacity of the mucosa of active inflammatory bowel disease (10, 11). We examined the effect of several PGs (PGE₁, PGE₂, PGI₂) and TXA₂ on the pattern of colonic mucosal blood flow in normal rats.

METHODS

Eicosanoids (PGE combined cyclodextrin: PGE₁-α-cyclodextrin and PGE₂-β-cyclodextrin), PGE metabolites (15-keto-PGE₁ and 15-keto-PGE₂), PGI₂ sodium salt and TXA₂ (ONO-3708, STA₂) were supplied by ONO Pharmaceutical Co., (Osaka, Japan). Male Wistar rats of approximately 300 g body weight were anesthetized with i. p. injection of pentobarbital (50 mg/kg). SHR (Spontaneous hypertensive rat) and WKY (Wistar Kyoto rat) rats were also used as control groups to examine sympathetic nerve activity. PGs or TXA₂ were administered, in normal saline, at an infusion rate of 0.1 ml/min, through a cannula inserted into the femoral vein. Control rats received the vehicle only (normal saline). We also monitored arterial blood pressure through a cannula inserted into the common cervical artery. Infusion of the drug continued for 30 min, and the experiment was followed by another period of 60 min of observation.

The tip of an optic fiber was attached to the caecal mucosa before the injection of eicosanoids. The colonic mucosal hemoglobin concentration index (IHb), a parameter of microcirculatory blood volume, and mucosal oxyhemoglobin saturation index (ISO₂), a parameter of mucosal oxygenation, were measured by a tissue spectrum analyzer TS-200 (Sumitomo Electric Industries, Ltd. Osaka, Japan). IHb and ISO₂ were calculated according to the equation provided by Sato et al. (12). These experiments were kinetically performed for 90 min commencing with the start of eicosanoids injection. IHb and ISO₂ were expressed as a percent change to the basal value before the administration of eicosanoids. The changes of IHb and ISO₂ caused by the eicosanoids injection were compared with the changes of those caused by the saline injection as the

control. Furthermore, the serum PGE₂ and cAMP (cyclic adenosine monophosphate) were measured using radio immune assay.

Data were expressed as mean \pm SEM. Statistical analysis was performed using Student's *t*-test, and a *p* value of <0.05 was considered to indicate the presence of a significant difference.

RESULTS

Effect of PG administration on colonic blood flow.

Injection of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ of PGE₁ caused a progressive increase in IHb and ISO₂. IHb increased maximally to $14.7 \pm 8.6\%$ at 80 min after the start of injection while ISO₂ was $15.6 \pm 8.0\%$ at 75 min. On the other hand, a dose of 1.0 $\mu\text{g}/\text{kg}/\text{min}$ or 10 $\mu\text{g}/\text{kg}/\text{min}$ of the same substance caused a decrease in systemic blood pressure and the level of IHb and ISO₂. However, under these conditions, IHb increased

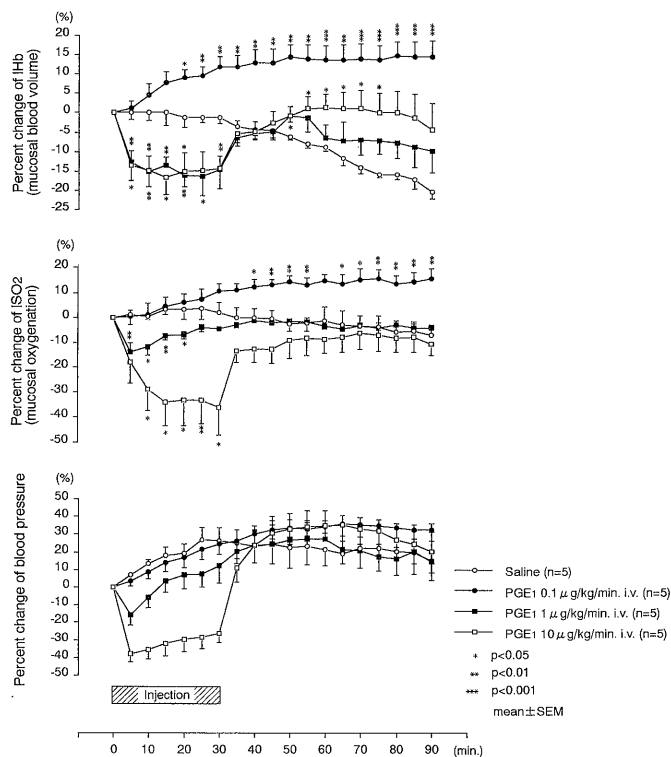


Figure 1. The effect of exogenous PGE₁ on colonic mucosal blood volume, mucosal oxygenation and blood pressure. PGE₁ was continuously injected for 30 min at a concentration of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ (closed circles), 1.0 $\mu\text{g}/\text{kg}/\text{min}$ (closed squares) and 10 $\mu\text{g}/\text{kg}/\text{min}$ (open squares). Open circles: saline injection to control rats. Solutions were administered using the same flow rate in the femoral vein of anesthetized rats. Index of colonic mucosal hemoglobin concentration (upper panel) and colonic mucosal oxyhemoglobin saturation (middle panel) were measured by a tissue spectrum analyzer during monitoring of blood pressure (lower panel) during 90 min.

slowly to the higher level than the control level and ISO₂ returned to the control level following the recovery of blood pressure (Fig. 1).

Infusion of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ of PGE₂ caused a gradual increase in IHb and ISO₂. The maximal increase of IHb was $10.7 \pm 4.5\%$ at 70 min after the start of infusion, while ISO₂ was $12.5 \pm 8.1\%$ at 85 min after the start of PGE₂ injection. When infused at 1.0 $\mu\text{g}/\text{kg}/\text{min}$ or 10 $\mu\text{g}/\text{kg}/\text{min}$, PGE₂ reduced systemic blood pressure and the level of IHb and ISO₂, but IHb and ISO₂ returned slowly to the control level following the recovery of blood pressure (Fig. 2). We also examined the effect of PGE metabolites, 15-keto-PGE₁ and 15-keto-PGE₂, at a dose of 5 $\mu\text{g}/\text{kg}/\text{min}$. Such dose failed to influence IHb. The injection of 1.0 $\mu\text{g}/\text{kg}/\text{min}$ of PGI₂ had an effect similar to that of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ of PGE₁, and did not change systemic blood pressure. The maximal increase of IHb was $11.5 \pm 8.2\%$ at 50 min while that of ISO₂ was $10.9 \pm 7.6\%$ at 85 min after the start of PGI₂ injection in a dose of 1.0 $\mu\text{g}/\text{kg}/\text{min}$. The level of IHb and ISO₂ did not change significantly during and after the administration of 0.1 $\mu\text{g}/\text{kg}/\text{min}$ of PGI₂. However, a severe hypotension and reduction in IHb and ISO₂ levels were observed during injection of 10 $\mu\text{g}/\text{kg}/\text{min}$ of PGI₂, but both IHb and ISO₂ increased slowly to the higher level than the control level following the recovery of blood pressure (Fig. 3).

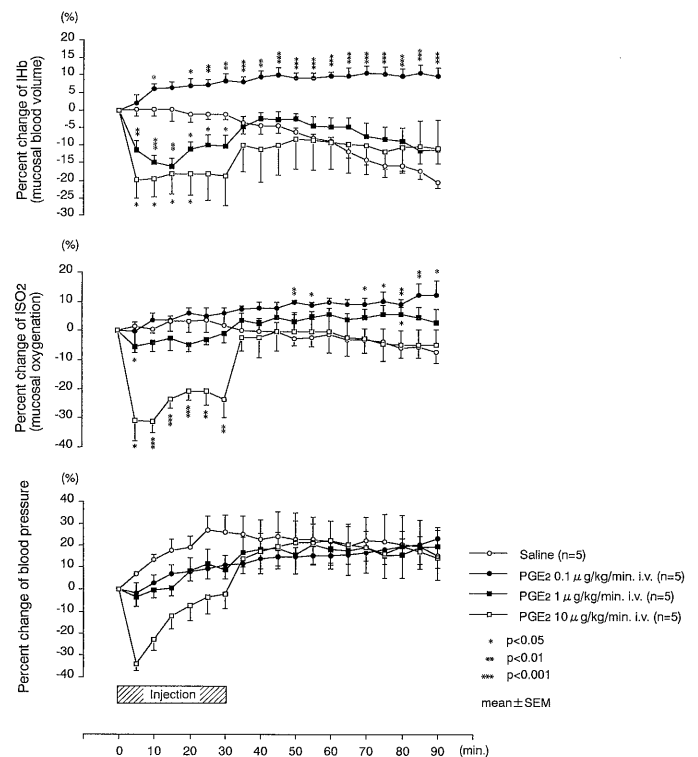


Figure 2. The effect of exogenous PGE₂ on colonic mucosal blood volume, mucosal oxygenation and blood pressure. The conditions were similar to those in Fig. 1.

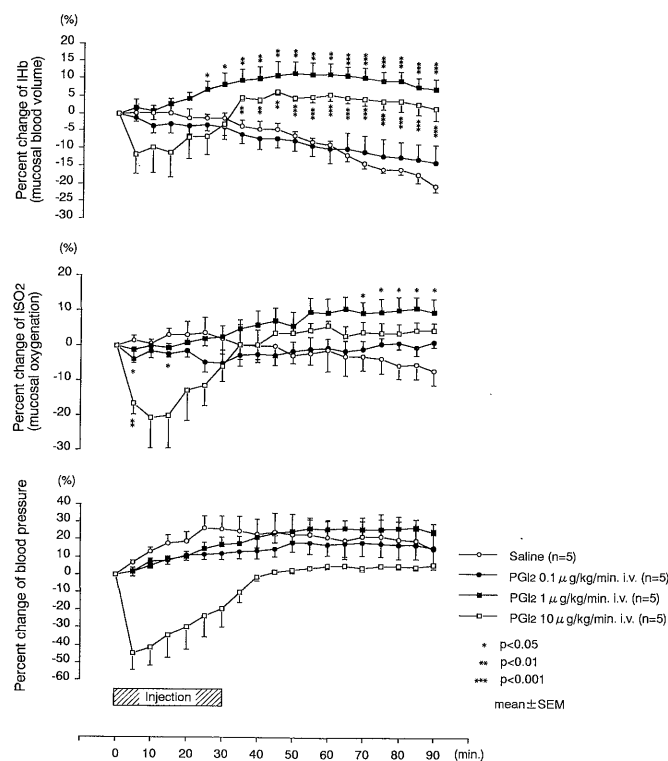


Figure 3. The effect of exogenous PGI₂ on colonic mucosal blood volume, mucosal oxygenation and blood pressure. The conditions were similar to those of Fig. 1.

Effect of TXA₂ administration on colonic blood flow.

Whereas the injection of 0.1 µg/kg/min of TXA₂ failed to influence systemic blood pressure, the drug reduced IHB significantly during injection to $-12.2 \pm 4.7\%$ at 30 min. The level of IHB returned, however, to the control level at 60 min after the commencement of TXA₂ injection. In contrast, injection of a larger dose of TXA₂ (1.0 µg/kg/min) caused a significant fall in systemic blood pressure and further reduced the level of IHB and ISO₂ (IHB, $-28.4 \pm 11.8\%$; ISO₂, $-46.5 \pm 24.6\%$, at 30 min after the start of TXA₂ injection). However, as the blood pressure started to gradually recover, IHB and ISO₂ also progressively increased (Fig. 4). Injection of 0.01 µg/kg/min of TXA₂ failed to have an effect on colonic blood flow. Injection of 10 µg/kg/min of TXA₂ caused a severe and rapid fall in systemic blood pressure and death. Furthermore, the simultaneous injection of 1.0 µg/kg/min of PGI₂ and TXA₂ was also examined. A combination of PGI₂ and TXA₂ showed compromised values with little or on change in blood pressure, IHB and ISO₂ (Fig. 5).

Kinetics of residual PGE₂ following the administration of PGE₂. Administration of PGE₂ (10 µg/kg/min) caused an initial decrease in IHB and ISO₂, but the level of both compounds increased slowly after the cessation of injection. The serum concentration of PGE₂ reached a peak level

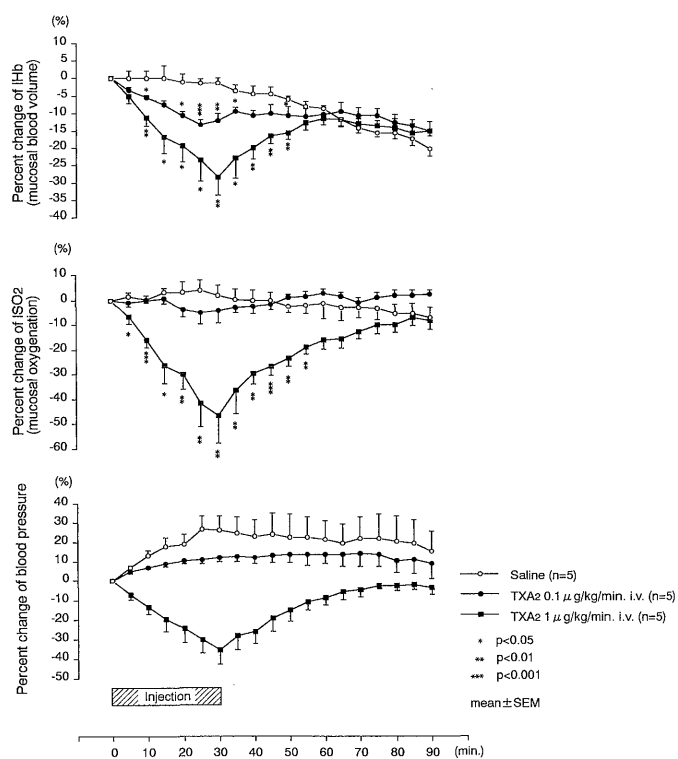


Figure 4. The effect of exogenous TXA₂ on colonic mucosal blood volume, mucosal oxygenation and blood pressure. The conditions were similar to those in Fig. 1.

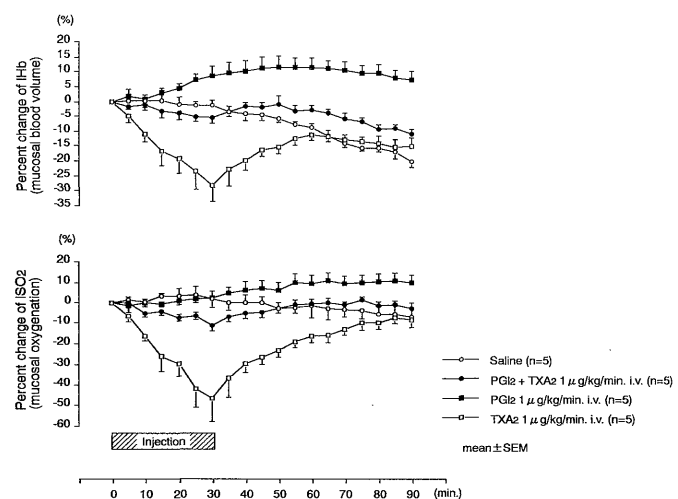


Figure 5. Effects of combination of PGI₂ and TXA₂ on colonic mucosal blood volume and mucosal oxygenation. The effect of 1.0 µg/kg/min of PGI₂ and a similar dose of TXA₂ administered together was compared with that of 1.0 µg/kg/min of PGI₂ and 1.0 µg/kg/min of TXA₂ injection, administered separately, on colonic mucosal hemoglobin concentration (upper panel) and colonic mucosal oxyhemoglobin saturation (lower panel).

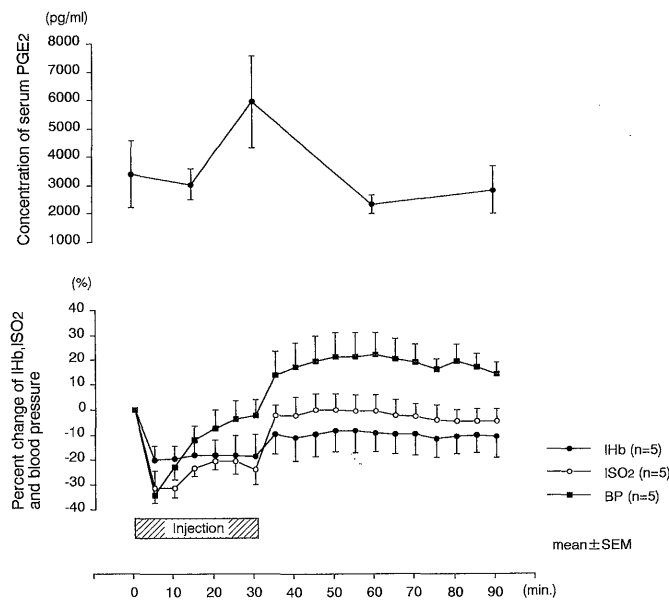


Figure 6. The concentration of serum PGE₂ following the administration of exogenous PGE₂ and the effect of such level on colonic mucosal blood volume, mucosal oxygenation and blood pressure. The concentration of serum PGE₂ was measured after the injection of 10 μg/kg/min of PGE₂ to confirm whether the residual PGE₂ (upper panel) influences recovery of colonic mucosal hemoglobin concentration, colonic mucosal oxyhemoglobin saturation and blood pressure (lower panel).

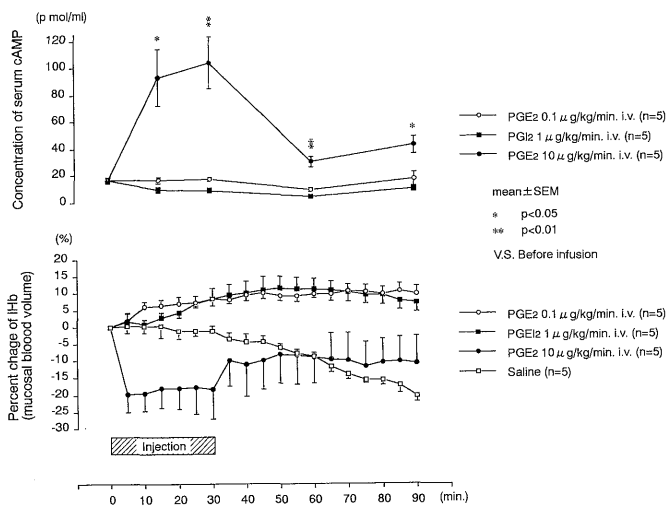


Figure 7. Serum cAMP level and effect on colonic mucosal blood volume after administration of exogenous PGE₂ and PGI₂. The concentration of serum cAMP (upper panel) was measured after the injection of 0.1 μg/kg/min and 10 μg/kg/min of PGE₂ and 1.0 μg/kg/min PGI₂ to examine whether cAMP influences the increase in colonic mucosal hemoglobin concentration (lower panel).

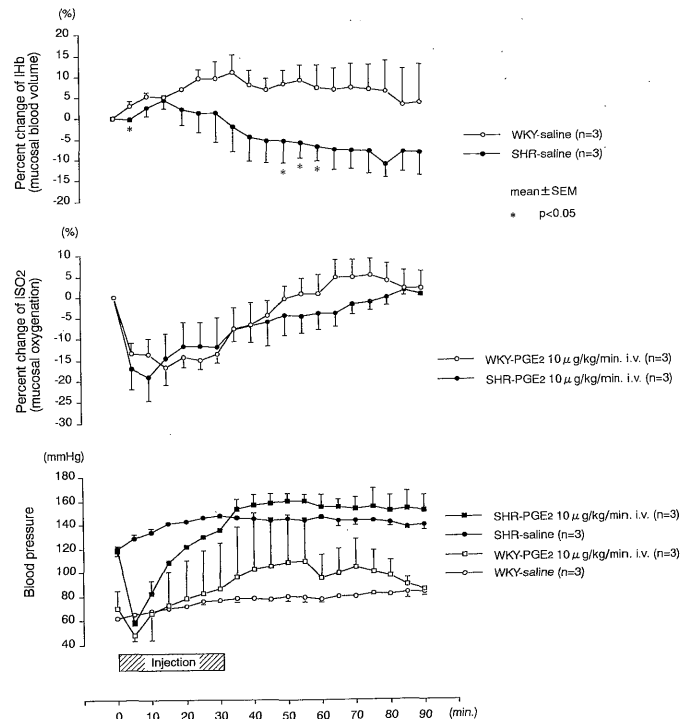


Figure 8. A comparison of the effect of PGE₂ on colonic blood volume and blood pressure between SHR and WKY rats. The effect of 10 μg/kg/min of PGE₂ injection was compared with saline injection in SHR and WKY to confirm the correlation between IHb of colonic mucosa and sympathetic nerve activity. Upper panel: colonic mucosal hemoglobin concentration during and after saline injection in SHR and WKY rats. Middle panel: colonic mucosal hemoglobin concentration during and after injection of 10 μg/kg/min of PGE₂ in SHR and WKY rats. Lower panel: blood pressure during and after saline and 10 μg/kg/min of PGE₂ injection in SHR and WKY rats.

at 30 min after the start of injection, then diminished to the initial value (Fig. 6).

Kinetics of serum cAMP following the administration of PGE₂ or PGI₂. Injection of PGE₂ (0.1 μg/kg/min) or PGI₂ (1.0 μg/kg/min) caused a gradual increase of IHB until 60 min after injection, but failed to influence the level of serum cAMP. However, a higher dose of PGE₂ (10 μg/kg/min) further reduced IHB during injection but the level returned slowly following cessation of PGE₂ injection. Injection of such a high dose of PGE₂ markedly elevated serum cAMP during infusion of PGE₂, but the level rapidly decreased after the end of injection, although it did not return to baseline value (Fig. 7).

Effect of PGE₂ administration on colonic blood flow in SHR and WKY rats. IHB was measured in hypertensive (SHR) and nonhypertensive rats (WKY) in order to examine the correlation between IHB and sympathetic nerve activity. As shown in Fig. 8, under control condi-

tions, IHb diminished in SHR rats compared with WKY rats. Administration of 10 $\mu\text{g}/\text{kg}/\text{min}$ of PGE_2 reduced IHb acutely in both groups of rats, but the level recovered gradually to the initial level following cessation of injection. There were no statistical differences between SHR and WKY rats.

DISCUSSION

The major finding of our study is a gradual increase in colonic mucosal blood volume caused by the administration of exogenous PGs (PGE_1 , PGE_2 and PGL_2). The increase was not due to changes in blood pressure. Furthermore, we also observed an increase in colonic mucosal volume with the administration of a high dose of PGs, without affecting the systemic blood pressure. Our results suggest that PGs may be one of the cytoprotective substances involved in increasing colonic mucosal blood flow.

Reflectance spectrophotometry can determine not only tissue blood volume but also the average oxy- and deoxyhemoglobin equilibrium in tissue by measuring the absorbance of hemoglobin and tissue oxygen saturation, respectively (13). The present study also demonstrated that exogenous PGs also increased ISO_2 , representing increased tissue oxygen saturation, without influencing blood pressure. Several investigators described a vasodilatory effect for PGs by demonstrating an increase in gastric mucosal blood flow (1, 2). In this regard, Dousa (14) reported that PG stimulates cAMP formation, which in turn accelerates the production of glycoprotein and glycosaminoglycan. It was also suggested that PG could provide mucosal cytoprotection by providing a sufficient level of tissue oxygen in mucosa. Thus, our results suggest a possible colonic mucosal cytoprotective role for exogenous PGs in addition to that already described in the gastric mucosa. Our conclusion is based on the finding that several PGs increased colonic mucosal blood flow and local tissue oxygen saturation.

TXA_2 induces platelet aggregation and constriction of vascular smooth muscles, while PGL_2 markedly suppresses platelet aggregation and dilates vascular smooth muscles. Thus, TXA_2 and PGL_2 play an important role in regulating mucosal circulation by maintaining homeostasis in the cardiovascular system (15). Our results demonstrated that PGL_2 increased colonic mucosal blood volume while maintaining a normal blood pressure, while TXA_2 reduced it. These results suggest that the colonic microcirculation is regulated by a balance between PGL_2 and TXA_2 .

Several studies have examined the effect of exogenous PGs on vascular smooth muscles (16-18). By using SHR and WKY rats in the present study, we were able to rule out any action of exogenous PG on the sympathetic nervous system, since no difference in colonic hemo-

dynamics was observed between the two groups of rats even when a high dose of PGE_2 was administered. Furthermore, our findings suggest that PGE metabolites (15-keto-PGE) have no effect on colonic blood flow. Residual PGE_2 did not influence the recovery of colonic mucosal blood flow in our study, because the serum concentration of PGE_2 diminished to the initial value following injection of a high dose of PGE_2 . Such recovery may be due to autoregulatory mechanisms acting after a reduction of blood pressure (19), and a change in the distribution of colonic blood flow. We failed to demonstrate increased levels of serum cAMP by PGE_2 or PGL_2 injection under normal level of systemic blood pressure, whereas serum cAMP increased during the administration of a high dose of PGE_2 . Although serum cAMP was too low to detect at normal blood pressure, it is possible that exogenous PGs may have increased cAMP, which may have dilated colonic mucosal capillaries.

IBD are characterized by ulceration or inflammation of the colonic mucosa and their etiology are still unknown. Patients with active ulcerative colitis have higher production of PGL_2 and TXA_2 metabolites (6-keto-PGF $_{1\alpha}$ and TXB_2) in colonic mucosa than normal subjects (10). Furthermore, abundant TXB_2 , and a low 6-keto-PGF $_{1\alpha}/\text{TXB}_2$ ratio are also described in Crohn's disease (11). These results suggest that deceleration of PGL_2 metabolism and acceleration of TXA_2 metabolism reduce colonic mucosal flow and diminish the cytoprotective capacity in patients with active IBD. Several investigators have described the protective effect of PGE in gastric mucosa (1, 20), and, in fact, PGE is used as a treatment for gastric ulceration. It is of interest that PGE_2 was initially regarded as an inflammatory factor in the mucosa of IBD, but only recently was re-considered as an anti-inflammatory substance inducing suppressor T cells (21). Sharon et al. (22) reported that the level of PGE_2 in rectal mucosa of patients with ulcerative colitis was higher than that found in a normal mucosa. In addition, Hulten et al. (23) reported that colonic mucosal blood flow increased during the active phase, whereas a little or no change in blood flow occurred during the remission phase in patients with ulcerative colitis and Crohn's disease. These results suggest that mucosal restoration in active IBD may possibly be due to the accumulation of endogenous PGE in colonic mucosa, increase in colonic blood flow and oxygenation, stimulation of cellular metabolism, and/or the transport of anti-inflammatory chemical mediators to tissues. The recent demonstration of an effective therapeutic role of PG in an experimental model of colitis (5,6), suggest that exogenous PGs may prove useful in the therapy of inflammatory bowel diseases.

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