Effects of Ecabet Sodium (TA-2711), a New Antiulcer Agent, on Gastrointestinal Mucosal Prostanoid Production and Morphology in Rats

Mine KINOSHITA,* a Hitoshi IWASAKI, Akira YASOSHIMA,* and Hajime TAMAKI*
Pharmacological Research Laboratory, a Research Laboratory of Drug Metabolism, Department of Pathology and Toxicology, b Tanabe Seiyaku Co., Ltd., 2–2–50, Kawagishi, Toda, Saitama 335, Japan. Received March 17, 1993

Effects of ecabet sodium (TA-2711), a locally acting antiulcer agent, on prostanoid production and the morphology of the rat gastrointestinal mucosa were studied in comparison with sucralfate. Ecabet, at therapeutic doses (25 and 100 mg/kg, p.o.), dose-dependently increased the gastric mucosal level of prostaglandin E, (PGE,); sucralfate (100 mg/kg, p.o.) showed a tendency to increase the PGE_2 level. In an ex vivo study, ecabet (25 and 100 mg/kg, p.o.) dose-dependently increased the capacity of the gastric mucosa to synthesize PGE_2 and PGI_2, without modifying thomboxane A_2 (TXA_2) synthesis, and the 100 mg/kg dose persisted for up to 3 h. Ecabet (400 mg/kg, p.o.) also significantly increased PGE_2 synthesis and there was a tendency to increase PGI_2 synthesis by the duodenal mucosa, without affecting TXA_2 synthesis. PGE_2 synthesis by the colonic mucosa was not affected, even at a high dose of ecabet (1000 mg/kg, p.o.). When the rat gastric mucosa was examined by light microscopy and scanning electron microscopy, ecabet (100 and 400 mg/kg, p.o.) had no morphological change to the gastric mucosa, while sucralfate (100 and 400 mg/kg, p.o.) produced apical rupture of the epithelial cells and subepithelial edema. The present study indicates that ecabet locally stimulates PGE_2 and PGI_2 production in the gastroduodenal mucosa and this effect is not attributable to a local irritant action accompanied by superficial epithelial damage.

Keywords ecabet sodium (TA-2711); sucralfate; gastric mucosa; PGE_2; PGI_2; local irritant action

Ecabet sodium (TA-2711, Fig. 1), a new locally acting antiulcer agent, is known to prevent the development of various experimentally induced gastric and duodenal ulcers and accelerate the healing of acetic acid-induced gastric ulcer in rats. It has been demonstrated that the mechanisms for the antiulcer effect of ecabet include antispasms in activity and enhancement of the gastroduodenal mucosal defensive factors such as gastric mucosal blood flow, gastroduodenal mucosal bicarbonate secretion and gastric mucosal adherent mucus. Mucosal prostaglandin (PG)-mediated processes are also assumed to be involved in the antiulcer action of ecabet, since the protective effect on ethanol-induced gastric lesions was inhibited by pretreatment with indomethacin. Endogenous PGs have been suggested to play an important role in maintaining the normal mucosal integrity of the stomach. In the present study, we investigated the effect of ecabet on prostaglandin production in the rat gastrointestinal mucosa in vivo, ex vivo and in vitro experiments. Stimulation of gastric mucosal PG formation was suggested to be one of the important mechanisms for the gastroprotective effects of some antiulcer agents such as sucralfate, colloidal bismuth and antacids. Oral administration of mild irritants such as 1 M NaCl and 20% ethanol also prevent the formation of gastric lesions induced by a necrotizing agent and stimulate gastric mucosal prostaglandin formation. Sucralfate has been reported to have an irritant effect on the gastric mucosa and cause microscopic damage of the superficial epithelial. Therefore, we also investigated the effect of ecabet on the morphology of the rat gastrointestinal mucosa by light microscopy and scanning electron microscopy.

Materials and Methods
Animals and Drugs Male Sprague-Dawley rats (Charles River, Japan), weighing 150–250 g, were used throughout the present study. Animals were fasted for 24 h before use, but were allowed access to water ad libitum. Ecabet sodium was synthesized in the Organic Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd. Other drugs were obtained from the following sources: sucralfate from Chugai Seiyaku Co., Ltd., cimetidine and indomethacin from Sigma. Radioimmunoassay kits for prostaglandin E_2 (PGE_2), 6-keto-prostaglandin F_1alpha (PGF_1alpha) and thromboxane B_2 (TXB_2) and [3H]PGE_2 (specific activity: 169.5 Ci/mmol) were purchased from New England Nuclear. Other chemicals were purchased from conventional commercial sources. In vivo experiments, drugs were either dissolved or suspended in distilled water in a volume of 5 ml/kg and were administered orally. In in vitro experiments, ecabet was dissolved in 0.15 M NaOH and diluted with a Krebs-Henseleit bicarbonate buffer (pH 7.4).

Measurement of PGE_2 Levels in Rat Gastric Mucosa (in Vivo) The test drugs were orally administered to rats, which were then killed 30 min later. The stomach was immediately removed, placed in ice-cold 0.9% NaCl, and cut open along the greater curvature. The gastric corpus wall was then placed between two glass slides and instantaneously frozen by immersion in hexane cooled in a dry ice-acetone bath. The frozen gastric mucosa was separated from its underlying muscle layer by pulling the two glass slides apart. Extraction and separation of mucosal PGE_2 were performed according to the methods described by Arakawa et al. The frozen gastric mucosa was homogenized in 2.5 ml methanol containing 10^{-4}M indomethacin. The homogenate was filtered and the filtrate evaporated at 37°C. Then, 3 ml CCl_4 and 5 ml 0.1 M phosphate buffer (pH 8.0) were added to the residue. After mixing, the aqueous layer was separated by centrifugation (3000 x g for 10 min at 4°C), and acidified to pH 3.5 with HCl. PGE_2 was extracted from the buffer with ethyl acetate. PGE_2, after separation by thin-layer chromatography, was measured by using the [3H]PGE_2 radioimmunoassay kits. The overall recovery of PGE_2 was estimated to be more than 80% using [3H]PGE_2 as a recovery marker.

Prostaglandin Synthesis by Rat Gastrointestinal Mucosa (ex Vivo) Mea-
urement of the capacity of the gastrointestinal mucosa to synthesize PGE_2, PGA_1, and TXA_2 was carried out as described by Boughton-Smith et al. After the oral administration of ecabet, the stomach, duodenum and colon were removed under ether anesthesia and immediately placed in ice-cold 0.9% NaCl. The gastric, duodenal and colonic mucosa were separated from the underlying muscle layer with fine scissors. The mucosa was transferred to an Eppendorf microtube, chopped with scissors for 1 min in 500 μl 0.1 M phosphate buffer (pH 7.4) at 4°C, and centrifuged at 12000 × g for 1 min (Eppendorf centrifuge 5412). The pellet was washed with the buffer, suspended in 500 μl of the same buffer, and shaken vigorously using a Vortex mixer (3000 rpm) for 1 min at room temperature. After addition of 40 μl 1% NaHCO_3 containing 10 μg indomethacin, the suspension was centrifuged at 12000 × g for 1 min. The concentration of PGE_2, 6-keto-PGF_1α (the stable metabolite of PGI_2) and TXB_2 (the stable metabolite of TXA_2) in the supernatant were determined using the specific 125I-radioimmunoassay kits.

**PGE_2: Release from Rat Gastric Mucosal Slices (in Vitro)** Experiments were carried out according to the method of Knapp et al. The stomach was removed under ether anesthesia, and immediately placed in ice-cold Krebs-Henseleit bicarbonate buffer having the following composition (mM): NaCl 120.0, NaHCO_3 25.0, CaCl_2 2.5, KCl 4.8, MgSO_4·7H_2O 1.2, KH_2PO_4 and glucose 10.0 (pH 7.4). The stomach was cut open along the greater curvature and the gastric corpus mucosa was separated from the underlying muscle layer with fine scissors. The mucosa was weighed, cut into pieces (2 × 4 mm), and then incubated in the Krebs-Henseleit bicarbonate buffer gassed with 95% O_2 and 5% CO_2 at 37°C. The incubation medium was replaced every 30 min and the amount of PGE_2 in the recovered medium was determined using the [125I]PGE_2 radioimmunoassay kit. After the first 30 min-incubation, the medium was changed to ecabet-containing buffer.

**Histological Evaluation of Gastric Mucosa** One hour after oral administration of ecabet and sulcrate, the animals were killed under ether anesthesia. The stomachs were removed and opened along the greater curvature.

For light microscopy, one half of the gastric wall was pinned to a polystyrol board and fixed in buffered 10% neutral formalin solution. After thorough fixation, 5-μm-thick paraffin sections of the gastric walls were prepared and stained with hematoxylin-eosin, Alcian blue and periodic acid-Schiff.

For scanning electron microscopy, the other half of the gastric wall was pinned to a polystyrol board and the mucosal surface was gently rinsed with Ringer’s solution. The tissues were then fixed in phosphate-buffered 1.2% paraformaldehyde–2.5% glutaraldehyde solution. The fixed tissues were dehydrated in a graded series of ethanol solutions and finally placed in isoamyl acetate. The dehydrated tissues were dried by liquid CO_2 substitution, placed on spinner stubs and coated with gold. These specimens were then examined in a scanning electron microscope (JEM-T220).

**Statistical Analysis** Statistical analysis was performed by the analysis of variance using Bonferroni’s method, and the paired Student’s t-test. A p value less than 0.05 was considered to be statistically significant. All data are expressed as means ± S.E.M.

**Results**

**Effect on PGE_2 Level in Rat Gastric Mucosa (in Vivo)** The basal level of PGE_2 in the gastric mucosa of control rats was found to be 13.9 ± 3.1 ng/g wet tissue (n = 7). Oral administration of 25 and 100 mg/kg of ecabet dose-dependently increased the mucosal PGE_2 level (Fig. 2). Sulcrate, at an oral dose of 100 mg/kg, tended to increase the PGE_2 level, but this increase was not statistically significant. Cimetidine (100 mg/kg, p.o.) had no influence on gastric mucosal PGE_2 level.

**Effect on Prostanoid Synthesis by Rat Gastrointestinal Mucosa (ex Vivo)** In the method used for measuring the capacity of mucosal tissue to synthesize prostanoids, the amounts of prostanoids in the final supernatant were found to be negligible without the mixing procedure. The amounts of PGE_2, PGA_1, and TXA_2 synthesized by the control rat gastric mucosa in response to mechanical stimulation (i.e. vortexing for 1 min at 3000 rpm) were 138.2 ± 17.2, 405.8 ± 38.0, and 99.7 ± 19.3 ng/g wet tissue/min, respectively. Prior treatment with ecabet (25, 100 mg/kg, p.o.) dose-dependently increased PGE_2 and PGA_1 synthesis without affecting TXA_2 synthesis (Fig. 3). The amounts of PGE_2 and PGA_1 produced in ecabet (100 mg/kg, p.o.)-treated rats were respectively 2.3 and 1.6 times higher than in the control group.

The duration of action of ecabet on ex vivo PGE_2 and PGA_1 synthesis is shown in Fig. 4. Ecabet, at an oral dose of 100 mg/kg, significantly (p < 0.01) increased both PGE_2 and PGA_1 synthesis 1 h after administration. The stimulatory effects on PGE_2 and PGA_1 synthesis were also observed at 3 h, but not 6 h after administration.
In the duodenal mucosa, oral administration of ecabet, at a dose of 400 mg/kg, produced a significant increase in ex vivo PGE\textsubscript{2} synthesis and tended to increase PGI\textsubscript{2} synthesis with no effect on TXA\textsubscript{2} synthesis (Fig. 5). In the colonic mucosa, even a high dose of ecabet (1000 mg/kg, p.o.) had no effect on ex vivo PGE\textsubscript{2} synthesis at 6 h after the dosing, when ecabet was thought to have reached the colon (control, 242.2 ± 40.5 ng/g wet tissue/min; ecabet, 276.5 ± 38.8 ng/g wet tissue/min, mean ± S.E.M., n = 6—8).

**Effect on PGE\textsubscript{2} Release from Rat Gastric Mucosal Slices (in Vitro)** Table I shows that ecabet (3—30 mg/ml) induced a concentration-dependent increase in PGE\textsubscript{2} release from gastric mucosal slices.

The osmolarity of the ecabet-containing buffer (30 mg/ml) was 450 mOsmol and was higher than that of Krebs-Henseleit buffer (280 mOsmol). To examine the contribution of hyperosmolarity to the ecabet-induced increase in PGE\textsubscript{2} release, the effect of a mannitol-containing buffer (450 mOsmol) was studied. With the mannitol-containing buffer no significant increase in PGE\textsubscript{2} release was observed (data is not shown).

**Histological Evaluation of Gastric Mucosa** Light microscopic studies demonstrated that the oral administration of ecabet (100 and 400 mg/kg) produced no histological change in the gastric mucosa (Fig. 6b shows a histological picture of the gastric mucosa in the rat treated with ecabet at a dose of 400 mg/kg). In contrast, edema of the lamina propria in the superficial zone of the oxyntic mucosa was seen in sucralfate (100 and 400 mg/kg, p.o.)-treated rats (Fig. 6c, d). The incidence of this histological change was dose-dependent: the incidence was 1/3 at a dose of 100 mg/kg, and 3/3 at a dose of 400 mg/kg. Scanning electron microscopy studies also demonstrated that sucralfate (100 and 400 mg/kg) produced ultrastructural damage to the surface epithelial cells, i.e. focal detachment and/or derangement of the surface epithelial cells (Fig. 7c, d). On the other hand, ecabet (100 and 400 mg/kg) did not affect the ultrastructure of the gastric mucosal surface epithelial cells (Fig. 7b shows a scanning electron micrograph of the gastric mucosa in the rat treated with ecabet at the dose of 400 mg/kg).

**Discussion**

In ex vivo experiments, mechanically induced PGE\textsubscript{2} and

---

**Fig. 4. Duration of the Stimulatory Effect of Oral Administration of Ecabet on the ex Vivo Synthesis of PGE\textsubscript{2} and PGI\textsubscript{2} by Rat Gastric Mucosa**

○, control; ●, ecabet (100 mg/kg). Each point represents the mean ± S.E.M. (n = 5—8). a) p < 0.05, compared with control.

**Fig. 5. Effect of Ecabet (100 and 400 mg/kg, p.o.) on the ex Vivo Synthesis of PGE\textsubscript{2}, PGI\textsubscript{2} and TXA\textsubscript{2} by Rat Duodenal Mucosa**

Rats were killed 1 h after oral administration of ecabet. The data are expressed as the means ± S.E.M. (n = 6—8). a) p < 0.01, compared with control.

---

**Table 1. Effect of Ecabet on PGE\textsubscript{2} Release from Rat Gastric Mucosa in Vitro**

<table>
<thead>
<tr>
<th>Conc. (mg/ml)</th>
<th>30—0</th>
<th>0—30</th>
<th>30—60</th>
<th>60—90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>141.0 ± 17.0</td>
<td>112.3 ± 15.1</td>
<td>126.6 ± 10.8</td>
<td>136.2 ± 14.5</td>
</tr>
<tr>
<td>Ecabet 3</td>
<td>139.5 ± 22.6</td>
<td>148.1 ± 25.4</td>
<td>160.3 ± 24.1</td>
<td>177.0 ± 20.3</td>
</tr>
<tr>
<td>Ecabet 10</td>
<td>137.8 ± 19.6</td>
<td>149.3 ± 24.8</td>
<td>171.5 ± 19.1</td>
<td>202.6 ± 22.6</td>
</tr>
<tr>
<td>Ecabet 30</td>
<td>162.6 ± 8.6</td>
<td>212.6 ± 35.3</td>
<td>255.7 ± 31.2</td>
<td>338.9 ± 67.5</td>
</tr>
</tbody>
</table>

Gastric mucosal slices were incubated (37°C) in Krebs-Henseleit bicarbonate buffer (pH 7.4). The buffer was replaced every 30 min and the amount of PGE\textsubscript{2} in the recovered buffer was measured by the 111\textsuperscript{In}-radiomunooassay kit. The ecabet-containing buffer was used from time 0. The data are expressed as the means ± S.E.M. (n = 6—7). a) p < 0.05, b) p < 0.01, compared with the amount of released PGE\textsubscript{2} during 30 min before application of the ecabet-containing buffer.
PGI₁ synthesis was found to be significantly enhanced in the gastric mucosa isolated from rats pretreated with ecabet. In addition, the oral administration of ecabet dose-dependently increased the PGE₂ level in rat gastric mucosa. These results suggest that ecabet-induced increases in *ex vivo* PGE₂ and PGI₁ synthesis reflects the PGE₂ and PGI₁ level in the gastric mucosa after the oral administration of ecabet. Boughton-Smith *et al.* also demonstrated that the chpped rat gastric mucosa generated prostanooids from an endogenous substrate following mechanical stimulation (*i.e.* shaking with a Vortex mixer) and the amount of generated prostanooids may reflect the level of activation of phospholipase A₂, cyclooxygenase or prostaglandin synthetase in the specimen.

The duration of the ecabet-induced increase in the capacity to synthetize PGE₂ and PGI₁ correlated well with the gastroprotective effect on the ethanol-induced lesions and the increasing effect on the amount of mucus adherent to the gastric mucosa reported by Onoda *et al.* PGE₂ and PGI₁ have been known to play an important role in the prevention of gastric injury through an enhancement of gastric mucosal blood flow and stimulation of mucus and bicarbonate secretion. Therefore, it is likely that the increase in gastric mucosal PGE₂ and PGI₁ levels contributes to the gastroprotective action of ecabet.

Ecabet, at an oral dose of 400 mg/kg, which gave an antiulcer effect on cysteamine-induced duodenal ulcer, produced a significant increase in *ex vivo* PGE₂ synthesis and exhibited a tendency to increase PGI₁ synthesis by the rat duodenal mucosa. It has been reported that in *in situ* experiments local application of ecabet enhanced acid-induced duodenal bicarbonate secretion, in parallel with an increase in PGE₂ release from the duodenum and this was inhibited by indomethacin. Therefore, the ecabet-induced increase in mucosal PGE₂ might be involved in the antiulcer effect of ecabet in the duodenum as well as in the stomach. On the other hand, a high dose of ecabet (1000 mg/kg, p.o.) had no effect on the capacity of the colonic mucosa to synthetize PGE₂ 6 h after oral administration, when ecabet was thought to have reached the colon. The effect of ecabet on the capacity to synthetize PGE₂ is different in the various regions of the gastrointestinal tract: the minimum dose of ecabet to give this effect was 25, 400 and >1000 mg/kg in stomach, duodenum and colon, respectively. Ito *et al.* indicated that ecabet had a high affinity for the gastric mucosa. It seems that the difference in the capacity to synthetize PGE₂ is due to the difference in affinity of ecabet for the gastrointestinal mucosa.

It was additionally found that ecabet had no effect on the capacity of the gastroduodenal mucosa to synthetize TXA₂. Stomach has been reported to have synthetic activity for PGI₁, PGE₂ and TXA₂. It has also been reported that PGI₁ and PGE₂ were more predominantly produced than TXA₂ in cultured gastric epithelial cells and enzymatically isolated gastric mucosal cells. From radioluminographic and microautoradiographic studies using [¹⁴C]ecabet, the radioactivity was found in the superficial epithelium and the surface of the gastric mucosa.
in the stomach. Thus, it seems reasonable that ecabet increases PGA and PGE formation without influencing TXA formation as a result of its distribution in the stomach. TXA, a potent vasoconstrictor, is known to have the ability to increase the susceptibility of the gastric mucosa to injury. On the other hand, PGA and PGE, potent vasodilators, protect the mucosa by enhancing gastric mucosal defensive factors. Therefore, the balance of these prostanoid production might be important in maintaining the gastric mucosal integrity. The ability of ecabet to increase mainly PGE and PGA formation is considered to be advantageous for gastric mucosal integrity.

In the present study, sucrallose was found to produce morphologic changes in the gastric mucosa: edema of the lamina propria, focal detachment and/or derangement of the surface epithelial cells, as well as a tendency to increase the PGE level. It has been previously reported that oral administration of sucrallose, at the doses giving gastroprotection, caused morphological damage of the gastric mucosa, which was also observed in the present study. The morphological change in the gastric mucosa after administration of sucrallose is considered to be the same as that after administration of mild irritants such as 1 M NaCl and 20% ethanol. Tarnawski et al. and Wallace et al. have suggested that the irritant effect accompanied by superficial epithelial damage may play an important role in the gastroprotective effect of sucrallose. On the other hand, ecabet at doses giving gastroprotection increased the capacity to synthesize PGE and PGA without any morphological change in the gastric mucosa. These findings indicate that the stimulatory effect of ecabet on gastric mucosal PG production is not due to a local irritant effect on the gastric mucosa.

It has been reported that a hypertonic solution increases the in vitro release of PGs from gastrointestinal mucosa. Therefore, the possible contribution of hyperosmolarity to the in vitro stimulatory effect of ecabet on gastric mucosal PG release was examined. While ecabet caused a concentration-dependent increase in PGE release from gastric mucosa, the mannitol-containing buffer with the same osmolarity (450 mM) exhibited no such effect. Thus, the stimulatory effect of ecabet on PGE release is not due to hyperosmolarity.

In conclusion, the stimulatory effect of ecabet on PGE and PGA formation in gastroduodenal mucosa may play an important role in its antiulcer effect through enhancement of the gastroduodenal mucosal defensive factors. At least, a local irritant effect on gastric mucosa is not likely to contribute to the stimulatory effect of ecabet on PGE and PGA formation. However, the precise mechanisms of the effect of ecabet on PGE and PGA formation in gastroduodenal mucosa are not clear at present and warrant further investigation.

Acknowledgments The authors would like to thank Dr. M. Ohtuka and Dr. K. Naito for their helpful suggestions throughout the study. The skillful technical assistance of Mr. M. Kimbo is gratefully acknowledged.
References