Acid-Promoted Epimerization of Arbaprostil, 15(R)-15-Methylprostaglandin E₂, Elicits Gastric Antisecretory Activities in Rats

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ABSTRACT—Gastric acid antisecretory activities of 15(R)-15-methylprostaglandin E₂ (arbaprostil) preincubated or not preincubated with 0.9% physiological saline, the pH of which was precisely adjusted to less than 4.30, were examined in pylorus-ligated rats, and compared with those of 15(S)-15-methylprostaglandin E₂ (15(S), epimer of arbaprostil). 15(S), unlike arbaprostil without preincubation, when s.c.-administered to rats significantly inhibited gastric acid secretion in a dose-dependent manner (30–300 µg/kg). However, arbaprostil preincubated at 37°C for 30 min with 0.9% saline, at pHs of 4.30, 2.75 and 1.20, respectively, showed the following order of pH-dependent antisecretory activities: 1.20 > 2.75 > 4.30. An increase in 15(S) formation from arbaprostil in a pH-dependent manner was also observed by radioisotopic experiments under the same incubation conditions using [³H]-labeled arbaprostil. The present result suggests that the gastric antisecretory effect of arbaprostil can be mainly explained in terms of the formation of 15(S) after oral administration.

It has been established that prostaglandin (PG) E₂ administered parenterally inhibits gastric acid secretion both in experimental animals and humans (1–3). The reduction of acid secretion appears to play an important role in the therapy of peptic ulcers (4–6). The clinical usage of PGE₂, however, is limited because the antisecretory effect of PGE₂ completely disappears when given orally (7). Since the rapid enzymatic metabolism at the 15-hydroxy position seems to play a key role in this degradation process, a methyl group was introduced into the carbon chain at that position to give 15(R)-15-methylprostaglandin E₂ (arbaprostil, Fig. 1). Arbaprostil possesses antisecretory effects after oral administration (8, 9), but we have inadvertently witnessed that the administration of this agent through other

routes (such as i.v., s.c. and i.d.) failed to reveal any significant antisecretory effect. To elucidate the cellular events underlying these observations, the present study was undertaken, under the working hypothesis that the epimerization of arbaprostil into the S-form (15(S)-15-methylprostaglandin E₂, Fig. 1) following oral administration could occur under the low pH condition, and that the S-form plays a determinant role in eliciting the antisecretory activities of arbaprostil.

To this end, we designed experiments first to evaluate whether arbaprostil preincubated at different pHs affect the potency in reducing gastric secretion in pylorus-ligated rats and then radioisotopically quantitated the amount of each isomer.

15(R)-15-methylprostaglandin E_2
(arbaprostil)

15(S)-15-methylprostaglandin E_2

Fig. 1. Chemical structures of arbaprostil and 15(S)-15-methylprostaglandin E_2 (15(S)).

MATERIALS AND METHODS

Preparation of animals

Male Sprague-Dawley rats (8 weeks of age) were deprived of food but allowed free access to water for 24 hr prior to the experiments. The abdomens of the animals were incised, and the pylorus of the rat was ligated under ether anesthesia. Four hours following the pylorus ligation, the animals were sacrificed, and the volume and acid concentration of the gastric contents were analyzed. Acid concentration was determined by automatic titration (Autoburette ABU-13, Radiometer, Copenhagen, Denmark), and acid output was expressed as $\mu\text{Eq}/4\text{ hr}$. Physiological saline (0.9%) containing ethanol (0.5%) with or without (control) the test drug was given s.c. (2 ml/kg) just after the pylorus ligation. The pH of the saline was adjusted by the addition of hydrochloric acid (4 N).

Experimental procedures

Arbaprostil not preincubated with acid and 15(S)-15-methylprostaglandin E_2 (15(S)): The animals were randomly assigned to seven groups (each group $n = 10$) as follows: Group 1: treated with 0.9% saline containing 0.5% ethanol (pH 7.40, control); groups 2, 3 and 4: treated with 30, 100 and 300 $\mu\text{g}/\text{kg}$ of arbaprostil, respectively; Groups 5, 6 and 7: treated with 30, 100 and 300 $\mu\text{g}/\text{kg}$ of 15(S), respectively.

Arbaprostil preincubated with acid: We decided to use incubation solutions of pH 4.30, 2.75, and 1.20 because Reele (10) reported that arbaprostil exhibited antisecretory activity under the acid environment of the stomach only at pHs less than five in humans. Arbaprostil was incubated as follows: the appropriate amount of arbaprostil in ethanol was added to 0.9% physiological saline, the pH of which had been adjusted to 4.30, 2.75 or 1.20. Then, each reaction mixture was incubated at 37°C for 30 min. The animals were assigned to ten groups (each group $n = 10$) as follows: Group 1: treated with 0.9% saline containing 0.5% ethanol (pH 1.20, control); groups 2, 3 and 4: treated with 30, 100 and 300 $\mu\text{g}/\text{kg}$ of arbaprostil preincubated at pH 4.30, respectively; Groups 5, 6 and 7: treated with 30, 100 and 300 $\mu\text{g}/\text{kg}$ of arbaprostil preincubated at pH 2.75, respectively; groups 8, 9 and 10: treated with 30, 100 and 300 $\mu\text{g}/\text{kg}$ of arbaprostil preincubated at pH 1.20, respectively.

Radioisotopic analysis

[^3H]Arbaprostil was dissolved into absolute ethanol and incubated at 37°C for 30 min in 0.9% physiological saline, the pH of which was precisely adjusted to 4.30, 2.75 or 1.20. The reaction mixture was then extracted with 8 ml of ethyl acetate. The resulting organic phase was evaporated to dryness with a stream of nitrogen. The residue was then dissolved in a small amount of ethanol and applied to a precoated silica gel thin-layer plate (60F $_{254}$, Merck & Company, Inc., Rahway, NJ, U.S.A.). [^3H]Arbaprostil, [^3H]15(S) and 15-methylprostaglandin A_2 were also applied to

the same plates as comparative controls. The plates were then developed in acetone-dichloromethane-acetic acid (20 : 79.5 : 0.5). The plates were dried and scanned for their radioactivity by a radiochromato-scanner (JTC-601, Aloka, Tokyo, Japan). The radioactivity in each zone was expressed as the percentage of the total counts per minute recovered for the entire lane. The position of 15-methylprostaglandin A₂ was visualized by spraying with 10% phosphomolybdic acid in ethanol.

Drugs

[³H]15(R)-15-methylprostaglandin E₂ ([³H]-arbabprostil), [³H]15(S)-15-methylprostaglandin E₂ (specific activity, 100 MBq/mg each), 15(R)-15-methylprostaglandin E₂ (arbabprostil), 15(S)-15-methylprostaglandin E₂ and 15-methylprostaglandin A₂ were gifts from the Upjohn Company (Kalamazoo, MI, U.S.A.).

Statistical analysis

Data are expressed as the mean $\pm$ S.E. Statistical analyses were done by Student's *t*-test; P values less than 0.05 were taken as significant.

RESULTS

Antisecretory effects of arbabprostil (not preincubated) and 15(S) in pylorus-ligated rats

The control volumes of gastric juice and acid output obtained from the pylorus-ligated animals treated with 0.9% saline (pH 7.40) were 6.1 ± 0.6 ml/rat and 520 ± 74.4 μ Eq/4 hr, respectively (Fig. 2). Subcutaneous administration of 15(S) significantly inhibited both parameters in a dose-dependent manner (30–300 μ g/kg). Arbabprostil, on the other hand, did not cause any significant effect on the parameters even at the highest dosage examined (300 μ g/kg).

Fig. 2. Effects of arbabprostil (not preincubated) and 15(S) on gastric acid secretion in pylorus ligated rats. Arbabprostil, 15(S) and 0.9% physiological saline containing 0.5% ethanol (pH 7.40, control) were administered s.c. immediately after the pylorus ligation. Each value represents a mean $\pm$ S.E. from ten rats per group. *P < 0.05, ***P < 0.001, compared with the control group treated with 0.9% saline.

Antisecretory effects of arbaprostil preincubated with acid in pylorus-ligated rats

As depicted in Fig. 3, arbaprostil preincubated at pH 1.20 has been shown to have the most potent activity among the conditions of preincubation used; its potency was almost equal to that of 15(S) (see Fig. 2). Furthermore, it was noted that the control values were not significantly different at pH 1.20 (6.5 ± 0.4 ml/rat and 594 ± 44.9 μ Eq/4 hr) from those at pH 7.40.

Radioisotopic analysis of [3 H]arbaprostil

Incubation of [3 H]arbaprostil under different pH conditions (pH 4.30, 2.75 and 1.20) at 37°C for 30 min was shown to give rise to three compounds possessing respective Rf values of 0.32 ([3 H]15(S)), 0.38 ([3 H]-arbaprostil) and 0.87 (15-methylprostaglandin A₂). The typical thin-layer chromatography

pattern shown in Fig. 4 indicates that [3 H]arbaprostil can be epimerized extensively to [3 H]15(S) at pH 1.20. A more precise estimate is obtained from the mean equivalence of the radio counts given in Table 1.

DISCUSSION

The present study demonstrates that s.c. administration of 15(S)-15-methylprostaglandin E₂ (15(S)) induces significant reductions in gastric acid secretion in pylorus-ligated rats, while 15(R)-15-methylprostaglandin E₂ (arbaprostil) failed to do so. It was noticed, however, that arbaprostil preincubated at 37°C for 30 min with 0.9% physiological saline, the pH of which was precisely adjusted to 4.30, 2.75 and 1.20, caused a marked increase in potency of anti-secretory activity in a pH-dependent manner.

The most likely reason for this increase, as

Fig. 3. Effects of arbaprostil preincubated with 0.9% physiological saline for 30 min at 37°C, the pH of which was adjusted to 4.30, 2.75 or 1.20, on gastric acid secretion in pylorus ligated rats. Arbaprostil preincubated at each pH and 0.9% physiological saline containing 0.5% ethanol (pH 1.20, control) were administered s.c. immediately after the pylorus ligation. Each value represents a mean $\pm$ S.E. from ten rats per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the control group treated with 0.9% saline.

Fig. 4. Acid-promoted epimerization of [^3H]arbabrostil at different pHs. [^3H]arbabrostil was incubated for 30 min at 37°C with 0.9% physiological saline, the pH of which was adjusted to 4.30, 2.75 or 1.20. The incubation mixture was extracted and applied to a silica gel thin-layer plate. The radioactivity of each lane was scanned by a radiochromatoscanner. The locations of authentic [^3H]15(S) (a: $R_f = 0.32$), [^3H]arbabrostil (b: $R_f = 0.38$) and 15-methylprostaglandin A_2 (c: $R_f = 0.87$) are each indicated by a black circle.

Table 1. Acid-promoted epimerizations of arbabrostil based on radioisotopic analysis

Experimental condition (pH)	Percent of total net counts		
	arbabrostil	15(S)	15-methyl PGA $_2$
4.30	65.0 $\pm$ 3.0	3.1 $\pm$ 0.3	4.0 $\pm$ 1.1
2.75	59.6 $\pm$ 2.7	10.2 $\pm$ 0.6	3.2 $\pm$ 0.7
1.20	24.3 $\pm$ 1.6	23.4 $\pm$ 1.8	17.8 $\pm$ 0.6

Each value represents the mean $\pm$ S.E. of 3 separate experiments.

compared to unincubated arbabrostil, appears to be the presence of the active S-form arising from acid-catalyzed epimerization. According to Robert and Yankee (11), it seems that

arbabrostil and 15(S) equilibrate in acid media. Furthermore, Merritt and Bronson (12) reported that the rate of epimerization of arbabrostil in vitro in aqueous solution is dependent on the pH and temperature of the solution.

In the present study, our radioisotopic analysis demonstrated that [^3H]arbabrostil is epimerized to [^3H]15(S) in acid medium at 37°C , and the rate of conversion increased with a decrease in pH of the preincubated solutions. Although the experiment at pH 1.20 also demonstrated the formation of [^3H]15-methylprostaglandin A_2 from [^3H]arbabrostil, 15-methylprostaglandin A_2 (300 $\mu\text{g}/\text{kg}$) given s.c. did not affect gastric acid secretion in pylorus-ligated rats (data not shown). Thus, it appears that arbabrostil develops a potent antisecretory action solely due to its epimerization to the active S-form.

Previously, Mizuta et al. (13) have reported that arbabrostil possessed a strong antisecretory activity when administered intragastrically in rats. According to Osawa et al. (14), [^3H]arbabrostil administered orally could be converted to [^3H]15(S) in rats; the ratio of [^3H]arbabrostil and [^3H]15(S) in the gastric contents is about 5 : 1 at 15 min and 2 : 1 at 30 min after oral administration of 25 $\mu\text{g}/\text{kg}$ of [^3H]arbabrostil. Reece (10) demonstrated that the gastric acid antisecretory activity of arbabrostil administered orally in humans is totally dependent on the gastric luminal pH, but significant antisecretory activity was not obtained when the gastric pH exceeded five. It is well-known that acid secretion, which is one of the significant factors in peptic ulceration (4–6), is higher in peptic ulcer patients than normal subjects during both the day and the night (4), so arbabrostil would be rapidly epimerized to the antisecretory 15(S) at the gastric mucosa after oral administration to peptic ulcer patients.

In addition to this antisecretory activity, Okabe et al. (15) and Kollberg et al. (16) have previously demonstrated the cytoprotective activity of arbabrostil following oral administration in rats and humans. Furthermore, 15(S) as well as arbabrostil at non-secretory doses protects the gastric mucosa against

grossly observable injury caused by absolute ethanol when given s.c. in order to diminish the effect of epimerization (H. Takanashi et al., unpublished data). These observations suggest that arbaprostil could show cytoprotective activity before and after epimerization by acid.

In summary, since such epimerizations occur solely under low pH conditions, this explains why there are gastric antisecretory effects following the oral administration but not the s.c. administration of arbaprostil. Coupling these antisecretory effects with previously demonstrated cytoprotective effects (15, 16), the potential value of orally administered arbaprostil for the treatment of peptic ulcer in clinical settings (17–20) is clear.

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