Biochemical Pharmacology, Vol. 42, No. 10, pp. 1875–1878, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

EFFECTS OF THE ENANTIOMERS OF LANSOPRAZOLE (AG-1749) ON ($H^+ + K^+$)-ATPase ACTIVITY IN CANINE GASTRIC MICROSOMES AND ACID FORMATION IN ISOLATED CANINE PARIETAL CELLS

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(*Received* 26 *February* 1991; *accepted* 19 *July* 1991)

Abstract—The effects of the enantiomers of 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]-sulfinyl]-1*H*-benzimidazole (lansoprazole, AG-1749) on acid formation in isolated canine parietal cells and ($H^+ + K^+$)-ATPase activity in canine gastric microsomes were investigated. Both the (+)-and the (−)-enantiomer of lansoprazole inhibited the acid formation stimulated by dibutyryl cyclic AMP (db-cAMP) in isolated canine parietal cells in a concentration-dependent manner with IC_{50} values of 59 and 82 nM, respectively. The enantiomers showed concentration-dependent inhibition of ($H^+ + K^+$)-ATPase with IC_{50} values of 4.2 and 5.2 μM, respectively. On the other hand, the IC_{50} values of lansoprazole for db-cAMP-stimulated acid formation and ($H^+ + K^+$)-ATPase were 59 nM and 2.1 μM, respectively. These results suggest that the two enantiomers of lansoprazole have antisecretory action due to inhibition of ($H^+ + K^+$)-ATPase.

Lansoprazole‡ (Fig. 1), a substituted benzimidazole, is an antisecretory agent three times as potent as omeprazole and its action is due to inhibition of ($H^+ + K^+$)-ATPase, the final step in the secretory process [1]. Our previous study revealed that lansoprazole inhibited ($H^+ + K^+$)-ATPase by reacting with SH groups of the enzyme after it had been transformed into active forms such as sulfenamide and disulfide derivatives via a unique rearrangement reaction within the acidic compartment of parietal cells [2, 3]. Although lansoprazole has a chiral center at the asymmetric sulfinyl group, it is normally synthesized as a racemate showing no optical rotation. In contrast, the active forms of lansoprazole such as the sulfenamide and disulfide derivatives do not have a chiral center. Therefore, it would be interesting to know whether the two enantiomers of lansoprazole are converted to the active forms within the parietal cells in a similar way. Sigrist-Nelson *et al.* [4] reported that the enantiomers of Ro 18-5364, a substituted benzimidazole, had identical effects on ($H^+ + K^+$)-ATPase activity in gastric microsomes. However, the inhibitory action of the enantiomers of substituted benzimidazoles on acid formation in the parietal cells has not yet been reported. In this study, effects of the (+)- and (−)-enantiomers on acid formation in the isolated canine parietal cells and on ($H^+ + K^+$)-ATPase activity in canine gastric microsomes were investigated.

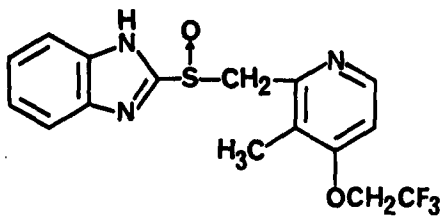

Fig. 1. Chemical structure of lansoprazole. Lansoprazole has a chiral center at the asymmetric sulfinyl group.

MATERIALS AND METHODS

Acid formation in isolated parietal cells. Three mongrel dogs (9.5–11.5 kg) of either sex were fasted for 24 hr before each experiment but had free access to water. The dogs were exsanguinated under pentobarbital anesthesia and their stomachs were removed. The fundic mucosa was bluntly dissected free from the underlying muscle layer. The tissue was minced with scissors and then exposed to collagenase (0.25 mg/mL) and EDTA to isolate the cells, according to the method of Soll [5]. A crude cell suspension was layered on the top of a preformed 50% Percoll gradient and the preparation was then centrifuged at 1000 rpm for 25 min, according to the method of Fryklund *et al.* [6]. The cell fraction with a density below 1.05 was collected and washed two or three times to remove the Percoll and was then suspended in Ca^{2+}-free Hanks' balanced salt solution (BSS) containing 1 mM EDTA, 10 mM HEPES and 0.2% bovine serum albumin. After being stained with Turk solution, parietal cells were identified by

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‡ Abbreviations: lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy) - 2 - pyridyl]methyl]sulfinyl] - 1*H* - benzimidazole; db-cAMP, dibutyryl cyclic AMP; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid.

their large size and concentric nuclei as observed under a light microscope (×200). Cell viability was tested by the trypan blue exclusion technique. The concentration of parietal cells was adjusted to $\sim 2 \times 10^6$ cells/mL and the cell suspension was used as the parietal cell-rich fraction (purity 50–70%; viability ~70%). Acid formation was measured as the accumulation of weak base [^{14}C]aminopyrine according to the method of Berglindh *et al.* [7]. The parietal cell-rich fraction ($\sim 4 \times 10^5$ cells/200 μL) was suspended in 2 mL of Earl's BSS containing 0.2 μCi [^{14}C]aminopyrine, 25 mM HEPES (pH 7.4), 0.2% bovine serum albumin (w/v) and various concentrations of test compound. A few minutes later, db-cAMP was added to a final concentration of 1 mM. The reaction mixture was incubated at 37° for 25 min under an atmosphere of 95% O_2/5% CO_2. Three aliquots (0.5 mL) of cell suspension were layered over 1 mL of Hanks' BSS in 1.5 mL-microfuge tubes followed by centrifugation at 9000 *g* for 1 min. The supernatant was discarded and the tip of each microfuge tube was cut off. The cell pellets were then digested with 0.5 mL of tissue solubilizer (NCS®). After a liquid scintillator was added, radioactivity was counted using a scintillation counter. The radioactivity in the cell pellet in the presence of 0.1 mM dinitrophenol was subtracted from the data to correct for trapped [^{14}C]-aminopyrine. The ratio of the concentration of [^{14}C]-aminopyrine accumulated in the parietal cells to that in the medium was used to evaluate the acid forming activity and was designated the aminopyrine ratio (AP ratio). The AP ratio was determined by the equation:

$$\text{AP ratio} = R_P/\text{parietal cell volume}/R_m$$

where R_P is the radioactivity accumulated in the cell pellet, R_m is the radioactivity per milliliter of incubation medium and the parietal cell volume is the product of the number of all parietal cells and the cell volume. The cell volume used was 3.1×10^{-9} cm^3, as determined by Soll [8]. The mean of AP ratios obtained from triplicate determinations using a single preparation is used as a representative value. As the AP ratio in the control group varied from experiment to experiment, percentage inhibition was calculated for each experiment and the results combined afterwards. Lansoprazole and the (+)- and (−)-enantiomers were all tested in each of three experiments.

$(H^+ + K^+)$-ATPase activity. $(H^+ + K^+)$-ATPase-rich gastric microsomes were partially purified according to the method of Wallmark *et al.* [9]. Five male mongrel dogs (10–15 kg) were fasted for 24 hr before each experiment but had free access to water. The dogs were exsanguinated under pentobarbital anesthesia and their stomachs were removed. The isolated canine stomachs were rinsed with tap water. The fundus was taken and placed in 3 M NaCl to remove superficial cells, cell debris, and mucus. The fundic mucosa was then scraped off. The mucosal scrapings were homogenized in a medium containing 0.25 M sucrose, 1 mM EDTA, and 5 mM Tris–HCl (pH 6.8) using a Polytron. The homogenate was differentially centrifuged to obtain microsomes. The crude microsomes were layered over 7.5% Ficoll (w/w) in 0.25 M sucrose and centrifuged at 100,000 *g* for 3 hr. The band appearing at the interface of the 0.25 M sucrose and the 7.5% Ficoll was collected, diluted with 0.25 M sucrose and centrifuged. The pellet was suspended in 0.25 M sucrose with a protein concentration of 0.5 mg/mL and stored at −20° until used. Protein concentration was measured according to the method of Lowry *et al.* [10] using bovine serum albumin as the standard. The activity of $(H^+ + K^+)$-ATPase was measured as follows: the reaction mixture contained, in a total volume of 1 mL, 70 mM Tris–HCl buffer (pH 6.8), 5 mM $MgCl_2$ and 20 μg/mL membrane protein with various concentrations of test compound in the presence or absence of 10 mM KCl and 10 μM valinomycin. The assay was carried out at 37°. After a preincubation period of 1 hr, the reaction was initiated by adding 2 mM ATP and the preparation was incubated for 20 min. The reaction was stopped by adding 1 mL of 12% trichloroacetic acid. Inorganic phosphate produced by ATP hydrolysis was determined according to the method of Fiske and Subbarow [11]. $(H^+ + K^+)$-ATPase activity was calculated from the difference between ATPase activities with or without potassium and valinomycin. The inhibitory effect of each compound was expressed as percentage inhibition with respect to the control $(H^+ + K^+)$-ATPase activity in each experiment and the values were combined afterwards. Lansoprazole and the (+)- and (−)-enantiomers were all tested using each of five microsomal preparations.

Chemicals and drugs. The following drugs were used: EDTA disodium salt, trichloroacetic acid, bovine serum albumin, 2,4-dinitrophenol (Wako Pure Chemicals, Osaka, Japan); Tris–ATP, valinomycin, collagenase type I (Sigma Chemical Co., St. Louis, MO, U.S.A.); db-cAMP (Boehringer Mannheim, F.R.G.). Lansoprazole was synthesized in our division and resolved into optically pure forms (99.9% each) by HPLC on a column of cellulose tris(3,5-dimethylphenylcarbamate). The specific rotation of the (+)- and (−)-enantiomers (c 1.1, chloroform) was +145° and −149°, respectively. All other reagents were the best grade available and were used without further purification. Test compounds were dissolved in methanol and then added to the reaction mixture.

Statistics. Data are expressed as means ± SE. IC_{50} values were calculated from the concentration–inhibition relationships by the method of least squares. Fiducial limits of the IC_{50} values were calculated according to Fieller's theorem [12].

RESULTS

Effects on acid formation stimulated by dibutyryl cyclic AMP in isolated canine parietal cells

Acid formation in isolated canine parietal cells was greatly stimulated by db-cAMP; the aminopyrine ratio was increased from 5 ± 4 to 96 ± 48 (N = 3). Lansoprazole (racemate) and the (+)- and the (−)-enantiomer of lansoprazole (10–1000 nM) suppressed the acid formation in a concentration-dependent manner. The IC_{50} values were 59, 59 and 82 nM, respectively (Table 1 and Fig. 2). The control AP ratio varied greatly from experiment to experiment,

Table 1. Effects of the enantiomers of lansoprazole on acid formation stimulated by db-cAMP in isolated canine parietal cells

Compound	Inhibition of acid formation IC_{50} (μM)
Lansoprazole	59 (42–84)
(+)-Enantiomer	59 (36–101)
(−)-Enantiomer	82 (47–171)

IC_{50} values were calculated from the concentration-inhibition relationships by the method of least squares. Experiments were done using three different cell preparations. Numbers in parentheses; 95% fiducial limits of IC_{50} values.

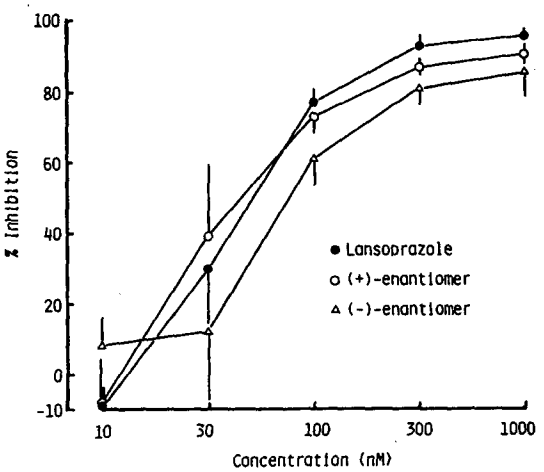

Fig. 2. Effects of the enantiomers of lansoprazole on acid formation stimulated by db-cAMP in isolated canine parietal cells. The AP ratio in the control group was 96 ± 48. The results are expressed as a % inhibition with respect to the acid formation in the control group. Data are means ± SE from three different experiments.

but similar concentration-dependency was observed for the two enantiomers in each experiment. Although a slightly greater potency was observed with the (+)-enantiomer, the difference was not significant.

Effects on $(H^+ + K^+)$*-ATPase activity*

The activity of $(H^+ + K^+)$-ATPase without exposure to lansoprazole or its enantiomers was 45.9 ± 5.3 μmol P_i/mg protein/hr (N = 5). Lansoprazole and the (+)- and the (−)-enantiomer of lansoprazole (1–30 μM) concentration-dependently inhibited the activity of $(H^+ + K^+)$-ATPase with IC_{50} values of 2.1, 4.2 and 5.2 μM, respectively (Table 2 and Fig. 3). Greater potency was observed with the (+)-enantiomer at 3 μM but inhibition by the two enantiomers did not differ significantly over the range of concentrations tested.

Table 2. Effects of the enantiomers of lansoprazole on $(H^+ + K^+)$-ATPase in canine gastric microsomes

Compound	Inhibition of $(H^+ + K^+)$-ATPase IC_{50} (μM)
Lansoprazole	2.1 (1.0–3.4)
(+)-Enantiomer	4.2 (2.2–7.1)
(−)-Enantiomer	5.2 (3.9–6.8)

IC_{50} values were calculated from the concentration-inhibition relationships by the method of least squares. Experiments were done using five different preparations. Numbers in parentheses; 95% fiducial limits of IC_{50} values.

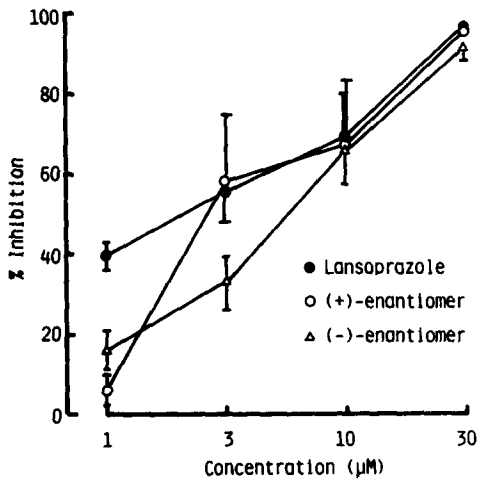

Fig. 3. Effects of the enantiomers of lansoprazole on $(H^+ + K^+)$-ATPase in canine gastric microsomes. $(H^+ + K^+)$-ATPase activity in the control group was 45.9 ± 5.3 μmol P_i/mg protein/hr. The results are expressed as % inhibition with respect to the control enzyme activity in each of five experiments. Data are the means ± SE from five experiments.

DISCUSSION

Lansoprazole has a chiral center at the asymmetric sulfinyl group, but it is normally synthesized as a racemate. In this study we compared the (+)- and (−)-enantiomers of lansoprazole. The (+)- and (−)-enantiomers inhibited $(H^+ + K^+)$-ATPase activity in isolated canine microsomes and acid formation stimulated by db-cAMP in isolated canine parietal cells. A slightly greater potency was observed with the (+)-enantiomer in both systems. However, as the difference was small and not statistically significant, the actions of the two enantiomers are considered to be identical in the two assay systems.

There was a great difference in the IC_{50} values for inhibition of acid formation and $(H^+ + K^+)$-ATPase. Similar results have been reported with omeprazole [13]. Substituted benzimidazoles seem to show a stronger effect in parietal cells than in enzyme preparations. This is due to (1) substituted benzimidazoles accumulating within the acidic compartment of the parietal cells because of their

weak base property and (2) conversion into active forms by an acid-catalysed rearrangement reaction [13].

In the experiment using microsomes, lansoprazole is gradually converted to active forms such as sulfenamide and disulfide derivatives which have no chiral center and the active forms inhibit $(H^+ + K^+)$-ATPase potently and immediately [2]. In this study, the two enantiomers inhibited $(H^+ + K^+)$-ATPase in gastric microsomes with almost the same potency. This is consistent with the report that the enantiomers of the sulfoxide agent Ro 18-5364 inhibit $(H^+ + K^+)$-ATPase with a similar concentration-dependency [4]. Therefore the (+)- and (−)-enantiomers of substituted benzimidazoles seem to be converted to their active forms in a similar way.

In isolated parietal cells, substituted benzimidazoles accumulate in the acid-forming space of the cells and are then activated [13]. In the present study, the effects of the (+)- and the (−)-enantiomer of lansoprazole on acid formation stimulated by db-cAMP in isolated parietal cells were almost identical. This indicates that the enantiomers of lansoprazole accumulate in the parietal cells and inhibit HCl production in a similar way.

From these results it is suggested that both enantiomers of lansoprazole have antisecretory action due to the inhibition of $(H^+ + K^+)$-ATPase and that the inhibitory effects of the two enantiomers are almost the same, at least in isolated parietal cells. Differences in pharmacodynamics and pharmacokinetics of the enantiomers remain to be studied.

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