Inhibition of Gastric H⁺,K⁺-ATPase Activity in Vitro by Dissolution Media of Original Brand-Name and Generic Tablets of Lansoprazole, a Proton Pump Inhibitor

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To investigate the inhibitory effect of a commercial proton pump inhibitor (lansoprazole) on the gastric proton pump H⁺,K⁺-ATPase in vitro, we used orally disintegrating (OD) tablets including original brand-name and generic tablets. In the course of the development of generic products, dissolution and clinical tests are necessary to ensure their bioequivalence to the original brand-name products; by contrast, there is almost no opportunity to demonstrate their activity in vitro. This study initially compared the similarity of the dissolution of test generic tablets with that of the original brand-name tablets. The dissolution tests for 15 and 30-mg lansoprazole tablets found their dissolution properties were similar. Subsequently, the dissolution media were sampled and then their effects on the H⁺,K⁺-ATPase activity were measured using tubulovesicles prepared from the gastric mucosa of hogs. We confirmed that the inhibitory effects of the generic tablets on H⁺,K⁺-ATPase activity were consistent with those of the original brand-name tablets. Furthermore, lansoprazole contents in each tablet estimated from their inhibitory effects were in good agreement with their active pharmaceutical ingredient content. To our knowledge, this is the first technical report to compare the in vitro biochemical activity of lansoprazole OD tablets between the original brand-name and generic commercial products.

Key words lansoprazole; H⁺,K⁺-ATPase; orally disintegrating tablet; dissolution property; generic product

Lansoprazole belongs to a group of drugs called proton pump inhibitors (PPIs). PPIs can specifically inhibit the gastric proton pump (H⁺,K⁺-ATPase) at the secretory surface of gastric parietal cells. 1,2 It is widely used for the treatment of active peptic ulcers, severe gastroesophageal reflux disease (GERD), and Zollinger–Ellison syndrome by suppressing gastric acid secretion. 3–4 From a pharmaceutical point of view, lansoprazole is assigned to class II drugs having low solubility and high permeability according to the biopharmaceutical classification system; it is a weak base (pKᵢₛ=8.87) and poorly soluble in water (3.2×10⁻² mg/mL at pH 7.0 and 25°C). Furthermore, lansoprazole is an acid-labile drug by nature; 5 thus, an enteric coating (pH-dependent coating) is essential for the oral dosage form. The absolute bioavailability of lansoprazole is sufficiently high: 91% for the 30-mg and 81% for the 15-mg enteric-coated formulations. 5

Currently, two different pharmaceutical forms of lansoprazole are available in clinical practice, 6 including capsule and orally disintegrating (OD) tablets. Both are classified into multiunit systems containing enteric-coated drug granules. 7–9 A multiunit system is considered to be superior to a single-unit system with respect to stability of biological action. This is in part because a multiunit system is less affected by the interdigestive migrating complex (IMC), which affects drug absorption. 7,10,11 Moreover, damage to the enteric coating of multiunit granules has less effect on the entire dose than it does on a single-unit system.

It is worth noting that the original brand-name lansoprazole OD tablet (Takepron OD), is the first OD product among commercial PPI pharmaceuticals. 6 OD tablets have recently been gaining much attention in the pharmaceutical industry because they are regarded as being patient-friendly dosage forms; they are easy to swallow and can substantially reduce the amount of water required for oral administration. In Japan, the patent of the Takepron lansoprazole OD tablet expired in 2005, and since then various generic OD tablets have been released on the market. For the development of a generic tablet, manufacturers need to demonstrate bioequivalence to the original brand-name product. The bioequivalence study for oral dosage forms includes an in vitro dissolution test and a human clinical study. 12 After confirming the dissolution similarity of the generic tablet to the original brand-name tablet, the bioequivalence study moves to the subsequent human clinical study to evaluate its therapeutic effect. This means that the effects of generic PPIs on H⁺,K⁺-ATPase activity in vitro have not been carefully evaluated during their development.

From these perspectives, this study focused on the inhibitory effect of the lansoprazole OD tablets on H⁺,K⁺-ATPase activity. The comparative study was conducted using the original brand-name tablet and a representative generic tablet. After confirming the similarity of dissolution of the test tablets, the solutions of the drugs were sampled and their inhibitory effects on H⁺,K⁺-ATPase activity were examined in vitro. We believe this is the first published report to compare lansopra-
prazole OD tablets between the original brand-name and generic commercial products in terms of the in vitro biochemical activity. The present study provides valuable information that furthers our understanding of these tablet qualities.

Experimental

Materials Lansoprazole was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Takepron OD tablets 15 and 30, original brand-name OD tablets, were from Takeda Pharmaceuticals (Osaka, Japan). Lansoprazole OD tablets 15 and 30 mg, generic tablets, were from Nichi-Iko Pharmaceuticals (Toyama, Japan). All other reagents were of analytical grade.

Dissolution Test The dissolution test was conducted using a dissolution tester (paddle method) (NTR-3000; Toyama Sangyo, Osaka, Japan) at 37 ± 0.5°C at a paddle rotation speed of 50 rpm with 900 mL of Japanese Pharmacopoeia XVII (JP 17) second fluid (pH 6.8, 0.05 M H1K2PO4 and 0.0236 M NaOH). Samples were withdrawn and filtered at the designated intervals. The concentration of lansoprazole was determined spectrophotometrically at 283 and 450 nm with a UV spectrophotometer (UV-1800; Shimadzu Seisakusho, Kyoto, Japan). To evaluate the similarity of the dissolution curves, a similarity factor (f2) was employed. This value is calculated from a logarithmic reciprocal square root transformation of the sum of the squared errors at all the points:

\[ f_2 = 50 \log \left( \frac{1}{n} \sum_{i=1}^{n} (F_i - S_i)^2 \right)^{1/2} \times 100 \]

The \( f_2 \) is a measure of the similarity in the percentage dissolution between the two curves. Further explanation is provided by Moore and Flanner.\(^{13} \) In this study, we obtained the dissolution ratio at 15, 30, 45, and 60 min to calculate \( f_2 \).

Hardness and Friability The hardness of the tablets was determined using a tablet hardness tester (Ogawa Seiki, Tokyo, Japan).

Friability was measured using a typical friability tester (TFT-1200; Toyama Sangyo). Approximately 6.5 g of the tablets was put into a friability tester, and then rotated at 25 rpm for 600 s. The obtained powders were sieved through a wire sieve with 500 µm holes (30 mesh) and the friability was calculated from the weight ratio of powder mass to the total tablet mass (approximately 6.5 g).

Disintegration Test The disintegration test was performed using a disintegration tester (NT-20H; Toyama Sangyo) and water (as solvent) at 37 ± 0.5°C. Disintegration time (DT) was defined as the interval required for the complete disappearance of a tablet or its particles from the tester net. DT was measured for three tablets of each formulation.

Measurement of Gastric H⁺,K⁺-ATPase Activity The tubulovesicles (TVs) of gastric parietal cells were prepared from isolated hog stomach obtained from Toyama meat center (Toyama, Japan). The procedure was fully described previously.\(^{14} \) The TV sample was freeze-dried and then stored in deep freezer at -80°C until used in the experiments. The H⁺,K⁺-ATPase activity was measured using a pyruvate kinase–lactate dehydrogenase-related system in which the hydrolysis of ATP was coupled with the oxidation of reduced nicotinamide adenine dinucleotide (NADH) in 2 mL of solution.\(^{15} \) The reaction mixture comprised 40 mM Tris–HCl (pH 6.8), 15 mM KCl, 3 mM MgSO₄, 1 mM ATP, 10 µg/mL valinomycin, 10 U/mL lactate dehydrogenase, 4 U/mL pyruvate kinase, 0.8 mM phosphoenolpyruvate, 0.2 mM β-NADH, 10% v/v of the dissolution media, and 60 µg of TVs. The decrease in the amount of NADH was measured using a Beckman DU 7500 UV-Vis spectrophotometer in the dual wavelength mode at 340 nm and 500 nm for 20 min at 25°C. Each specimen was tested in triplicate.

Results and Discussion

In the initial phase of this study, we investigated the dissolution properties of the test tablets. Because the lansoprazole granules in the OD tablets have an enteric polymer coating, this study monitored the dissolution behavior in the JP 17s fluid (pH 6.8). After about 15 min of lag time, all test tablets showed rapid drug dissolution (Fig. 1). The drug dissolution eventually reached over 90% by 120 min. The \( f_2 \) values between the original brand-name and generic tablets were calculated for both 15 and 30-mg tablets. The \( f_2 \) is widely used to compare the similarity between two different dissolution curves.\(^{13,16,17} \) In general, an \( f_2 \) value of 50 or greater (50–100) is regarded as being similar. This is based on the fact that the average difference between the two dissolution curves is less than 10% when the \( f_2 \) value is more than 50. Furthermore, the Japanese guidelines for bioequivalence studies of generic products describes the principles to judge the similarity of dissolution properties between test and reference tablets.\(^{18} \) According to the guidelines, the acceptance criteria for the test generic tablets are defined as follows. For the dissolution test at pH 6.8, “the average dissolutions of the test product are within those of the reference product ±15% at two appropriate time points when the average dissolutions of the reference product are around 40 and 85%.” Or \( f_2 \) value is not less than 42.” The calculated \( f_2 \) values were sufficiently high to ensure the dissolution similarity: 53.7 and 50.8 for 15 and 30-mg tablets, respectively. Therefore, for both 15 and 30-mg lansoprazole tablets, the dissolution properties of the test generic tablet were proved to be similar to the original brand-name tablet. We also investigated the dissolution similarity between 15 and 30-mg tablets for the original brand-name and generic tablets. The observed \( f_2 \) values were 54.4 and 55.7 for generic and original brand-name tablets, respectively. Thus, we confirmed that the test tablets possessed similar dissolution properties regardless of different lansoprazole contents.

An in vitro dissolution test is considered as a major surrogate for ensuring in vivo performance and product quality of oral dosage forms. In particular, it has been a powerful tool with which to evaluate whether products with acceptable quality are consistently manufactured. In the U.S.A., after the drug products are approved, quality defect is a problem that may pose a potential hazard to the public health and must be submitted to the Food and Drug Administration (FDA) via field-alert reports (FARs). Dissolution failures of oral drug products are included into the FARs. Sun et al. recently assessed 389 dissolution failures in FARs submitted to the FDA from 2005 to 2014.\(^{19} \) From the analysis, they found that dissolution failures occurred more frequently in modified-release (MR) drug products than those in immediate-release (IR) drug products. Furthermore, it is worth noting that there was no significant relationship between the frequency of dissolution failure and manufacturers; no significant difference in frequency of the
dissolution failure was observed between original brand-name and generic products. The lansoprazole OD tablets are classified as MR drug products. From this, there is a higher risk of dissolution failure than in ordinary IR drug products.

In addition to the dissolution property, we evaluated the other tablet properties. As shown in Table 1, hardness of the generic tablet was significantly higher than that of the original brand-name tablet. In accordance with the higher hardness, DT of the generic tablet was significantly longer. Although there was no difference in friability between the original brand-name and generic tablets, higher values were observed from 30-mg tablets. That is probably because of the larger tablet size; the weight of 30-mg tablets is twofold that of 15-mg tablets, resulting in an increase in the tablet size with higher lansoprazole content.

In the next phase of this study, the effects of the solutions of dissolved lansoprazole OD tablets on the activity of gastric H⁺,K⁺-ATPase were examined in vitro. For this experiment, the intracellular TVs of gastric parietal cells were prepared from hog stomach. H⁺,K⁺-ATPase, a nonelectrogenic H⁺,K⁺-antiporter, is abundantly expressed in the TVs. After the dissolution test for each tablet at pH 6.8 for 120 min, the dissolution media were sampled and then their efficacy for inhibiting the ATP-hydrolyzing activity of H⁺,K⁺-ATPase was examined. There was no significant difference in the inhibitory effects between the original brand-name and generic tablets (Fig. 2). In addition, H⁺,K⁺-ATPase activity steadily decreased.
with higher lansoprazole content in the tablets; % changes in H⁺,K⁺-ATPase activity were 60.2±0.6 and 38.2±3.8% for 15 and 30-mg generic tablets, respectively. We further estimated the availability of lansoprazole contained in each tablet for H⁺,K⁺-ATPase activity. For this purpose, the calibration curve, \( y = 97.19 \times x^{-1.47}/(5.68^{-1.47} + x^{-1.47}) \), between lansoprazole concentration and H⁺,K⁺-ATPase activity was obtained by using standard solutions dissolving a designated amount of lansoprazole in the JP 17s fluid (Fig. 3a). The IC₉₀ value was calculated to be 5.5µM and was similar to that reported previously.²⁴,²⁵ The lansoprazole contents in each tablet were calculated in terms of H⁺,K⁺-ATPase activity based on a calibration curve. As shown in Fig. 3b, the lansoprazole contents calculated from the H⁺,K⁺-ATPase activity were highly consistent with the amount of active pharmaceutical ingredient contained in each tablet. For example, the dissolution media collected from 15 and 30-mg generic tablets showed H⁺,K⁺-ATPase activities corresponding to 13.5±0.2 and 25.5±3.0µg of lansoprazole, respectively. It indicates that appropriate response of the lansoprazole can be expected, regardless of tablet excipients. In a preliminary experiment, we found that the excipients slightly increased the solubility of lansoprazole; the generic tablet was disintegrated in a small volume of the JP 17s fluid and then the lansoprazole concentration in the saturated solution was measured. The observed value amounted to 54µg/L (data not shown); this concentration was a little bit higher than the solubility (32µg/L at pH 7.0). However, in this case, such a slight increase in the solubility had no effect on the H⁺,K⁺-ATPase activity.

The bioequivalence of the lansoprazole OD tablets has been reported by Shimatani et al.²⁶ They investigated the acid-suppressive effect after repeated administration of various 15-mg OD tablets according to a crossover study in eight healthy Helicobacter pylori-negative CYP2C19 extensive metabolizers. Their study employed original brand-name tablets and three different generic tablets including the same excipients. In a preliminary experiment, we found that the excipients in the tablets did not have an influence on their H⁺,K⁺-ATPase inhibitory activities. We believe that these findings could offer profound insight into the manufacturing of the lansoprazole OD tablets.

**Conclusion**

The present study provides enhanced technical knowledge concerning the quality of commercial lansoprazole OD tablets. As well as similar dissolution curves, inhibitory effects of the generic tablets on H⁺,K⁺-ATPase activity were completely consistent with those of the original brand-name tablets. Furthermore, we confirmed that the excipients in the tablets did not have an influence on their H⁺,K⁺-ATPase inhibitory activities. We believe that these findings could offer profound insight into the manufacturing of the lansoprazole OD tablets.

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**Conflict of Interest**

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