ABSTRACT

We compared two lactase preparations derived from *Aspergillus orizae* (AOL) and *Penicillium multicolor* (PML) for stability in the stomach and overall enzymatic activity in 10 asymptomatic subjects with lactase deficiency. The subjects were given 10,000 FCC units of either AOL or PML 30 min prior to or simultaneously with 300 ml of milk. Gastric juice was withdrawn through a nasogastric tube immediately after and every 15 min for 60 min, and breath was sampled before and every 15 min for 6 h after the milk ingestion. When lactase was given simultaneously with the milk, gastric juice lactase activity and galactose concentration were significantly higher than the control levels. When lactase preparations were given 30 min prior to the milk, neither lactase activity nor galactose was detected in the gastric juice. The pH of the gastric juice was about 6.0 after the milk ingestion. Breath hydrogen did not increase when milk was ingested simultaneously with enzymes, but did increase if enzymes were given 30 min prior to milk ingestion. There were no significant differences in lactase activity, galactose concentration in gastric juice, or breath hydrogen when AOL and PML were compared. In conclusion, with exogenous lactase, digestion of lactose begins in the stomach when pH is raised to 6.0 by the buffering action of milk. Lactase preparations are effective assessed by breath hydrogen analysis in asymptomatic individuals with lactase deficiency if the enzymes are given simultaneously with milk.

Key Words: lactase deficiency, lactose intolerance, galactose, lactase, breath hydrogen

INTRODUCTION

Average milk intake in Japan and probably in other Asian countries is extremely low. We have shown previously that average consumption of milk is 200 ml per day among Japanese, which is 1/3 the amount consumed by Americans. Since we have many patients with osteoporosis, we recommend a diet richer in milk for its calcium content. The problem is, however, that almost all Japanese and Asians are lactase deficient. While 20% of Japanese are lactase intol-
erant, others generally without symptoms cannot tolerate large amounts of milk.\textsuperscript{1,2} To increase milk intake in our population, it is necessary to adopt some means to reduce the lactose content in milk. Prehydrolyzed milk with lactase is available in Japan, but it is not popular because of its sweet taste and limited availability. Several lactase preparations are available that require a medical prescription. The most practical way of making lactase preparations widely available may be to sell them on an over-the-counter (OTC) basis. However, the effectiveness of lactase preparations has not been studied in asymptomatic lactase-deficient subjects.

Enzymes including lactase have an optimal pH for activity. Many lactase preparations act optimally at pH 5.0,\textsuperscript{3,4} which is close to postprandial gastric pH.\textsuperscript{5} We have recently shown that lipase preparations taken with a meal start to act from the early gastric phase, probably because postprandial gastric pH rises to the optimal pH of lipase.\textsuperscript{6} If the same is true for lactase, gastric digestion of lactose will start from the stomach. If we give a lactase preparation that is stable at a lower pH, the preparation could remain active in the stomach in the fasting state, long before lactose-containing food products are ingested.

In the present study, we addressed the three following questions: Do lactase preparations stable at a relatively low pH remain active in the stomach for a relatively long period of time? Does gastric digestion of lactose occur as a result of the lactase preparation? Are lactase preparations effective in asymptomatic subjects with lactase deficiency?

SUBJECTS AND METHODS

Subjects
Ten people volunteered as study subjects. They were divided into two groups of five each. The first group (1 woman and 4 men, 23 to 25 years of age) was designated for gastric digestion study. The other group (2 women and 3 men, 20 to 42 years of age) was designated for breath hydrogen analysis. All subjects were healthy, had no history of gastrointestinal disease, and had not used any antibiotic for at least 3 months. In preliminary experiments, subjects were given 300 ml of milk, which contains about 18 g of lactose, and showed breath hydrogen levels of over 20 ppm. They tolerated ingestion of 300 ml of milk well and reported no subjective symptoms. The subjects were considered lactase deficient but not lactose intolerant.\textsuperscript{7} Written informed consent was obtained from all subjects, and the study was approved by the Human Research Committee of the Research Center of Health, Physical Fitness and Sports, Nagoya University.

Methods
The lactase preparations used in this study were based on lactase produced by \textit{Aspergillus oryzae} or \textit{Penicillium multicolor} (AOL and PML, respectively); both were generously supplied by Amano Enzyme Co. (Gifu, Japan). The characteristics of the enzymes are given in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>\textit{A. oryzae}</th>
<th>\textit{P. multicolor}</th>
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</thead>
<tbody>
<tr>
<td>Optimum pH</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>pH stability</td>
<td>4.0–7.0</td>
<td>2.5–7.0</td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>50°C</td>
<td>60°C</td>
</tr>
<tr>
<td>Thermal stability</td>
<td>&lt; 50°C</td>
<td>&lt; 60°C</td>
</tr>
<tr>
<td>Km for lactose</td>
<td>0.035 mol/L</td>
<td>0.011 mol/L</td>
</tr>
</tbody>
</table>
Both preparations (10,000 FCC units) were dissolved with 10 ml of distilled water before administration. The dose of the lactase preparations was determined from the \textit{in vitro} study as that to digest about 18 g of lactose. In the gastric digestion experiment, 34.2 g of skim milk powder containing 18.3 g of lactose was dissolved in 300 ml of distilled water. In the breath hydrogen analysis experiment, 300 ml of non-skimmed milk was used.

\textit{Gastric digestion protocol}

This study was conducted using a crossover design. After subjects fasted overnight, a nasogastric tube was inserted and they participated in one of the following tests: AOL given 30 min prior to milk ingestion; AOL given with milk; PML given 30 min prior to milk ingestion; PML given with milk; maltodextrin given with milk (as a control).

Ten milliliters of gastric juice was aspirated immediately after the subject swallowed the milk and every 15 min thereafter for 60 min. The pH of the gastric juice was determined and the specimen was divided into two parts of 5 ml each. One sample was used to determine lactase activity, which was measured by the FCC method at pH 4.5 with O-nitrophenol-\(\beta\)-D-galactopyranoside as a substrate. The other sample was used for galactose and HPLC analysis for mono- and di-saccharides. Enzymatic activity was inactivated by addition of 0.15 ml of 6N HCl in these samples. Galactose was measured with galactose test kits (Boehringer Mannheim, Tokyo). For HPLC analysis, 2.0 ml of acetonitrile was added and mixed to 0.5 ml of a 5-fold distilled water dilution of the supernatant obtained by centrifuging the inactivated enzyme samples. The mixture was put through a membrane filter and 30 \(\mu\)l of the filtrate was used for analysis. Since we did not know the actual gastric volume at any given time, mono- and di-saccharide values were divided by the original lactose value and showed as the percentage remaining in the stomach. Lactase activity and galactose concentrations were standardized by this rate.

\textit{Breath hydrogen analysis}

After an overnight fast, subjects were subjected to the same protocol as that for the gastric juice analysis. Breath samples were collected in a commercially available collection bag before milk ingestion and every 15 min after ingestion for 6 h. Lunch (a 100 g hamburger patty with coffee or tea) was provided at 4 h after the test was started. Hydrogen was measured with a gas chromatograph (MicroLyzer model 12i, Quintron Instruments, Milwaukee, WI, USA). The area under the curve (AUC) was calculated from the time breath hydrogen increased by 3 ppm over the baseline (oro-cecal transit time; OCTT) until the end of the experiment. Since the OCTT varied from subject to subject, the AUC was expressed per hour.

\textit{Statistical analysis}

Data are expressed as mean \(\pm\) standard error. The paired \(t\)-test was used for paired data and Bonferroni’s method was applied after analysis of variance (ANOVA) for multiple comparisons.

\section*{RESULTS}

\textit{Gastric digestion}

When lactase was given simultaneously with milk, lactase activity and galactose concentrations in gastric juice were significantly higher than under control conditions (Figs. 1 and 2). Lactase activity then decreased gradually to the control level. Galactose concentration increased to a maximum by 15 min and remained high. There were no significant differences in lactase
Fig. 1  Lactase activity in gastric juice. Mean ± SE values are shown. Open squares and open triangles indicate lactase from *A. orizae* (AOL) and *P. multicolor* (PML), respectively, given 30 min prior to milk ingestion, and solid squares and solid triangles indicate AOL and PML, respectively, given simultaneously with milk ingestion. Open circles indicate control values. (* p < 0.05 vs. control)

Fig. 2  Galactose concentrations in gastric juice. Mean ± SE values are shown. Open squares and open triangles indicate lactase from *A. orizae* (AOL) and *P. multicolor* (PML), respectively, given 30 min prior to milk ingestion, and solid squares and solid triangles indicate AOL and PML, respectively, given simultaneously with milk ingestion. Open circles indicate control values. (* p < 0.05 vs. control and # p < 0.05 vs. time 0)
GASTRIC DIGESTION OF LACTOSE

Fig. 3  pH of gastric juice. Only mean values are shown. Open squares and open triangles indicate lactase from *A. orizae* (AOL) and *P. multicolor* (PML), respectively, given 30 min prior to milk ingestion, and solid squares and solid triangles indicate AOL and PML, respectively, given simultaneously with milk ingestion. Open circles indicate control values.

Fig. 4  Breath hydrogen concentrations. Only mean values are shown. Open squares and open triangles indicate lactase from *A. orizae* (AOL) and *P. multicolor* (PML), respectively, given 30 min prior to milk ingestion, and solid squares and solid triangles indicate AOL and PML, respectively, given simultaneously with milk ingestion. Open circles indicate control values.
activity or galactose concentrations when AOL and PML were compared. When the lactase preparations, either AOL or PML, were given 30 min prior to the milk, lactase activity and galactose were not detected in the gastric juice. The gastric juice pH increased to 6.0 (mean; 5.8 – 6.3) soon after the ingestion of milk, remained high for at least 30 min, and then slowly decreased to below 4.0 (mean; 2.5 – 3.4) at 60 min (Fig. 3). There were no significant differences in pH values between any of the subjects under any condition.

Breath hydrogen

The breath hydrogen concentration did not increase when AOL or PML was given simultaneously with milk (Fig. 4). The AUC per hour was significantly lower when AOL or PML was given simultaneously with milk than in the control condition (p<0.05, Fig. 5). When lactase preparations were taken 30 min prior to the ingestion of milk, the breath hydrogen concentration increased and the AUCs did not differ from the AUC in the control condition. There was no significant difference in the AUCs between the preparations with AOL and PML.

**DISCUSSION**

The lactase preparations used in this study were derived from *A. oryzae* and *P. multicolor*. Both lactase preparations have similar enzymatic characteristics, such as an optimal temperature and pH for activity, and temperature stability. The pH stability, however, differs somewhat, in that PML is stable between 2.5 and 7.0 and AOL between 4.0 to 7.0. Because of this difference, it is possible that PML survives longer than AOL in the acidic gastric juice. In the present experiment, however, both enzymes lost their activity when administered 30 min prior to the milk ingestion. The pH in the fasting gastric juice might have been below 2.5, but this
was not measured. In our previous study with 84 subjects, fasting gastric juice pH in subjects younger than 40 years old was 1.3 to 2.1.\(^7\) Biller et al.\(^9\) suggested that lactase from A. oryzae might be more effective than that from Kluyveromyces lactis because of its increased stability over wider ranges of pH and temperature. However, they studied children with lactose intolerance, and the pH of gastric juice may be higher in children than in adults.

In an in vitro study, AOL but not PML lost its activity within 30 min in an acidic environment. Thus, we chose administration 30 min before as well as immediately before milk ingestion. Since both enzyme preparations given 30 min prior to milk ingestion became inactive, it does not seem necessary to conduct a further study with a longer period before milk ingestion.

The digestion of lactose by exogenous lactase starts from the gastric phase. This is analogous to lipid digestion by exogenous lipase. If we give lipase with a fatty meal, fat digestion starts in the stomach and the meal empties faster from the stomach.\(^1\) In this study, pH in the stomach increased to 6.0 after milk ingestion, creating an ideal environment for lactase activity. If lactase is given simultaneously with milk, pH stability is not a major problem in gastric digestion of lactose.

It is well documented that lactase preparations are effective for patients with lactose intolerance.\(^3,4,9-13\) From our breath hydrogen data, it seems reasonable to consider that milk will not cause symptoms in the subjects with lactose intolerance if sufficient amounts of lactase preparation are given. The dose should be 10,000 FCC units for 300 ml of milk. The effectiveness of lactase preparations, however, given to individuals with lactase deficiency but no gastrointestinal symptoms is not well demonstrated. Since there are no symptoms, it has been difficult to assess the effect of lactase preparations. With breath hydrogen analysis, it becomes possible to assess the effect of lactase preparations in such persons. When undigested lactose in milk enters the colon it is rapidly fermented to short chain fatty acids by colonic bacteria, liberating CO\(_2\), hydrogen, and, in some people, methane. The hydrogen diffuses into the blood and is exhaled in the breath. Thus, after ingestion of milk, measurement of hydrogen concentration of the breath indicates the amount of lactose that has entered the colon. As expected, both our lactase preparations were effective when they were given simultaneously with milk. They were ineffective when given 30 min prior to the milk.

About 20% of the population in Japan is lactose intolerant.\(^1,2\) The majority of Japanese are lactase deficient but display no clinical symptoms.\(^1,2\) Breath hydrogen in all subjects in our study increased over 20 ppm after ingestion of 300 ml of milk, thus indicating lactase deficiency.\(^7\) The average milk intake of adults in Japan is about 200 ml per day, which is far less than that of Europeans and Americans. Usually 75% of an individual’s calcium is obtained from dairy products. In the Japanese, calcium intake is very low in terms of the daily nutritional requirement. Thus, it is suggested by many nutritionists as well as in the mass media that Japanese should consume more milk to prevent osteoporosis. Since the Japanese and others who are lactase deficient cannot tolerate large amounts of milk, milk should be taken with lactase preparations in one form or another. Although administration of lactase preparations may increase milk intake in asymptomatic lactase deficient subjects, there are no data demonstrating this to our knowledge. To increase milk intake, however, administration of lactase preparations may be the best choice. Any type of lactase preparation will be useful if taken simultaneously with milk, and such preparations should be provided on an OTC basis to increase individual milk intake in countries like Japan.

In conclusion, digestion of lactose starts from the stomach by exogenous lactase when pH is raised to 6.0 by the buffering action of milk. Lactase preparations are effective for asymptomatic individuals with lactase deficiency if taken simultaneously with milk.
REFERENCES


