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ORIGINAL COMMUNICATION

Effect of exogenous β-galactosidase in patients with lactose malabsorption and intolerance: a crossover double-blind placebo-controlled study

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Objective: To evaluate the efficacy of the addition to milk, 5 min and 10 h before its consumption, of a lactase obtained from *Kluyveromyces lactis* in lactose malabsorbers with intolerance.
Design: Double-blind, placebo-controlled, crossover study.
Setting: University Hospital.
Subjects: In total, 11 male and 19 female (aged from 18 to 65 y, mean age 43.3 y) lactose malabsorbers with intolerance participated.
Interventions: Each patient underwent three H_2 breath tests, in a random order. We used 400 ml of cow's semiskimmed milk as substrate and a β-galactosidase obtained from *K. lactis*. The test A was carried out adding to the milk the enzyme (3000 UI), 10 h before its consumption; the test B was performed adding the β-galactosidase (6000 UI) 5 min before milk ingestion and the test C was made using placebo. We evaluated the maximum breath H_2 concentration, the cumulative H_2 excretion and a clinical score based on intolerance symptoms (bloating, abdominal pain, flatulence and diarrhoea).
Results: Our study showed a significant reduction of the mean maximum H_2 concentration after both test A (12.07 ± 7.8 p.p.m.) and test B (13.97 ± 7.99 p.p.m.) compared with test C (51.46 ± 16.12 p.p.m.) (ANOVA $F = 54.33$, $P < 0.001$). Similarly, there was a significant reduction of the mean cumulative H_2 excretion after both test A (1428 ± 1156 p.p.m.) and test B (1761 ± 966 p.p.m.) compared with test C (5795 ± 2707 p.p.m.) (ANOVA $F = 31.46$, $P < 0.001$). We also observed a significant reduction of the mean clinical score after both test A (0.36 ± 0.55) and test B (0.96 ± 0.85) compared with test C (3.7 ± 0.79) (ANOVA $F = 106.81$, $P < 0.001$). Moreover, with regard to the mean clinical score, there was a significant reduction after test A with respect to test B (Bonferroni's $P = 0.03$).
Conclusions: Our study shows that in lactose malabsorbers with intolerance, the lactase obtained from *K. lactis* can represent a valid therapeutic strategy, with objective and subjective efficacy and without side effects.
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Introduction

Lactose malabsorption is characterised by deficiency of lactase, an intestinal enzyme that hydrolyses lactose to its components galactose and glucose (Gilat *et al*, 1972). It is a very common condition, reaching 70% in southern Italy (Gudmand-Hoyer, 1994). High concentrations of lactase are physiologically present in neonates; nevertheless, postweaning, a genetically programmed and irreversible reduction of its activity occurs in the majority of the world's population (Wang *et al*, 1998).

The presence in the colonic lumen of malabsorbed lactose does not necessarily result in gastrointestinal symptoms.

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When this condition is related to uncomfortable clinical manifestations as bloating, abdominal pain and diarrhoea, 'lactose intolerance' occurs (Shaw & Davies, 1999). Worldwide, up to 50% of malabsorbers exhibits symptoms (Rosado *et al*, 1984). Nowadays, the usual behaviour for this condition consists of the avoidance of milk and dairy products from the diet. However, this restriction leads to a reduction of intake of substances such as calcium, phosphorus and vitamins, and may associate with decreased bone mineral density (Solomons *et al*, 1985a, b; Di Stefano *et al*, 2002). To overcome these limits, in the last years, several approaches have been studied: drugs that increase contact time between enzyme and substrate, either delaying orocoecal transit (ie loperamide) (Szilagyi *et al*, 1996, 2000) or delaying gastric emptying (ie propantheline) (Peuhkuri *et al*, 1999); continuous lactose consumption to induce colonic adaptation (Briet *et al*, 1997); substitutes for milk (Swagerty *et al*, 2002); yogurt and probiotics for their bacterial β-galactosidase activity (Onwulata *et al*, 1989; Saltzman *et al*, 1999); addition of exogenous lactase to the milk before its consumption (Solomons *et al*, 1985a, b; Barillas & Solomons, 1987; Corazza *et al*, 1992; Lin *et al*, 1993). In particular, replacement therapy resulted in an efficacious strategy; nevertheless, only few double-blind and/or placebo-controlled trials have been performed.

We have evaluated, in a double-blind, placebo-controlled, crossover study, the efficacy of the addition to the milk, 10 h and 5 min before its consumption, of lactase obtained from *Kluyveromyces lactis* in lactose malabsorbers with intolerance.

Patients and methods

We enrolled 30 patients (11 male, 19 female; aged from 18 to 65 y, mean age 43.3 y), referred to our Day Hospital of Internal Medicine and Gastroenterology because of symptoms compatible with lactose intolerance, and who were lactose H_2 breath test positive. Each patient underwent, in a random order, three H_2 breath tests. An interval of at least 72 h was allowed among successive tests, to avoid the effect of colonic acidification. We used 400 ml of cow's semi-skimmed milk as substrate (containing about 20 g of lactose), and a β-galactosidase obtained from *K. lactis* (Silact, Sofar SpA, Trezzano Rosa, MI, Italy).

The test A was performed adding the enzyme to the milk, with mild mixing, 10 h before its consumption (preincubated): the temperature of milk during incubation was 4°C; the test B was carried out adding the β-galactosidase 5 min before milk ingestion (mealtime) and the test C was made using placebo.

Concentration of β-galactosidase was 5000 U/ml; 0.3 ml corresponds to 1 drop of used enzyme. While in test A, we used 3000 U (2 drops) of lactase, and in test B we added 6000 U (4 drops). These quantities were chosen considering the units able to hydrolyse, in the two different preparations, at least 70% of present lactose, as suggested by the manufacturer. One neutral lactase unit is the quantity of enzyme that incubated at 25°C and pH 7.5 with *o*-nitrophenyl-β-D-galactopyranoside produces 1 μmol of *o*-nitrophenyl per minute. Test C was performed using aspartame, a sweetening substance not able to digest milk, because galactose and glucose are more sweet than intact lactose.

An investigator prepared milk with either enzyme or placebo in numbered containers, identical in shape and colour. Another blinded investigator administered treated milk to the patients. Enrolled subjects did not have any information about the content of milk.

The evening before the test, all subjects consumed a meal of only rice, meat and olive oil to avoid the probable influence, on the basal H_2 values, of a prolonged gas production due to the presence of nonabsorbable or slowly fermentable material in the colonic lumen. After an overnight fast, and a mouthwash with chlorhexidine to eliminate the possible early hydrogen peak due to the fermentation of the ingested sugar by oropharyngeal bacteria, patients received the milk with either enzyme or placebo. Smoking and physical exercise were forbidden 1 h before and throughout the test. Sampling of alveolar air was performed by means of a commercial device, which allows the first 500 ml of dead space air to be separated and discarded, while the remaining 700 ml of end-alveolar air is collected in a gas-tight bag (Gasampler Quintron, Milwaukee, WI, USA). Subjects were instructed to avoid deep inspiration and not to hyperventilate before exhalation.

For the analysis of H_2 concentration in air samples, we used a dedicated gas chromatograph (Model 12i, Quintron Instrument, Milwaukee, WI, USA). Breath samples were taken at fasting and every 30 min for 4 h after milk ingestion. Hydrogen concentrations were expressed in parts per million (p.p.m.). An increase in H_2 concentration of at least 20 p.p.m. above the basal value was considered indicative of lactose malabsorption. We evaluated the maximum H_2 concentration and the cumulative H_2 excretion, the latter obtained using the formula for the sum of areas of consecutive trapezoids, in accordance with Kotler *et al* (1982).

For 8 h following milk ingestion, all subjects kept a diary where they recorded the eventual occurrence of intolerance symptoms whose severity was indicated by a score. Considered symptoms were bloating (absent = 0; mild = 1; moderate = 2; severe = 3), abdominal pain (absent = 0; mild = 1; moderate = 2; severe = 3), flatulence (absent = 0; mild = 1; moderate = 2; severe = 3) and diarrhoea (absent = 0; present = 3). For every patient, a cumulative index was calculated by the sum of the partial score of each symptom. Obtained data were collected by a blinded investigator and were analysed by a blinded statistician.

Statistical analysis

Statistical analysis was performed by means of one-way analysis of variance (ANOVA). *Post hoc* comparison was

assessed with Bonferroni's correction. A *P*-value of 0.05 or less was considered significant. Data are expressed as mean ± standard deviation (s.d.).

Results

No statistical difference was found among the baseline H_2-concentration before test A (2.77 ± 1.98), test B (2.7 ± 1.6) and test C (4.31 ± 3.4) (ANOVA F = 5.05, $P = 0.008$). Our study showed a significant reduction of the mean maximum H_2 concentration after the addition of lactase to milk, respectively, 10 h (12.07 ± 7.8 p.p.m.) and 5 min (13.97 ± 7.99 p.p.m.) before its consumption with respect to placebo (51.46 ± 16.12 p.p.m.) (ANOVA F = 54.33, $P < 0.001$) (Figure 1); the reduction percentage was 77 and 73%, respectively.

Similarly, there was a significant reduction of the mean cumulative H_2 excretion when the lactase was added to milk both 10 h (1428 ± 1156 p.p.m.) and 5 min (1761 ± 966 p.p.m.) before its consumption with respect to placebo (5795 ± 2707 p.p.m.) (ANOVA F = 31.46, $P < 0.001$) (Figure 2); the reduction percentage was 76 and 70%, respectively.

The curves of breath hydrogen displaying half-hour intervals with mean (± s.d.) obtained in test A, test B and test C are shown in Figure 3.

We also observed a significant reduction of mean clinical score after the addition of lactase to milk either 10 h (0.36 ± 0.55) or 5 min (0.96 ± 0.85) before its consumption compared with placebo (3.7 ± 0.79) (ANOVA F = 106.81, $P < 0.001$) (Figure 4); the reduction percentage was 90 and 75%, respectively.

Finally, no differences in the mean maximum H_2 concentration and in the mean cumulative H_2 excretion were found with preincubated milk or adding enzyme at mealtime (Figures 1 and 2); instead, about the mean clinical score, there was a significant reduction when lactase was added 10 h rather than 5 min before milk consumption (Bonferroni's $P = 0.03$ group A *vs* group B) (Figure 4).

Discussion

The use of exogenous β-galactosidase in lactose malabsorbers was shown to be efficacious and without any side effects. Initially, this approach was judged as not practical because of the necessity to add the enzyme some hours before milk consumption (Rask Pedersen *et al*, 1982; Onwulata *et al*, 1989; Lin *et al*, 1993). Other studies, carried out to resolve this matter, have demonstrated the efficacy of the lactase also when added at mealtime (Solomons *et al*, 1985a, b; Barillas & Solomons, 1987; Corazza *et al*, 1992). Nevertheless, until now just a few double-blind and/or placebo-controlled trials have been performed. For this reason, although replacement therapy with lactase does not represent a novel strategy, we designed a double-blind, placebo-controlled, crossover study using two different prehydrolysed prepara-

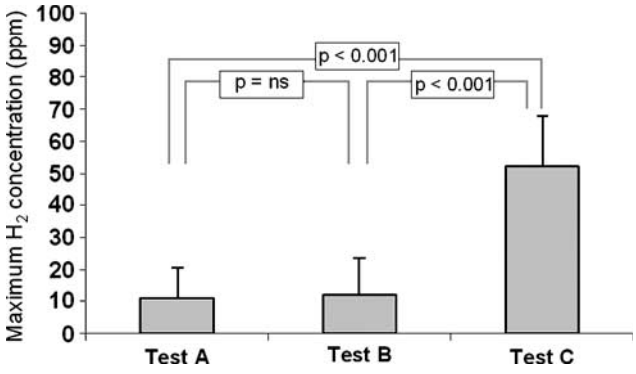

Figure 1 Comparison of the mean maximum H_2 concentration obtained in test A (preincubated), test B (mealtime) and test C (placebo). Statistical comparison was performed by means of ANOVA; *P*-values are calculated considering Bonferroni's correction.

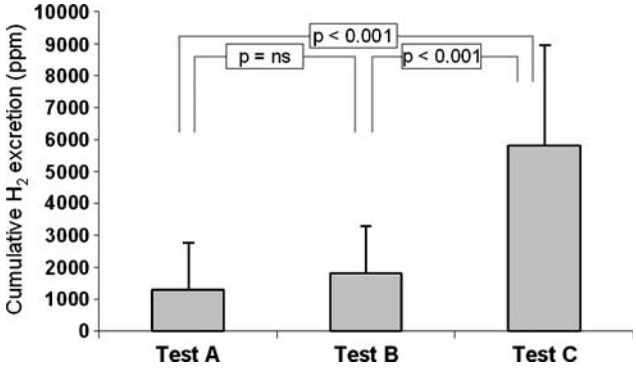

Figure 2 Comparison of the mean cumulative H_2 excretion obtained in test A (preincubated), test B (mealtime) and test C (placebo). Statistical comparison was performed by means of ANOVA; *P*-values are calculated considering Bonferroni's correction.

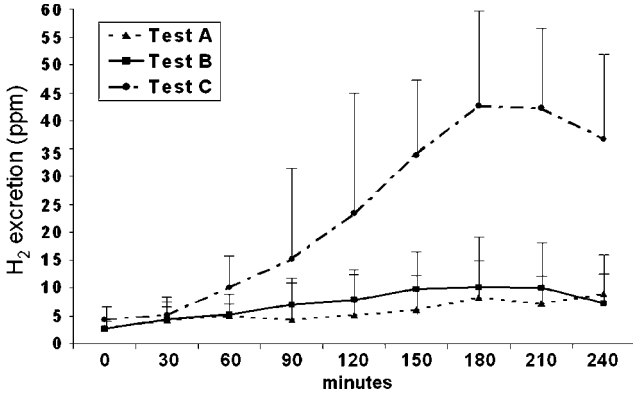

Figure 3 Comparison of the curves of breath hydrogen displaying half-hour intervals with mean (± s.d.) obtained in test A (preincubated), test B (mealtime) and test C (placebo).

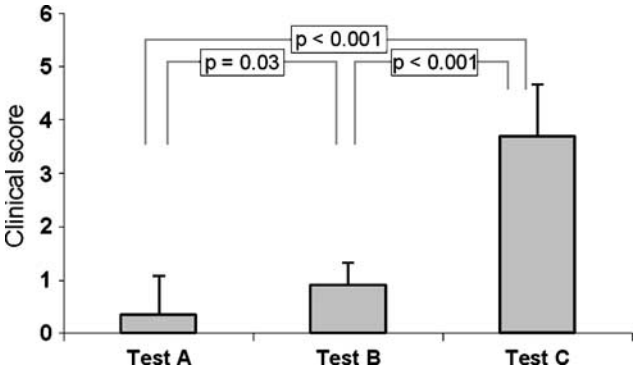

Figure 4 Comparison of the mean clinical score obtained after test A (preincubated), test B (mealtime) and test C (placebo). Statistical comparison was performed by means of ANOVA; *P*-values are calculated considering Bonferroni's correction.

tions obtained adding exogenous lactase 10 h before or at mealtime.

We found a significant reduction of the H_2 production and symptoms score when the milk was prehydrolysed by β-galactosidase with respect to placebo. In particular, both preparations were efficacious in reducing the mean maximum H_2 concentration and the mean cumulative H_2 excretion. These results do not fully resemble the previous studies and this can be explained by some observations: firstly, by the different enzyme origin. Comparative studies had already shown the major efficacy of the lactase derived from *K. lactis* with respect to the enzyme obtained from *Aspergillus niger* (Rosado *et al*, 1984; Solomons *et al*, 1985a, b). In this way, we could explain the results obtained by Corazza *et al*. In fact, they added a lactase derived from *A. niger* to the milk at mealtime and obtained a lower reduction both of maximum H_2 concentration (about 38%) and cumulative H_2 excretion (about 43%) than us (Corazza *et al*, 1992). The dose of enzyme is another factor that should be considered. Lami *et al* (1988), in a double-blind study, found a percentage reduction of the H_2 maximal peak of about 39% if 2000 UI of lactase from *K. lactis* was added at mealtime, and of about 58% when 1000 UI of the same enzyme was added 12 h before milk consumption. We can speculate that a linear relationship exists between the dose of lactose and the units of β-galactosidase required to improve digestion. So, our better results could be explained by the three-fold quantity of lactase that we used.

Also regarding clinical aspects, our study showed very satisfactory results. In particular, we found a significantly symptoms score reduction of both milk-lactase preparations compared with placebo; moreover, we have found a slight significant improvement when lactase was added 10 h rather than 5 min before milk consumption. It is not easy to explain the significance of this difference about subjective parameter. In our opinion, despite this little difference, we can advise the use of the enzyme also at mealtime, because of its greater practicality.

The clinical aspect should be considered more relevant than the H_2 excretion; in fact, in malabsorber patients with intolerance, since there are no known adverse effects of lactose maldigestion other than acute gastrointestinal symptoms, the major end point is to resolve the clinical picture. Just for this reason, the treatment is reserved exclusively for intolerant subjects (Suarez *et al*, 1995). Moreover, there is emerging evidence that malabsorbed lactose could act as a prebiotic and so could be beneficial against some lower intestinal diseases (Szilagyi, 2002).

Low-lactose/lactose-free products should be strongly suggested in malabsorber patients with intolerance to avoid the common behaviour of limiting intake of milk and dairy products. Certainly, the already on-sale lactose-free milk is more practical than addition of exogenous β-galactosidase. On the other hand, the possibility to use the lactase makes feasible milk consumption also when lactose-free milk is not available and when intolerant subjects are away from home (ie restaurant, cafeterias, home of friends, etc.). Moreover, other commercially available preparations of lactase (in capsules or tablets) could be very useful and easy to use, overall for solid dairy products. Nevertheless, previous studies have shown that these preparations were less effective than prehydrolysed milk, probably because of gastric inactivation of the enzyme (Onwulata *et al*, 1989), and more expensive (Suarez *et al*, 1995).

Finally, even though our diary was not specifically addressed to evaluate the palatability, none of our patients reported about taste alteration of the milk treated with exogenous lactase, as previously described (Onwulata *et al*, 1989).

Conclusions

In lactose malabsorbers with intolerance, the use of the prehydrolysed milk with the β-galactosidase obtained from *K. lactis* results in an effective and rational therapeutic approach, accepted by patients, without side effects, with objective satisfactory results (reduction of H_2 excretion) and with reduction of the gastrointestinal symptoms.

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