Research Article

Effects of Exogenous Lactase Administration on Hydrogen Breath Excretion and Intestinal Symptoms in Patients Presenting Lactose Malabsorption and Intolerance

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Objective. To establish whether supplementation with a standard oral dose of Beta-Galactosidase affects hydrogen breath excretion in patients presenting with lactose malabsorption. Methods. Ninety-six consecutive patients positive to H2 Lactose Breath Test were enrolled. Mean peak H2 levels, the time to reach the peak H2, the time to reach the cut-off value of 20 ppm, the cumulative breath H2 excretion, the areas under the curve, and a Visual Analogical 10-point Scale for symptoms were calculated. Genotyping of the C/T-13910 variant was carried out. Results. Following the oral administration of Beta-Galactosidase, in 21.88% of the cases, H2 Lactose Breath Test became negative (Group A), while mean peak H2 levels (74.95 ppm versus 7.85), \( P < 0.0000 \), in 17.71% (Group B) were still positive, with the H2 level 20 ppm above the baseline, but the peak H2 levels were significantly lower than those observed at the baseline test (186.7 ppm versus 66.64), \( P < 0.0000 \), while in 60.41% (Group C) they were still positive with the peak H2 levels similar to those observed at the baseline test (94.43 versus 81.60 ppm). All 96 individuals tested presented the C/C-13910 genotype nonpersistence. Conclusions. The response to oral administration of Beta-Galactosidase in patients with symptoms of lactose malabsorption presents a significant variability.

1. Introduction

Lactose malabsorption (LM), intolerance (LI) are a common condition affecting a large proportion of the world’s population [1]. The most common cause of lactose intolerance is lactase deficiency, a decreased production of the enzyme lactase in the small intestinal villi. In normal conditions, lactose is broken down in the small intestine by lactase, to glucose and galactose [2]. Lactase-deficient individuals are not able to cleave this disaccharide and may become symptomatic following the ingestion of lactose. In LM, undigested lactose is fermented by the colonic flora causing, in many subjects, symptoms of LI such as diarrhoea, bloating, nausea, borborygmi, and abdominal pain.

Several treatment approaches have been proposed over the last few years [3], namely, addition of exogenous lactase to intact milk [4, 5], low-lactose milk [6], yogurt, and probiotics, due to their bacterial lactase activity [7–9] and pharmacological and nonpharmacological strategies that can prolong the contact time between enzyme and substrate delaying gastrointestinal (GI) transit time [10] and lactose administration for colonic adaptation [11]. Enzyme-replacement treatment with microbial exogenous lactase (obtained from yeasts or fungi) represents a possible strategy for primary lactase deficiency. However, while several studies have confirmed the efficacy of solid lactase preparations in reducing both H2 excretion and symptoms [12, 13], other comparative studies have shown that these preparations are significantly less effective than prehydrolysed milk, probably due to the gastric enzyme inactivation [14]. Since LI management with Beta-Galactosidase oral supplements remains unclear and data regarding their efficacy in reducing the H2 breath concentration are inadequate, the aim of the present investigation was to assess whether supplementation with a standard oral dose of Beta-Galactosidase obtained from Aspergillus oryzae affects hydrogen breath excretion and GI symptoms in lactose intolerant patients.
2. Materials and Methods

2.1. Patients. After approval of our Ethical Committee, we have selected, between January 2011 and June 2011, 96 consecutive patients (80 females and 16 males, overall mean age 38.0 years, range 18–65) who attended to the Gastroenterology Outpatient Unit of University of Cagliari, Italy, for the presence of GI symptoms, abdominal pain, nausea, bloating, and borborygmi, following lactose ingestion and were evaluated for LM by means of H2 Lactose Breath Test (H2 LBT). All patients were positive to H2 Lactose Breath Test and were considered eligible for the study following a detailed explanation regarding the investigation. All patients agreeing to take part, following a detailed explanation regarding the investigation, signed an informed consent form before admission. Exclusion criteria were age <18 or >65 years, diagnosis of neoplasia, inflammatory bowel disease, previous GI surgery, history of allergy to milk proteins, lack of compliance, history of liver, kidney, lung, heart, metabolic, or neurological disorders, treatment with laxatives, antibiotics, and prokinetics, or any other treatment known to affect the colonic flora or motility in the month prior to the study. Patients were interviewed regarding GI symptoms (abdominal pain, nausea, bloating, and diarrhoea) and completed a questionnaire, including items regarding demographic data [e.g., sex, age, and body mass index (BMI)].

2.2. H2 Lactose Breath Test. The H2 LBT was performed using a breath gas analyzer Model 12i MicroLyzer Plus (Quintron Instruments, Milwaukee, WI, USA). Basal breath specimens were obtained after overnight fasting; the day before each breath test, the patients avoided eating slowly absorbed carbohydrates (bread, pasta, or fibre) in order to avoid delayed exhalation of hydrogen in the breath [15]. Cigarette and/or cigar smoking and physical exercise were not permitted within the 12 hours before the test in order to prevent hyperventilation and consequent changes in hydrogen content in the breath. Before starting the test, patients rinsed their mouths with an antiseptic wash (Chlorhexidine 0.05% 20mL), followed by tap water, to avoid a rapid hydrogen peak due to the effect of oral bacteria on lactose. After having evaluated the baseline H2 breath concentration, the patients swallowed 25 gr of lactose (equivalent of the lactose content in 500 mL of cow’s milk) dissolved in 300 mL of water. Over a 4-hour period, breath samples were collected at 30-minute (min) intervals (from 8 a.m. till 12 noon) by having the subjects exhale into a mylar foil gas two-bag system while the patient was in a sitting position [16, 17]. The H2 LBT, in agreement with the last International Guidelines (see, e.g., Rome Consensus Conference, 2007), was considered positive for lactose malabsorption if the H2 concentration, in the exhaled air, exceeded 20 parts per million (ppm) above the baseline during the monitoring period [18, 19]. Mean peak H2 levels (ppm), the time to peak H2 (min), and the time to reach the cut-off value of 20 ppm were calculated [20, 21]. Also, to better standardize data collection and further strengthen the hypothesis of our study, we evaluated the cumulative breath H2 excretion (ppm). We also assessed the value of the areas under the entire curve (overall concentration of exhaled H2 in 4 hours). Two H2 LBTs were carried out in each patient, the initial test and the test following intervention (15000 Units of an acid-resistant Beta-Galactosidase obtained from Aspergillus oryzae), and were administered 1 hour before lactose intake. As pointed out by the manufacturer, one tablet (7500 Units) is able to hydrolyse 16 gr of lactose; thus, 2 tablets (equal to 15000 Units) should be sufficient to hydrolyse 25 gr of lactose contained in the solution administered to the patient. To avoid the effect of colonic acidification, the mean time interval between the baseline test and following the intervention was 8 days (range 9-10 days) [22].

2.3. Gastrointestinal Symptoms. On the day of the test, during the 8 hours after substrate ingestion, all patients were asked to rate four symptoms (abdominal pain, nausea, bloating, and borborygmi) using a Visual Analogical 10-point Scale (VAS) (0, no symptoms, to 10, the severity of the symptom). For each patient, the VAS was calculated for each symptom as well as the cumulative VAS by adding together the single symptom VAS score of levels.

2.4. Genotyping. DNA was isolated from EDTA-blood using a Qiaprep blood DNA Extraction kit (Qiagen, Hilden, Germany). Briefly, 200 mL EDTA-blood was treated with protease for 15 min at 56°C followed by addition of AL lysis buffer and ethanol. The mixture was passed through a spin column and washed according to the manufacturer’s instructions. DNA was eluted with 100 mL LAE buffer and quantified on agarose gel using lambda DNA as the standard. The DNA fragment spanning C/T-13910 variants were amplified using the forward primer [5#-GGA TGC ACT GCT GTG ATG AG-3#] and reverse primer [5#-CCC ACT GAC GTA TCC TCG TG-3#] to also include positions.

Both sequencing and RFLP were carried out by using this PCR product, which was done without knowledge of the clinical data and the results of the LTT and LBT.

2.5. Sequencing. The PCR product was sequenced using an automated DNA Sequencer (ABI3100 Applied Biosystems, Ipswich, MA, USA) with the forward primer to read 400 base pairs (bps) in one direction. When necessary, the result was reconfirmed by sequencing the other strand with the reverse primer. All sequence data could be read with a high confidence level from 213830 to 214190 bps (i.e., 361 bps spanning the C/T-13910 upstream of the LCT locus). MCM6 reference gene sequence (GenBank reference sequence) was used because this SNP lies within the MCM6 locus, which is the neighbouring from the gene upstream of the LCT locus.

2.6. Restriction Fragment Length Polymorphism. The PCR product (300 ng) was digested with 1-2 Units of BsmFI restriction enzyme (New England Biolabs, Foster City, CA, USA) and 1-reaction buffer B in a 30 mL reaction volume. The reaction mixture was incubated at 65°C for 4 hours and then electrophoresed on a 2.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light (302 nm). On the basis of the sequence information around the 13910 upstream position of the LCT locus, the expected band
2.7. Statistical Analysis. The statistics data were analysed using SPSS software. We performed a test of normality on the quantitative variables mean peak H2 levels (ppm), the time to peak H2 (min), and the cumulative breath H2 excretion (ppm) to define the type of test for the hypothesis testing. Tests of normality were significant; therefore, we proceeded with nonparametric tests for testing hypotheses about the variables mean peak H2 levels (ppm), the time to peak H2 (min), the time to reach the cut-off value of 20 ppm, and the cumulative breath H2 excretion (ppm). Scores of abdominal pain, nausea, bloating and diarrhoea compared before-after and were tested with the Kruskal-Wallis test. To avoid spurious assessment of statistical significance between groups differences, we proceeded to analyze the data with ANOVA. In particular, we tested simultaneously Groups A, B, and C before and after oral intake of Beta-Galactosidase for the mean peak H2 levels (ppm), the time to peak H2 (min), the time to reach the cut-off value of 20 ppm, and the cumulative breath H2 excretion (ppm).

3. Results

Following the oral administration of tilactase, in 21/96 (21.88%) H2 LBT became negative (Group A, Figure 1), while mean peak H2 levels (74.95 versus 7.85 ppm) \( P < 0.0000 \). In 17/96 (17.71%) Group B, Figure 2) were still positive with H2 levels 20 ppm above the baseline but the mean peak H2 levels were significantly lower than those observed at the baseline test (186.7 versus 66.64 ppm) \( P = 0.0006 \); furthermore, the time to reach the cut-off value of 20 ppm (Figure 6) was significantly longer in A than that observed in B (141.42 versus 100.58 min), \( P = 0.02 \), and Group C (141.42 versus 115.34 min), \( P = 0.03 \), and the time to reach the peak H2 levels (Figure 7) was significantly longer in A than in B (205.71 versus 153.75 min), \( P = 0.001 \), and Group C (205.71 versus 183.10 min), \( P = 0.002 \). No statistically significant result was achieved by the analysis of the areas under the entire curve before and after Beta-Galactosidase administration.

All 96 individuals tested presented the C/C-13910 genotype, which is the polymorphism for the Sardinian population associated with lactase nonpersistence [23].

3.1. Effect of Tilactase on Symptoms. Following the oral tilactase consumption, a significant reduction in the mean

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**Figure 1**

**Group A. Mean peak H2 levels**

**Figure 2**

**Group B. Mean peak H2 levels**

**Figure 3**

**Group C. Mean peak H2 levels**
Table 1: Visual analogical scale for symptoms.

<table>
<thead>
<tr>
<th></th>
<th>Abdominal pain</th>
<th>Bloating</th>
<th>Nausea</th>
<th>Diarrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Beta-Galactosidase</td>
<td>2.54</td>
<td>5.02</td>
<td>1.62</td>
<td>0.88</td>
</tr>
<tr>
<td>After Beta-Galactosidase</td>
<td>2.07</td>
<td>3.42*</td>
<td>1.09</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Beta-Galactosidase</td>
<td>3.61</td>
<td>5.29</td>
<td>0.73</td>
<td>1.53</td>
</tr>
<tr>
<td>After Beta-Galactosidase</td>
<td>3.50</td>
<td>5.11</td>
<td>0.14*</td>
<td>0.53*</td>
</tr>
<tr>
<td><strong>Group C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Beta-Galactosidase</td>
<td>3.68</td>
<td>5.35</td>
<td>1.55</td>
<td>1.74</td>
</tr>
<tr>
<td>After Beta-Galactosidase</td>
<td>2.78*</td>
<td>4.47*</td>
<td>1.13</td>
<td>1.00*</td>
</tr>
</tbody>
</table>

*P = 0.02

4. Discussion

Results of the present study indicate that oral administration of 15000 Units (600 Units × gr lactose) of Beta-Galactosidase obtained from fermentation of Aspergillus oryzae, followed by ingestion of a water lactose solution, in lactose malabsorber

clinical scores for abdominal pain, bloating, and diarrhoea was observed in Group C; in Group B, a significant reduction in the mean clinical score resulted in nausea and diarrhoea, while in Group A a significant reduction in the mean clinical score resulted in bloating (Table 1).
individuals, with genetically related hypolactasia with the
C/T-13910 variant and GI symptoms of L1, is effective in sig-
nificantly decreasing the mean peak H2 levels and the cumu-
lative values of breath H2 excretion in approximately 40% of
subjects (Groups A and B), while, in 60% of subjects (Group
C), the breath H2 excretion levels do not change with respect
to the baseline test. The value of the areas under the entire
curve could play a predictive role regarding the test response
after administration of lactase in order to understand how
a greater or lesser concentration of global H2 breath could
affect the outcome of the test in relation to the amount
of lactase administered. However, no significant result was
observed after statistical analysis. Regarding the effect of
tilactase ingestion on GI symptoms, a significant reduction
in the symptom score with the exception of nausea was
observed. It is noteworthy that these data indicate a signifi-
cant variability in the responses to oral Beta-Galactosidase.
Our study, in agreement with other works, has shown that
there is no direct correlation between symptoms and H2
breath excretion. We also observed that the clinical response
after administration of a standard dose of lactase does not
correlate directly with the H2 concentration in exhaled air.
This fact is particularly evident in Group C in which, after
taking lactase, any substantial change is not observed with
regard to the mean peak H2 levels and the cumulative breath
H2 excretion values. Also, though interesting, unfortunately,
our work did not take into account body weight and BMI
value of patients; in fact, many studies have been conducted
with the aim of evaluating the relationship between body
weight and the dose required for a positive effect of tilactase.
A possible explanation for the interindividual differences
could be the effect of variations in the degree of lactose
digestion (LD) [24], of the potential gastric inactivation of
the enzyme [25], of the intestinal motility patterns [26],
or of the gastric emptying [27]. At the baseline test, some
significant differences between the groups were present; for
instance, in Group A, we detected a longer time to reach
the cut-off value of 20 ppm and the time to reach the peak
H2 levels. In Group A, a delay in the orocecal transit time
could possibly be a possible explanation for the longer time
to reach the cut-off value as well as the time to reach the peak
H2 levels. A delay in the small intestine transit time suggests
that longer exposure between the Beta-Galactosidase and the
lactose in the intestinal lumen could contribute to improve
the LD [28–31]. Furthermore, the decrease in intestinal transit
time, prolonging the action of the Beta-Galactosidase in the
intestinal lumen, decreases, in turn, the osmotic load of the
lactose which, as a nonabsorbable sugar, could accelerate
the intestinal transit time reducing the time available for
lactose hydrolysis [30–32]. However, the transit time is not
the only explanation behind the different responses obtained
in the three groups after taking lactase. As discussed, other
mechanisms, not better known and currently unconvincing,
could play a role.

In Group B, following Beta-Galactosidase, the H2 LBT
was still positive, with H2 levels 20 ppm above baseline, and
the peak H2 levels and the cumulative breath H2 excretion
were, however, significantly lower than those observed at
the baseline H2 LBT. Of note in this group is the fact that,
at baseline H2 LBT, the mean peak levels of H2 were signifi-
cantly higher than those at Groups A and C. In this group, the
significant but partial response to Beta-Galactosidase could
be due to a lower concentration of the epithelial enzyme,
and therefore higher levels of exhaled H2 could be achieved
by the larger amount of lactose reaching the colonic lumen
where it is fermented by the flora in the colon. In these
hypotheses, a higher dose of oral Beta-Galactosidase could be
a useful tool for increasing the hydrolysis of ingested lactose
in the small bowel, thus reducing the amount of undigested
lactose reaching the large intestine. However, the high levels
of exhaled H2, observed in Group B, could also be the result
of the colonic bacteria Beta-Galactosidase activity or the
amount of methanogenic bacteria present in the colon. The
hydrogen produced following lactose ingestion, by lactose-
tolerant patients, is likely, at different rates, oxidised by
methanogenic bacteria; therefore, it could be argued that,
in Group B, nonsignificant amounts of H2 are consumed
by methanogenic and/or sulphate-reducing bacteria. There-
fore, an interindividual variability in the microbiota and in
the colonic bacteria Beta-Galactosidase activity is possibly
involved in determining the difference in the amount of H2 in
the lactose colonic fermentation. For these reasons, in some
cases, an oral supplementation of oral lactase (over 15000
Units) could reduce the concentration of H2 expired in no
responder patients (Group C) or modify the severity of symp-
toms in Groups A and C. In approximately 60% of patients
(Group C), the oral administration of Beta-Galactosidase
was not effective in decreasing breath H2 excretion. The
“resistance” to the oral Beta-Galactosidase observed in these
patients could be the result of inactivation of the exogenous
enzyme. The Beta-Galactosidase, in order to effectively main-
tain the enzymatic activity in the conditions usually found
in the gastrointestinal tract such as gastric acidity and bile
centrations, requires the mechanical protection of the
enzyme during the gastric passage and against the action
of the bile [33]. It has been demonstrated that gastric acid
reduces bacterial lactase activity in 20–60 min [34]. If the
mechanical protection of the enzyme is disgregated in the
gastric lumen, the acid pH could reduce the action of the
residual Beta-Galactosidase. Gastric emptying and intestinal
transit should also be taken into consideration; a fast gastric
emptying and intestinal transit time with consequently a
shorter contact time between enzyme and substrate could
reduce the carbohydrate absorption. Studies that evaluated
the effect of metoclopramide and propantheline on lactose
digestion revealed that propantheline-induced prolongation
of gastric emptying improves lactose tolerance as measured
by breath H2 concentration compared to metoclopramide
[35]. It has already been hypothesized that the levels of breath
H2 excretion might influence the occurrence of symptoms
following lactose ingestion [36, 37].

In this respect, in the present series, the decrease in H2
levels in the breath does not seem to affect the symptom
scores; in fact, no significant positive correlation was found
between the peak H2 levels and the total symptom score, in
particular for abdominal pain and nausea.

The present data are in agreement with those by di Stefano
et al. [38] who found no correlation between the severity of
symptoms and the level of breath H2 excretion. The causes of the GI symptoms in lactose intolerant patients are not clearly understood. Several factors could contribute to the development of symptoms, for instance, psychological factors [39], functional GI disorders, visceral sensitivity, or bowel motor abnormalities [40]. This would appear to suggest that, in addition to the digestion of lactose in the small intestine, other factors may influence the onset of lactose intolerance symptoms. Recently, the involvement of colonic factors has been hypothesized [41–44]. The balance between the ability of the colonic microbiota to ferment lactose and the ability of the colon to remove the fermentation metabolites would influence the onset of lactose intolerance, making it either more severe or less severe. A low lactose fermenting capacity of the colonic microbiota, which leads to inefficient removal of malabsorbed lactose and/or its intermediate fermentation metabolites, e.g., glucose and galactose) or to a low absorption capacity of the colon or a low SCFA/gas-metabolizing capacity of the colonic microbiota which leads to poor removal of fermentation metabolites, may contribute to the development of symptoms. Although, following oral Beta-Galactosidase administration, an improvement in some GI symptoms, bloating in Group A and nausea and diarrhea in Group B, was obtained, our findings show that, particularly in patients not presenting a decrease in the H2 levels following oral Beta-Galactosidase administration (Group C), a more extensive improvement in abdominal symptoms (abdominal pain, bloating, and diarrhea) was observed. At the moment, we have no security if the improvement in the severity of symptoms observed in Group C is the result of a placebo effect or different metabolic response to lactase. Our results indicate that oral administration of 15000 Units of Beta-Galactosidase or different metabolic response to lactase. Our results indicate that oral administration of Beta-Galactosidase from Aspergillus niger in adult lactose malabsorption: a double-blind crossover study, Alimentary Pharmacology and Therapeutics, vol. 6, no. 1, pp. 61–66, 1992.


