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Clinical Trials Study

Efficacy of incremental loads of cow's milk as a treatment of lactose malabsorption in Japan

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Abstract

BACKGROUND

Lactose intolerance (LI) is commonly seen in East Asian countries [1]. Several literatures showed that lactose or milk loading has been used as a treatment of lactose malabsorption (LM) in Western countries, but there were no reports regarding this type of treatment in Japan. As lactose or milk loading requires ingestion of large amounts of lactose within a short period, this was considered to be too harsh for Japanese people because of their less habitual milk consumption (175 mL per day in average [2]) than western people. In this study, we demonstrated lactose tolerance acquisition in suitable way for Japanese.

AIM

The aim of this study was to examine the efficacy of lactose (cow's milk) loading treatment in patients with LM.

METHODS

Individuals with abdominal symptoms induced by milk or dairy products (LI symptoms) were identified by a questionnaire. A 20g lactose hydrogen breath test (20gLHBT) was carried out to confirm LM diagnosis and to evaluate co-existence of

small intestinal bacterial overgrowth (SIBO). Respondents diagnosed with LM were selected as study subjects and were treated with incremental loads of cow's milk, starting from 30mL and increasing up to 200mL at 4 - 7days intervals. After the treatment, changes in symptoms and LM diagnostic value of 20gLHBT were investigated. Stool samples pre- and post-treatment were examined for changes in intestinal microbiota using 16S rRNA. Informed consent was obtained prior to each stage of the study.

RESULTS

In 46 subjects with LI symptoms (10-68 years old, mean age 34 years old) found by questionnaire, 35 subjects (76.1%) were diagnosed with LM by 20gLHBT, and 6 subjects had co-existing SIBO.

The treatment with incremental cow's milk was carried out in 32 subjects diagnosed with LM (14-68 years old, median age 38.5 years old).

The mean period of the treatment was 41 ± 8.6 days. Improvement of symptoms was observed in 29 subjects (90.6%) [95% confidence interval: 75.0 - 98.0%].

Although 20gLHBT indicated that 10 subjects (34.5%) were found to have improved diagnostic value of LM, no change was observed in 16 subjects (55.2%).Analysis of the fecal intestinal microbiota showed a significant increase in *Blautia* in 7 subjects who became symptom-free after the treatment ($P = 0.0313$).

CONCLUSION

Lactose malabsorption was diagnosed in approximately 75% of the subjects who had LI. Incremental loads of cow's milk was regarded as a useful treatment for LM without affecting everyday life.

INTRODUCTION

Self-reported lactose intolerance (LI) affects approximately 45% of the Japanese population, according to survey in 2015 [3]. The average daily milk consumption by Japanese people was found to be around 175mL, indicating less habitual milk consumption than that of western countries, in spite of the nutritional benefit [2]. Current adjuvant treatment of lactose intolerance is self-administration of commercialized lactose-degrading enzyme before consuming milk or dairy products, yet its effect has been limited. Literature from other countries reported that colonic adaptation by daily milk or lactose consumption reduced LI symptoms in patients who also suffered lactose malabsorption (LM) [1], but patients who underwent this treatment were required to ingest large volumes of milk within a short period, and was considered to be too harsh for Japanese.

On the contrary, the abdominal symptoms can also be induced by psychological conditions, which should be ruled out from the lactose-induced symptoms [4,5]. To resolve this issue, LM is diagnosed non-invasively by the lactose hydrogen breath test (LHBT). In order to distinguish psychogenic symptoms, a single-blind comparative study (SBCS) is conducted on subjects with self-reported LI, as well as LHBT to diagnose LM. For subjects diagnosed LM, lactose tolerance acquisition treatment is conducted in a suitable way for Japanese, followed by the assessment of the treatment efficacy.

As other studies have reported intestinal microbiota changes when clinical symptoms are alleviated by daily milk intake [6], the analysis of intestinal microbiota is also conducted to assess the changes before and after the treatment.

MATERIALS AND METHODS

1. Subjects

A questionnaire survey was undertaken by Japanese people aged between 10 and 70 to identify subjects with abdominal symptoms due to cow's milk and dairy products consumption. The questionnaire asked for the amount of milk and dairy products that

caused abdominal symptoms and the severity of the symptoms; and milk allergy or other underlying diseases as exclusion criteria(Figure 1).

This study was approved by the Tokyo Women's Medical University Ethics Committee. Informed consent was obtained from subjects prior to beginning each stage of the study.

2. Clinical examinations

1) Diagnostic studies

A 200mL single-blind comparative study was conducted in order to identify abdominal symptoms caused by cow's milk (Study A) , and 20g-lactose hydrogen breath test (20g LHBT) was performed to diagnose with LM within these subjects (Study B). Study A and Study B were carried out separately, with a minimum one-week interval.

Study A: 200mL single-blind comparative study (SBCS)

Lactose-reduced milk (LRM) (contained approximately 1.9g of lactose/ 200mL) and general milk (GM) (unadjusted milk: contained approximately 9.8g of lactose/ 200mL), were used as the test materials of the study. The subjects started from ingesting 200mL of the test material (LRM or GM) after fasting, and abdominal symptoms, including bloating, abdominal pain, borborygmi, gas, diarrhea, were recorded for up to 3 h after the intake. Symptom severity was also recorded and classified into 5 grades, using visual analog scales (0: absence, 1: trivial, 2: mild, 3: moderate, 4: severe).

These two trial tests were separately performed with at least one week interval.

Outcomes of this study were evaluated and classified into 3 groups based on the characteristic of symptoms as follows.

(1) More obvious symptoms induced by GM than with LRM.

(2) Symptoms induced by LRM or unclear difference between two materials (unevaluable group).

(3) No symptoms induced by either material.

Study B: Lactose challenge test: 20g-lactose hydrogen breath test (20g LHBT)

The subjects were required to fast overnight, at least 5 h prior to the lactose challenge. At the start of LHBT, the subject exhaled into a gas collection bag, followed by ingestion of 20g lactose dissolved in approximately 150mL water. Breath samples were then collected at 30-minute intervals for 3 h (7 times in total). Abdominal symptom severity was also recorded during the test. The breath hydrogen concentration was measured by using MicroLyzer 12i (QuinTron Inst. Co. Inc., USA).

The diagnostic criterion for LM was set as 20ppm or more hydrogen level from the baseline.

In addition, diagnostic evaluation of small intestinal bacterial overgrowth (SIBO) was considered to indicate that the elevated breath hydrogen concentration and abdominal symptoms coexisted within 60 minutes from the start of the test.

2) Stool collection for analysis of intestinal microbiota.

Stool samples were collected from the subjects before and after the treatment to evaluate changes in intestinal microbiota. The stool samples were appropriately stored frozen until DNA extraction and microbiota profiling using the V4 region of the 16S rRNA [7], performed by Bioengineering Lab. Co., Ltd... An increase or decrease of intestinal microbiota population change before and after the treatment was evaluated by comparing each bacterium occupancy rate out of total bacteria.

3. The treatment method for LM: incremental loads of cow's milk

The subjects identified with LM were required to start the treatment immediately after completing the diagnostic studies. Subjects began taking 30mL of general milk around the same time every day on empty stomach, and the amount of milk was gradually increased by 30mL after 4 - 7days. If they were anxious about abdominal symptoms, they were allowed to maintain the same dosage up to 7 days. During the treatment period, subjects were required to record their general conditions, amount of

milk ingested, and symptoms. Subjects were instructed to avoid taking any other milk or dairy products on an empty stomach, except for the milk supplied for the study, otherwise dairy products were allowed in small amounts during or after meals. Throughout the treatment, subjects were also instructed to avoid taking confounding medicines such as antibiotics, probiotics, prebiotics, antidiarrheal agents, and intestinal regulators.

All subjects were informed about LM treatment protocol and consent was obtained prior to starting the treatment. Participants were also given the right to withdraw from the study at any time.

Doctors (authors) routinely monitored the progress of each subject fortnightly during the treatment period *via* phone or e-mail correspondence. Study participants were obliged to report any decline in their physical condition and follow care instructions from the physician where needed.

After the subjects succeeded in taking 200mL of milk for more than 4 days, a final examination was conducted to evaluate the efficacy of the treatment, described as below.

Evaluation of the therapeutic effect of the incremental cow's milk treatment

The subjects were required to return their completed questionnaire to their doctor after the completion of the treatment.

(1) Degree of symptoms improvement after the treatment

0: no symptoms, 1: trivial symptoms, 2: mild symptoms but improved, 3: moderate symptoms but improved, 4: no improvement .

(2) Capable volume of milk tolerated without anxiety about abdominal symptoms.

1: up to 50 mL, 2: up to 100 mL, 3: up to 150 mL, 4: up to 200 mL

4. Final examinations immediately after the treatment

After completion of the treatment, 20g LHBT was performed to examine changes in lactose tolerance before and after the treatment.

In addition, stool was also collected at the end of the study from participants, in order to identify changes in the intestinal microbiota by methods described previously.

5. Statistical analysis

Values were presented as mean ± standard deviation (SD).Fisher's exact test, paired t-test or Wilcoxon test was applied wherever appropriate. A two-sided p value of <0.05 was considered to be statistically significant. Logistic regression analysis was also applied to the 95% confidence interval (CI).All statistical analyses were performed using JMP.

RESULTS

1. Subjects

Following the questionnaire held between July 2017 and December 2019 regarding abdominal symptoms caused by lactose consumption, 55 subjects were recruited and 9 subjects were excluded according to the exclusion criteria, some of which refused to participate to this study (Figure 2). Hence, 46 subjects aged 10 - 68 (mean age: 34.0 years old, male/female: 16/30) participated in the study upon informed consent.

The amount of milk that the subjects recognized abdominal symptoms during their daily lives was found to be: 100mL in 9 subjects (19.6%), 150mL in 4 subjects (8.7%), 200mL in 19 subjects (41.3%), and 250mL or more in 7 subjects (15.2%). Five subjects (10.9%) did not answer as they were avoiding milk consumption. The remaining 2 subjects (4.3%) had abdominal symptoms induced by other dairy products, such as fresh cream.

2. Results of clinical examination

1) Diagnostic studies

Study A) 200mL single-blind comparative study (200mL SBCS)

The result consisted of (1) more obvious symptoms induced by general milk than lactose-reduced milk (tested positive) in 22 subjects (47.8%); (2) unevaluable symptoms

in 20 subjects (43.5%) (Symptoms induced by lactose-reduced milk in 16 subjects and unclear difference between two materials in 4 subjects); (3) no symptoms induced by either material (tested negative) in 4 subjects (8.7%)(Figure 3).

Study B) Diagnosis with LM from 20g LHBT and evaluation of SIBO

Thirty-five out of 46 subjects (76.1%) were diagnosed with LM.

Moreover, abdominal symptoms appeared at early stage (within 60 minutes from the start of the test) in 6 out of 35 subjects, suggesting that SIBO correlated with rise of breath-hydrogen.

Furthermore, reliability of the LM diagnosis by SBCS was also assessed. Setting the LM diagnosis by 20g LHBT as the gold standard, the diagnosis precision by SBCS was 80.8% (sensitivity 86.4%, specificity 50.0%).

2) The characteristics seen in LHBT among the group of unevaluable subjects classified based on the result of SBCS

The onset of abdominal symptoms during the LHBT in the unevaluable group was investigated and summarized in Table 1.

Abdominal symptoms appeared within 30 minutes after lactose ingestion (early onset of symptoms) in 9 out of 14 unevaluable subjects diagnosed with LM (tested positive in LHBT) (64.3%). On the other hand, early onset of symptoms was found in 5 out of 6 unevaluable subjects diagnosed with non-LM (tested negative in LHBT) (83.3%). Overall, 14 out of 20 subjects (70.0%) in the unevaluable group had early onset of abdominal symptoms from LHBT.

3. Results of the treatment with incremental loads of milk for LM

The treatment study was conducted on 32 out of 35 subjects who received a definitive diagnosis of LM, after excluding 3 subjects: 2 subjects regarded as inappropriate and 1 subject did not agree to the informed consent.

The age distribution was 14 - 68 years, with a median age of 38.5 years (male: female = 8:24).

The treatment period was 29 - 66 days (mean 41±8.6 days). All 32 subjects were compliant with treatment regime and completed the study schedule.

1) Evaluation of symptoms improvement

After the treatment, "no symptoms", "trivial symptoms", "mild symptoms but improved", "moderate symptoms but improved", and "no improvement" indicated in 7 (21.9%), 9 (28.1%), 8 (25.0%), 5 (15.6%), and 3 subjects (9.4%), respectively (Figure 4). Thus, symptoms were estimated to have improved in 29 out of 32 subjects in total (90.6%) [95% confidence interval: 75.0-98.0%].

Volume of milk which could be tolerated without anxiety of abdominal symptoms was classified into 3 capacity volumes: 200mL in 15 subjects (51.8%), 150mL in 7 subjects (24.1%), and 100mL in 7 subjects (24.1%).

2) Comparison of diagnostic values for LM by 20g LHBT before and after the treatment

Therapeutic effect was also evaluated by using objective data of LHBT on 29 subjects who showed symptoms improvement (Figure 5). Changes were defined based on 15ppm difference in diagnostic value before and after the treatment.

A decrease of more than 15ppm was seen in 10 subjects (34.5%), indicative of an improvement after the treatment. An increase of more than 15ppm was observed in 3 subjects (10.3%), whereas difference of 15ppm or less, meaning no change, was seen in 16 subjects (55.2%).

4. Result of intestinal microbial analysis before and after the treatment

Fecal microbiota was assessed on 29 subjects who had therapeutic effects.

1) There was no significant change in total bacterial occupancy before and after the treatment. However, there was a trending increase in *Lachnospiraceae Blautia* (median

+0.65, P = 0.0789), and a trending decrease in *Lachnospiraceae [Ruminococcus]* (median -0.50, P = 0.0773).

2) Change in bacterial occupancy rate based on degree of symptom's improvement.
There was a significant increase of *Blautia* in 7 subjects who became symptom-free after the treatment (P = 0.0313). (Figure 6)
On the other hand, the change of diagnostic values of LHBT on the 7 subjects after the treatment varied: decreased (improved) in 2 subjects, had no change in 3 subjects, and increased in 2 subjects.

DISCUSSION

It has only been 50 years since LI was recognized and scientifically analyzed. Recently, LI is defined as a clinical syndrome characterized by abdominal symptoms after lactose consumption. However, LI needs to be distinguished from lactose maldigestion or malabsorption, which are also subclinical conditions, where LM can also be indicative of inefficient absorption of lactose caused by primary and secondary decrease of lactase activity or other intestinal conditions. Diagnosis of LI requires comparison with inert placebo, endorsed by a National Institute of Health (NIH) conference [1, 6, 8, 9].

Lactose hydrogen breath test is currently considered as the gold standard for diagnosing LM, symptoms in this test are observed in a dosage-dependent manner.

Recently, there have been many studies that apply a 20-25g lactose dosage, as a more realistic dosage in LHBT for diagnosing LM [10]. Thus, 20g of lactose was used in this study.

Our previous study showed the prevalence of LM diagnosed by 20g LHBT was 52% among 31 subjects (Japanese adults), regardless of the presence of subjective symptoms caused by milk or dairy products consumption [11]. Of all the subjects with self-reported LI symptoms, 76.1% were diagnosed with LM, suggesting that, one quarter of the subjective symptoms may not be directly linked to LM. Furthermore, LM was

distinguished from symptoms of self-reported LI by 200mL SBCS. In our study, 43.5% were found to be unevaluable, revealing that abdominal symptoms are often influenced by psychogenic conditions.

On diagnosing LM by LHBT, in cases where oro-cecal transit time is within a normal range, the symptoms are believed to appear in 50-100 minutes after lactose ingestion. An increase in breath hydrogen is observed at least 60 minutes after lactose intake, peaking at around 120-150 minutes, indicating that breath hydrogen correlates with symptom onsets [12]. The "early onset of symptoms" was defined as appearance of abdominal symptoms within 30 minutes after lactose ingestion in LHBT, and accordingly, 70% of subjects in the unevaluable group tested by SBCS, had early onset of symptoms, suggesting a brain-gut interaction.

Moreover, the study led to assume that 6 out of 35 subjects diagnosed with LM were also suspected to have SIBO. Lactulose hydrogen breath test has been widely used to detect SIBO, while it does not have indicative criteria of SIBO. Lactose HBT, on the other hand, can be useful for SIBO detection as an increase in breath hydrogen can be detected within 90 minutes after lactose ingestion. Thus, LM with SIBO can be distinguished from LM alone (by observing a peak of hydrogen after 90 minutes) [13]. However, a study of patients with chronic diarrhea in China, which applied HBT with 10g-lactulose loading and 20g-lactose loading, reported that SIBO was more prevalent in patients with LI than those with LM. In this case, several overlapping pathological conditions were suspected [6, 14].

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder classified by Rome IV. IBS is characterized by abdominal pain associated with abnormal bowel habit, but IBS patients can also suffer from other GI and non-GI symptoms, including psychological symptoms and psychiatric comorbidity [15]. Some studies in China reported that 80-85% of the patients with diarrhea-predominant IBS also had LM [16, 17].

Despite some limitations on evaluating IBS or SIBO, the LHBT provides many key pieces of information, such as transition of breath hydrogen and symptoms onset

during the test. Therefore, non-invasive 20g LHBT was believed to be useful not only for diagnosing LM, but also for examining the cause of LI symptoms.

In Western countries, there are various methods of lactose load to treat LM, such as daily dose of 34g lactose for 2 wk [5], incremental milk intake starting from 118mL (4oz) up to 708mL (8oz) in 6 days [18], and incremental lactose intake starting from 0.3-0.6g/kg with adding 0.2g/kg/day (max 1.0g/kg) for 10-17days [19]. In these studies, some participants refused to continue the treatments due to severe abdominal symptoms from lactose intake. In some reports from Europe, 12g or less lactose was reported to be well tolerated with minimal or no symptoms [8, 9, 20], though even this low amount of lactose may still be intolerable for Japanese people. In our study, 9 out of 46 subjects had subjective symptoms caused by drinking 100mL of milk (approximately 10g of lactose), according to the questionnaire of self-reported LI symptoms. Hence, we started from 30mL of milk intake and gradually increased the amount in every 4-7 days until 200mL could be ingested successively. As a result, all subjects completed the treatment schedule without dropping out.

The mean treatment period was 41 days. After the treatment, 91% of the subjects showed improvement in their abdominal symptoms and 76% were able to drink 150-200mL of milk at a time without anxiety of abdominal symptoms. These outcomes suggested that our original treatment for LM with ordinary milk was effective for Japanese patients without affecting quality of life. In addition, this treatment could be widely applied to Asian and African people suffering from LM [6].

Comparing the diagnostic values of LM by 20g LHBT, undertaken before and after the treatment, abdominal symptoms improved only in one-third of the subjects and no change was seen in half of the subjects, suggesting that colonic adaptation was insufficient to see changes in diagnostic values regardless of improved symptoms. This could be due to limitations of this study such as lack of dietary restrictions except for milk, maximum amount of milk set at 200mL, and insufficient sample size.

Some reports hypothesized that reduced symptoms were related to lactose adaptation of colonic bacteria, while other clinical studies reported that lactose induced growth of

Bifidobacteria and *Lactobacillues* in intestinal microbiota [21,22]. Even though such bacteria were not observed in our study, it was interesting that there was significant increase of fecal *Blautia* in 7 subjects who became symptom-free after the treatment. It is known that fecal *Blautia* is likely to decrease in patients who have obesity, liver diseases, and diabetes [23]. A fecal microbiota analysis in another study also had an interesting finding that *Blautia* significantly increased among subjects with LM after daily intake of 250 mL of whole milk for 4 wk [24]. Therefore, an increase of fecal *Blautia* found in our study indicated a favorable intestinal environment.

CONCLUSION

The treatment by incremental loads of ordinary cow's milk was useful in treating LM without affecting quality of life. As three-fourths of the subjects with LI symptoms in our study were further diagnosed with LM and showed improved lactose tolerance post-treatment, this treatment may also benefit people with LI symptoms but unknown LM status.

ARTICLE HIGHLIGHTS

Research background

Self-reported lactose intolerance (LI) has been known to have high prevalence in Asian people. However, there has been no recent report in Japan regarding the prevalence of lactose malabsorption (LM). Some literatures showed that colonic adaptation by daily milk or lactose ingestion reduced LI symptoms in patients with LM, but such treatment has not been reported in Japan.

Research motivation

According to literature from Western countries, patients with LM who underwent milk or lactose loading therapy were required to ingest large volumes of milk within a short period. Applying the same treatment to Japanese people was considered high risk for abdominal symptoms during the treatment, due to less habitual consumption of milk

than western people. In this study, we implemented an original method of milk loading without affecting daily life of study subjects.

Research objectives

The aim of this study was to examine the efficacy of incremental cow's milk loading for treating patients with LM.

Research methods

We selected subjects with LI symptoms from a questionnaire, and the selected subjects underwent a 20g lactose hydrogen breath test (20gLHBT) for diagnosis of LM. We then conducted the treatment of incremental loads of cow's milk on the subjects diagnosed with LM, starting from 30mL and increasing up to 200mL at 4-7 days intervals. After the treatment, improvement of symptoms and LM diagnostic value of LHBT were investigated. Stool samples of pre- and post-treatment were examined for changes in intestinal microbiota using 16S rRNA.

Research results

By LHBT, LM was diagnosed in 35 out of 46 subjects with LI (76%) selected by questionnaire. Improvement of abdominal symptoms after the treatment was seen in 29 out of 35 subjects with LM (91%). The diagnostic value measured in LHBT before and after the treatment improved in 10 out of 29 subjects with reduced symptoms (35%), and no change was observed in 16 subjects (55%). Analysis of fecal microbiota showed a significant increase of *Blautia* in 7 subjects who became symptom-free after the treatment.

Research conclusions

Incremental loads of cow's milk that are commercially available was a useful treatment for LM without affecting daily lives of Japanese people.

Research perspectives

The incremental loads of cow's milk can be widely utilized for LM patients, as well as improving their QOL. We would like to further verify efficacy of the same treatment in a longer term study.

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