原　　著

Lactobacillus reuteri Tablets Suppress Helicobacter pylori Infection ―A Double-blind Randomised Placebo-controlled Cross-over Clinical Study―

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Abstract

We studied the effect of *Lactobacillus reuteri* strain SD2112 Tablets Reuterina (ERINA Co., Inc.), in suppressing *H. pylori* urease activity and to use the urea breath test (UBT) as a marker for the burden of infection. Method 1：Assessment of UBT and *H. pylori* density. Subjects were 33 *H. pylori*-positive patients from whom were obtained gastric biopsy specimens by upper gastrointestinal endoscopy. The correlation between UBT and *H. pylori* density was investigated. Individual UBT was established for each patient. Patients were divided by *H. pylori* density was 3 groups：Group I (low-density), Group II (moderate-density), and Group III (high-density). The individual UBTs were then correlated to the established *H. pylori* quantity. Method 2：Assessment of suppressive effect of *L. reuteri* on *H. pylori* urease activity. Subjects were 40 asymptomatic volunteers with an UBT exceeding 15‰, randomly allocated to four groups： Subjects in Group A underwent active treatment for 4 weeks (period 1) and placebo treatment for the following 4 weeks (period 2). These in Group B underwent treatment in reverse order. Those in Group C underwent placebo. Group D consisted of volunteers with negative UBT undergoing active treatment for the full 8 weeks. Result 1：UBT was 11.6±2.0‰, 22.1±2.6‰, and 35.4±7.6‰ in Groups I, II, and III, showing UBT that increased significantly (I vs. II：p＜0.01 and I vs. III：p＜0.05) based on *H. pylori* density. Result 2：Significant differences were seen in the decrease in UBT before versus after medication in Groups A and B. In Group A, lower UBT was maintained until the end of the full 8-week period. The overall decrease in UBT due to medication with *L. reuteri* Tablets was 69.7±4.0% (p＜0.05). Conclusion：Administration of *L. reuteri* Tablets ［Reuterina (ERINA Co.,Inc.)］ significantly decreased UBT in *H. pylori*-positive subjects, demonstrating that *L. reuteri* suppresses *H. pylori* urease activity and *H. pylori* density.

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Introduction

Helicobacter pylori has been recognized as a cause of gastric mucosal injury. The treatment of first choice in the management of *H. pylori*-positive peptic ulcer is eradication of the organism, but this fails, in 10-20% of patients undergoing eradication[1)〜5)]. A decline in eradication due to increased drug-resistant *H. pylori* has become a

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worldwide issue. No definitive treatment policy for refractory cases, in whom eradication of *H. pylori* has failed, has been established. A large number of asymptomatic *H. pylori*-infected subjects have no clinical need for eradication, yet, this group of "healthy" subjects should be considered at risk to develop disease if infection remains untreated[6]. Probiotic treatments designed to improve disordered indigenous intestinal bacterial flora are drawing increased attention. Of these, lactic acid bacteria have been reported to suppress *H. pylori* gastritis by reducing *H. pylori* density. We studied the effect of one of these probiotics, *Lactobacillus reuteri* strain SD2112 Tablets [*L. reuteri* tablet, Reuterina (ERINA Co., Inc.)], in suppressing *H. pylori* urease activity and used the urea breath test (UBT) as a marker for the burden of infection.

Materials and Methods

1. UBT assessment

UBT was used for diagnosing *H. pylori* infection. Fasting subjects were instructed to orally take a ^{13}C-urea preparation (UBIT Tablet 100mg, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), in the early morning with 100 mL of water while sitting. Exhalation specimens were collected on two occasions, before and 20 minutes after oral administration and UBT was conducted with ^{13}C-urea measurement (UbiT-IR300 ; Otsuka Electronics Co., Ltd., Osaka, Japan). The formula used to calculate ^{13}C-urea was : $\Delta^{13}C = \delta^{13}C$ (after administration of UBIT Tablets) $- \delta^{13}C$ (before administration of UBIT Tablets). Based on the results of large-scale prospective clinical trials in Japan[7], a 2.5% cut off value for UBT was used. UBT exceeding 2.5% was taken as positive. It was shown elsewhere[8] that the reproducibility of UBT increases with time. Detailed assessment of this report revealed a trend for UBT to be unstable at or above 50‰. Another report[9] has shown marked variation in urease activity not be attributable to technical diagnostic reasons, although instability terded to occur when urease activity was high. Based on these reports, we studied UBT 8 weeks before medication and established UBT value as a standard, compared to UBT, the standard vs before medication. Subjects in whom the difference in UBT standard versus before was 50‰ or higher were excluded from the present analysis because of low reliability.

2. Correlation of UBT to *H. pylori* density

Informed consent was obtained from 33 *H. pylori*-positive patients (age : 28-63 years ; mean : 49.2±9.90 years ; 30 males and 3 females) undergoing upper gastrointestinal endoscopy at Kyorin University Hospital. Gastric biopsy specimens were obtained and individual UBT was established for each patient. Biopsy samples were collected from two sites on the greater gastic curvature, the antrum and the upper corpus. Each specimen was immediately embedded in Seed Tube HP (Eiken Chemical, Tokyo, Japan) and stored at 4℃. Each biopsy specimen was placed in 1mL of buffered sodium chloride peptone solution (Nissui, Tokyo, Japan), and inoculated onto Skirrow's media (Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) at 37℃ for 5-7 days under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). The amount of *H. pylori* was expressed as described elsewhere[10]. The correlation between UBT and bacterial density was investigated. Patients were divided into three groups by *H. pylori* density : Group I [less than 1×10^5 colony forming units (cfu) /mL], Group II (between 1×10^5 and up to 5×10^5 cfu/mL), and Group III (more than 5×10^5 cfu/mL). Patients in these groups were matched for age, gender and disease. The individual UBT was then correlated to the established *H. pylori* density.

3. Assessment of suppressive effect of *L. reuteri* on *H. pylori* urease activity

Informed consent was obtained from 179 asymptomatic volunteers (age : 18-65 years ; mean : 44.5±10.5 years ; 84 males and 95 females). Of 40 volunteers in the study, 5 were *H. pylori*-negative and 35 were *H. pylori*-positive (47.8±10.7 years ; 19 males and 21 females) .We studied UBT four times ; 8weeks before, before, 4 weeks after and 8 weeks after medication. Volunteers with an UBT exceeding 15% were randomly allocated to either Group A, B or C. Group A (n = 15, 48.1±13.5 years) and Group B (n = 15, 48.9±7.05 years) entered a randomised, double-blind crossover study where the effects of *L. reuteri* tablets (active ingredient 10^8 Colony Forming Units of *L. reuteri*, strain ATCC 55730) was compared to the effect of identical placebo tablets. Placebo tablets contained no *L. reuteri*, and the isomalt content was slightly higher to compensate for weight. The daily dose was 4 tablets. Group A received active treatment for 4 weeks (period 1) and placebo treatment for the following 4 weeks (Period 2). Subjects in Group B received the study treatment in the reverse order, i.e., placebo tablets for 4 weeks

Fig. 1 Correlation between UBT and *H.pylori* density
  * p＜0.05 (Wilcoxon signed-ranks' test)
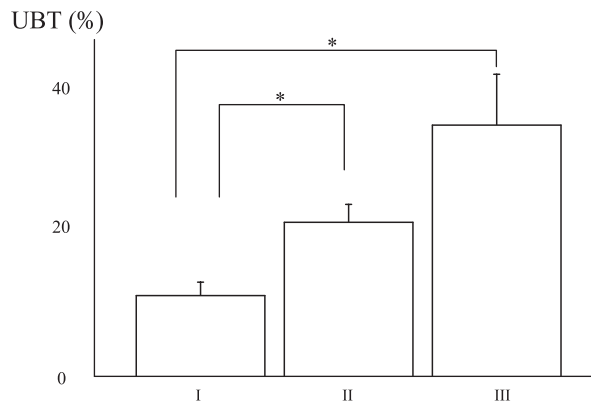
(Period 1) and *L. reuteri* tablets during the following 4 weeks (Period 2). Group C (n＝5, 48.8±11.6 years) was also part of the randomised double-blind study, but received placebo for the full 8 weeks. The purpose of this group was to assess the natural intraindividual variation in UBT measurement over time. To measure true variations in UBT, volunteers had to have an initial UBT of 15‰ or more. Group D (n＝5, 42.6±9.39 years) consisted of volunteers with a negative UBT and were treated for the full 8 weeks with 4 tablets daily of *L. reuteri* tablets. The purpose of this group was to ascertain that *L. reuteri* per se did not influence UBT assessment. Exclusion criteria were chronic diseases, those receiving oral medications such as antacids and antibiotics ; yogurt consumption, and participation in other clinical studies within the preceding 4-week period.

4. Analysis

All data obtained was expressed as mean standard error of the mean (SEM). The significance of difference between groups was analyzed using the Wilcoxon test, and software used was Stat view ver. 5.0 for Macintosh. The study protocol was submitted to the Kyorin University Ethical Committee for review and approval.

Results

1. UBT and *H. pylori* density

As shown in Figure 1, UBT was 11.6±2.0‰ (mean±SEM), 22.1±2.6‰ and 35.4±7.6‰ in Groups I (low density group : $0.4×10^5±0.1×10^5$ cfu/mL), II (moderate density group : $2.5×10^5±0.4×10^5$ cfu/mL) and III (high density group : $8.8×10^5±0.7×10^5$ cfu/mL), showing that UBT increased significantly (I vs. II : $p＜0.01$ and I vs. III : $p＜0.05$) with to bacterial density.

2. Suppressive effect of *L. reuteri* Tablets on *H. pylori*

（a）Variation in UBT

As shown in Figures 2a through -2d, significant ($p＜0.05$) differences were seen in the decrease in UBT before versus after 4 weeks of medication in Group A (n＝13) and before versus after 8 weeks of medication in Group B (n＝11), whereas no significant difference was seen in Group C (n＝5) or D (n＝5).

（b）UBT decrease

The decrease in UBT during the period that subjects took *L. reuteri* Tablets was studied in 24 subjects in Groups A and B [6 subjects were disqualified and excluded from the study]. Decrease was assessed based on UBT before and after 4 weeks of medication in Group A and before and after 8 weeks of medication in Group B. In the comparison with before medication, UBT changed 65.3±5.8% after 4 weeks of medication ($p＜0.05$) in Group A, and 74.9±5.2% after 8 weeks of medication ($p＜0.05$) in Group B, i.e., UBT differed significantly. No significant difference was seen in the decrease in Group C or D. Figure 3 shows UBT variation during oral medication with *L. reuteri* Tablets in all Group A and B subjects. The overall decrease in UBT due to medication with *L. reuteri* Tablets was 69.7±4.0% ($p＜0.05$) during this period, i.e., the difference was significant.

（c）Number of individuals responding to treatment.

All in all, 19 of the 24 subjects responded favourably to treatment with *L. reuteri* tablets whereas only 10 responded to placebo treatment ($p＜0.01$, Fisher's exact test). It is noteworthy that 7 of the 10 who responded favourably to the placebo treatment belonged Group A, indicative that they could be "late" responders to *L. reuteri*.

Discussion

There are arguments for and against the correlation between *H. pylori* density and UBT. Some reports[11)12)]

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Fig. 2   Changes in UBT in Group A (a), B (b), C (c) and D (d). UBT treatment differs in Group A and B. For Group A (n = 13), UBT was before versus 4 weeks after medication. For Group B (n = 11), UBT was before versus 8 weeks after medication. Group C (n = 5), and Group D (n = 5).
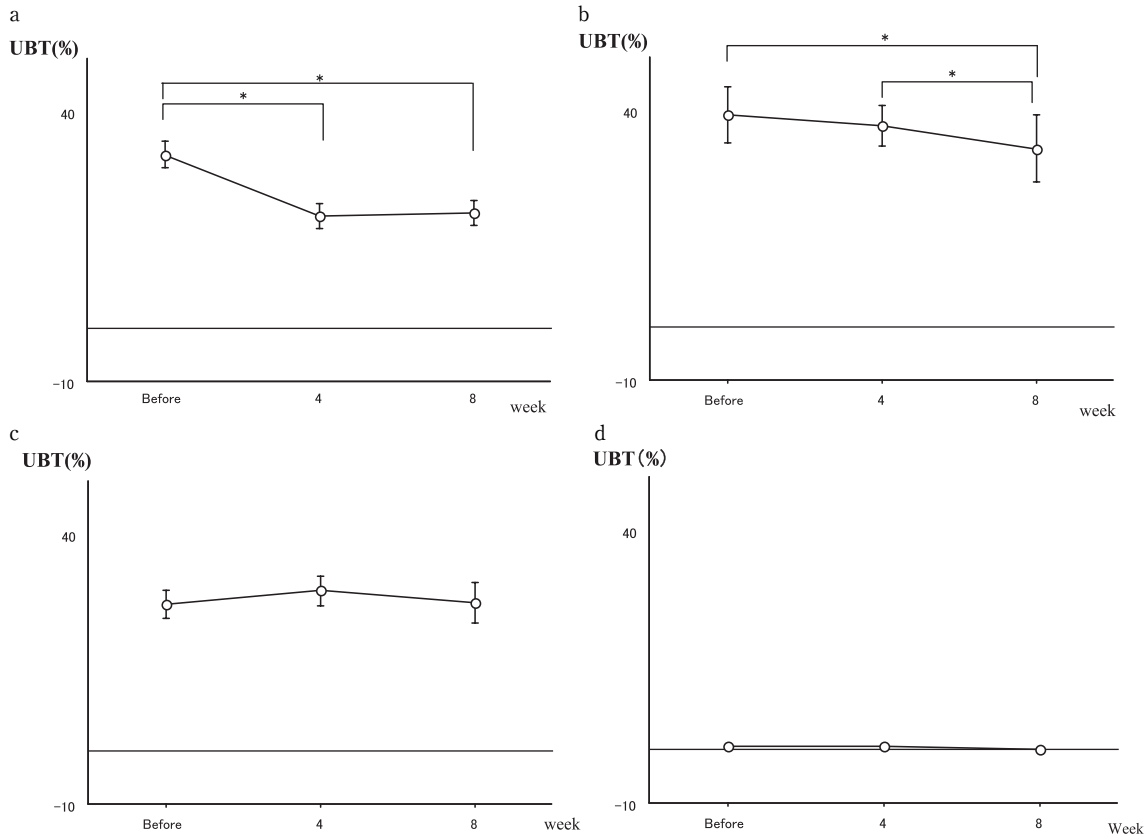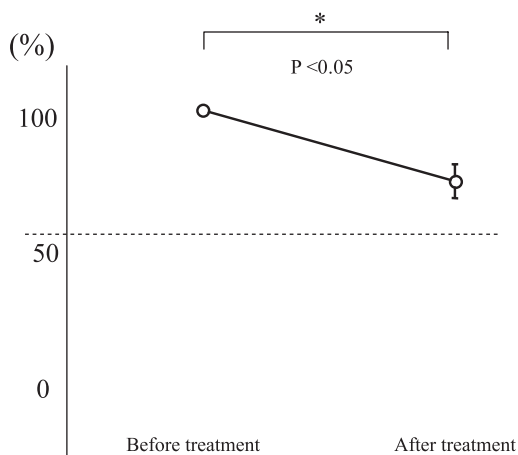

Fig. 3   Reduction in UBT from the beginning of oral medication (n = 24) with *L. reuteri* Tablets to the end in all subjects.

have indicated a significant correlation between *H. pylori* density determined histologically and UBT, while others[9] have shown no correlation between these factors. We compared the possible correlation between *H. pylori* density and UBT in three groups with different bacterial densities, i.e., low, moderate, and high. As shown in Figure 1, a significant difference was seen in UBT among these three groups, suggesting a correlation between bacterial density and UBT. Regarding the etiology of gastric mucosal injury, some reports[13)14] have indicated that ammonia is produced by *H. pylori* urease activity and that reversed hydrogen ion diffusion produces associated injury. Another report[9] discussing the correlation between *H. pylori* urease activity and the gastritis score, while yet another[15] showed that UBT is useful for evaluating density and activity

of *H. pylori* and the severity of gastritis. We showed a significant correlation between *H. pylori* density and the gastritis score[10]. When *H. pylori* density increased, the pathogen also presumably increased and induced gastric mucosal injury in the host.

Probiotics are increasingly being used as dietary supplements, to promote health, and to treat of digestive organ diseases[16)17]. *Lactobacillus gasseri* OLL 2716 (LG21), a probiotic, is reported to decrease UBT, the pepsinogen I to II ratio, and serum *H. pylori* antibody titer in *H. pylori*-positive patients[18]. LG21 is believed to suppress *H. py-*

lori density. We used UBT to assess the suppressive effect of *L. reuteri* on *H. pylori* in healthy volunteers. As shown in Figure 2a, administering *L. reuteri* Tablets in Group A resulted in significantly decreased UBT at the end of the first-4 week period and this lower UBT value was maintained until the end of the full 8-week period, during the latter half of which subjects took placebo Tablets. In Group B, UBT decreased during *L. reuteri* administration (Figure 2b). Consistent with these results, some reports[19)20)] have shown that *L. johnsonii* La1 strain (*Lactobacillus*), administered to mice, survived for a long time in the intestine even after administration was discontinued. In the present study, *L. reuteri* showed prolonged survival in the stomach and the intestine[21)] even after the administration of *L. reuteri* Tablets was discontinued.

L. reuteri, which is a common intestinal bacterium in vertebrates, is known to be nonpathogenic and very safe. It is reported to produce an antibiotic-like substance and to adhere to the digestive tract, suppressing the attachment of pathogenic bacteria to the digestive tract mucosa[22)]. This antibiotic-like substance, reuterin (3-hydroxypropionate aldehyde), is from anaerobically growing organisms and is produced in the presence of glycerol[23)]. It has a broad antibacterial spectrum, which includes Gram-positive bacteria, Gram-negative bacteria, yeasts and other fungi, according to one report[24)]. It is reported[25)] that *L. reuteri* inhibits *in vitro* attachment of *H. pylori* to the gastric mucosa and that *L. reuteri* inhibits the growth of *H. pylori in vitro*. Decreased UBT with *L. reuteri* administration, in the present study, may be explained by an action of reuterin on *H. pylori* involving indirect inhibition of attachment of *H. pylori* to the gastric mucosa, suppressing of *H. pylori* density.

H. pylori infection is known to stimulate both the gastric mucosa and lymphocytes, promoting the secretion of cytokines including interleukin (IL)-8 and TNF-α, and to cause gastritis. *H. pylori* infection induces inflammation in gastric mucosa and that causes gastritis. In the above discussion on LG21, IL-8 production was suppressed by LG21 in vitro. There are also reports[26)] showing that *Lactobacillus* suppresses these cytokines and ameliorates inflammatory intestinal diseases and allergic reactions. *L. reuteri* ATCC 55730 given exogenously in tablet form leads to a significant colonisation of the human gastric antrum[21)], the major site of *H. pylori* infection. Thus, a close interaction between *L. reuteri* and the pathogen can be expected *in vivo* after administration of the probiotic. These reports raise the possibility that *L. reuteri* Tablets suppress *H. pylori*-derived gastritis by suppressing gastric mucosal cytokines.

Conclusions

Administration of *L. reuteri* Tablets 〔Reuterina (ERINA Co.,Inc.)〕 significantly decreased UBT in *H. pylori*-positive subjects, demonstrating that *L. reuteri* suppresses *H. pylori* density. It is hoped that, *L. reuteri* Tablets can be used both to prevent asymptomatic *H. pylori* positive subjects to develop clinical symptoms and to improve gastritis, and also improve symptom and gastritis in *H. pylori* infected patients, in whom *H. pylori* eradication has failed.

Acknowledgement

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Lactobacillus reuteri 含有プロバイオティクスの *Helicobacter pylori* 抑制効果に関する検討

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　この研究は，*Lactobacillus reuteri* strain SD 2112 株（*L. reuteri*）の *H. pylori* 抑制効果の検討である．今回，我々は *L. reuteri* 含有整腸剤［*L. reuteri* 錠，Reuterina（ERINA CO., INC.）］の効果について検討を行った．【検討1】H. pylori 菌量と UBT 値の相関：*H. pylori* 陽性消化性潰瘍患者の 33 名を，Group I（低菌量群），Group II（中菌量群），Group III（高菌量群）の3群に分類し，*H. pylori* 培養と同時に尿素呼気試験（UBT）を行った．【検討2】*L. reuteri* の *H. pylori* 抑制効果：*H. pylori* 陽性者 35 名と陰性者5名に，二重盲検法による交差試験で *L. reuteri* 錠または placebo 錠を内服させた．UBT は基準値として測定した内服開始8週間前の値の他，内服開始前，開始後4週間および8週間に検査した．【成績1】*H. pylori* 菌量と UBT 値の間には，正の相関が見られ，Group I と Group III で有意差を認めた（p＜0.05）．【成績2】UBT は，*L. reuteri* 錠の内服期間（A 群＋B 群）で，前値 100％ から 69.7±4.0％（p＜0.05）と有意に減少した．一方で，C 群（placebo 内服群）・D 群（*H. pylori* 陰性 *L. reuteri* 錠内服群）では変動は認めなかった．【結論】*L. reuteri* 含有整腸剤の内服により，UBT 値は有意に減少した．また，*L. reuteri* は *H. pylori* 菌量も減少させる可能性が示唆された．