Efficacy and safety of *Lactobacillus reuteri DSMZ17648* in patients infected with *Helicobacter pylori* and not having absolute indications for eradication therapy

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Abstract. The study of efficiency and safety of two modes of 28-day monotherapy with *Lactobacillus reuteri DSMZ17648* in patients infected with Helicobacter pylori who have no absolute indications for eradication therapy, was conducted. The level of Helicobacter pylori colonization in the stomach mucous membrane was estimated by the data of 13C-ureas respiratory test. The level and stage of gastritis were estimated by the data of morphological *research by OLGA system.*
Keywords: Lactobacillus reuteri DSMZ17648, insemination with *Helicobacter pylori,* 13C-ureas respiratory test, OLGA.

Helicobacter pylori (HP) is one of the most common human infections. The prevalence of this infection in Moscow is 60.7-88% [1,2], in St. Petersburg is 63.6% [3], in Eastern Siberia it reaches 90% [4, 5].

Eradication (elimination) of HP is strictly recommended for patients with gastric ulcer and duodenal ulcer, gastric MALT-lymphoma, atrophic gastritis, as well as the patients, who underwent gastric resection due to gastric cancer and the relation in the first degree to the patient with gastric cancer; in addition, eradication is indicated at the request of an infected HP patient after consultation with a doctor [6].

The management of patients infected with HP without symptoms or with manifestations of functional dyspepsia, with chronic non-atrophic gastritis is the subject of discussion. Previously, it was believed that such patients have no absolute indications for HP eradication. However, the Kyoto consensus, published in September 2015, views chronic gastritis associated with HP as an infectious disease and recommends eradication, since the elimination of HP can lead to a complete recovery of the gastric mucosa. In contrast, chronic inflammation with prolonged persistence of HP leads to the development of atrophic gastritis [8,9] and a number of subsequent changes in the gastric mucosa leading to cancer (Correa cascade) [10]. HP is the most important risk factor for gastric cancer; its eradication is recognized as the most promising strategy to reduce morbidity of gastric cancer [11,12].

However, the widespread use of antibiotics to eliminate HP is associated with significant problems. Among them, there is a decrease in the effectiveness of treatment due to the formation of HP resistance to antibiotics used both in HP eradication schemes and at the population level [13-16]. The Kyoto consensus [7] noted concerns about the negative impact of eradication therapy on human health, in particular, the increase in allergies or obesity, violation of the composition of the intestinal microbiota. In addition, a significant increase in the use [7] of antibiotics in the conditions of mass eradication of HP will inevitably lead to an increase in the resistance of another dangerous microflora that poses a threat to humans.

In the stomach is a well-adapted niche-specific microbiological community. The use of probiotics is considered as an alternative or addition to eradication therapy or even as a preventive strategy [17]. The Maastricht IV/ Florence Consensus stated that “some probiotics and prebiotics added to eradication schemes show promising results in reducing the incidence of side effects” [18].

Probiotic cultures of various origins are being studied. Among them, the most promising are Lactobacillus reuteri (L. reuteri), which have anti-HP activity [18]. As has been shown, L. reuteri are resistant to both acid (pH = 2.0) and bile (4%), have a direct inhibitory effect on HP, which is based on the production of bacteriocins, H2O2 and reuterin. Reuterin has an antibacterial effect against gram-negative and gram-positive microorganisms. In addition to reuterin, L. reuteri produces such powerful antimicrobial compounds as reutericin-6 and reutecycline that affect gram-positive bacteria (which may help reduce the frequency of damage to the microbiota of the intestine under conditions of antibacterial therapy).

It is noted that L. reuteri reduces the adhesion of HP to the epithelial cells of the stomach, has antioxidant activity, plays an important role in stabilizing the barrier function of the stomach and reduces inflammation of the mucous membrane [17]. The unique ability of L. reuteri DSMZ17648 to specifically bind to HP cells has been proven, forming co-aggregates that are naturally excreted from the body, resulting in a decrease in the level of colonization of HP in the stomach [19].

From August 2014 to March 2016, a clinical study was conducted on the basis of the Central Research Institute of Gastroenterology of the Loginov Moscow Clinical Scientific Center, the purpose of which was to evaluate the effectiveness and safety of 28-day intake of probiotic bacteria L. reuteri DSMZ17648 (Helinorm®) in patients with revealed HP, not having absolute indications for eradication therapy.

The main objectives of the study:

• assessment of the dynamics of HP contamination according to the 13C Urea Breath Test after using probiotic bacteria L. reuteri DSMZ17648;

• assessment of the morphological dynamics of the severity and stage of gastritis using the system OLGA during treatment;

• assessment of the dynamics of clinical manifestations of the disease and the incidence of adverse effects of therapy.

The study was approved by the Decision No.3/2014 of the Local Ethics Committee at the Loginov Moscow Clinical Scientific Center on July 4, 2014.

Materials and methods

The study involved patients aged 18 to 60 years with the presence of HP, confirmed by the 13C Urea Breath Test (13C-UBT), who signed the informed consent, approved by the Local Ethics Committee at the Login Moscow Clinical Scientific Center.

The study did not include patients:

1) with severe pain and dyspeptic syndrome;

2) having absolute indications for eradication therapy (gastric ulcer and duodenal ulcer, atrophic gastritis, relatives of patients with gastric cancer (relation in the first degree), patients with gastric cancer, patients with gastric MALT-lymphoma);

3) taking antibiotics, proton pump inhibitors, H2-blockers, bismuth preparations within 30 days prior to the visit;

4) with neoplasms;

5) in past medical history of clinically significant allergic reactions;

6) in past medical history of severe clinically significant neurological, cardiovascular, gastrointestinal, hepatic, renal, immune and other diseases;

7) patients with mental illness, which, according to the investigator, make unacceptable the patient's participation in the study;

8) pregnant women and during lactation;

9) patients who are inclined to refuse to study and follow the doctor's orders.

From the study were excluded the patients who refused to further participate in the study, patients with a deterioration in objective indicators of the condition, patients who had diseases requiring administration of drugs affecting the evaluation of the effectiveness of therapy (antibiotics, proton pump inhibitors, H2-blockers, bismuth preparations) as well as patients who have serious adverse events.

Patients with atrophy of the gastric mucosa, first identified during the study, were not excluded from the study, but after completing the study in accordance with the protocol, they were offered eradication therapy using antibiotics and follow-up in accordance with international recommendations.

Study design

The patients were divided into two groups: the 1st group received monotherapy with Helinorm® at a dose of 200 mg (1 capsule) per day for 28 days, the 2nd group - 200 mg (1 capsule) 2 times a day for 28 days.

The level of contamination HP was determined using the 13C-UBT, which was performed prior to the start of taking Helinorm®, 2 weeks and 4 weeks after initiation of therapy.

Test 13C-UBT is as follows: the patient is invited to exhale air into the container. Capacity after exhalation immediately clogged. Then the patient drinks a test solution (carbamide enriched in the isotope of carbon 13C) and after half an hour again exhales the air into another container. In the presence of HP in the stomach of the examined patient, urea is hydrolyzed with release of carbon dioxide labeled 13C, which enters the blood and is excreted from the body through the lungs. In the absence of HP, this does not occur. The isotopic composition of carbon in the exhaled air is analyzed by a spectrometer (analyzer) before and after taking urea to determine the 13C / 12C ratio. If the change in this ratio in the second sample exceeds 2%, then it is concluded that bacteria are present.

Before initiation of therapy and after its completion, esogastroduodenoscopy (EGDS), sampling for biopsy (3 in the antrum and 2 in the body of the stomach) and morphological examination to assess the severity and stage of gastritis using the system OLGA (Operative Link for Gastritis Assessment) [20]. Under the severity of gastritis understand the severity of inflammation of the mucous membrane (total inflammatory infiltration with neutrophilic leukocytes and mononuclear cells) with an integrative assessment of the severity of changes in the body and the antrum of the stomach. The severity reflects the risk of developing atrophic gastritis. Under the stage of chronic gastritis understand the severity of the deformation of the glandular structures characteristic of the body and the antrum of the stomach. The stage reflects the risk of developing gastric cancer [8].

Patients kept a diary in which they daily noted the fact of taking the drug, evaluated the severity of symptoms on a 3-point scale (pronounced symptom: 3 points; moderately pronounced symptom: 2 points; mild symptom, which manifested from time to time: 1 point; no symptoms: 0 points) and indicated possible adverse events.

The screening included 72 patients with the previously suspected presence of HP (Fig. 1). In 12 of them, according to the results of the test 13C-UBT was obtained a negative result. 1 patient refused to conduct EGD and discontinued participation in the study before the start of therapy. In all, 59 patients received the drug and started the treatment. The average age of patients was 45.5 ± 13.2 years.

1 patient in the 2nd group was excluded from the study before the 3rd visit, since during a morphological examination he was diagnosed with gastric MALT-lymphoma. After 2 weeks of therapy, 49 patients were exanimate with test 13C-UBT. After 4 weeks of therapy, the dynamics of HP contamination according to the results of the test 13C-UBT was evaluated in 23 patients of the 1st group and in 27 patients of the 2nd group. Morphological dynamics was evaluated in 20 patients in the 1st group and in 21 patients in the 2nd group.

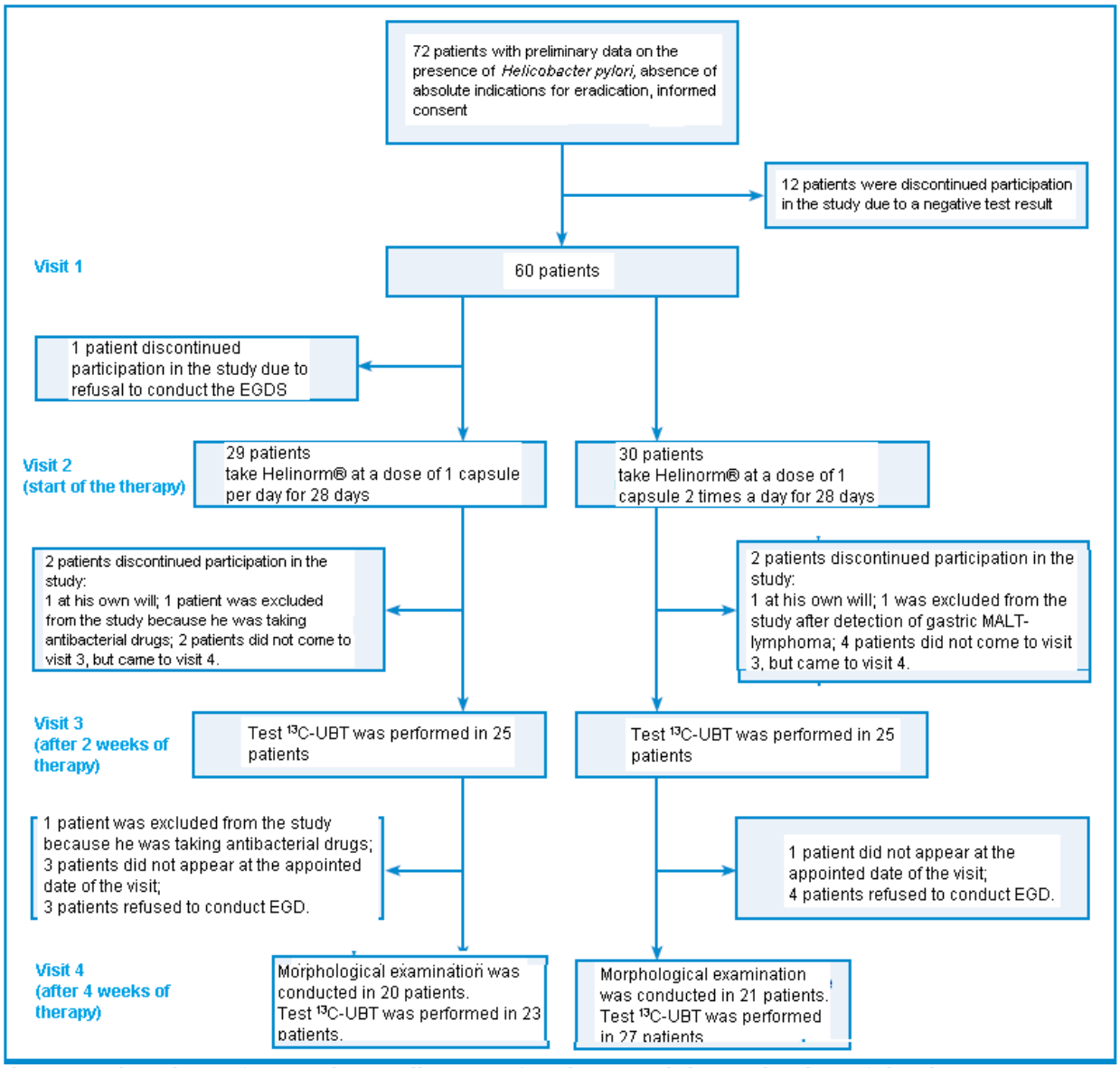

Figure 1. Flowchart of screening. Follow-up of patients and the evaluation of the therapy effectiveness during visits.

Table 1

Dynamics of the results of test ^{13}C-UBT on the 14th and 28th days of therapy with Helinorm®

Dynamics	After 14 days of therapy				After 28 days of therapy			
	1st group (n = 25)		2nd group (n = 24)		1st group (n = 23)		2nd group (n = 27)	
Decrease	14	56.0%	13	54.2%	13	56.5%	19	70.4%
No change	4	16.0%	4	16.6%	3	13.1%	3	11.1%
Increase	7	28.0%	7	29.2%	7	30.4%	5	18.5%

Study results

Quantitative assessment of H. pylori contamination according to the results of 13C Urea Breath Test

When comparing the quantitative data of the results of test 13C-UBT before therapy and on the 14th day of therapy, a positive trend (a decrease of more than 1%) was observed in 56% of patients in the 1st group and in 54.1% in the 2nd group, as well as on the 28th day of therapy in 56.5% of patients in the 1st group and in 70.4% in the 2nd group (Table 1).

When assessing the dynamics of contamination after 28 days of therapy, it was noted that in the 1st group, the average value did not change (10.2 ± 5.2% before therapy and 9.9 ± 7.6% after therapy, p = 0.424), While in the 2nd group, this value significantly decreased (12.2 ± 7.3% before therapy and 7.9 ± 6.6% after therapy, p = 0.02).

Table 2

Dynamics of the severity of gastritis in the system OLGA by the 28th day of therapy with Helinorm®

Dynamics	1st group (n = 20)		2nd group (n = 21)	
Decrease	5	25%	6	28.6%
No change	14	70%	12	51.1%
Increase	1	5%	3	14.3%

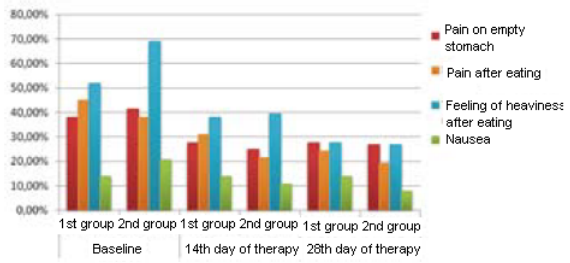

Figure 2. The proportion of patients with the manifestation of symptoms prior to therapy, as well as on the 14th and 28th days of therapy with Helinorm® (%)

Morphological assessment of inflammation signs in the gastroduodenal zone (according to the system OLGA)

When assessing the morphological dynamics after 28 days of therapy, a decrease in the degree of gastritis in the 1st group in 25% of patients was shown; in the 2nd group in 28.6% of patients (Table 2).

According to the results of the morphological examination, the stage III gastritis was diagnosed in 6 patients, while the stage IV in 7 patients. When analyzing the EGDS protocols, it was noted that only 1 patient with stage IV described a "mucosa is motley, hyperemic with foci of atrophy". This fact demonstrates the urgent need for the widespread introduction of morphological examination according to the system OLGA into clinical practice.

Dynamics of clinical symptoms of the disease

Against the background of treatment in the 1st and 2nd groups, there was a decrease in the proportion of patients who had dyspepsia symptoms, namely epigastric pain on empty stomach and after eating, feeling of heaviness after eating. The proportion of patients with nausea did not change in the 1st group, but decreased in the 2nd group (Fig. 2). Also, by the 28th day of treatment, there was a significant decrease in the severity of pain and feeling of heaviness after eating (Table 3).

Thus, there was a decrease in both the proportion of patients with symptoms of dyspepsia and the severity of these symptoms.

No adverse events were observed during treatment.

Discussion

The presented data allow us to conclude that the 28-day intake of L. reuteri DSMZ17648 is accompanied by a decrease in gastric mucosa contamination by HP (according to test 13C-UBT), however, the effectiveness depends on the frequency of intake: when taken once a day, 51.9% patients showed positive dynamics, when taken twice a day, respectively, 70.4%. It was shown that in the majority of patients of the 1st group who demonstrated the response to treatment, a positive response was observed already by the 14th day of therapy. With a double intake, an increase in efficiency was observed by the 28th day. It should be especially noted that only with the double intake of Helinorm there was a significant decrease in the average rate of HP contamination (12.2 ± 7.3%, after treatment 7.9 ± 6.6%, p = 0.02).

Against the background of monotherapy, positive dynamics of dyspepsia symptoms was observed, both a decrease in the proportion of patients with symptoms and a decrease in their severity. Regardless of the frequency of administration, a decrease in the degree of inflammation was observed in 25-28.6% of cases.

H. Mehling et al. (2013) during a blind, placebo-controlled study in people infected with HP, without clinical symptoms of disease, showed that against the background of the dried cells of L. reuteri DSMZ17648, there was a significant decrease in the level of colonization of the stomach. The level of HP contamination was determined using a 13C-Urea Breath Test before and after 14 days of taking the drug. A significant decrease in HP contamination was found in the group receiving L. reuteri DSMZ17648 (daily dose of 2 x 1010 non-viable cells in two doses, after breakfast and dinner), but not in the placebo group. The response was significantly more pronounced with a high initial rate contamination of HP [19].

According to a placebo-controlled study conducted by C. Holz et al. (2014) in asymptomatic patients infected with HP (47 pairs of twins and 34 patients infected with HP), there was a marked decrease in the 13C-Urea Breath Test in the group of L. reuteri DSMZ 17648 (-4.9 ± 7.8, in the placebo group: - 0.6 ± 5.3, p = 0.026), reflecting the reduction of contamination of the stomach by HP. The drug was also administered in two doses, after breakfast and dinner [21].

Table 3

The severity of symptoms on a 3-point scale before the start of therapy, on the 14th and 21st days of Helinorm® taking

Symptoms		Pain on empty stomach	Pain after eating	Feeling of heaviness after eating	Nausea
Baseline	1st group	0.8 ± 1.0	0.8 ± 1.0	0.9 ± 0.9	0.2 ± 0.5
	2nd group	0.7 ± 1.0	0.6 ± 1.0	0.9 ± 0.7	0.3 ± 0.6
	P value	0.376	0.324	0.483	0.278
14th day of therapy	1st group	0.4 ± 0.6	0.4 ± 0.8	0.6 ± 0.9	0.2 ± 0.5
	$P_{1\text{-}1}$ to baseline	0.065	0.099	0.179	0.480
	2nd group	0.4 ± 0.8	0.3 ± 0.7	0.5 ± 0.7	0.1 ± 0.3
	$P_{2\text{-}2}$ to baseline	0.151	0.106	0.047	0.153
	$P_{1\text{-}2}$ on the 14th day	0.422	0.311	0.340	0.288
28th day of therapy	1st group	0.4 ± 0.6	0.3 ± 0.7	0.4 ± 0.8	0.1 ± 0.4
	$P_{1\text{-}1}$ to baseline	0.055	0.035	0.034	0.396
	2nd group	0.4 ± 0.7	0.2 ± 0.5	0.4 ± 0.7	0.0 ± 0.2
	$P_{2\text{-}2}$ to baseline	0.110	0.034	0.011	0.057
	$P_{1\text{-}2}$ on the 28th day	0.49	0.253	0.441	0.114

Our data on the reduction of HP contamination when taking L. reuteri DSMZ17648 are comparable to the results published by Mehling and Holz; In addition, we noted positive clinical, and in quarter of observations - also positive morphological dynamics. The latter fact deserves attention and, obviously, further studies are required to determine the predictors of a positive morphological response of L. reuteri DSMZ17648 used as a monotherapy, a strain considered as an alternative to eradication therapy or as a means of prevention.

A large evidence base has been accumulated on the use of L. reuteri as an additional component of eradication therapy, which increases efficacy and reduces the incidence of adverse events. Thus, during a double-blind, placebo-controlled, randomized clinical study, it was shown that adding L. reuteri increases the effectiveness of a 14-day triple scheme of eradication (omeprazole 40 mg/day, amoxicillin 2000 mg/day, clarithromycin 1000 mg/day) by 8.6% (74.3% versus 65.7% in the placebo group), reduces the incidence of adverse events, primarily disorders of taste and diarrhea ($p = 0.002$), provides a significant intergroup difference in the increase in the quality of life (GSRS questionnaire) and a decrease in the severity of inflammation [22].

In another randomized clinical study, the effect of L. reuteri on the effectiveness of second-line therapy (esomeprazole 40 mg/day, levofloxacin 1000 mg/day, amoxicillin 2000 mg/day for 7 days) was evaluated. In the group of patients who received this treatment in combination with L. reuteri, the efficacy was 80% versus 60% in the placebo group ($p = 0.038$). Multivariate analysis showed that L. reuteri is a factor that ensures the effectiveness of eradication: OR = 3,055 (95% CI; 1,146-8,150; p = 0,026). In patients treated with L. reuteri, the incidence of adverse events significantly decreased, primarily nausea ($p <0.001$) and diarrhea ($p <0.004$) [23].

The data presented demonstrate the promise of assessing the effect of L. reuteri DSMZ17648 on the effectiveness and frequency of adverse events after use of eradication schemes.

Thus, it has been shown that 28-day monotherapy with L. reuteri DSMZ17648 (Helinorm®) reduces the contamination of the gastric mucosa by Helicobacter pylori in a dose-dependent manner. Regardless of the multiplicity of intake of the drug, in a quarter of patients was noted a decrease in the degree of inflammation in the system OLGA. By the 28th day of treatment, there was a decrease in both the proportion of patients with symptoms of dyspepsia and the severity of these symptoms.

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