Chapter

Alpha-Glutamyl-Tryptophan in the Treatment of Chronic Atrophic Gastritis, Associated with *Helicobacter pylori*

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Abstract

To evaluate the effectiveness of alpha-glutamyl-tryptophan as a cytoprotector in comparison with the control group (placebo) as part of the complex therapy of chronic atrophic *H. pylori* (HP)-associated gastritis. A total of 121 patients with chronic atrophic HP-associated gastritis were observed in 3 research centers. Before and after treatment blood test "Gastropanel", stomach endoscopy with biopsies of atrophied mucosa for histological examination, rapid urease test for *H. pylori* detection, and daily pH-metry were performed. After HP eradication, according to randomization, the study drug (n = 61) or placebo (n = 60) was administered twice a day, in the morning 20–30 minutes before meals and in the evening before bedtime for 28 days. Alpha-glutamyl-tryptophan intake is associated with a statistically significant increase in acidity index according to pH-metry ($p = 0.001$), an increase in the ratio of pepsinogen I/pepsinogen II ($p = 0.003$), decrease in the level of gastrin-17 ($p = 0.005$), increase in the number of glands per 1 mm^2 of the gastric mucosa ($p = 0.028$). Alpha-glutamyl-tryptophan in the treatment of chronic atrophic HP-associated gastritis has a superior regenerative effect compared with placebo, and promotes the restoration of acid-forming and pepsin-forming functions of the stomach.

Keywords: chronic atrophic gastritis, *Helicobacter pylori*, alpha-glutamyl-tryptophan, atrophy, stomach

1. Introduction

In accordance with the current standards for the treatment of chronic gastritis, eradication of *Helicobacter pylori* is an effective strategy to reduce the risk of developing stomach cancer [1–4]. However, a large percentage of *H. pylori*-infected patients get to the primary doctor's appointment already at the stage of not superficial active gastritis, but at the stage of atrophic gastritis, when the risk of cancer becomes higher [5–8]. Data on the possibility of reverse development of atrophy and intestinal metaplasia after eradication of infection are contradictory [1, 9]. Even if the pathogen is successfully

eradicated in patients with chronic atrophic gastritis, regression of atrophy does not always take place. Already the Maastricht Consensus III introduced the term "point of no return", after which *H. pylori* eradication no longer gives a significant preventive effect on the development of stomach cancer [10]. This "point of no return" is considered to be the presence of severe atrophy and intestinal metaplasia. Thus, in a meta-analysis of 12 studies involving 2658 patients conducted by J. Wang et al., it was shown that H. pylori eradication reduces atrophy in the stomach body ($p = 0.006$), but not in its antrum ($p = 0.06$), and does not affect intestinal metaplasia in these departments (respectively, $p = 0.42$ and $p = 0.76$) [11]. Consequently, the changes described in the Correa cascade can progress even in the absence of a microorganism, which once again emphasizes the need for additional measures to preserve the structure and function of the gastric mucosa at the stage of atrophy development. Therefore, it is extremely important to look for ways of primary cancer prevention by including in the complex therapy of this category of patients drugs for the restoration of the gastric mucosa, i.e., having a regenerative effect [12]. One of such medicine is alpha-glutamyl-tryptophan. The pharmacological properties of alpha-glutamyl-tryptophan revealed during experimental [13] and clinical [14] studies indicate the effect of this peptide on the unified pathogenetic mechanisms of inflammatory diseases of the gastrointestinal tract: it is effective against chronic gastritis accompanied by atrophic processes of the gastric mucosa [15].

The aim is to evaluate the effectiveness of alpha-glutamyl-tryptophan as a cytoprotector in comparison with the control group (placebo) as part of the complex therapy of chronic atrophic *H. pylori*-associated gastritis.

2. Materials and methods

2.1 Patients population

We included in the study 152 adult patients with a history of diagnosis of "chronic atrophic gastritis" in three research centers. A total of 121 patients met all inclusion criteria: signed informed consent (before the start of the study procedures); men and women aged 40–70 years; previously diagnosed chronic atrophic gastritis; the presence of complaints and symptoms characteristic of chronic atrophic gastritis; confirmed diagnosis of atrophic gastritis associated with *H. pylori* infection by endoscopic and histological examination; hypo- or anacidity according to pH-metry (pH greater than 5.0); negative pregnancy test; use of any methods of contraception during the entire time of participation in the study. Four weeks before participation in the study some drugs are prohibited to use: cytostatic, immunosuppressive, hormonal (hormone-like), antimicrobial or sedative effects, as well as drugs or biologically active additives for the treatment of inflammatory diseases of the stomach, duodenum (bismuth-containing drugs, proton pump inhibitors, antacids, gastric secretion or motility stimulants, antiemetics, laxatives, etc.), liver and pancreas (hepato-pancreatoprotectors, enzymes, choleretics, cholekinetics, etc.), nonsteroidal anti-inflammatory drugs (NSAIDs).

2.2 Diagnostic methods

Before and after treatment complex diagnostic examination was performed for all patients.

Evaluation of the results of daily pH-metry. In the course of the study, the data of the daily pH-metry of the stomach was analyzed twice (before the appointment of the studied drug during the screening period and after taking the course of the studied drug). The analysis included an assessment of the parameters: the minimum pH value, the average pH value, the aggressiveness index, and the acidity index.

Evaluation of the results of histological examination. Morphological (morphometric) analysis of the biopsy data of the gastric mucosa taken from the center of the atrophy zone twice (before the appointment of the test drug during the screening period and after taking the course of the study drug/placebo). The analysis included an assessment of such parameters as the number of glands per 1 mm^2 of the gastric mucosa, the depth of the glands of the gastric mucosa, and the number of lining cells per 100 epithelial cells of the gastric mucosa.

Evaluation of the dynamics of laboratory parameters. "Gastropanel" is a comprehensive study with the determination of a number of biochemical markers associated with gastric secretion and regenerative processes in the gastric mucosa, as well as antibodies to *H. pylori*. The "Gastropanel" complex was performed according to the standard protocol of the manufacturer (BioHit, Finland) and included an assessment of the level of pepsinogen I (PG I), pepsinogen II (PG II), gastrin-17, and the IgG titer to *H. pylori*. In addition to analyzing data on the content of the listed compounds, the PG I/PG II ratio was calculated and analyzed to assess the degree of atrophy. The study was conducted twice (before the appointment of the study drug during the screening period and after the end of the course of taking the study drug/placebo). The ratio of PG1/PG2 decreases linearly with increasing severity of atrophic gastritis.

Verification of the presence of H. pylori infection was made using several methods:

1. A biochemical rapid urease test.

2. Histological examination (Romanovsky-Giemsa staining).

3. A blood test for the presence of antibodies to IgG *H. pylori* was performed in a complex of laboratory tests "Gastropanel".

2.3 Treatment

The order of randomization: patients who met the inclusion criteria and did not have non-inclusion criteria were randomized into two groups: the main and control in a ratio of 1:1. Then performing the randomization procedure, the random number method was used. A double-blind method was used: neither the patients nor the research doctor knew which drug alpha-glutamyl-tryptophan or placebo the study participant received. These measures made it possible to minimize the influence of the human factor and contributed to obtaining more reliable data on the safety and effectiveness of the drug. The study drug and placebo were identical in packaging, labeling, and appearance.

The therapy in the study consisted of two stages:

1. A standard course of eradication therapy with Omeprazole 20 mg two times a day 10 days; amoxicillin 1000 mg two times a day for the first 5 days; clarithromycin 500 mg two times a day for next 5 days.

2. After *H. pylori* eradication, according to randomization, the study drug (n = 61) or placebo (n = 60) was administered twice a day, in the morning 20–30 minutes before meals and in the evening before bedtime for 28 days.

In the patients included in the study, the use of other medications and non-drug treatments other than the use of eradication therapy and the study drug/placebo was not allowed for the treatment of the underlying disease (atrophic gastritis).

During the study, all possible adverse events were recorded.

3. Statistical data processing

The statistical software package "Statistica 12.0 for Windows" was used for statistical analysis. The value $p<0.05$ is taken as the level of statistical significance. The data, the distribution of which corresponded to normal, were presented in the form of arithmetic averages indicating standard square deviations ($M \pm \sigma$). The data, the distribution of which differed from the normal, were presented in the form of median and quartile intervals (Mediana: [25 quartile; 75% quartile]). The Lillefors criterion was used to determine the degree of difference between the distribution and the normal one.

Due to the small sample size, nonparametric criteria were used in most cases to assess the dynamics of indicators between visits. The dynamics of the indicators were evaluated using the Wilcoxon criterion, as well as the McNemar criterion. If necessary, Bonferroni's correction for the multiplicity of comparison was used. The reliability of the differences between the indicators of dependent groups for parametric data with a normal distribution was evaluated using the Student's t-test for dependent samples.

The results of each assessment were considered as statistical indicators of the effect. In the future, the difference in the proportion of patients with a positive effect was calculated according to the primary (main variable) and secondary efficiency parameters, and a two-sided 95% confidence interval was built for this difference. To assess the effectiveness of the studied drug, a statistical analysis of the population according to the protocol (PP) was used.

The study was conducted in strict accordance with the ethical principles of the Helsinki Declaration of the World Medical Association of 1964, as amended in 2013, in accordance with the international standards of the Guidelines for Good Clinical Practice (ICH GCP R2) and other necessary regulatory documents in force on the territory of the Russian Federation.

4. Results

4.1 Data of histological examination

One of the main morphological signs of atrophic gastritis is a decrease in the number of glands of the gastric mucosa, as well as a violation of their histological structure. Therefore, an important indicator for assessing the regenerative effect of alpha-glutamyl-tryptophan can be considered an increase of 26.1% in the number of glands per 1 mm^2 while taking the study drug (**Figure 1**).

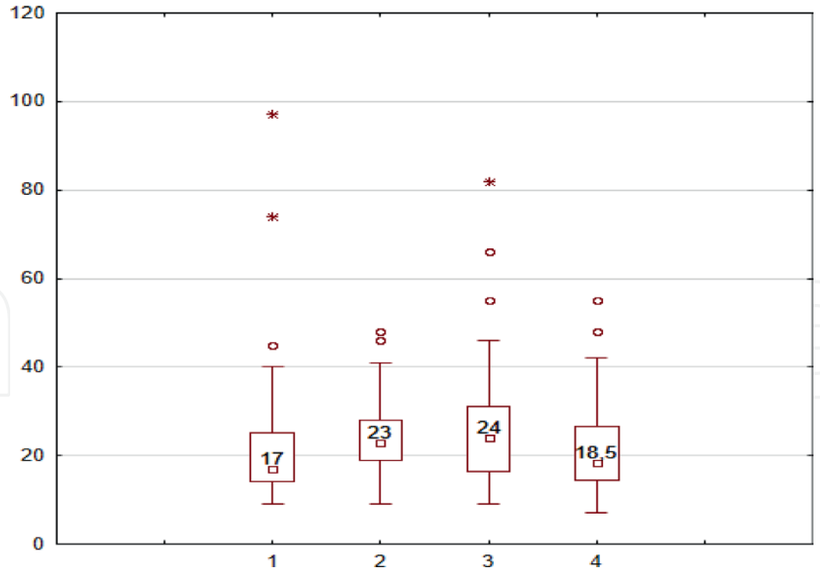

Figure 1.
Diagram of the scale of the dynamics of the number of glands per 1 mm² of the gastric mucosa during the administration of the alpha-glutamyl-tryptophan and placebo.

The medians, 25 and 75% quartiles, outliers of values, and minimum and maximum values of the indicator are presented

Along the abscissa axis of the treatment group:

1. results of alpha-glutamyl-tryptophan group before treatment

2. results of alpha-glutamyl-tryptophan group after treatment

3. results of placebo group before treatment

4. results of placebo group after treatment

On the ordinate axis: the number of glands per 1 mm^2 of the gastric mucosa in the atrophy zone

The diagram shows that taking of alpha-glutamyl-tryptophan leads to the statistically significant ($p = 0.028$) increase in the number of glands per 1 mm^2 of the gastric mucosa in comparison with the initial screening indicators. In the group of patients taking placebo, on the contrary, there was a decrease in the number of glands per 1 mm^2 of the gastric mucosa after the end of treatment in comparison with screening indicators, which had no statistical significance of differences. Intergroup comparison of the final treatment parameters showed that after a course of therapy in patients taking the alpha-glutamyl-tryptophan, the number of glands per 1 mm^2 of the gastric mucosa was statistically significantly higher compared with the results in the placebo group ($p = 0.013$).

4.2 Data of daily pH-metry

The average pH value after taking of study drug was shift in the acidic side by 1.59 times compared to the initial values from 4.3 [2.6; 6.1] to 2.7 [1.6; 4.7] ($p = 0.001$). No statistically significant dynamics of the mean pH was obtained in placebo group: from 4.35 [2.4; 6.1] to 3.6 [1.8; 5.0].

The value of the acidity index increased statistically significantly after taking the study drug as compared with the initial values by 5.44 times (from 8.95 [0; 50.33] to 48.70 [0; 110.90]), (p = 0.001), and in comparison with the placebo group by 2.94 times (from 22.80 [0; 63.62] to 16.55 [0; 77.68]), (p = 0.034) (**Figure 2**). In the placebo group, a decrease in the acidity index was noted, which indicates the absence of regression of atrophy even against the background of successful eradication of *H. pylori*.

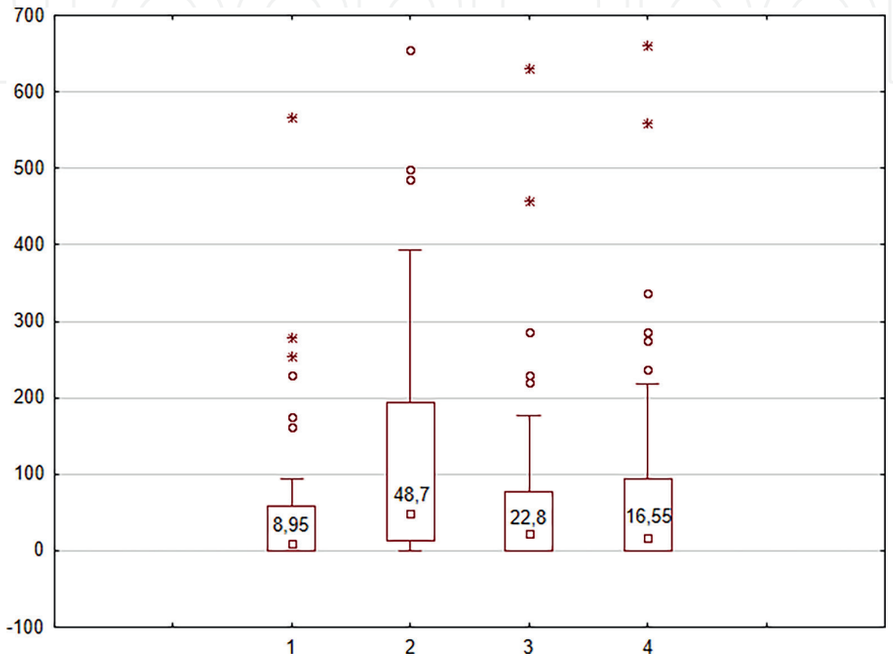

Figure 2.
Diagram of the magnitude of changes in the acidity index of gastric juice during the administration of alpha-glutamyl-tryptophan and placebo.

The medians, 25 and 75% quartiles, outliers of values, and minimum and maximum values of the indicator are presented

Along the abscissa axis of the treatment group:

1. results of alpha-glutamyl-tryptophan group before treatment

2. results of alpha-glutamyl-tryptophan group after treatment

3. results of placebo group before treatment

4. results of placebo group after treatment

On the ordinate axis: stomach acidity index, units

A statistically significant increase in the aggressiveness index by 1.48 times was revealed after taking the study drug when comparing the results before and after treatment (from 4.8 [3.1; 6.2] to 3.25 [1.7; 5.0]), ($p < 0.001$). For the placebo group, the same indicator was 1.18 times (parameter change from 4.9 [2.7; 6.2] to 4.15 [2.0; 5.5]). The effect of the studied drug on the value of the aggressiveness index was more pronounced and stable than that of placebo.

There were no statistically significant changes in the minimum pH of gastric juice during the administration of both the study drug and placebo.

The results obtained indicate that alpha-glutamyl-tryptophan has a clinically significant moderate positive effect on increasing the acidity of gastric juice, i.e. restoring the functional activity of the glandular apparatus of the gastric mucosa in patients with chronic atrophic gastritis, associated with *H. pylori*. These changes in acid secretion can be regarded as an effect that occurs indirectly due to the restoration of the morphological structure of the gastric mucosa. It should be understood that a relatively short course of the drug was used in the study, which, nevertheless, revealed a statistically significant positive effect on the indicators of acid secretion in this category of patients.

4.3 "Gastropanel" data

When evaluating the results of the Gastropanel complex of laboratory examinations, we saw a statistically significant ($p = 0.003$) 1.45-fold increase in the ratio of pepsinogen I /pepsinogen II (PG I/PG II): 9.33 [5.5; 12.2] was revealed in comparison with the initial data (6.43 [4.17; 9.6]) in alpha-glutamyl-tryptophan group and the absence of similar differences in the placebo group (before taking placebo: 7.01 [4.55; 10.9], after taking placebo: 8.03 [4.8; 11.6]) (**Figure 3**).

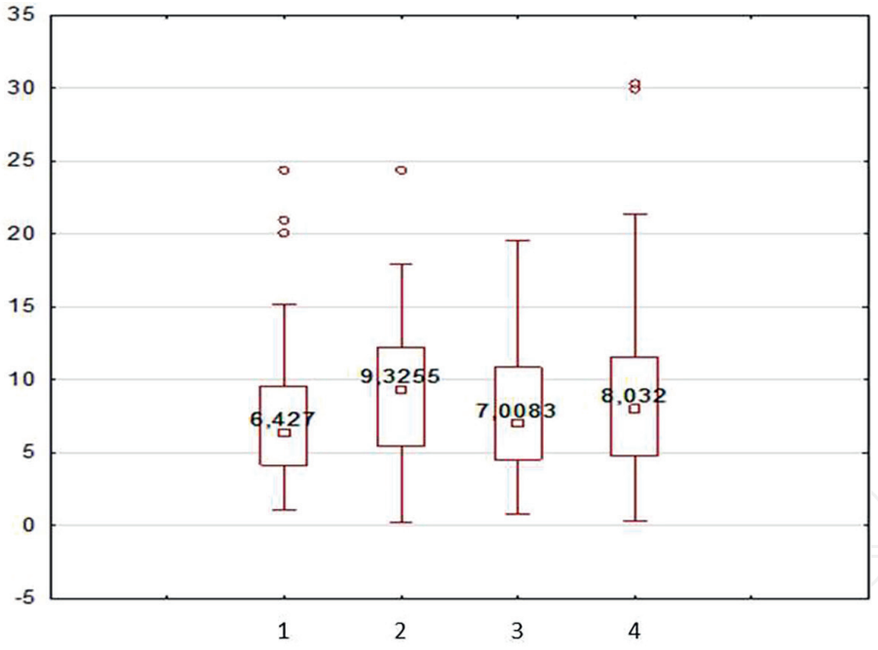

Figure 3.
Diagram of the range of values of the PGI/PGII coefficient in the process of taking alpha-glutamyl-tryptophan and placebo.

The medians, 25 and 75% quartiles, outliers of values, and minimum and maximum values of the indicator are presented

Along the abscissa axis of the treatment group:

1. results of alpha-glutamyl-tryptophan group before treatment

2. results of alpha-glutamyl-tryptophan group after treatment

3. results of placebo group before treatment

4. results of placebo group after treatment

On the ordinate axis: values of the PGI / PGII coefficient, units

The restoration of the normal PGI / PGII ratio indirectly indicates the restoration of pepsin-forming function of the stomach and is an important point in the pathogenetic treatment of chronic atrophic gastritis, since such an effect can be classified as anti-oncogenic.

We found a statistically significant ($p = 0.005$) 1.75-fold decrease in the level of gastrin-17 in the blood (pmol/L) (2.9 [1.4; 8.6]) in comparison with the initial data (5.1 [1.85; 14.0]) after taking the alpha-glutamyl-tryptophan and the absence of similar differences in the placebo group (before taking placebo: 5.13 [1.38; 16.9]; after taking placebo: 3.14 [1.28; 15.1]) (**Figure** 4). This indicates a decrease in inflammation associated with *H. pylori* invasion. That is, it can be a manifestation of both regenerative and anti-inflammatory effects of alpha-glutamyl-tryptophan.

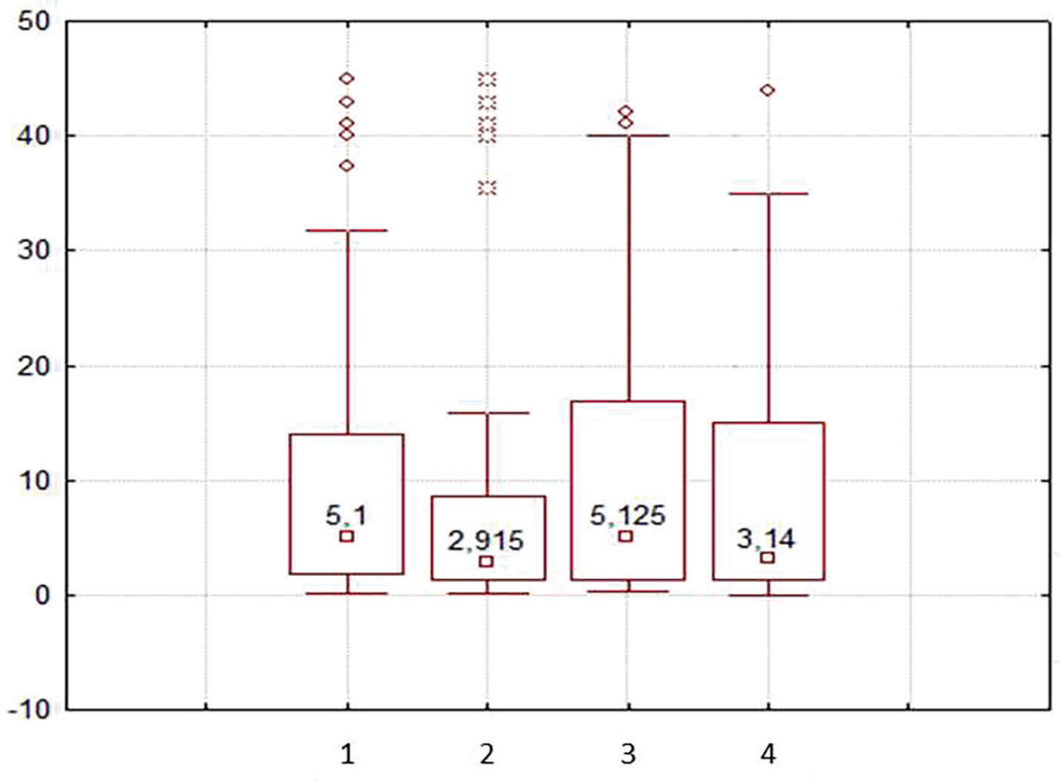

Figure 4.
Diagram of the range of gastrin-17 levels in venous blood (pmol/l) in the process of taking alpha-glutamyl-tryptophan and placebo.

The medians, 25 and 75% quartiles, outliers of values, and minimum and maximum values of the indicator are presented

Along the abscissa axis of the treatment group:

1. results of alpha-glutamyl-tryptophan group before treatment

2. results of alpha-glutamyl-tryptophan group after treatment

3. results of placebo group before treatment

4. results of placebo group after treatment

On the ordinate axis: gastrin-17 level in venous blood, pmol/l

4.4 Estimation of adverse events

In the study, 45 adverse events (AE) were registered. Of these, 20 were on the background of taking the study drug, 12 were on the background of taking a placebo, 13 occurred before the taking of the study drug/placebo. An intergroup comparison of the number of AE detected during the period of taking the study drug and placebo revealed no statistically significant differences ($\chi 2 = 1.55$; $p = 0.213$) in the amount of AE in the study groups. No any serious AE was detected in the study.

5. Conclusion

According to the results of the study, it can be concluded that the study drug alpha-glutamyl-tryptophan, in comparison with placebo, has superior regenerative efficiency (an increase in the number of glands per 1 mm^2 of the gastric mucosa, an increase in the acidity index and a shift in the average pH value to the acidic side according to the daily pH-metry, an increase in the ratio of PG I / PG II and a decrease in the level of gastrin-17 in the blood according to the Gastropanel complex). The study drug promotes the restoration of acid-forming and pepsin-forming functions of the stomach in the treatment of chronic atrophic gastritis in patients aged 40–70 years. It can be assumed that the study drug, due to the restoration of anatomical and functional parameters of the gastric mucosa, also decreases the pro-oncogenic processes associated with the development of atrophy.

Therefore, it can be recommended to include alpha-glutamyl-tryptophan in the complex therapy of chronic atrophic gastritis, associated with *H. pylori* and for primary prophylaxis of gastric cancer. In the future, it is important to conduct studies with the estimation of alpha-glutamyl-tryptophan efficacy in the treatment of chronic atrophic gastritis not associated with *H. pylori*, peptic ulcer disease (*H. pylori*-associated and not associated), chronic non-atrophic gastritis (*H. pylori*-associated and not associated) for improving regeneration of gastric mucosa.

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