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Original Article

Study of compound bismuth and magnesium granules on clearance of helicobacter pylori infection in KM mice

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Abstract: Aim: To investigate the eradication therapy, mucosal protective effect and its mechanism of compound bismuth and magnesium granules on Helicobacter Pylori (H. pylori) infection in KM mice. Methods: HP infection model in KM mice was established by gavage with HP standard strain NCTC 11637. KM mice were randomly divided into five groups: the normal control group, the model group, the compound bismuth and magnesium granules, the PPI triple therapy group, the PPI triple therapy + compound bismuth and magnesium group. After administration for two weeks, the gastric antrum tissues were taken for rapid urease test and Warthin Starry silver stained to determine HP infection. The gastric mucosal injury and changes of cell ultrastructure were observed by HE staining and electron microscopy. Immunohistochemistry was used to detect the protein expression of IL-8, IL-10, TNF-α and VEGF. Results: The clearance rates in each treatment group were significantly higher than those in the model group. At the same time, there had varying degrees of improvement on inflammation of the gastric mucosa in the treatment groups compared with that in the model group. The IL-8 and TNF-α expression in each treatment group were lower than those in the injury group. The VEGF and IL-10 expression in each treatment group had no significant decrease. Conclusion: Compound bismuth and magnesium granules had cleaning effect on HP infection in KM mice and therapeutic effect on HP-induced gastric mucosal injury. The mechanism may be associated with inhibition of related inflammation factors.

Keywords: Compound bismuth and magnesium granules, helicobacter pylori, clearance, KM mice models

Introduction

Helicobacter pylori (*H. pylori*) are one of the causes for the most common chronic infection in humans, and closely associated with a variety of gastrointestinal diseases [1]. A number of large-scale epidemiological intervention studies show that eradication of H. pylori can prevent gastric cancer and reverse precancerous lesions such as gastric atrophy and intestinal metaplasia [2, 3]. The rate of H. pylori infection is 50% [4] as well as a high incidence of gastric cancer. Improving the success rate of H. pylori eradication therapy in China has more significance. The antibiotic resistance of H. pylori is growing with the extensive development of H. pylori eradication therapy, leading to the continuous decline of the eradication rates of short-PPI triple therapy [5]. Therefore, to find a new path to deal with H. pylori resistance and improve H. pylori eradication rate have become a top priority [6]. In recent years, the role of Chinese medicine in the eradication of H. pylori attracts more attention. Compound bismuth and magnesium granules is a new combination of chemical and traditional Chinese medicine preparations, its clinical efficacy has been widely validated, but few studies are on its suppression of H. pylori. This study discusses the eradication role of the compound bismuth and magnesium granules for H. pylori, as well as the treatment for gastric mucosal injury caused by H. pylori infections through establishing KM mice models of H. pylori infection.

Material and methods

Experimental animals

Male KM mice of SPF class with age of 6 week's old, weight of 18~22 g were provided by the

Table 1. Helicobacter pylori clearance rates in each group

	Group	n	Negative	Clearance rate
I	Normal control group	13	13	
II	Model group	12	0	
III	Medium-dose compound bismuth and magnesium granules group	10	6	60.0%a
IV	PPI triple therapy group	11	8	72.7%a
V	Compound bismuth and magnesium granules + PPI triple therapy group	11	10	90.9%a

Note: "a" indicates *P*<0.05 versus the model group.

Table 2. EDS of gastric mucosa in each group

	Group	n	EDS ($\bar{x}$±s)
I	Normal control	13	1.154±0.376
II	Model group	12	2.833±0.718a
III	Compound bismuth and magnesium granules	10	1.700±0.483b
IV	PPI triple therapy group	11	1.454±0.522b
V	Compound bismuth and magnesium granules + PPI triple therapy group	11	1.273±0.467b

Note: "a" indicates P<0.01 versus the normal control group. "b" indicates P<0.01 versus the model group.

Beijing Weitonglihua company. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Aeropace center hospital.

Preparation of H. pylori bacteria

After the recovery and passage, NCTC 11637 strain was densely streaked to culture in medium containing 10% sheep blood under the condition of 37°C, placed in micro-aerobic environment for 72 hours, scraped helicobacter pylori in the Petri dishes before administering for one hour, mixed with Brucella broth to a concentration of 1×109 CFU/mL.

Preparation of animal model and method of administration

The experimental animals were randomly divided into five groups: the normal control group, the model group, the compound bismuth and magnesium granules, the PPI triple therapy group, the PPI triple therapy + compound bismuth and magnesium group. In addition to the control group, the animals in the other groups were adaptive feeding for four days, the mice were fasted food and water for 12 hours, then 0.5 ml H. pylori bacteria solution was gavage for each rat, fasted for 6 hours, administrated H. pylori bacteria solution every other day with a total of 5 times. Animals in the control group and model group were given an equal volume of distilled water, animals in other groups were administered in accordance with the grouping, once a day for 5 days, fasted water and food 6 h before and 1 h after administration.

Specimen collection and testing

The mice were fasted for 12 h after administration, then killed by cervical dislocation, the stomachs were fetched with disinfection, cut the skin stomach along the greater curvature of the stomach cavity, three pieces of tissues were taken from the gastric antrum, one for urea enzyme experiments, the other two were saved in 4% formaldehyde and glutaraldehyde for pathology, histopathology and ultrafine immunohistochemical examination.

Detection index

H. pylori infection and cleaning situation: diagnosis of H. pylori infection by silver staining and rapid urease test were both positive.

Sinus mucosa pathology and ultrafine pathological changes were observed HE staining and electron microscopy.

Gastric mucosal epithelial damage was calculated by epithelia damage scoring (EDS) [7]: 1 point indicated normal mucosa, mucosal sur-

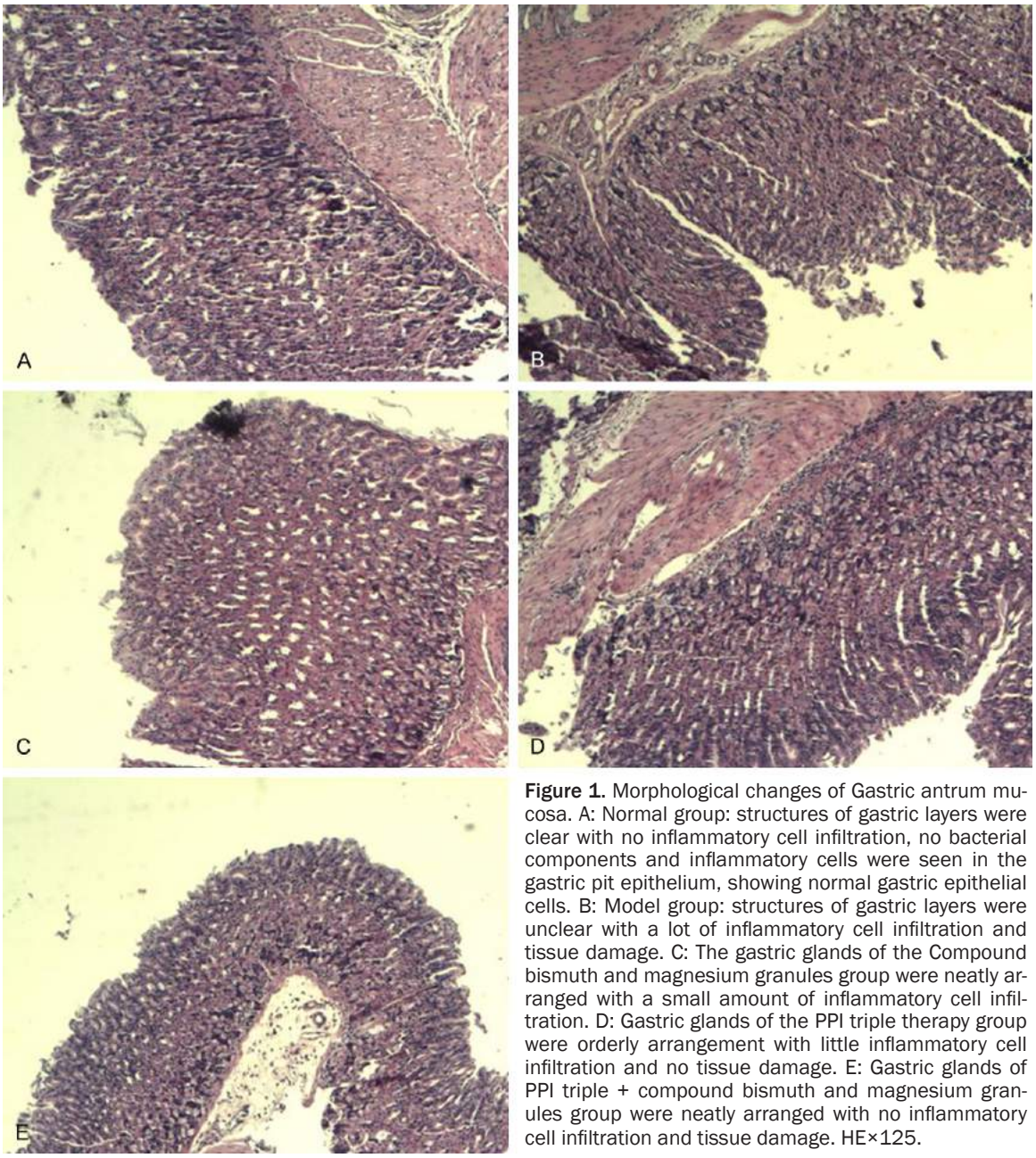


Figure 1. Morphological changes of Gastric antrum mucosa. A: Normal group: structures of gastric layers were clear with no inflammatory cell infiltration, no bacterial components and inflammatory cells were seen in the gastric pit epithelium, showing normal gastric epithelial cells. B: Model group: structures of gastric layers were unclear with a lot of inflammatory cell infiltration and tissue damage. C: The gastric glands of the Compound bismuth and magnesium granules group were neatly arranged with a small amount of inflammatory cell infiltration. D: Gastric glands of the PPI triple therapy group were orderly arrangement with little inflammatory cell infiltration and no tissue damage. E: Gastric glands of PPI triple + compound bismuth and magnesium granules group were neatly arranged with no inflammatory cell infiltration and tissue damage. HE×125.

face cells damage was expressed as 2 points, 3 points was considered to be damage involving glandular cells, and 4 points meant erosion, bleeding or ulceration of mucosal erosion.

Cytokines testing: the expression of IL-8, IL-10, TNF-α and VEGF protein were qualitatively observed under an optical microscope, and localization was performed.

Statistical analysis

SPSS 17.0 was used for statistical analysis, measurement data was expressed as "$\bar{x} \pm s$", the comparisons between the data of the two groups were performed using independent samples T-test, ANOVA was used for comparisons among the groups when the variance was homogeneous, while rank test was used when the variance was heterogeneous, and Fisher exact probability test for enumeration data.

Results

Cleaning situation of H. pylori infection in each group

H. pylori clearance rates of each group were shown in **Table 1**. After giving appropriate treat-

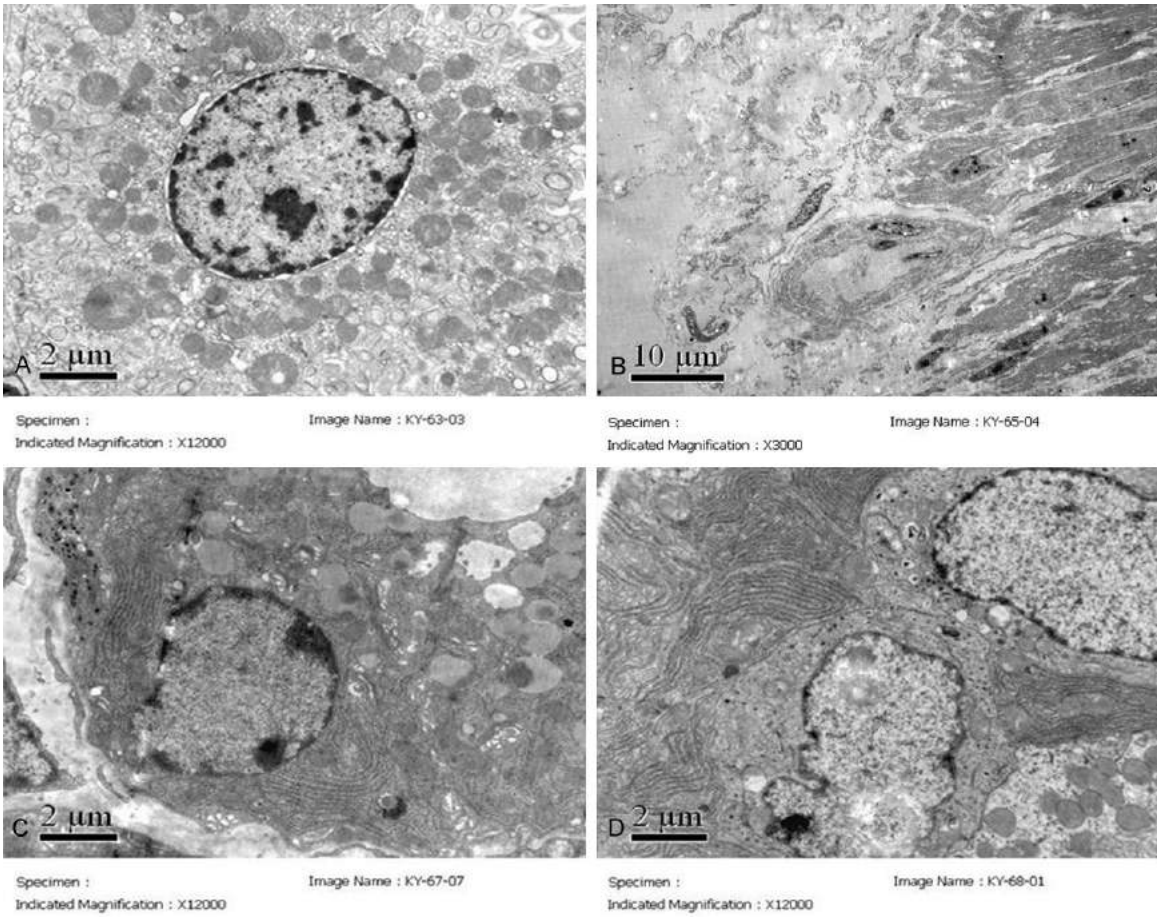


Figure 2. Supermicro pathological changes. A: The basic structure was clear and abundant endoplasmic reticulum and secretory granules in the normal control group. B: The model group: microvilli was sparse and loss, mitochondria and endoplasmic reticulum were heavily swelled and expanded. The basic structure was unclear, some formed vacuoles, and phagolysosomes increased. C: The basic structure of the compound bismuth and magnesium granules group and the PPI triple therapy group was similar to that in normal control group ,showing tiny amount of secretory granules. D: Cells in the PPI triple therapy + compound bismuth and magnesium group were tightly packed with almost normal ultrastructure, showing abundant endoplasmic reticulum and secretory granules.

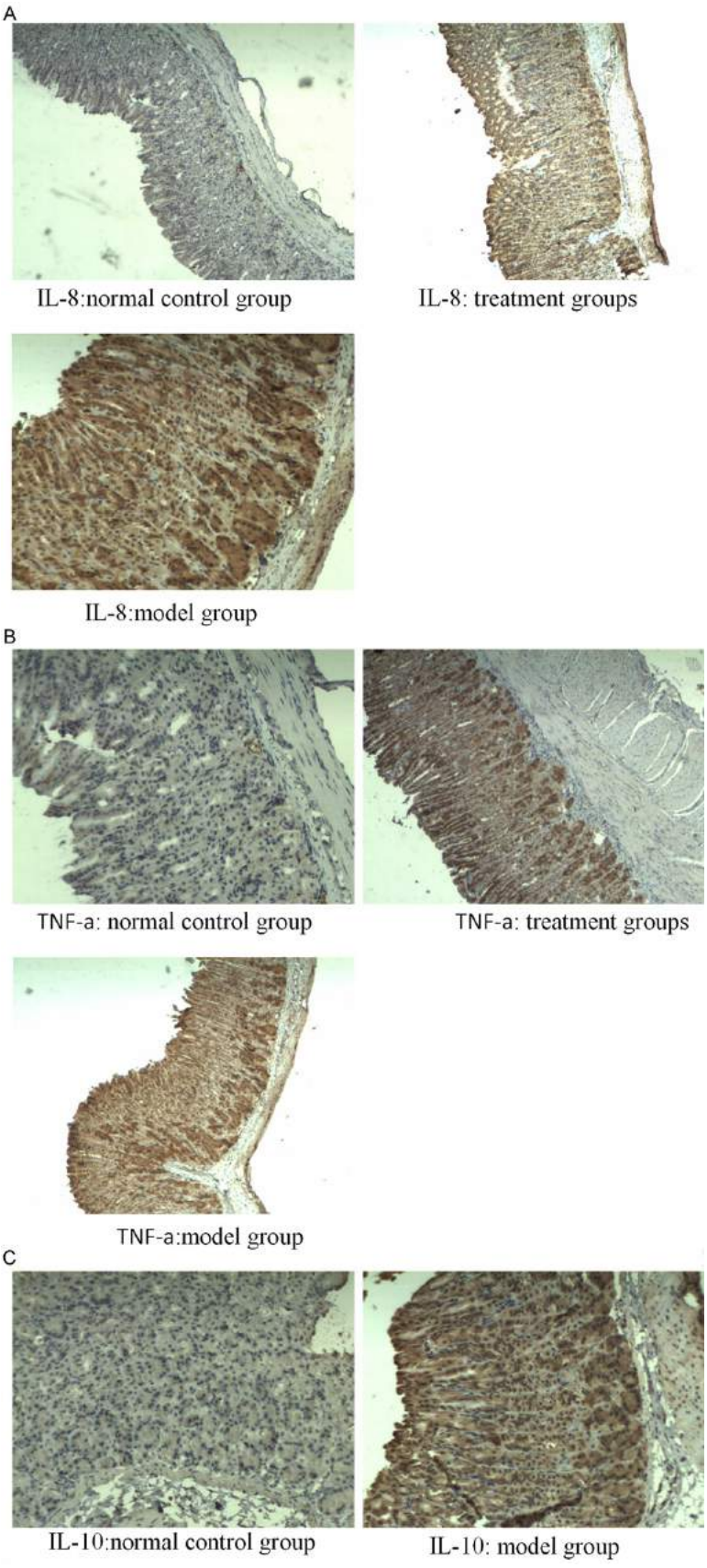
ment to each treatment groups, the cleaning rates of each treatment group were significantly higher than that of the model group.

Gastric mucosal injury

Points of mucosal injury in each groups were shown in **Table 2**. EDS of the model group (2.833±0.718) was significantly higher than that of the control group (1.154±0.376) ($P<0.01$). the EDS of each treatment groups were significantly lower than that of the model group ($P<0.05$, $P<0.01$).

Morphological changes of gastric antrum mucosa

Normal group: structures of gastric layers were clear with no inflammatory cell infiltration, no bacterial components and inflammatory cells were seen in the gastric pit epithelium, showing normal gastric epithelial cells (**Figure 1A**). Model group: superficial mucosal inflammatory exudates with a amount of bacterial components and epithelial shedding was appeared (**Figure 1B**). Submucosal edema was obvious, during which floating neutrophils and lymphocytes were visible. The small blood vesseis near the muscle hyperplasia of the lamina propria were hyperplasia and congestion, large amounts of neutrophils, lymphocytes and plasma cells were seen in the mucous layer and submucosa. Compound bismuth and magnesium granules group: the gastric glands were more orderly arrangement with a small amount of inflammatory cell infiltration (**Figure 1C**). The PPI triple therapy group: Gastric glands of the



PPI triple therapy group were orderly arrangement with little inflammatory cell infiltration and no tissue damage (**Figure 1D**). Gastric glands of PPI triple + compound bismuth and magnesium granules group were neatly arranged with no inflammatory cell infiltration and tissue damage (**Figure 1E**).

Supermicro pathological changes

Observation results by electron microscopy showed that the basic structure was clear and abundant endoplasmic reticulum and secretory granules in the normal control group (**Figure 2A**). The cell gap widened significantly in the model group, microvillus was sparse and loss, mitochondria and endoplasmic reticulum were heavily swelled and expanded. The basic structure was unclear, some formed vacuoles, and phagolysosomes increased (**Figure 2B**). The basic structure of the compound bismuth and magnesium granules group and the PPI triple therapy group was similar to that in normal control group, showing tiny amount of secretory granules (**Figure 2C**). Cells in the PPI triple therapy + compound bismuth and magnesium group were tightly packed with almost normal ultrastructure, showing abundant endoplasmic reticulum and secretory granules (**Figure 2D**).

Immunohistochemistry

IL-8, TNF-α, IL-10 and VEGF in normal gastric mucosa and submucosa were negatively or weakly positively expre-

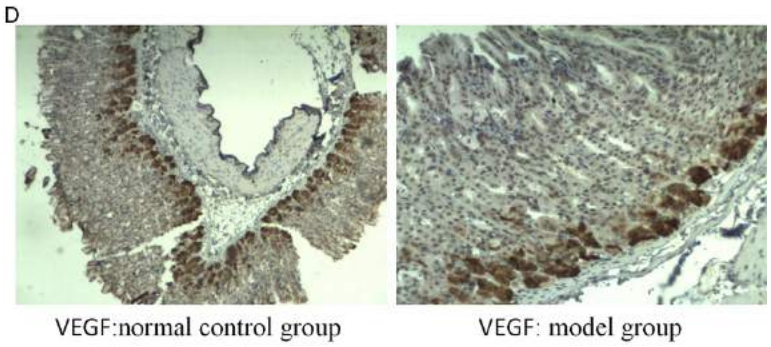


Figure 3. Immunohistochemistry analysis. A, B: The expressions of IL-8 and TNF-α in normal gastric mucosa and submucosa are negative or weakly positive, while in model group were obviously enhanced. There expression levels in treatment groups were lower than in model group and higher than in control group. C, D: IL-10 and VEGF expression in model group was higher than that of the normal control group, but no significant difference appeared between the treatment groups and the model group.

ssed. Compared with the normal control group, expression in the model group significantly increased (**Figure 3A-D**). the IL-8 and TNF-α expression of each treatment groups was significantly lower than that of the model group. IL-10 and VEGF expression in model group was higher than that of the normal control group, but no significant difference appeared between the treatment groups and the model group (**Table 3**).

Discussion

H. pylori were the most common chronic infections for mankind, and closely associated with a variety of gastrointestinal diseases. A number of large-scale epidemiological intervention studies showed that eradication of *H. pylori* could prevent gastric cancer and reverse precancerous lesions such as gastric atrophy and intestinal metaplasia [8]. The rate of *H. pylori* infection was 50% as well as a high incidence of gastric cancer. Improving the success rate of *H. pylori* eradication therapy in China had more significance. The antibiotic resistance of *H. pylori* was growing with the extensive development of *H. pylori* eradication therapy, leading to the eradication rates of short-PPI triple therapy continue to decline [9]. Therefore, to find a new path to deal with *H. pylori* resistance and improve *H. pylori* eradication rate have become a top priority. In recent years, a number of studies showed that bismuth [10, 11] could increase the sensitivity of *H. pylori* to antibiotics, increasing bismuth on the basis of the standard triple therapy could improve the *H. pylori* eradication rates [12-14]. Bismuth + PPI plus two antimicrobial agents was confirmed the most important program in the "fourth national consensus *H. pylori* infection treatment" [15]. Moreso, the role of traditional Chinese medicine received more and more attention [16-18]. The traditional Chinese medicine was applied to treat *H. pylori* infection, and a certain effect was achieved.

Compound bismuth and magnesium granules was a new combined preparations of bismuth and traditional Chinese medicine including main components such as bismuth aluminate, heavy magnesium carbonate, sodium bicarbonate, licorice extract powder, Francis buckthorn bark, fennel powder, aloe and Shichangpu. The efficacy was widely clinically proven. Did compound bismuth and magnesium granules had scavenging on *H. pylori*? Whether it could improve antibiotic resistance of *H. pylori* as some bismuth? These were all lack of systematic research.

This study showed that gastric bismuth magnesium particles had a certain role in suppressing and killing to *H. pylori* in mice in vivo. The eradication rates to *H. pylori* in the bismuth magnesium particles were 60%, with significant difference compare to the model group. Compound bismuth and magnesium granules in combination with PPI triple therapy could significantly increase the clearance rate of *H. pylori* in mice.

H. pylori infecting gastric mucosa may induce the local expression upregulation of the series of cytokines such as IL-4, IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-γ and EGF [19, 20]. These cytokines formed a complex network of immune inflammation to play an important role in *H. pylori*-related gastric mucosal damage mechanisms [21, 22]. The repair process of gastric mucosa not only needed to fill the mucosa missing, but also rebuilt the submucosal tissue structure. VEGF had the role of promoting proliferation of angiogenesis, epithelial cells, and cell protec-

Table 3. Cytokine expression between groups

	Group	n	IL-8 Positive (%)	IL-10 positive (%)	VEGF positive (%)	TNF-α positive (%)
I	Normal control	13	7.7 (1/13)	15.4 (2/13)	15.4 (2/13)	15.4 (2/13)
II	Model	12	83.3 (10/12)	66.7 (8/12)	58.3 (7/12)	91.7 (11/12)
III	Compound bismuth and magnesium granules	10	30.0 (3/10)a	50.0 (5/10)	40.0 (4/10)	40 (4/10)a
IV	PPI triple therapy group	11	27.3 (3/11)a	36.4 (4/11)	36.4 (4/11)	36.4 (4/11)a
V	Compound bismuth and magnesium granules + PPI triple therapy group	11	18.2 (2/11)a	27.3 (3/11)	27.3 (3/11)	18.2 (2/11)a

Note: "a" indicates P<0.05 versus the model group.

tion, as well as promoting gastrointestinal mucosal epithelial hyperplasia and maintaining the mucosa complete, which were important factors to promote ulcer healing [23]. It had been reported that *H. pylori* infection could promote the expression of VEGF [24]. The results showed that the expression of IL-8, IL-10, TNF-α and VEGF in the model group was significantly stronger than that in the normal control group. the IL-8 and TNF-α expression of each treatment groups was significantly lower than that of the model group. The protein positive rate of Il-10 and VEGF had no significant differences in each treatment groups and the model group, which may be caused by the association of Il-10 and chronic gastritis-related activities, while was nothing to do with the severity of gastric mucosal lesions. But the repair process of gastric mucosa was more complex and needed longer time. So the positive rate of VEGF protein in each group had no obvious differences.

In this study, the results of HE staining, staining under electron microscopy and EDS integration indicated that compound bismuth and magnesium granules had a significant protective effect on the *H. pylori*-induced gastric mucosal injury. Gastric mucosal barrier included protective factors such as mucus barrier, mucus and bicarbonate secretion, mucosal blood flow, epidermal growth factor, prostaglandins and hexosamine [25]. The machanisms of compound bismuth and magnesium granules prevented *H. pylori*-induced gastric mucosal injury through these kinds of factors remained to be further studied.

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Disclosure of conflict of interest

None.

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