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Mechanism of Weiwei granules in the treatment of chronic active *Helicobacter pylori* gastritis with atrophy based on the TLR4/NF-κB/COX-2 inflammatory signaling pathway

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Summary. Objective. Our paper aimed to elucidate the mechanism of Weiwei granules in the treatment of *Helicobacter pylori* (Hp)-positive chronic atrophic gastritis (CAG) based on the TLR4/NF-κB/COX-2 inflammatory signaling pathway.

Methods. Hp-positive CAG patients were randomized into the control group (treated with quadruple therapy) or the observation group (treated with Weiwei granules based on the control group). The clinical efficacy, Hp clearance rate, and efficacy of traditional Chinese medicine (TCM) symptoms were compared between the two groups after six months of treatment. The scores of various histopathology variables, serum levels of inflammatory factors (interleukin-6 [IL-6], interleukin-8 [IL-8], and tumor necrosis factor-alpha [TNF-α]), gastrin-17 (G-17) and motilin (MTL), pepsinogen (PG) I and PG II, as well as serum levels of gastrointestinal hormone endothelin (ET), epidermal growth factor (EGF), and calcitonin gene-related peptide (CGRP), were compared between the two groups before and after treatment. TLR4, NF-κB, and COX-2 mRNA levels were compared in gastric mucosal tissues before and after treatment in the two groups.

Results. After treatment, the clinical efficacy, Hp clearance rate, and efficacy of TCM symptoms of patients in the observation group were higher than those in the control group. After treatment, the scores of various histopathology variables, serum levels of inflammatory factors (IL-6, IL-8, and TNF-α), gastrointestinal hormones (ET and EGF), and the expression levels of TLR4, NF-κB, and COX-2 mRNA in the gastric mucosal tissues were lower and G-17, MTL, CGRP, and PG I levels were higher in the observation group than in the control group.

Conclusion. Weiwei granules can effectively improve Hp-positive CAG patients and reduce the expression levels of TLR4, NF-κB, and COX-2

Key words: Weiwei granules, *Helicobacter pylori*, Chronic atrophic gastritis, TLR4, NF-κB, COX-2

Introduction

Chronic atrophic gastritis (CAG), usually described as gastric mucosal atrophy, is an asymptomatic condition that can develop into gastric cancer (GC) in some patients (Li et al., 2018b). This histological change might be caused by an autoimmune-induced response against parietal cells or their components or has a link to infection with *Helicobacter pylori* (Hp) (Rodriguez-Castro et al., 2018). Hp is a chief human pathogen that results in chronic and progressive gastric mucosal injury and is a significant inducer of gastric atrophy and even cancer (Lahner et al., 2018). As reported, anti-Hp treatment is a vital therapeutic approach for treating CAG, and other therapies, consisting of symptomatic pain relief, gastric mucosa protection, proton pump inhibitor administration, as well as Vitamin C supplementation, have been applied in CAG therapy (Li et al., 2017). Nevertheless, the optimal treatment method still needs to be further verified.

Recently, doctors in traditional Chinese medicine (TCM) have placed more emphasis on the exploration of CAG pathogenesis and have achieved certain progress. Since TCM has the double-layer function of treatment and rehabilitation and has fewer side effects, natural TCM preparations have been increasingly accepted by patients with gastric diseases. For instance, a recent article unveiled that Sijunzi decoction, as a Chinese classical formula, may have a very good therapeutic

effect on CAG patients (Gan et al., 2017). Even though Qinghuayin (QHY), also a Chinese formula, is broadly utilized in CAG therapy under TCM theories of resolving dampness and clearing heat (Li et al., 2018a), for our work, we selected another Chinese formula, namely Weiwei granules, for the CAG therapy.

Toll-like receptor 4 (TLR4) was initially discovered as a possible binding receptor for Hp on gastric epithelial cells, and a functional TLR4 polymorphism has relevance within the progression of premalignant abnormalities such as hypochlorhydria and gastric atrophy (Hold et al., 2007). Cyclooxygenase 2 (COX-2) is lowly expressed under normal conditions whereas it is elevated at sites of inflammation (Tiwari et al., 2019). Upregulation of COX-2 has been observed in Hp-infected gastric mucosa (Konturek et al., 2000). A marked weakening of NF-kappa B (NF-κB) binding and the production of free radicals are observed in COX-2 overexpressed cells (Hahm et al., 2002). Li et al. found that TLR4, NF-κB, and COX-2 levels are increased via CAG model establishment and reduced by the administration of QHY (Li et al., 2018a). However, the mechanism of Weiwei granules in the treatment of Hp-positive CAG patients based on the TLR4/NF-κB/COX-2 inflammatory signaling pathway remains to be elucidated, which is the main purpose of our research.

Materials and methods

Ethical approval

This study obtained approval from the ethics committee of Changchun University of Chinese Medicine (approval number: 20200306). Written consent was acquired from all participants.

Study subjects

The 76 patients observed in this study were from the Department of Gastroenterology of Changchun University of Chinese Medicine. All cases were diagnosed as CAG by electronic gastroscopy and pathological examination at the time of treatment, and these patients also had Hp infections. Inclusion criteria: (1) The research subject signed an informed consent form and was informed and agreed to accept the research on this topic; (2) Patients who were diagnosed with CAG by electronic gastroscopy and pathological examination and also combined with Hp infection at the time of consultation in our hospital, were selected. Exclusion criteria: (1) Patients with other gastrointestinal diseases, such as severe peptic ulcer bleeding, and gastric mucosa pathology suggestive of malignant disease; (2) Patients with cardiac, cerebral, renal, hepatic, or other system (e.g., hematopoietic)-associated serious primary diseases, and psychiatric patients; (3) Patients with a pathologic diagnosis of severe heterogeneous hyperplasia or suspected malignancy; (4)

Patients who were concurrently using other drugs to treat the disease, or who were allergic to the drugs used in the subject; (5) Patients who did not take the medication as prescribed and could not judge the efficacy or had incomplete information; (6) Pregnant and lactating women; (7) Patients who were allergic or had a history of allergy to multiple medications; or who were known to be allergic to the medications in this study; (8) Persons under 18 and over 65 years of age. The enrolled patients were randomly divided into an observation group or a control group using the random number table method, with 38 cases in each group.

Diagnosis of Hp infection

For the diagnosis of Hp infection, according to the therapeutic judgment criteria established by the Fourth Chinese National Consensus Report on the management of *Helicobacter pylori* infection in 2012 (Chinese Society of Gastroenterology et al., 2013), Hp infection could be diagnosed if one of the following three items was met: (1) Stained tissue section rapid urease test (RUT) and bacterial culture of gastric mucosal tissue, one of which was positive; (2) positive 13C- or 14C-urea breath test (UBT); (3) positive *H. pylori* stool antigen (HpSA) detection. In this experiment, we mainly used (1) and (2).

Western medical diagnostic criteria for CAG

Western medical diagnostic criteria for CAG were based on the "Chinese Consensus on Chronic Gastritis" developed at the annual National Consensus Conference on the Diagnosis and Treatment of Chronic Gastritis, the "Consensus Opinions on the Diagnosis and Treatment of Chronic Atrophic Gastritis in Traditional Chinese Medicine" developed by the Spleen and Gastrointestinal Diseases Branch of the Chinese Association of Traditional Chinese Medicine, and "Guidelines for Clinical Research of New Traditional Chinese Medicines (Trial)" developed annually. The diagnosis of CAG required a combination of endoscopic and patho-histological diagnoses.

Scoring criteria for gastroscopy diagnosis

The main endoscopic manifestations of CAG were granular mucosal surface, flattened mucosal wrinkles, submucosal vascular permeability, and gray intestinal nodules in some areas, which were graded as:

grade I: fine granular, partially permeable blood vessels, and solitary gray intestinal epithelial nodules;

grade II: moderately granular, with continuous uniformly permeable vessels and multiple gray intestinal epithelial chemosis nodules;

grade III: coarse granules, the disappearance of folds, vessels up to the surface, and diffuse gray intestinal epithelial chemosis nodules.

Scoring criteria for pathologic diagnosis

The histopathological biopsy of CAG included five indicators: chronic inflammation, activity, atrophy, intestinal metaplasia, and intraepithelial neoplasia. The histological changes of chronic inflammation, activity, atrophy, intestinal metaplasia, and intraepithelial neoplasia were observed following the New Sydney Gastritis Classification (Stolte and Meining, 2001) and Vienna Classification (Schlemper et al., 2000). The primary variables were atrophy, intestinal metaplasia, and intraepithelial neoplasia, and the secondary variables were chronic inflammation and activity. Each variable was categorized as "normal", "mild", "moderate", and "severe", with higher scores assigned to the primary variables, such as "0, 3, 6, and 9" points, and "0, 1, 2, and 3" points to the secondary variables, respectively.

Diagnostic criteria for TCM symptoms

The diagnostic criteria for TCM symptoms refer to the diagnostic criteria of the Guidelines for Clinical Research of New Chinese Medicines. Primary symptoms: (1) vague pain or distension in the stomach cavity; (2) distension and fullness of the stomach. Secondary symptoms: (1) noise in the stomach; (2) poor appetite and dullness; (3) fatigue; (4) inhibited defecation; (5) halitosis; (6) nausea and vomiting; (7) dryness of the mouth; (8) bitterness of the mouth. Tongue pulse: The tongue was red with little fluid, or purple and dark, or had strange spots and fatigue points, cracks, lack of coating, or yellow and greasy coating, and the pulse was thin or wet. Tongue and pulse phases were required, along with one primary and two secondary conditions. Each symptom was categorized as "none", "mild", "moderate", and "severe", with higher scores assigned to the primary symptom, such as "0, 2, 4, and 6" points, and "0, 1, 2, and 3" points to the secondary symptom, respectively.

Treatment methods

Patients in the control group were treated with quadruple therapy, and administrated rabeprazole enteric-coated tablets (Xinhua Pharmaceutical (GaoMi) Co., Ltd., Shandong, China; national medicine permission number: H20080683; 10 mg/d, 2 times/d, orally before meals), amoxil capsules (Kerui Pharmaceutical (Group) Co., Ltd., Chongqing, China; national medicine permission number: H50020726; 1000 mg/d, 2 times/d, orally after meals), furazolidone tablets (RunHong Pharmaceutical Co., Ltd., Henan, China; national medicine permission number: H41020309; 100 mg/d, 2 times/d, orally after meals), and bismuth potassium citrate tablets (Livzon Pharmaceutical Group Inc., Guangdong, China; national medicine permission number: H10900084; 0.6 g/d, 2 times/d). The patient carried out a course of treatment for three months and took a total of two courses of treatment.

Patients in the observation group were treated with Weiwei granules based on the control group. Weiwei was taken twice a day, 20 g each time, with boiled water 0.5h before meals. The formula was as follows: Dang Shen (*Codonopsis Pilosula*) and Huang Qi (*Astragalus Mongholicus*) (15 g each), Bai Zhu (*Rhizoma Atractylodis Macrocephalae*), She She Cao (*Herba Hedyotis Diffusae*), Huang Jing (*Rhizoma Polygonati*), Gou Qi (*Fructus Lycii*), Ban Zhi Lian (*Sculellaria Barbata*), Mai Dong (*Radix Ophiopogonis*), and Shi Hu (*Dendrobium nobile*) (7.5 g each), San Qi (Pseudo-ginseng), E Zhu (*Curcuma Zedoary*), and San Leng (*Rhizoma Sparganii*) (5 g each). The patient carried out a course of treatment for three months and took a total of two courses of treatment.

Observational indicators

1. Symptomatic observation, gastroscopy, and pathohistological examination were performed once before treatment and once within one week after six months of treatment. The clinical efficacy in patients from the two groups was compared. Criteria for evaluating the efficacy of TCM symptoms: with the Nimodipine method, the efficacy index was calculated with (pre-treatment points - post-treatment points)/pre-treatment points × 100%. Criteria for determining clinical symptoms: cured: symptoms and signs disappeared or basically disappeared, efficacy index≥95%; significant effective: significant improvement in symptoms and signs, 70%≤efficacy index<95%; effective: significant improvement in symptoms and signs, 30%≤efficacy index<70%; ineffective: no significant improvement in symptoms and signs, or aggravation, efficacy index<30%. Determination criteria of clinical efficacy: clinical recovery: basic disappearance of clinical symptoms and signs, gastroscopic review of the chronic inflammation of the mucosa was significantly improved to a mild degree, and histopathologic examination confirmed normalization or disappearance of glandular atrophy, intestinalization, and dysplasia; significant effective: the disappearance of major clinical symptoms and signs, gastroscopy review of chronic inflammation of the mucosa was improved, and histopathologic examination confirmed that glandular atrophy, intestinalization, and dysplasia returned to normal or decreased by two grades; effective: the main symptoms and signs were significantly reduced, gastroscopic review of the mucosal lesion paradoxically shrunk by more than 1/2, and histopathological examination confirmed that chronic inflammation was reduced by one grade, glandular atrophy, intestinalization, and dysplasia were reduced; ineffective: failed to meet the above criteria for effectiveness, or deteriorated.

2. The Hp clearance rate after treatment was recorded in both groups. 14C-UBT was performed four to five weeks after six months of treatment. The patient was instructed to take one 14C urea capsule on an empty

stomach with about 20 mL of cool boiled water, and after sitting still for 15 min, the patient was instructed to blow into the expiratory card at a uniform rate (being careful not to suck inwards during this process until the color of the indicator changes from orange-red to yellow). The air collection card was inserted into the HUBT-20 plug-in Hp detector for automatic detection, and the result was printed after 5 min. Values for Hp infection: carbon 14 urea radioactivity ≥ 50 was positive for Hp; ≤ 25 was negative; and 25-50 was indeterminate. If the measurement result was negative, i.e., no Hp was detected by 14C-UBT, then Hp clearance was considered. Hp clearance rate=number of Hp-negative cases/total number of cases examined $\times$ 100%.

3. Fasting peripheral venous blood was collected before treatment and within one week after six months of treatment and serum was isolated. Serum inflammatory factor levels were compared between the two groups. Interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) were measured by enzyme-linked immunosorbent assay (ELISA) using kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

4. Fasting peripheral venous blood was collected before treatment and within one week after six months of treatment and serum was isolated. The levels of gastrin and motilin were compared between the two groups. Serum gastrin-17 (G-17) and serum motilin (MTL) were measured by ELISA using kits from JianglaiBio (Shanghai, China).

5. Fasting peripheral venous blood was collected before treatment and within one week after six months of treatment and serum was isolated. The pepsinogen content of patients in the two groups was compared. Levels of pepsinogen (PG) (PG I and PG II) were measured by the latex-enhanced immunoturbidimetric method.

6. Fasting peripheral venous blood was collected before treatment and within one week after six months of treatment and serum was isolated. The immunoradiometric fluorescence method was employed to evaluate the changes in serum gastrointestinal hormone endothelin (ET), epidermal growth factor (EGF), and calcitonin gene-related peptide (CGRP)

levels before and after treatment.

7. Adverse reactions (rash, diarrhea, dry mouth, abdominal pain, lethargy, etc.,) of patients were recorded.

8. Gastric mucosal tissues were harvested before treatment and within one week after six months of treatment. The levels of TLR4, NF-κB, and COX-2 mRNA in gastric mucosal tissues were compared.

qRT-PCR

An appropriate amount of gastric mucosa was made into tissue homogenate and total RNA was extracted using TRIzol reagent (Invitrogen). The concentration of these RNA samples was estimated with an Epoch UV spectrophotometer. The RNA obtained was reverse transcribed into cDNA using the PrimeScript RT reagent Kit with a gDNA Eraser (TaKaRa, Dalian, China). cDNA was detected with SYBR Green (TaKaRa) upon amplification with gene-specific primers (Table 1) and amplified in a fluorescent PCR instrument. We obtained cycle threshold (Ct) data and used the $2^{-\Delta\Delta Ct}$ method to standardize data against GAPDH expression levels.

Statistical methods

SPSS 22.0 software was implemented to analyze the data. Measurement data were described by means±standard deviations, which were processed by the t-test. Enumeration data were expressed as the number of cases, which were processed by the chi-square test. If data showed a P-value<0.05, the difference was statistically significant.

Results

General data

A total of 76 patients were enrolled in this research, including 38 patients in the control group and 38 patients

Table 1. Primer sequences for genes.

Gene	Primer sequence (5'-3')
TLR4	F: 5'-TTGAGCAGGTCTAGGGTGATTGAAC-3' R: 5'-ATGCGGACACACACACTTTCAAATA-3'
NF-κB	F: 5'-TGCACCTAGCTGCCAAAGAAGGA-3' F: 5'-TCTGCTCCTGCTGCTTTGAGAA-3'
COX-2	F: 5'-GTCTGATGATGTATGCCACAATCTG-3' R: 5'-GATGCCAGTGATAGAGGGTGTTAAA-3'
GAPDH	R: 5'-TGACTTCAACAGCGACACCCACT-3' R: 5'-GACTGAGTGTGGCAGGGACT-3'

F, forward; R, reverse.

Table 2. Comparison of patients' general data between the control (quadruple therapy: rabeprazole, amoxil, furazolidone, bismuth potassium citrate) and observation group (Weiwei granules based on the control group) for the treatment of chronic active *Helicobacter* gastritis with atrophy.

	Control group (n=38)	Observational group (n=38)	P
Age (years)	48.79±6.77	49.05±6.62	0.865
Gender [n (%)]			0.637
Male	22 (57.89)	25 (65.79)	
Female	16 (42.11)	13 (34.21)	
BMI (kg·m^{-2})	23.39±2.25	23.15±2.39	0.654
Disease duration (years)	6.50±2.44	6.24±2.77	0.662
Smoking history [n (%)]	7 (18.42)	10 (26.32)	0.583
Diabetes history [n (%)]	3 (7.89)	5 (13.16)	0.711
Cardiovascular disease [n (%)]	8 (21.05)	6 (15.79)	0.768

BMI, body mass index.

in the observation group. The general data of patients in the two groups were compared; the results revealed that there were no notable differences in age, sex ratio, body mass index, disease duration, smoking history, diabetes history, and cardiovascular disease of patients between the control and observation group (all *P*>0.05; Table 2).

Clinical efficacy

In terms of clinical efficacy, the total effective rate of patients was 76.32% in the control group, and 94.74% in the observation group. The difference in the total clinical efficacy of patients in the two groups was found to be statistically significant (*P*<0.05; Table 3).

Hp clearance rate

After treatment, 28 patients in the control group were Hp negative with 14C-UBT, and the Hp clearance rate was 73.68%; 34 patients in the observation group were Hp negative with 14C-UBT, and the Hp clearance rate was 89.47%; there was a higher Hp clearance rate in the observation group than in the control group (*P*<0.05;

Table 3).

Efficacy of TCM syndromes

In terms of the efficacy of TCM syndromes, the total effective rate was 94.74% in patients from the observation group and 68.42% in the control group. The difference between the two groups was found to be statistically significant, with the observation group higher than the control group (*P*<0.05; Table 3).

Pathohistological conditions

Before treatment, the difference between the control and observation group in terms of points for each pathohistological variable (chronic inflammation, activity, atrophy, intestinal metaplasia, and intraepithelial neoplasia) was not statistically significant (all *P*>0.05). After treatment, the histopathology of patients in both groups improved compared with that before treatment; the comparison of points for each variable in the observation group with the control group after treatment showed a statistically significant difference, with the

Table 3. Comparison of clinical efficacy, Hp eradication rate, the efficacy of traditional Chinese medicine syndromes, and pathohistological scores in patients between the control (quadruple therapy: rabeprazole, amoxil, furazolidone, bismuth potassium citrate) and observation group (Weiwei granules based on the control group) for the treatment of chronic active *Helicobacter* gastritis with atrophy.

		Control group (n=38)	Observation group (n=38)	*P*
Clinical efficacy [n (%)]				
Cured		3 (7.89)	5 (13.16)	
Significant effective		15 (39.48)	17 (44.74)	
Effective		11 (28.95)	14 (36.84)	
Ineffective		9 (23.68)	2 (5.26)	
Total effective rate (%)		76.32	94.74	0.022
Helicobacter pylori eradication rate [n (%)]				
Negative		28 (73.68)	34 (89.47)	
Positive		10 (26.32)	4 (10.53)	
Eradication rate (%)		73.68	89.47	0.033
Efficacy of traditional Chinese medicine syndromes [n (%)]				
Clinical recovery		6 (15.79)	18 (47.38)	
Significant effective		7 (18.42)	9 (23.68)	
Effective		13 (34.21)	9 (23.68)	
Ineffective		12 (31.58)	2 (5.26)	
Total effective rate (%)		68.42	94.74	0.003
Pathohistological scores (points)				
Atrophy	Before treatment	5.21±1.65	5.16±1.76	0.893
	After treatment	3.74±1.93*	2.76±1.48*	0.016
Intestinal metaplasia	Before treatment	3.58±2.06	3.53±1.83	0.907
	After treatment	3.03±2.26*	2.10±1.52*	0.041
Intraepithelial neoplasia	Before treatment	3.84±2.32	3.95±2.40	0.846
	After treatment	3.26±2.45*	2.24±1.60*	0.034
Chronic inflammation	Before treatment	1.68±0.84	1.91±0.85	0.141
	After treatment	1.45±1.00*	0.89±0.73*	0.008
Activity	Before treatment	2.05±0.80	2.03±0.79	0.886
	After treatment	1.47±1.01*	0.95±0.73*	0.011

**P*<0.05 vs. before treatment.

observation group being superior to the control group (all *P*<0.05; Table 3). Pathological examination results are shown in Figure 1. Before treatment, inflammatory cell infiltration was seen in the gastric mucosal tissues, the number of intrinsic glands was significantly reduced and decreased in size, and the inflammation of the mucosa and the shape of the glands were improved after treatment.

Adverse effects

Adverse reactions occurred in the control and observation group. The incidence of adverse reactions in the control group was 18.41% (one case of rash, two cases of diarrhea, two cases of dry mouth, one case of abdominal pain, and one case of lethargy); the incidence of adverse reactions in the observation group was 21.05% (two cases of rash, three cases of diarrhea, two cases of dry mouth, one case of abdominal pain, and one case of lethargy). The difference in the incidence of adverse reactions between the control and observation

group was not statistically significant (*P*>0.05).

Serum inflammatory factor levels and gastrin and motilin levels

Before treatment, no significant difference was witnessed in IL-6, IL-8, TNF-α, G-17, and MTL levels between the control and observation group (all P>0.05). After treatment, lower IL-6, IL-8, and TNF-α levels, together with higher G-17 and MTL levels, were observed in both the control and observation group; however, these differences in levels before and after treatment were greater in the observation group than in the control group (all *P*<0.05; Table 4).

Gastrointestinal hormone levels and pepsinogen levels

Before treatment, gastrointestinal hormone (ET, EGF, and CGRP) and pepsinogen (PG I and PG II) levels were not notably different between the control and observation group (all P>0.05). After treatment,
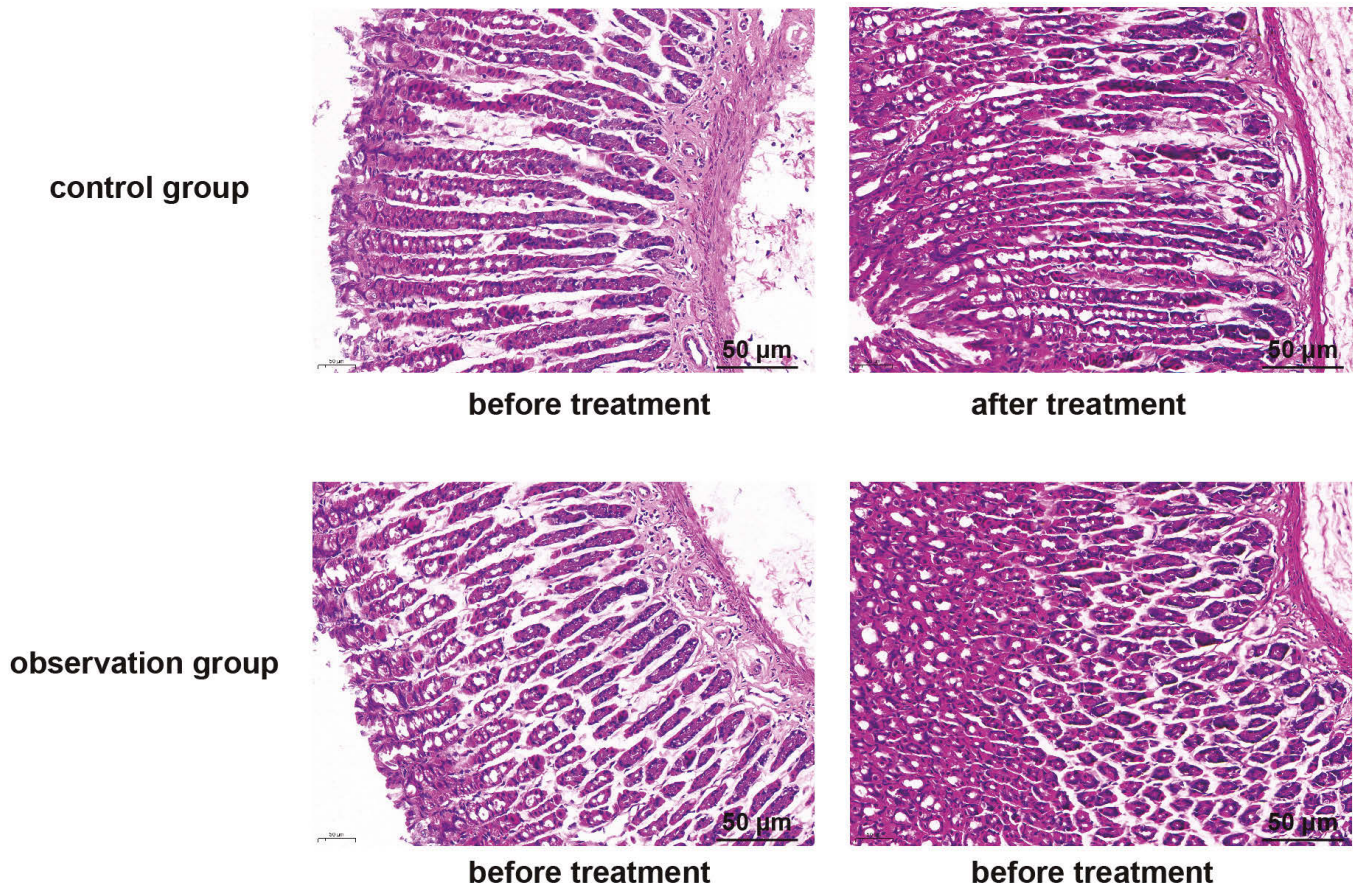

Fig. 1. Pathological examination results between the control (quadruple therapy: rabeprazole, amoxil, furazolidone, bismuth potassium citrate) and observation group (Weiwei granules based on the control group) for the treatment of chronic active *Helicobacter* gastritis with atrophy. Before treatment, inflammatory cell infiltration was seen in the gastric mucosal tissues, and the number of intrinsic glands was significantly reduced and decreased in size, and the inflammation of the mucosa and the shape of the glands were improved after treatment. HE staining, ×200.

gastrointestinal hormone levels (ET and EGF) were decreased and CGRP and PG I were increased in both the control and observation group; a greater reduction in ET and EGF, together with more elevated CGRP and PG I levels were seen in the observation group compared with the control group (all *P*<0.05; Table 5).

TLR4, NF-κB, and COX-2 expression levels

Before treatment, the expression levels of TLR4, NF-κB, and COX-2 exhibited no differences between the two groups (all *P*>0.05). After treatment, the expression levels of TLR4, NF-κB, and COX-2 decreased in the

two groups, with a greater decrease in the observation group (all *P*<0.05; Table 6).

Discussion

Chronic atrophic gastritis is described as the reduction or the vanishment of the original gastric glands, which could be substituted for pseudo-pyloric or intestinal metaplasia (Park et al., 2021). This condition could contribute to an enhanced risk of gastric cancer due to changes in the intragastric microenvironment (Lahner et al., 2019). Even with the rapid progression of modern medical therapy for CAG, no specific treatment

Table 4. Comparison of serum inflammatory factor levels and gastrin and motilin levels in patients between the control (quadruple therapy: rabeprazole, amoxil, furazolidone, bismuth potassium citrate) and observation group (Weiwei granules based on the control group) for the treatment of chronic active *Helicobacter* gastritis with atrophy.

Indicator	Control group (n=38)		Observation group (n=38)	
	Before treatment	After treatment	Before treatment	After treatment
IL-6 (pg/mL)	19.16±2.46	11.78±1.73*	19.05±2.64	6.68±0.94*#
IL-8 (pg/mL)	22.46±2.59	17.65±1.62*	22.48±2.06	10.75±1.53*#
TNF-α (pg /mL)	42.50±5.77	30.39±4.25*	43.15±5.39	21.85±3.57*#
G-17 (ng /L)	289.81±32.46	360.53±43.39*	292.26±31.79	399.16±49.38*#
MTL (ng /L)	175.86±18.13	218.27±31.18*	173.85±17.31	252.86±35.94*#

IL-6, interleukin-6; IL-8, interleukin-8; TNF-α, tumor necrosis factor-α; G-17, gastrin-17; MTL, motilin. * *P*<0.05 vs. before treatment; # *P*<0.05 vs. control group.

Table 5. Comparison of gastrointestinal hormone and pepsinogen levels in patients between the control (quadruple therapy: rabeprazole, amoxil, furazolidone, bismuth potassium citrate) and observation group (Weiwei granules based on the control group) for the treatment of chronic active *Helicobacter* gastritis with atrophy.

Indicator	Control group (n=38)		Observation group (n=38)	
	Before treatment	After treatment	Before treatment	After treatment
ET (ng /L)	56.16±6.46	40.78±5.73*	57.05±6.64	36.15±5.11*#
EGF (ng /mL)	4.46±0.59	3.65±0.42*	4.48±0.60	2.74±0.35*#
CGRP (ng /L)	27.62±6.14	32.99±5.49*	27.75±6.32	39.38±6.14*#
PG I (µg/L)	75.73±8.06	97.35±10.14*	73.91±8.41	125.66±13.66*#
PG II (µg/L)	12.98±3.14	12.36±3.68	12.84±3.67	12.76±3.55

ET, endothelin; EGF, epidermal growth factor; CGRP, calcitonin gene-related peptide; PG I, pepsinogen I; PG II, pepsinogen II. * *P*<0.05 vs. before treatment; # P<0.05 vs. control group.

Table 6. Comparison of TLR4, NF-κB, and COX-2 expression in patients between the control (quadruple therapy: rabeprazole, amoxil, furazolidone, bismuth potassium citrate) and observation group (Weiwei granules based on the control group) for the treatment of chronic active *Helicobacter* gastritis with atrophy.

Indicator	Control group (n=38)		Observation group (n=38)	
	Before treatment	After treatment	Before treatment	After treatment
TLR4	3.76±0.46	2.78±0.33*	3.75±0.44	1.57±0.29*#
NF-κB	5.76±0.89	2.85±0.42*	5.48±0.86	2.34±0.35*#
COX-2	3.49±0.43	2.17±0.41*	3.41±0.46	1.21±0.33*#

TLR4, Toll-like receptor 4; nuclear factor-kappa B, NF-κB; cyclooxygenase-2, COX-2. * *P*<0.05 vs. before treatment; # *P*<0.05 vs. control group.

has been observed in clinical practice. Based on this, we undertook this research to elucidate the mechanism of Weiwei granules in the treatment of Hp-positive CAG patients.

TCM, on the one hand, improves clinical symptoms and, on the other, controls CAG progression and alleviates gastric mucosal precancerous lesions, thus diminishing the prevalence and mortality of GC (Ma et al., 2022). At present, many articles have revealed the therapeutic effects of different TCM prescriptions for CAG. For example, the Wei-Wei-Kang-Granule (WWKG) is very suitable for CAG treatment, with no significant side effects (Lin et al., 2012). In the meantime, the Zuojin pill (ZJP) is used for treating CAG clinically, and its pharmacological functions and potential mechanisms in CAG therapy have been clarified (Wen et al., 2022). In our work, we observed that Weiwei granules based on quadruple therapy alleviated inflammatory reactions (reduced IL-6, IL-8, and TNF-α levels), effectively eradicated Hp, and had good efficacy and high safety in treating chronic active *Helicobacter* gastritis with atrophy. In TCM theory, CAG belongs to stomach pain and abdominal distention resulting from Qi deficiency and blood stasis. Weiwei granules are a Chinese patent medicine made based on years of experience, which has the effects of invigorating qi and blood circulation, tonifying spleen and Yin, and detoxification. The formula of Weiwei granules was as follows: Dang Shen (*Codonopsis Pilosula*) and Huang Qi (*Astragalus Mongholicus*) (15 g each); Bai Zhu (*Rhizoma Atractylodis Macrocephalae*), She She Cao (*Herba Hedyotis Diffusae*), Huang Jing (*Rhizoma Polygonati*), Gou Qi (*Fructus Lycii*), Ban Zhi Lian (*Sculellaria Barbata*), Mai Dong (*Radix Ophiopogonis*), and Shi Hu (*Dendrobium nobile*) (7.5 g each); and San Qi (Pseudo-ginseng), E Zhu (*Curcuma Zedoary*), and San Leng (*Rhizoma Sparganii*) (5 g each). Dang Shen, Huang Qi, and Bai Zhu strengthen the spleen and benefit the vital energy. The four herbs, Gou Qi, Mai Dong, Huang Jing, and Shi Hu, nourish the yin and benefit the stomach. Together with the first three herbs, they replenish the deficiencies of the spleen and stomach and restore the function of the middle jiao for transportation. San Qi, San Leng, and E Zhu improve blood circulation and disperse stasis, while She She Cao and Ban Zhi Lian clear away heat and toxic material. The latter five herbs are used to drive out evil spirits, resolve blood stasis, and detoxify toxins. The combination of all these medicines works to benefit the qi to strengthen the spleen, nourish and benefit the stomach, and also activate blood circulation and remove blood stasis, as well as clearing heat and detoxification. All of these drugs work together correlatively to achieve the treatment of CAG. Unfortunately, there are few studies on Weiwei granules in CAG therapy, thus requiring further clinical verification in the future.

In this paper, we measured the levels of serum inflammatory factors (IL-6, IL-8, and TNF-α), PG I and PG II, G-17, and motilin, and gastrointestinal hormones (ET, EGF, and CGRP) to validate the therapeutic effect of Weiwei granules. G-17 has potential diagnostic power because it displays good specificity for CAG diagnosis (Wang et al., 2016). Motilin induces the production of pepsin in gastric master cells, as well as the generation of pancreatic polypeptide and somatostatin, which is linked to disturbed gastrointestinal motility (Al-Missri and Jialal, 2022). A low PG I/II ratio could be utilized for detecting atrophic gastritis, and the PG I/II ratio is relevant in nutritional and metabolic disorders (Arinton, 2010). ET is a strong stimulator of the release of neuropeptides in neurogenic inflammation (Camara et al., 2012). EGF exerts protective effects against gastric congestion and tracheal transfusion via capsaicin-sensitive afferent neurons (Matsumoto et al., 2001). CGRP has anti-platelet aggregation, anti-apoptosis, anti-proliferation, anti-oxidant, as well as anti-senescence properties, revealing that CGRP is a vital endogenous substance protecting against high-risk diseases (Luo et al., 2013). The findings of our research disclose that Weiwei granule treatment contributed to reduced levels of serum inflammatory factors, ET, and EGF, and elevated levels of G-17, motilin, PG I, and CGRP.

Furthermore, our paper also highlights that Weiwei granule treatment could reduce TLR4, NF-κB, and COX-2 levels and that Weiwei granules functioned in CAG via the TLR4/NF-κB/COX-2 inflammatory signaling pathway. Similarly, it was previously suggested that Qinghuayin (QHY) administration could reduce the expression levels of TLR4, NF-κB, and COX-2 in CAG (Li et al., 2018a). Another study demonstrated that ZJP exerts therapeutic effects on Hp-positive CAG by blocking the JMJD2B/COX-2/VEGF axis and the HMGB1/NF-κB pathway (Wen et al., 2022). Moreover, a Chinese article has underlined that Wenweishu Capsule treatment can improve histopathological changes, alleviate gastric cavity signs, reduce serum levels of inflammatory factors and the protein levels of NF-κBp65, IκBα, and COX2, highlighting that Wenweishu Capsules might alleviate gastric mucosal injury in rats with CAG (Zhang et al., 2020). All these findings confirm the significance of the TLR4/NF-κB/COX-2 axis in the participation of TCM formulations in CAG treatment.

In summary, this paper suggests that Weiwei granules can effectively improve Hp-positive CAG patients and reduce the expression levels of TLR4, NF-κB, and COX-2. The TLR4/NF-κB/COX-2 axis might be a vital mechanism for the functions of Weiwei granules. This paper validates the properties of Weiwei granules as an adjuvant treatment method against CAG, nevertheless, its inner mechanisms warrant further elucidation. Additionally, further expansion of the sample size is needed to enrich our findings in the future.

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