Exploration of Tuina Effect and Mechanism in Chronic Atrophic Gastritis Rats

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ABSTRACT

Background: *Chronic atrophic gastritis is a very common condition in the general clinical practice of gastroenterology. Tuina treatment is widely used clinically. The aim of our study was to investigate the mechanisms of tuina in rats with chronic atrophic gastritis.*

Methods: *Fifty-six specific pathogen-free grade rats were employed for our study. They were divided into 5 groups and treated differently. Body weight was recorded every week, and their small intestine propulsive ratio was measured after intragastric administration of carbon powder. Histopathological observation of gastric tissues was performed using hematoxylin and eosin staining. The levels of motilin and gastrin in serum were detected by enzyme-linked immunosorbent assay, and the expression levels of Bcl-2 and cytochrome C were measured by the western blot assay.*

Results: *There was no significant difference in body weight and small intestine propulsive ratio between the chronic atrophic gastritis model group and the tuina group ($P > .05$). However, we can see some significant changes in histomorphology after treatment with tuina. For example, the atrophy of gastric mucosal epithelium and glands had improved, and the inflammatory cells infiltrating the lamina propria were decreased significantly. Moreover, the level of gastrointestinal hormone GAS was increased ($P < .001$), and there was no statistically significant difference in motilin, Bcl-2, and cytochrome-c after treatment with tuina ($P > .05$).*

Conclusion: *Our research demonstrated the effectiveness of tuina treatment on chronic atrophic gastritis with a possible underlying mechanism that affected the secretion of gastric acid, which could provide some useful information for clinical application.*

Keywords: *Apoptosis, chronic atrophic gastritis, gastrointestinal hormones, tuina*

INTRODUCTION

Chronic atrophic gastritis (CAG), defined as the disappearance of the normal glands after suffering repeated damage, with or without intestinal metaplasia,[1] is considered as an important precancerous disease in the development of gastric cancer.[2] It is a kind of progressive digestive disease, and occurs in 25.8% of patients with gastritis.[3] The incidence of CAG is influenced by multiple factors, such as *Helicobacter pylori* infection, the living environment, irregular diet, heredity, age, and autoimmunity. The clinical symptoms include stomach distention, stomach ache, anemia, and depression. As a result, it continues to be a significant public health burden, which severely impacts the quality of human life.[4]

Currently, *H. pylori* eradication, acid suppression, and vitamin supplementation are commonly used to treat CAG. Though traditional Chinese drugs,[5] western medicine,[6,7] acupuncture,[8] and other therapies have emerged in recent years, there are still some deficiencies, and the treatment may be inefficient, harmful, and expensive. Therefore, it is important and necessary to search for an effective treatment for CAG. Tuina, which has been used for a thousand years, is a typical Chinese way for treating disease. Currently, it is increasingly applied in clinical practice because of its low cost and safety.[9] The therapy method of tuina is a series of orderly manual techniques (pressing, kneading, knocking, and friction) acting on the surface of the body. The selection of manual acupoint is based on the zang-fu organs and the meridian theory of traditional Chinese medicine (TCM). By pressing these acupoints, tuina can increase the distribution of blood and warmth to the distal regions,[10] thereby unblocking meridians and achieving Yin and Yang balance. What's more, research has demonstrated that tuina can significantly shorten transit time, reduce abdominal distension, increase bowel movements, and promote the return of normal bowel function.[11] However, there has

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been no research exploring the mechanism of tuina in the treatment of CAG. Gastric contraction has been well-documented to have strong association with plasma motilin (MTL) level,[12] and the primary physiological function of gastrin (GAS) is to stimulate the secretion of gastric acid.[13] With CAG occurrence, the serum levels of MTL and GAS would change.[14] In addition, a previous study indicated that abnormal apoptosis of gastric epithelial cells was involved in the progression of CAG.[15] Bcl-2 is an anti-apoptotic protein, and cytochrome C (cyt-c) is an important protein in the mitochondrial electronic respiratory chain. They all participate in the process of apoptosis.[16]

To explore the underlying mechanism of tuina in the treatment of CAG, we conducted a research study in 56 specific pathogen-free (SPF) grade rats by observing their body characteristics and measuring the levels of gastrointestinal hormones and apoptosis-related proteins. Our study may provide a theoretical foundation for clinical application of tuina in CAG treatment.

MATERIALS AND METHODS

Animals

Fifty-six SPF-grade rats (8 weeks old, 200 ± 20g) were provided by the Animal Experiment Center of Xinjiang Medical University. They were given compound feeds, and housed in an air-conditioned animal room (room temperature, 22.5 ± 2.5°C; relative humidity, 65%). All rats were allowed to acclimatize to the environment for a week before the experiment was started. Our study was performed in compliance with the guidance for the Care and Use of Laboratory Animals formulated by the National Institutes of Health, and was approved by the Ethics Committee of Xinjiang Medical University. The need for informed consent was not applicable to our study.

Main Points

- *There were significant changes in histomorphology; the atrophy of gastric mucosal epithelium and glands was improved; and the inflammatory cell infiltration in the lamina propria was decreased significantly, after treatment with tuina.*
- *The gastrointestinal hormone level of gastric increased after treatment with tuina.*
- *There was no statistically significant difference in apoptosis-related proteins of Bcl-2 and cytochrome C (cyt-c) between the model group and the tuina group.*

Tuina Stroking

The manual techniques of tuina were as follows: The rats were first grabbed by the fur on their back, to the degree that they could be easily controlled. Next, they were placed in the supine position and the abdomen was pressed with the fingers, with a light intensity of stimulation. Then, the abdomen was rubbed clockwise for 8 minutes.

CAG Model Establishment and Treatment

After habituation for a week, 6 rats were randomly selected for the preliminary experiment (3 for the control group and 3 for the model group), and the remaining 50 rats were divided into 5 groups (n = 10) including a control group, model group, drug group, (Omeprazole 0.44 mg/kg, Clarithromycin 6.92 mg/kg, and Metronidazole 7.6 mg/kg; triple-drug therapy[17]), the tuina group, and the drug + tuina group.

The CAG rats in the model group were freely administered *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG, 100 μg/L, MB0455, Meilunbio, Shanghai, China) combined with irregular feeding (alternating between one day of sufficient food and one day of fasting) for 12 weeks.[18] Rats in the control group could freely access normal food and water for 12 weeks, and then they were perfused with normal saline. The other 3 groups were treated with corresponding therapies (drug group: triple-drug therapy once a day; tuina group: rub the abdomen clockwise for 8 minutes a day; drug + tuina group: triple-drug therapy combined with tuina) in the next 70 days after the model established. Finally, 0.8 mL carbon powder suspension was administered by gastric gavage, and then the rats were sacrificed after 20 minutes. The study design is shown in Figure 1.

Observation of Animal Body Index

Animal weight was recorded during the process of model establishment and treatment. Additionally, the small intestine propulsive ratio was calculated according to the moving distance of carbon powder. The calculation formula was as follows: small intestine propulsive ratio = (carbon powder moving distance/total length of ileum–cecum) × 100%.

Histopathological Observation (H&E)

The gastric antrum tissues were processed as follows: (1) The tissues were collected from the rats of each group. (2) The tissues were washed with normal saline. (3) Tissues were fixed with 10% neutral buffered formalin and embedded in paraffin. (4) Tissues were stained with

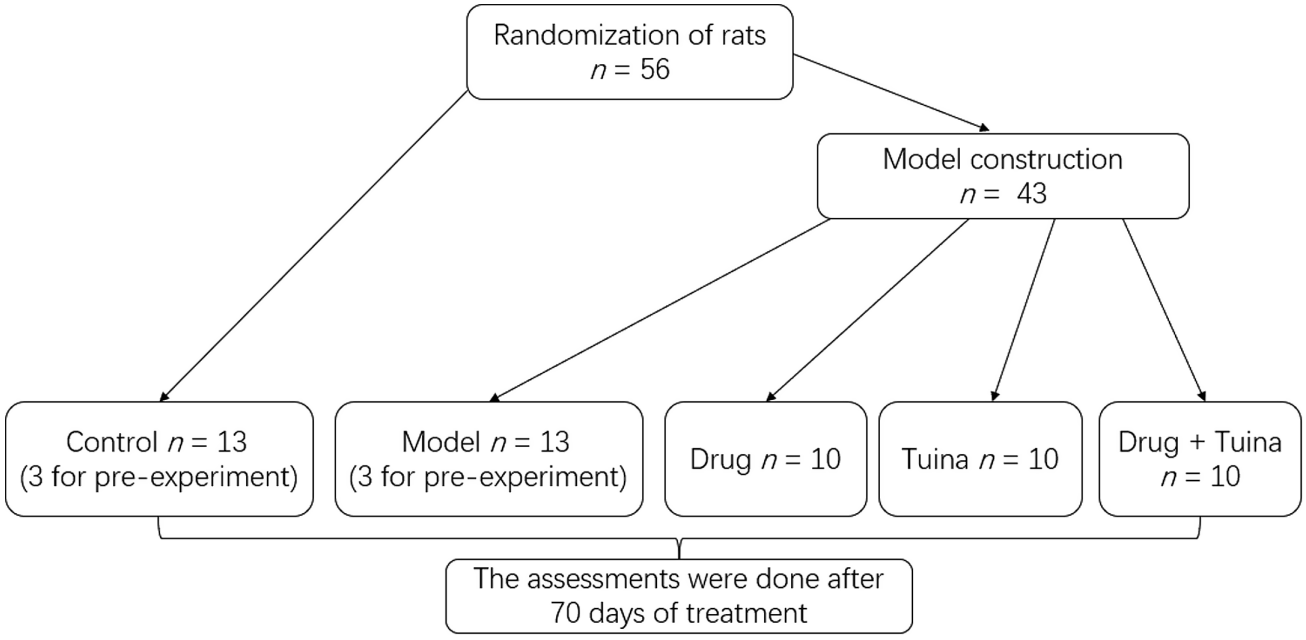

Figure 1. Study design of the work.

H&E. Finally, we observed and analyzed the tissues using a light microscope.

Enzyme-Linked Immunosorbent Assay (ELISA)

The abdominal aortic blood of rats in each group was collected, centrifuged (4°C, 3000 rpm, 10 minutes) to collect the serum, and then stored at −20°C. Serum MTL and GAS levels were determined using a commercial ELISA kit (Wuhan Huamei Biotech Co. Ltd., Wuhan, China) accordig to the manufacturer's instructions, respectively. The optical density (OD) values were recorded and then used to calculate the corresponding protein content.

Western Blot Assay

The total proteins in the gastric tissues of all rats were extracted using radio immunoprecipitation assay (RIPA) lysis buffer with a phosphatase inhibitor cocktail, and the protein concentrations were detected with the bicinchoninic acid assay kit (Beyotime, Jiangsu, China) in compliance with the manufacturer's instructions. Protein samples were separated on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. After blocking with 5% non-fat dry milk at room temperature for 1 hour, the membranes were incubated with primary antibodies overnight at 4°C. The primary antibodies were anti-cytochrome C (bs-0013R, Bioss Antibodies, Woburn, MA, USA), anti-Bcl-2 (bs-4563R, Bioss), and anti-β-actin. Then, all blots were incubated with fluorescent secondary antibody at room temperature for 1 hour. Finally, the protein bands were visualized using western blotting detection kits, and Image J software was used to analyze the results.

Statistical Analyses

All experimental data were displayed as mean ± SD. The Statistical Package for Social Sciences (SPSS), version 19.0 software (SPSS Inc.; Chicago, IL, USA) was applied to statistical analyses. Statistical comparisons were made by one-way analysis of variance (ANOVA). GraphPad Prism 5.0 was used as an assistant drawing tool. A value of $P < .05$ was considered to be a significant difference.

RESULTS

Body Weight and Small Intestine Propulsive Ratio

In the period during 19-22 weeks, although an increase was observed in the average body weight in the other 4 groups compared with the model group, it was not statistically significant ($P > .05$, Figure 2). The weight data are shown in Supplementary Table 1. In terms of the small intestine propulsive ratio, though we found that the ratio was decreased in the model group compared to the control group, there was no significant difference between the model group and the other 3 intervention treatment groups ($P > .05$, Figure 3), which indicated that the 3 therapies did not improve the small intestinal peristaltic function.

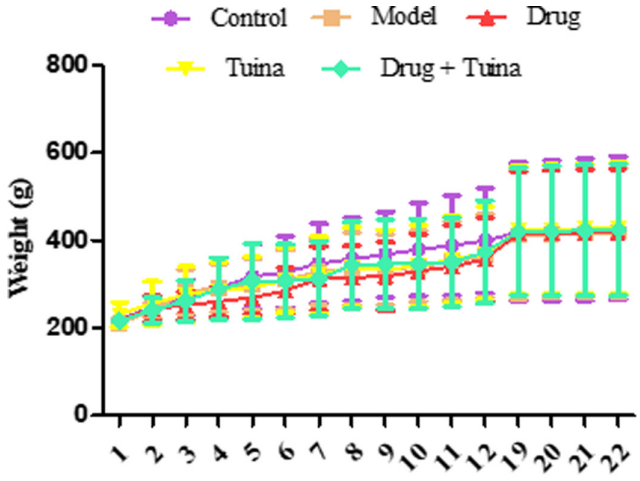

Figure 2. Changes in mean body weight during the experimental process. Control – rats received no treatment; Model – rats were treated with MNNG; Drug – rats were treated with MNNG and triple-drug therapy; Tuina – rats were treated with MNNG and tuina; Drug + Tuina – rats were treated with MNNG, tuina, and triple-drug therapy. MNNG, N-methyl-N′-nitro-N-nitrosoguanidine.

Histological Lesions of Gastric Tissues in CAG Rats

Gastric tissues were obtained from the rats, and H&E staining was employed to detect the pathological changes to evaluate the therapeutic efficacy on CAG rats. As shown in Figure 4, the mucosal epithelium of rats in the control group was complete, with orderly arrangement of the glands, no hyperplasia and metaplasia in the lamina propria, and no change in the muscular layer and stroma of the mucosa. In the model group, there were some changes in the mucosa and glands, such as the thinning of the mucosal layer, atrophy of the glands, incomplete glandular duct, and significantly acute and chronic inflammatory cells infiltrating the lamina propria, indicating that the CAG rat model was successfully established. Furthermore, after tuina treatment, the thickness of the mucosa had increased slightly; moreover, the degree of gland atrophy had decreased, and the infiltration of inflammatory cells in the lamina propria had also decreased. In addition, the atrophy of the gastric mucosal epithelium and glands had recovered, and the inflammatory cells had decreased significantly in the drug and drug + tuina groups.

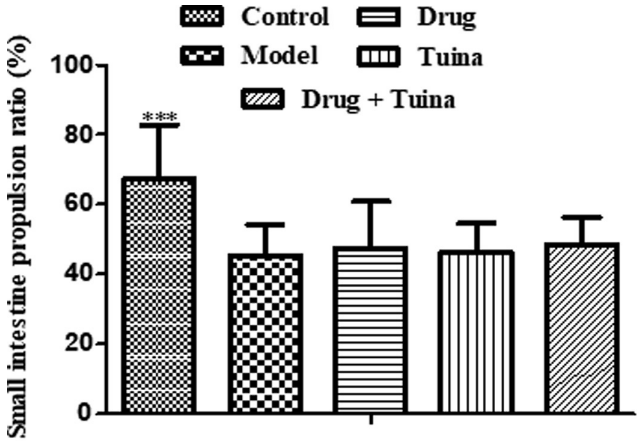

Figure 3. Effect of different treatments on the small intestine propulsive ratio in CAG rats. Control – rats received no treatment; Model – rats were treated with MNNG; Drug – rats were treated with MNNG and triple-drug therapy; Tuina – rats were treated with MNNG and tuina; Drug + Tuina – rats were treated with MNNG, tuina, and triple-drug therapy. ***$P < .001$ vs model group. CAG, chronic atrophic gastritis; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

Tuina Affected the Level of Gastrointestinal Hormones in Serum of CAG Rats

After measuring the MTL and GAS levels of gastrointestinal hormones in the serum of each group (Figure 5a, Figure 5b), we found that the content of GAS in serum was reduced, whereas MTL was increased in the CAG model group compared with the control group. After tuina treatment, the level of GAS had increased ($P < .001$), while there was no statistic difference in the level of MTL ($P > .05$).

Expression of Bcl-2 and Cyt-c in CAG Rats

Finally, analyzing the apoptosis-related proteins of Bcl-2 and cyt-c, we found that the increased Bcl-2 expression in the stomach tissues of CAG model group was reduced in the drug and drug + tuina groups ($P < .05$, Figure 6a), and a decrease was also noticed in the tuina group. However, the difference was not statistically significant ($P > .05$, Supplementary Table 2). Similarly, the expression of cyt-c had increased in the model group, after treatment with drug and drug + tuina, the expression level had reduced ($P < .05$), but there was no statistically significant difference in rats treated with tuina alone ($P > .05$, Figure 6b). The expression of Bcl-2 and cyt-c proteins is shown in Figure 6c. Our results indicate that there is no statistically significant difference in apoptosis-related proteins of Bcl-2 and cyt-c with tuina treatment.

DISCUSSION

A disease caused by numerous combined risk factors, CAG is considered as an significant precancerous disease in the development of gastric cancer.[19] Our previous study has demonstrated that tuina treatment could alleviate the symptoms of gastritis clinically,[20] though its mechanism is still unclear. Therefore, we conducted a study on

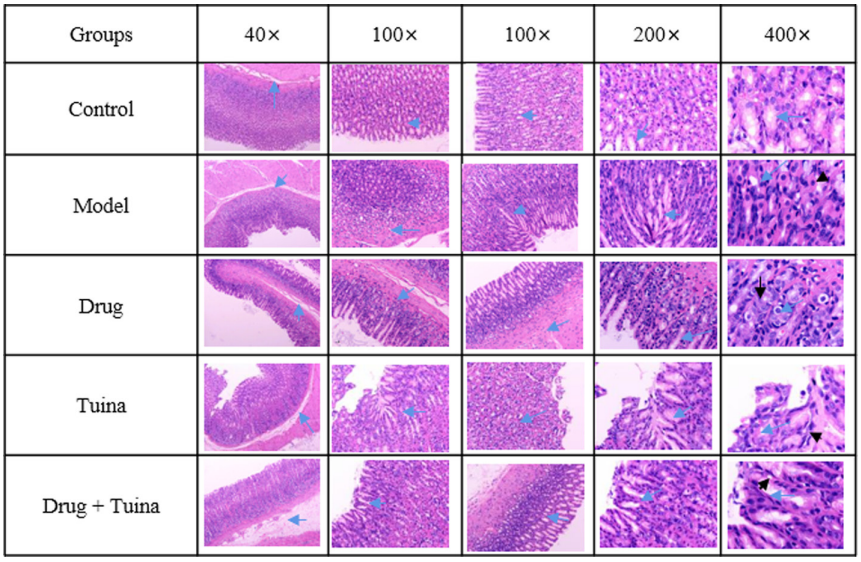

Figure 4. Pathological changes in the gastric mucosa of CAG rats. Gastric mucosa was stained with H&E, and inflammatory changes were observed under 40×, 100×, 200×, and 400×. No significant pathological changes were found in the control group. Significant pathological changes were found in the model group, which include thinning of lamina propria, reduction in gland cells, and infiltration of inflammatory cells. These pathological changes were reduced in the 3 treatment groups. Control – rats received no treatment; Model – rats were treated with MNNG; Drug – rats were treated with MNNG and triple-drug therapy; Tuina – rats were treated with MNNG and tuina; Drug + Tuina – rats were treated with MNNG, tuina, and triple-drug therapy. CAG, chronic atrophic gastritis; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

CAG rats to explore how it works. Finally, we found that there was no significant difference either in body weight or in the small intestine propulsive ratio between the CAG model group and the tuina group. However, there was an increased secretion of gastrin, and the pathological changes of the gastric tissue were improved after treatment with tuina.

Weight loss, depression, slow movement, and dull fur are the main symptoms of CAG in rats.[21] Bai Yu et al[22] also showed in their study that compared with the CAG group, the weight of the treatment groups increased significantly. The mechanism may be that atrophic gastritis may reduce circulating ghrelin levels, resulting in a decrease in the number of ghrelin-producing cells, which

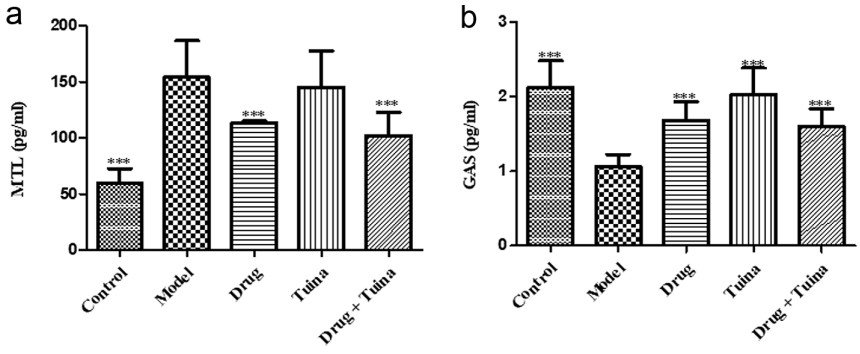

Figure 5. Effect of different treatments regulated the level of gastrointestinal hormones in serum of CAG rats. The levels of gastrointestinal hormones including (a) MTL and (b) GAS were measured by the respective ELISA kits. Control – rats received no treatment; Model – rats were treated with MNNG; Drug – rats were treated with MNNG and triple-drug therapy; Tuina – rats were treated with MNNG and tuina; Drug + Tuina – rats were treated with MNNG, tuina, and triple-drug therapy. ***$P < .001$ vs. model group. CAG, chronic atrophic gastritis; MTL, motilin; GAS, gastrin; ELISA, enzyme-linked immunosorbent assay; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

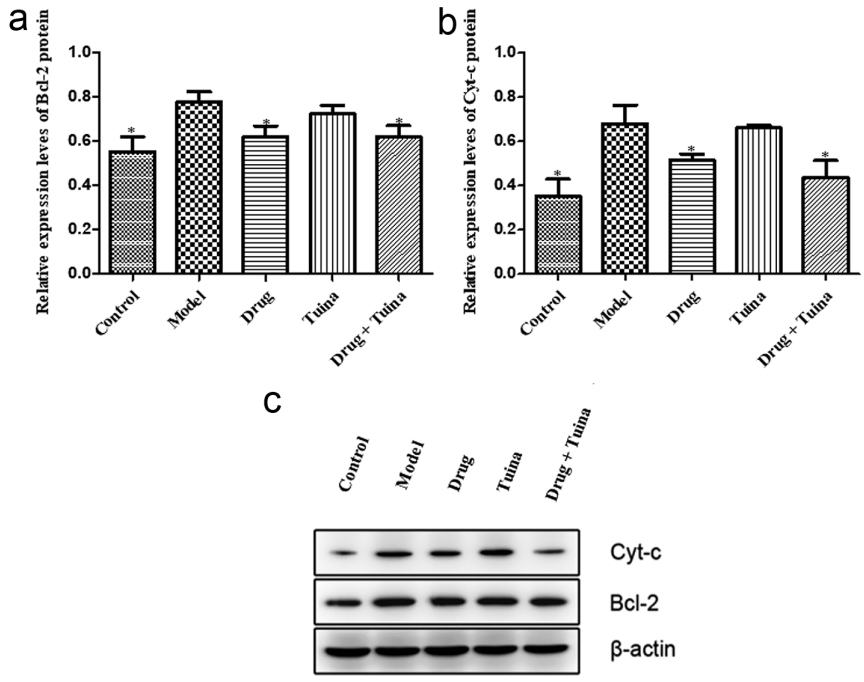

Figure 6. Different treatments affected the expression of apoptosis-associated proteins in the gastric tissues of CAG rats. (a) Relative mRNA expression of Bcl-2; (b) Relative mRNA expression of cyt-c; (c) Protein expression of genes in each group. Control – rats received no treatment; Model – rats were treated with MNNG; Drug – rats were treated with MNNG and triple-drug therapy; Tuina – rats were treated with MNNG and tuina; Drug + Tuina – rats were treated with MNNG, tuina, and triple-drug therapy. *$P < .05$ versus model group. CAG, chronic atrophic gastritis; cyt-c, cytochrome C; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

indirectly affects the body weight.[23] After treatment by drug or other methods, the atrophy of oxyntic glands may be recovered and the ghrelin level increased, which then results in weight gain. We have a strong feeling that the short duration of the intervention is the main reason for the negative results of our study.

As for the pathological changes of gastric tissue, CAG is characterized by atrophy of gastric mucosal epithelium and glands, thinning of gastric mucosa, and reduced number of glands.[24] Our study demonstrated that tuina treatment could reduce the inflammation, decrease the number of atrophic glands, and recover the morphology of gastric mucosa, which indicated that tuina was effective in histology.

Subsequently, we evaluated the effectiveness of tuina on gastrointestinal hormones of GAS and MTL. Gastrin, synthesized in the pyloric mucosal G cells, is the main hormonal stimulant of acid secretion during the ingestion of food, and is also a trophic hormone that maintains the integrity of gastric mucosa. Simultaneously, it may play a role in carcinogenesis.[13] Our results showed that the serum level of GAS was reduced in the CAG group and increased after treatment with tuina, which was consistent with the result of Ji Zhang.[14] We guess that it maybe because the atrophic gastric mucosa lead to a decrease in the number of G cells, which decrease the level of GAS. Several studies have reported that GAS can be used as a functional marker in the state of the gastric mucosa,[25] which can prove the effect of tuina in our study to a certain extent. At the same time, GAS could promote the contraction of the pyloric sphincter, slow down gastric emptying, and then reduce the small intestine propulsive ratio. Although there was no statistic difference between the model group and the other 3 intervention treatment groups, we can see that the ratio of the model group is lower than control group from our results. Motilin, produced by endocrine cells of the proximal small intestine, is a gastroprokinetic hormone,[26] and its function is to stimulate gastrointestinal smooth muscle contraction. Normally, MTL levels increase cyclically every 90 to 120 minutes, and this cycle could be broken after the ingestion of a meal.[27] Clinical studies have found that the MTL levels in patients with gastritis are higher than normal,[28] which is in accordance with our results.

Previous studies have demonstrated that the abnormal apoptosis of gastric mucosa was confirmed as an important factor in the development of CAG.[15] We conducted our study with 2 common apoptosis-related factors, Bcl-2 and cyt-c. The Bcl-2 family plays an important role in programmed cell death. Changes in the quantitative relationship between Bax and Bcl-2 activated the mitochondrial apoptotic pathway,[29] and then cyt-c, is released from the mitochondrial intermembrane space to the cytoplasm,[30] which induces caspase activation, ultimately resulting in programmed cell death.[31] Our western blot analysis showed that there was no statistically significant difference in Bcl-2 and cyt-c between the tuina group and the model group. However, we found that the expression levels of the 2 proteins were reduced in drug and drug + tuina groups, which indicated that tuina treatment did not reverse the process of apoptosis in our study.

In conclusion, our research showed that tuina could repair pathological lesions of gastric mucosa, increase the serum level of GAS, and then improve the symptoms of CAG. Our findings reveal the mechanism of tuina and provide a theoretical basis for clinical application. Moreover, there are some shortcomings in our study, such as the short duration of the intervention, and some impact factors such as Bax and caspase are not referred. Therefore, it is necessary to conduct further in-depth research.

Ethics Committee Approval: Our study was performed in compliance with the guide for the Care and Use of Laboratory Animals formulated by the National Institutes of Health, and was approved by the Ethics Committee of Xinjiang Medical University.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Y.Q.; Design – X.M.; Writing Manuscript – Z.X.

Declaration of Interests: The authors have no conflict of interest to declare.

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Supplementary Table 1. Mean Body Weights During the Experimental Process

Weeks	Control group (mean ± SD)	CAG Model group (mean ± SD)	Drug group (mean ± SD)	Tuina group (mean ± SD)	Drug + Tuina group (mean ± SD)
1 week	219.392 ± 14.687	211.192 ± 12.846	218.090 ± 9.592	230.800 ± 28.781	216.410 ± 11.403
2 weeks	248.631 ± 26.520	240.377 ± 16.823	245.190 ± 28.211	256.790 ± 49.651	240.920 ± 28.095
3 weeks	270.846 ± 39.373	283.492 ± 51.274	252.110 ± 32.314	277.260 ± 63.387	262.110 ± 46.369
4 weeks	291.753 ± 53.835	291.685 ± 54.510	261.070 ± 34.624	285.670 ± 66.727	289.750 ± 71.403
5 weeks	317.854 ± 72.970	299.815 ± 58.777	269.540 ± 38.837	293.670 ± 69.809	307.350 ± 86.190
6 weeks	328.462 ± 81.691	310.869 ± 67.668	285.660 ± 52.736	309.110 ± 74.367	307.620 ± 83.587
7 weeks	347.769 ± 90.117	328.423 ± 75.444	313.320 ± 72.860	320.840 ± 87.402	312.870 ± 85.241
8 weeks	357.339 ± 94.954	336.846 ± 82.195	316.050 ± 70.683	338.870 ± 90.811	343.380 ± 99.286
9 weeks	368.477 ± 97.954	333.562 ± 79.934	317.790 ± 78.136	334.150 ± 89.951	336.260 ± 102.018
10 weeks	379.562 ± 106.743	340.569 ± 80.319	330.360 ± 84.384	342.070 ± 92.731	346.750 ± 102.601
11 weeks	389.062 ± 114.914	353.169 ± 88.850	340.940 ± 92.693	357.210 ± 98.282	350.470 ± 102.676
12 weeks	399.715 ± 119.060	365.162 ± 94.653	357.120 ± 95.189	370.550 ± 106.252	373.840 ± 117.686
19 weeks	419.490 ± 157.810	413.080 ± 146.187	416.340 ± 141.792	423.670 ± 146.653	420.840 ± 146.426
20 weeks	422.340 ± 159.822	414.600 ± 145.644	417.070 ± 142.877	424.950 ± 147.416	421.950 ± 147.937
21 weeks	425.560 ± 162.158	417.270 ± 146.813	418.000 ± 143.030	427.000 ± 148.401	422.770 ± 149.300
22 weeks	428.920 ± 163.052	418.520 ± 147.051	419.240 ± 144.382	428.250 ± 149.084	424.510 ± 149.976

CAG, chronic atrophic gastritis. 1-12 weeks: the process of CAG model building. 19-22 weeks: the process of 3 different therapies.

Supplementary Table 2. Effect of Different Treatments Affected the Expression Level of Bcl-2 in the Gastric Tissues of CAG Rats

Groups	Bcl-2 ($\bar{x} \pm s$)
Control (n = 2)	0.553 ± 0.068
Model (n = 3)	0.779 ± 0.046
Drug (n = 3)	0.621 ± 0.048
Tuina (n = 3)	0.723 ± 0.039
Drug + Tuina (n = 3)	0.620 ± 0.050

CAG, chronic atrophic gastritis.