Efficacy of Banxia Xiexin decoction in a rat model of chronic atrophic gastritis

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Abstract

OBJECTIVE: To investigate the effectiveness of Banxia Xiexin decoction (BXD) in a rat model of chronic atrophic gastritis (CAG).

METHODS: Sixty 6-week-old healthy Wistar rats (30 males, 30 females) were used in the present study. A rat model of CAG was successfully established using the combined active immunization/ethanol/sodium deoxycholate method. BXD was prepared from a mixture of seven Chinese herbs, and was intragastrically administered to CAG rats at three different doses (6, 12, and 24 g·kg⁻¹·d⁻¹). After 24 weeks, the rats were euthanized, and gastric tissue specimens were collected. Gastric mucosal specimens were stained with hematoxylin and eosin for histological examination to evaluate the degree of inflammation and morphological changes. Immunohistochemical staining was performed to examine the mucosal expression of proliferating cell nuclear antigen. Serum gastrin levels were measured using radioimmunoassay. The expression of Notch signaling-associated genes was examined by quantitative polymerase chain reaction and Western blot assay.

RESULTS: BXD at all three doses significantly reversed the adverse effects generated by CAG in rats. Compared with control rats, the CAG rats who were administered BXD had an accelerated growth rate, reduced inflammatory cell infiltration, improved gastric mucosal morphology, augmented thickness of the gastric mucosa, increased number of gastric glands, enhanced mucosal expression of proliferating cell nuclear antigen, and elevated serum gastrin levels.

CONCLUSION: BXD has a therapeutic effect in a rat model of CAG by targeting the Notch signaling pathway, thereby blocking the CAG from progressing to early gastric cancer.

INTRODUCTION

Gastric cancer is the fourth-most common cause of cancer-related death worldwide, and is the sec-
ond-most common cause of cancer-related death in 
China. Furthermore, gastric cancer in China accounts 
for approximately 43% of the total annual number of 
cases in the world. Although the pathogenesis of 
gastric cancer is not fully understood, it has been well 
established that chronic atrophic gastritis (CAG) is a 
major contributor to the development of gastric can-
cer, as CAG is both an important precursor lesion and 
an intermediate step in the carcinogenesis cascade 
of gastric cancer. In China, as many as 29.7% of CAG 
cases transform into gastric cancer annually. Therefore, 
the successful treatment of CAG may help pre-
vent the progression to gastric cancer. CAG is a progressive digestive disease characterized by 
the loss of normal glandular epithelium in the stomach 
lining as a result of ulceration, erosion and chronic in-
flammation, leading to mucosal injury and dysfunc-
tion. CAG resulting from Helicobacter pylori infec-
tion has commonly been treated with antibiotics such as 
amoxicillin, metronidazole and furazolidone. However, antibiotic administration may give rise to 
long-term persistence of antibiotic resistance in these 
bacterial populations. Thus, there is an urgent need 
to develop other effective therapeutic options for 
the management of CAG, especially for CAG with an auto-
immune origin.

Banxia Xiexin decoction (BXD) is an ancient Chinese herbal medicine that is clinically used by Chinese do-
tors to treat functional dyspepsia by harmonizing the 
stomach and intestines. BXD consists of water-sol-
uble extracts from seven traditional Chinese herbs, in-
cluding Banxia (Rhizoma Pinelliae), Huangqin (Radix 
Scutellariae Baicalensis), Ganjiang (Rhizoma Zingiberis), 
Danshen (Radix Salviae Miltiorrhizae), Huanglian (Rhi-
zoza Coptidis), Dazao (Fructus Jujubae), and Gancao (Radix Glycyrrhizae). The herbal ingredients, ingre-
dient ratios, and/or oral dose of BXD are modified in 
accordance with the disease severity and patient symp-
toms. However, it remains largely unknown whether 
BXD plays a role in the treatment of CAG. The present study aimed to investigate the effectiveness of 
BXD in a rat model of CAG.

MATERIALS AND METHODS

Animals
Sixty 6-week-old healthy Wistar rats (30 males, 30 fe-
nacles) were purchased from Shanghai SLAC Laborato-
ry Animal Co., Ltd. (Shanghai, China). These rats were 
 housed under specific pathogen-free conditions 
(room temperature, (25 ± 2 °C; relative humidity, 
50%-65%; 12-h light/12-h dark cycle) with good ven-
tilation and free access to food and water. All rats were 
acclimated for 1 week, and weighed before the experi-
ment. The animal experiments were approved by the 
Animal Ethics Committee of Zhejiang Chinese Medi-
cal University, and were performed in compliance with 
the Guide for the Care and Use of Laboratory Animals 
formulated by the National Institutes of Health.

BXD preparation
All seven crude herbs were purchased from the Clinical 
Department of Zhejiang Chinese Medical University. 
The crude herbs [9 g of Banxia (Rhizoma Pinelliae), 9 g 
of Huangqin (Radix Scutellariae Baicalensis), 6 g of 
Ganjiang (Rhizoma Zingiberis), 30 g of Danshen (Radix 
Salviae Miltiorrhizae), 3 g of Huanglian (Rhizoma Coptidis), 30 g of Dazao (Fructus Jujubae), and 9 g of 
Gancao (Radix Glycyrrhizae)] were mixed in 10-fold 
mass of water (approximately 1000 mL) for 30 min, 
followed by heating for 45 min. The liquid herbal ex-
tracts were then leached, before being concentrated via 
thermal evaporation. The extracts were prepared at a fi-
nal concentration of 2.4 g/mL, and stored at 4 °C until use.

CAG rat model and animal experiments

A rat model of CAG was established as previously de-
scribed. Briefly, rats were actively immunized by sub-
cutaneous injection of 0.3 mL of saline gastric mucosal homogenates from rats (antigen) emulsified with 
an equal volume of complete Freund’s adjuvant. After 30 d, 
the immunization was repeated once. The immunized 
rats were then fed 20 mmol/L of sodium deoxycholate 
solution instead of water every day, and were intragas-
trically administered 60% ethanol once every 10 d (2 mL 
per rat) during the initial 4 weeks. From weeks 5-16, 
the rats were alternately fed 10 mmol/L of sodium de-
oxycholate solution and 30% ethanol, instead of water, 
every 7 d. The 60 rats were randomly divided into the following 
six groups: control group, CAG group, low dose BXD 
group, medium dose BXD group, high dose BXD 
group, and folic acid (FA) group (positive control); 
each group contained 10 rats (five males and five fe-
nacles). FA was used as a positive control because FA re-
portedly has therapeutic value for CAG. The control 
group were fed with water, instead of sodium deoxy-
cholate or ethanol solution, throughout the initial 
16 weeks, and were subsequently intragastrically ad-
ministered saline, instead of BXD or FA, for an addi-
tional 8 weeks. The CAG group were intragastrically ad-
ministered saline, instead of BXD or FA, for 8 weeks. 
In the BXD groups, CAG rats were intragastrically ad-
ministered BXD at doses of 6 (low dose), 12 (medium 
dose), and 24 g·kg⁻¹·d⁻¹ (high dose) for 8 weeks. In 
the FA group, CAG rats were intragastrically adminis-
tered FA at a dose of 5 mg·kg⁻¹·d⁻¹ for 8 weeks.

Histological analysis
At the end of the study (week 24), all rats were 
weighed and euthanized by carbon dioxide asphyxia-
tion, followed by cervical dislocation. The gastric tis-
tues were harvested, and mucosal samples were ob-
tained by cutting along the lesser curvature from the
Inflammation is a characteristic of CAG. Inflammation grading was performed to determine whether BXD plays a protective role in preventing gastric inflammation in CAG rats. In accordance with the criteria of the Houston Symposium in 1996, the degree of inflammation was semiquantitatively graded as: grade 0, no inflammation and undetectable leukocyte infiltration in the gastric mucosa; grade 1, mild inflammation and sparse leukocyte infiltration at the bottom of the gastric glands or in the upper mucosa; grade 2, moderate inflammation and obvious leukocyte infiltration in the entire mucosa; grade 3, severe inflammation and densely packed leukocytes in the entire mucosa. The inflammation grade was determined based on the mean grade of 10 randomly selected fields in a section observed under a Nikon 80i microscope (Nikon, Tokyo, Japan). The thickness of the gastric mucosa was then measured, and the number of mucosal glands was counted using Image-Pro Plus 5.0 software (Media Cybernetics, Rockville, MD, USA). The mean mucosal thickness and mean number of mucosal glands per mm² calculated in the sections obtained from 10 rats in each group were used to assess the mucosal morphology. Inflammation grading and morphological changes were evaluated by two independent experienced pathologists in a double-blind manner.

Immunohistochemical staining
CAG is also characterized by increased proliferation rates of epithelial cells in the gastric mucosa, which is responsible for replacing the injured gastric glandular epithelium. Therefore, immunohistochemical staining was performed to determine whether BXD affected the mucosal expression of proliferating cell nuclear antigen (PCNA), a proliferation marker, in CAG rats. Gastric tissue sections were dehydrated, blocked with 10% goat serum, permeabilized with 0.01% Triton X-100 in PBS, and incubated with the primary antibody against PCNA (#13110S; Univ-Bio, Shanghai, China) at 4 °C overnight, followed by incubation with horseradish peroxidase-conjugated secondary antibody (Univ-Bio) for 1 hour at room temperature. The sections were then counterstained with hematoxylin. The staining was visualized under a Nikon 80i microscope using 3,3′-diaminobenzidine. Staining intensity was quantified by histomorphometric analysis using Carl Zeiss Imaging Systems (Carl Zeiss Microimaging GmbH, Jena, Germany).

Measurement of serum gastrin levels
Gastrin is a peptide hormone that stimulates gastric acid secretion and gastric motility. Hence, serum gastrin levels were measured to assess the gastroprotective effects of BXD. Immediately after euthanasia, 1 mL of blood was collected by cardiac puncture from each rat, and the serum was obtained by centrifugation at 1000 × g for 10 min. Serum gastrin levels were measured using a 125I CAS Radioimmunoassay kit (North Institute of Biological Technology, Beijing, China) in accordance with the manufacturer’s instructions.

Western blot analysis
CAG is a complex process that involves the activation of a variety of signaling pathways, among which the activated Notch signaling pathway plays a central role. Therefore, the present study evaluated whether BXD regulated the Notch signaling in CAG. A mortar and pestle was used to grind a portion of fresh gastric tissue (approximately 200 mg) to fine powder in liquid nitrogen, followed by protein extraction using RIPA buffer (50 mmol/L Tris-HCl, pH 7.4, 1% Triton X100, 0.25% wt/vol sodium deoxycholate, 150 mmol/L of NaCl, 1 mmol/L of EGTA, 0.1% SDS) containing protease inhibitors. The protein concentration was measured using the BCA Protein Assay Reagent (Lianke Biotech Co., Ltd., Zhejiang, China). The lysates were denatured by boiling with SDS and 2-mercaptoethanol solution, resolved on 10% SDS-PAGE, and transferred onto a PVDF membrane (Bio-Rad, Hercules, CA, USA). Then, the membrane was blocked with 5% non-fat dry milk in TBST, incubated with primary antibodies in TBST overnight at 4 °C, and incubated with horseradish peroxidase-conjugated secondary antibody (Univ-Bio) for 1 hour at room temperature. The detection was performed using enhanced chemiluminescence (Lianke Biotech Co., Ltd.). Antibodies against Notch1 (#AF1057, Univ-Bio), Notch2 (#AF1190, Univ-Bio), Hes1 (#11988, Cell Signaling Technology Inc.), Jagged1 (#AF599, Univ-Bio), and GAPDH (#Mab5465, Cell Signaling Technology Inc.) were used for immunoblotting.

Real-time quantitative Polymerase Chain Reaction (qPCR)
Total RNA was extracted from the powdered gastric tissues using a TaKaRa MiniBEST Universal RNA Extraction Kit (Branch of TaKaRa Bio Group, Liaoning, China) in accordance with the manufacturer’s protocol. The cDNA was synthesized from 1 µg of total RNA using a HiScript II Q RT SuperMix kit (Vazyme Biotech Co., Ltd., Jiangsu, China). The qPCR was performed on a CFX384™ qPCR thermocycler (Bio-Rad) using the ChamQ™ SYBR® qPCR Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China). The relative mRNA expression levels were evaluated using the 2-ΔΔCt method by normalizing to β-actin. The primers used for the qPCR are shown in Table 1.

Statistical analysis
Statistical analyses were performed using SPSS 17.0 (SPSS Statistics for Windows, version 17.0, released
RESULTS

BXD promoted the growth of CAG rats
There was a significant increase in body weight gain in the low, medium, and high dose BXD groups compared with the CAG group (Table 2). Furthermore, the high dose BXD group had a significantly greater body weight gain compared with the FA group (Table 2).

BXD reduced inflammation in CAG rats
The CAG rats had significantly higher inflammation grades than the control group (P < 0.01). Furthermore, the inflammation grades were significantly lower in the low, medium, and high dose BXD groups compared with the CAG group (all P < 0.01). However, the inflammation grade did not differ among the three BXD groups (P > 0.05). Moreover, the BXD groups had similar inflammation grades to the FA group (P > 0.05) (Figure 1).

BXD improved the gastric mucosal morphology in CAG rats
Compared with the corresponding control groups, both male and female CAG rats exhibited morphological abnormalities in the gastric epithelium and mucosa (Figure 2A). Furthermore, the low, medium, and high dose BXD treatments effectively reversed these morphological alterations, as evidenced by regularly arranged gastric epithelial cells and a dense and orderly distribution of gastric glands in the mucosa in the BXD groups (Figure 2A).

There was a significant increase in mucosal thickness in the three BXD groups compared with the CAG group (all P < 0.01; Figure 2B). Furthermore, the high dose BXD group had a significantly thicker mucosal layer than the FA group (P < 0.01; Figure 2B). The BXD groups also had a significantly greater number of gastric glands than the CAG group (all P < 0.01; Figure 2B). Collectively, these results revealed that BXD dose-dependently improved the gastric mucosal morphology in CAG rats.

BXD decreased the mucosal expression of PCNA in CAG rats
PCNA was weakly expressed in the gastric mucosa of CAG rats. BXD decreased the mucosal expression of PCNA in CAG rats dose-dependently.

Table 1 PCR primers used in this study

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer sequence</th>
<th>Amplicon length (bp)</th>
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<tr>
<td>β-actin</td>
<td>Forward: 5'-TGTTGCCCTAGACTTCGAGCA-3'</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CCATACCCAGGAAGGAAGGT-3'</td>
<td></td>
</tr>
<tr>
<td>Notch1</td>
<td>Forward: 5'-GTGAGTGAGATGGACTGGAC-3'</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-GGAAGGGATTTGTCGTCAGC-3'</td>
<td></td>
</tr>
<tr>
<td>Notch2</td>
<td>Forward: 5'-CTGCCCTGGAATAAGATGGA-3'</td>
<td>115</td>
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<td></td>
<td>Reverse: 5'-CTGCCATTTGTTTACACAGC-3'</td>
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<tr>
<td>Jagged1</td>
<td>Forward: 5'-CTGAGGACTACGAGGGCAAG-3'</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-CCCTTCAGAGTATCGTGG-3'</td>
<td></td>
</tr>
<tr>
<td>Hes1</td>
<td>Forward: 5'-ACTGCACTGACAGCGATACGC-3'</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5'-GGAAGGCGACACTGCGTATGG-3'</td>
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</tr>
</tbody>
</table>

Notes: * Internal control. PCR: polymerase chain reaction.

Table 2 Growth changes in rats with induced CAG treated with BXD or FA (g, x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body weight before the treatment</th>
<th>Body weight after the treatment</th>
<th>Body weight gain</th>
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<tr>
<td>Control</td>
<td>10</td>
<td>238±28</td>
<td>312±31</td>
<td>74±32ab</td>
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<tr>
<td>CAG</td>
<td>10</td>
<td>198±23</td>
<td>206±21</td>
<td>8±4a</td>
</tr>
<tr>
<td>Low dose BXD</td>
<td>10</td>
<td>196±22</td>
<td>234±30</td>
<td>39±14b</td>
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<tr>
<td>Medium dose BXD</td>
<td>10</td>
<td>194±23</td>
<td>240±32</td>
<td>46±21</td>
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<tr>
<td>High dose BXD</td>
<td>10</td>
<td>194±23</td>
<td>261±27</td>
<td>67±12ab</td>
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<tr>
<td>FA</td>
<td>10</td>
<td>199±23</td>
<td>234±28</td>
<td>35±11</td>
</tr>
</tbody>
</table>

Notes: the control group and CAG group were intragastrically administered saline at a dose of 5 mg · kg⁻¹ · d⁻¹ for 8 weeks. The low, medium, and high groups were intragastrically administered BXD at doses of 6 (low dose), 12 (medium dose), and 24 g · kg⁻¹ · d⁻¹ (high dose) for 8 weeks. In the FA group, CAG rats were intragastrically administered FA at a dose of 5 mg · kg⁻¹ · d⁻¹ for 8 weeks. CAG: chronic atrophic gastritis; BXD: Banxia Xiexin decoction; FA: folic acid. *P < 0.01 vs the CAG group; **P < 0.01 vs the FA group.
The control group and CAG group were intragastrically administered saline at a dose of 5 mg·kg⁻¹·d⁻¹ for 8 weeks. The low, medium and high groups were intragastrically administered BXD at doses of 6 (low dose), 12 (medium dose), and 24 g·kg⁻¹·d⁻¹ (high dose) for 8 weeks. In the FA group, CAG rats were intragastrically administered FA at a dose of 5 mg·kg⁻¹·d⁻¹ for 8 weeks. CAG: chronic atrophic gastritis; BXD: Banxia Xiexin decoction; FA: folic acid. Gastric tissues were stained with hematoxylin and eosin, and the inflammation grade was determined in accordance with the degree of leukocyte infiltration in the gastric mucosa. \( P < 0.01 \) vs the control group; \( P < 0.01 \) vs the CAG group; \( P > 0.05 \) vs the FA group; \( n = 10 \) rats in each group.

**BXD enhanced serum gastrin levels in CAG rats**

The three BXD groups had significantly greater serum gastrin levels than the CAG group (all \( P < 0.01 \)). Furthermore, the serum gastrin levels in the BXD groups were comparable to those in the FA group (\( P > 0.05 \)) (Figure 4).

**BXD inhibited the Notch signaling pathway in CAG rats**

The mRNA expressions of Notch1, Notch2, Notch1/2 target gene Hes1, and Notch1/2 ligand Jagged1 were...
DISCUSSION

The present study focused on the role of BXD in the treatment of CAG, a precursor lesion of gastric cancer. As patients infected with Helicobacter pylori (H. pylori) frequently develop CAG, animal models of H. pylori infection have been extensively used to study CAG. However, the development of H. pylori-induced CAG is an extremely slow process that may last for the lifetime of an individual. Therefore, it is challenging to accurately mimic the human pathophysiology of CAG in animal models. Previous findings have shown that the CAG observed in a rat model of H. pylori infection is less severe than that observed in humans. In addition, these animal models are not suitable for studies of chronic autoimmune atrophic gastritis that are not associated with H. pylori infection. Instead, in the present study, CAG was induced via a combination of factors, including active immunization and the administration of ethanol and sodium deoxycholate. This model may mimic the initiation, development and progression of human CAG within a relatively short period of time (24 weeks).

The present study found that BXD significantly improved the morphology and function of the gastric mucosa (Figures 1, 2 and 4), suggesting that BXD has gastroprotective effects. Moreover, BXD did not function in a strictly dose-dependent manner (Figures 1-4), indicating that a low dose of BXD (6 g·kg⁻¹·d⁻¹) is enough to protect against CAG in rats. This may provide valuable information that can be used to determine the optimal dosage of BXD that is both safe and effective for treating CAG in humans. However, although the present results showed that BXD plays a protective role in CAG, the possible adverse effects of...
BXD on other tissues and organs, such as the kidney, liver and heart, were not examined. Thus, further studies are required to objectively evaluate the systemic effects of BXD.

Similarly to other Chinese herbal medicines, BXD comprises a mixture of a variety of crude herbs. Hence, it may be challenging to investigate the precise mechanism of BXD in treating CAG. Notch signaling is implicated in the pathogenesis of gastric cancer. As CAG is a precursor lesion of gastric cancer, the downregulation of Notch by BXD in tumor tissues.

In conclusion, our findings show that BXD has a therapeutic effect in a rat model of CAG. This therapeutic effect was achieved by targeting the Notch signaling pathway, thereby blocking the progression of CAG to early gastric cancer.

REFERENCES
2 Liang D, Liang S, Jin J, Li D, Shi J, He Y. Gastric cancer burden of last 40 years in North China (Hebei province): a population-based study. Medicine (Baltimore) 2017; 96 (2); e5887.


