Protective effect and mechanisms of Weining granule on N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric cancer in rats

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OBJECTIVE: To investigate the protective effect and molecular mechanisms of Weining granule on N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric cancer in rats.

METHODS: A total of sixty healthy male wistar rats were randomly divided into five groups, including control group (CG), gastric cancer model group (MG), low-dose Weining granule treated group (LWT), medium-dose Weining granule treated group (MWT), and high-dose Weining granule treated group (HWT). Except the control group, the other groups were treated with MNNG to establish a rat model of gastric cancer. Low-dose Weining granule treated group, medium-dose Weining granule treated group, and high-dose Weining granule treated group were fed 9.0, 18.0 and 36.0 g/kg Weining granule, respectively. Histopathologic and molecular biologic technology were adopted to determine the protective effect of Weining granule on MNNG-induced gastric cancer in rats. The pathological changes of gastrointestinal tissue were observed. Meanwhile, the differential expression of proliferation, apoptosis and angiogenesis markers were determined, including proliferating cell nuclear antigen (PCNA), pokemon, cyclin D1, B-cell lymphoma-2 (Bcl-2), caspase-3, phosphatase and tensin homolog (PTEN) and vascular endothelial growth factor (VEGF).

RESULTS: After the MNNG treated, the pathological changes of stomach tissue were improved noticeably, including the intestinal metaplasia and atypic hyperplasia. The experiment was completed in 58 rats (96.67%). As compared with gastric cancer model group, the general states of rats were improved significantly after treated with different dose Weining granule. Moreover, treatment with different doses of Weining granule could inhibit the protein and mRNA expression of PCNA, pokemon, cyclin D1, B-cell lymphoma-2 (Bcl-2), caspase-3, phosphatase and tensin homolog (PTEN) and vascular endothelial growth factor (VEGF).

CONCLUSION: Weining granule could improve gas-
Gastric cancer is one of the most commonly diagnosed cancer, and the third leading cause of cancer-related deaths in the world.1 It’s incidence vary dramatically across different countries, and nearly 50% of the gastric cancer happened in East Asia, especially China.2 Among the clinical treatments on gastric cancer, surgical resection seems to be the unique curative treatment in the management of gastrointestinal cancer. Chemotherapy and/or surgery could improve quality of life and prolong survival for unrespectable gastric cancer, but the 5-year overall survival (OS) for gastric cancer is still lower than 30%.3,4 Therefore, an effective drugs to gastric cancer is warranted.

The pathogenesis of gastric cancer is a multistep and multi-factorial process. Gastric cancer, intestinal metaplasia and atypical proliferation are the common precancerous lesion of gastric cancer.5 The development of gastric cancer is associated with excessive cell proliferation, apoptosis suppression, as well as sustained angiogenesis. The dynamic balance between gastric epithelial cells proliferation and apoptosis is of great importance for maintaining gastric mucosal integrity. Activation of proto-oncogene, inhibition of cancer suppressive factor or excessive proliferation of gastric epithelial cells, and suppression of apoptosis signaling pathway are key in the development of gastric cancer. In addition, the progression and metastasis of gastric cancer are closely correlated to tumor angiogenesis. Angiogenesis promotes the malignant growth and metastasis of gastric cancer cells.6

Traditional Chinese prescriptions or herbs have been identified as potential anti-cancer agents in China.7 Weining granule is a complementary and alternative medicine used for gastric cancer treatment in Traditional Chinese Medicine (TCM). The prescription contains four TCM ingredients: two strengthen-spleen and replenish-Qi compounds [Huangqi (Radix Astragi Mongolici) and Baihuasheshecao (Herba Hedyotidis)] and two blood-activating and stasis-eliminating compounds [Ezhu (Rhizoma Curcumae Phaeocaulis) and Gouqizi (Fructus Lycii)]. According to the pharmacology research, Huangqi (Radix Astragali Mongolici) could promote the apoptosis of human gastric carcinoma cell line BGC-823;8 the main components of Ezhu (Rhi-

MATERIALS AND METHODS

Drug and reagents

Weining granule (No. 20130612) was prepared by the National medicine research and development center of Ruikang Hospital Affiliated to Guangxi university of Chinese Medicine. MNNG was purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG was purchased from Shanghai San- goon Biotech Co., Ltd. (Shanghai, China). Antibodies against PCNA, cyclin D1, p53, c-myc, apoptosis (caspase-3, B-cell lymphoma-2 (Bcl-2), phosphatase and tensin homolog (PTEN)) and metastasis (vascular endothelial growth factor (VEGF)) were purchased from Cell Signaling Technology. The Trizol reagent kit was purchased from Invitrogen (Carlsbad, CA, USA) the Syb-Green PCR Master Mix from Applied Biosystems.

Animals

The study was conducted in accordance with the guidelines for animal experiments of Guangxi Medical University. Forty male Wistar rats weighing (180 ± 20) g, age of 4-6 weeks old, were obtained from the Laboratory Animal Centre of the Guangxi Medical University.
(the qualified production number is SCXK- 2012-0002). Rats were maintained in animal facility at a temperature ([21 ± 1] °C) and humidity (40%-50%) under a 12 h light/dark cycle. Rats had free access to regular chow pellets and tap water.

**Experimental protocol**

After 1-week of acclimatization, rats were randomly divided into five groups, including control group (n = 10), gastric cancer model group (n = 20), low-dose Weining granule treated group (n = 10), medium-dose Weining granule treated group (n = 10), and high-dose Weining granule treated group (n = 10). The gastric cancer model group were treated with MNNG in the drink, while low-dose Weining granule treated group, medium-dose Weining granule treated group, and high-dose Weining granule treated group were treated with MNNG in drink and 9.0, 18.0 and 36.0 g/kg of Weining granule in the feed, respectively. Except the control group, all rats received MNNG at a concentration of 180 μg/mL drinking water ad libitum for 56 weeks. The models were built referenced to previous study. To enhance gastric cancer development, the following measures were applied: (a) 56 °C 10% sodium chloride (10 mL/kg) weekly by gavage; (b) 0.85% sodium deoxycholate by gavage at the afternoon of eating day, while 40% alcohol (10 mL/kg) by gavage at the afternoon of fasting day (fasting for one day after two-days normal eating); (c) clipping tails of the rats by forceps weekly. The general health of all rats were monitored closely during the study period. At the end of 56th week, all rats were killed by cervical dislocation. Then a thorough necropsy was made, and the gastric antrum was scrutinized for lesions. Autopsy was performed on animals that died before the end of the experiment to determine the cause of death and the presence of gastric tumors. The stomach was also opened along the greater curvature and carefully examined. Part of tissue samples were fixed in 10% buffered formaldehyde for hematoxylin-eosin (HE) staining study, and the remaining specimens were kept in − 80 °C until RNA and protein extraction. All dissections were performed by investigators blinded to the different treatment groups. The number, size and location of lesions were documented.

**Histological changes of gastric cancer mucosal tissue**

Gastric cancer mucosal tissue samples were immersed into 10% neutral buffered formalin for 24 h, passed and embedded in paraffin. The paraffin blocks were then sectioned by 5 μm thickness for HE staining and examined under light microscope by a pathologist who was unaware of the treatment groups. In each case, 9 serial sections were used for HE stains.

**Real-time polymerase chain reaction (PCR) analysis**

Total RNA was extracted using Trizol reagent according to the manufacturer’s instructions and verified by visualization of the 28S/18S ribosomal RNA ratio on 1.8% agarose gel electrophoresis. First-strand cDNA was synthesized using 1 μg RNA, in which 0.2 μL oligo dT was added at 70 °C for 5 min, and then placed on ice for 1 min, then 0.5 μL MMLV reverse transcriptase (200 IU/μL), 0.5 μL RNasin (40 IU/μL), 2 μL RT buffer, 0.2 μL forward primer, 0.2 μL downstream primer, and 0.1 μL dNTPs (10 mmol/L) were added at 37 °C for 60 min. The reverse transcriptase was inactivated at 95 °C for 3 min. The PCR products were then separated on 1.5% agarose gels. As showed in Table 1, the primer sequences of caspase-3, Bcl-2, cyclin D1, PTEN, PCNA, VEGF, pockmon, and β-actin were synthesized by Shanghai Sinopharm Chemical Reagent Co., Ltd. The real-time PCR reactions were performed using iQ™ SYBR®Green Supermix kit according to the manufacturer’s instructions (Bio-Rad). RNA was amplified using the ABI Prism 7500 Sequence Detection system (Applied Biosystems, Carlsbad, CA, USA). The relative expression ratio of mRNA in gastric tissues was quantified by the 2^−△△Ct method using the cycle threshold value and normalized to the β-actin product from the same sample.

**Western blot analysis**

Firstly, 50 mg of the gastric tissues were homogenized in 400 μL mixture of radio-immunoprecipitation assay (RIPA) buffer with 4 μL PMSF on ice for 30 min, centrifuged at 1000 rpm for 10 min at 4 °C. Subsequently, collected the supernatants and quantified the total proteins by the BCA assay. Then the proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the gels were electrophoretically transferred onto polyvinylidene difluoride membrane using a Trans-Blot SD apparatus (Bio-Rad). The membranes were blocked at 4 °C overnight, followed by incubation with anti-PCNA (1: 300 dilution), anti-cyclin D1 (1: 300 dilution), anti-pokemon (1: 300 dilution), anti-caspase-3 (1: 300 dilution), anti- Bcl-2 (1: 300 dilution), anti-PTEN (1: 300 dilution), anti-VEGF (1: 300 dilution), or anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1: 300 dilution) antibodies and washed 3 times (over 10 min) with TBS before incubating with the HRP conjugated secondary antibodies at a dilution of 1: 1000 for 1 h at room temperature. Visualization of the protein bands was achieved by the chemiluminescence method, and the films were developed and fixed. GAPDH was used as an internal reference.

**Statistical analysis**

SPSS software version 17.0 (SPSS, Chicago, IL, USA) was used to perform all the statistical analyses. All experiments were performed ≥ 3 times with triplicate measurements and data were expressed as mean ± standard deviation ( x ± s). Statistical differences between groups were analyzed using the R software (version 3.2.5, http://www.r-project.org/). A P value less than 0.05 were considered statistically significant difference.
RESULTS

General observations
Before the termination of the experiment two rats died in groups CG and MG, respectively. The cause of death was not found by histological observation, so excluded the two rats from the statistics. As we observed, rats in the group CG showed a fat body and glossy hair coat, good appetite, granular stool, reddish tail, and agile activity. While rats in the group MG exhibited listlessness, emaciation, loss of appetite, loose stools, pale tail, loose and lusterless of hair coat, lazy activities and unresponsive, or palpable enclosed mass. Treated with different doses of Weining granule significantly improved above mentioned symptoms at different degrees.

Gross pathological changes of gastric mucosa
Rats in the group CG showed integrative gastric cavity without deformation, elastic gastric wall, pinkish and glossy mucosa with greater mucus on the lining, smooth and regular mucosal fold, while rats in the group MG showed deformation of gastric cavity, thickened stomach wall with low elasticity, flattened or irregular folds, gastric mucosal hyperemia or punctate hemorrhage, rough incanous gastric mucosa or yellow stained mucosa, hyperplastic nodules or antral ulcer defects. After treated with different doses of Weining granule, rats in groups LWT, MWT and HWT showed pink and smooth surface gastric mucosa, regular arrangement of mucosal folds, without obvious hyperplastic nodules and ulcers.

Histopathological examination
Biopsy of rat gastric tissues were observed under optical microscope. The tissues from group CG appeared structurally integrated mucosa epithelial cells, without exfoliated cells or mucosal defect and regular arranged the gastric gland of tunica mucosa; the eosinophil infiltration and little inflammatory cell infiltration were highlight in lamina propria, and the muscularis mucosa hyperplasia wasn’t found. After treated with the MNNG, the pathological changes in the rats of group MG showed incomplete, rough, and erosive gastric mucosa; different shape or size of epithelial cells with multifocal atrophy; different degrees of degeneration or necrosis of the exfoliated cells (mainly lymphocytes, neutrophils, eosinophils and a few mononuclear cells); disordered glands, different sizes of the glands in basal region, and a few of share wall; mucosal layer extending to the mucous membrane. Treated with different doses of Weining granule, the tissues exhibited intact mucosa, regular arranged epithelial cells, no intestinal metaplasia, and a few inflammatory cell infiltration (mainly eosinophil; the amount of inflammatory cells was obviously lower than the model groups).

Proliferation-related genes expression
PCNA, cyclin D1, and pokemon gene levels in gastric tissues were determined by real time PCR and Western blot analysis. Compared with the group CG, PCNA, cyclin D1, andpokemon mRNA expressions were significantly higher in the group MG (all $P < 0.01$). Treated with Weining granule, the PCNA, cyclin D1, and pokemon mRNA expression significantly decreased.

Table 1 Primer sequences of the targeted gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
<th>Amplification products (bp)</th>
</tr>
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<tbody>
<tr>
<td>Caspase-3</td>
<td>CATGCACATCCCTCCTCCTCCTG</td>
<td>CCCACTCCCAGTCATTCTCCTT</td>
<td>158</td>
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<tr>
<td>Bcl-2</td>
<td>CTTCAGGGATGGGTTGGAACTT</td>
<td>CAGGCTCCGTATGGCATTGCCGTT</td>
<td>174</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>AACTTTCCCTCTCGTCTACCG</td>
<td>GACCAGCTTTTCTCCTTCCACCT</td>
<td>184</td>
</tr>
<tr>
<td>PTEN</td>
<td>GGAAAGGACGGACTGTTGTA</td>
<td>TGCCACTGTTTGTAATCCCA</td>
<td>197</td>
</tr>
<tr>
<td>PCNA</td>
<td>GAAGTTTTTCTGAGTGTGGGG</td>
<td>ACAGTGGAGGCTGTTTGTG</td>
<td>164</td>
</tr>
<tr>
<td>VEGF</td>
<td>TCGAGGAAAGGGAGGTC</td>
<td>TTTAATCCAGCTGCTCCG</td>
<td>180</td>
</tr>
<tr>
<td>Pockmon (ZBTB7A)</td>
<td>TGTTGCTATCTGTGGAGGAGG</td>
<td>GTTGGCGAGATGACGACGG</td>
<td>175</td>
</tr>
<tr>
<td>β-actin</td>
<td>CACCGCGAGATGACGAGGC</td>
<td>CCCATACCCGACGACCACACC</td>
<td>154</td>
</tr>
</tbody>
</table>

Notes: Bcl-2: B-cell lymphoma-2; PTEN: phosphatase and tensin homolog; PCNA: proliferating cell nuclear antigen; VEGF: vascular endothelial growth factor.
with a dose-dependent manner when compared to the gastric cancer model group (all \( P < 0.01 \)) (Figure 1A).

PCNA, cyclin D1, and pokemon levels were quantified and expressed relative to the corresponding intensity of the \( \beta \)-actin bands. PCNA, cyclin D1, and pokemon protein expressions were significantly increased in the group MG when compared to the group CG (all \( P < 0.01 \)). Treated with Weining granule, the PCNA, cyclin D1, and pokemon protein expression significantly reduced with a dose-dependent manner (all \( P < 0.01 \)).

**Apoptosis-related genes expression**

As shown in Figure 2A, the RT-PCR analysis indicated that caspase-3 and PTEN mRNA expressions were significantly decreased and Bcl-2 mRNA expression was significantly increased in the gastric cancer model group compared to the control group (all \( P < 0.01 \)). After treated with Weining granule, the mRNA expression of caspase-3 and PTEN significantly increased, while the Bcl-2 decreased with a dose-dependent manner than the gastric cancer model group (all \( P < 0.01 \)).

Caspase-3, Bcl-2, PTEN protein levels were quantified and expressed relative to the corresponding intensity of the \( \beta \)-actin bands. Caspase-3 and PTEN protein expressions were significantly decreased, while Bcl-2 protein expression was significantly decreased in the gastric cancer model group than the control group (all \( P < 0.01 \)).

Weining granule can significantly increase caspase-3 and PTEN protein expression, and decrease Bcl-2 protein in a dose-dependent manner than the gastric cancer model group (all \( P < 0.01 \)).

**Expression of angiogenesis-related genes**

As shown in Figure 3A, VEGF mRNA expression was significantly higher in the group MG when compared to the group CG (\( P < 0.01 \)). After treated with Weining granule, the VEGF mRNA expression significantly decreased in a dose-dependent manner.

![Graph A](image1.png)

**Figure 1** Effect of Weining granule on PCNA, cyclin D1, and pokemon expression

A: PCNA, cyclin D1, and pokemon mRNA expressions were measured by the real time PCR (\( n = 3 \)). Results are expressed relative to \( \beta \)-actin. B: relative protein level of PCNA, cyclin D1, and pokemon (\( n = 3 \)). Low-dose Weining granule treated group, medium-dose Weining granule treated group, and high-dose Weining granule treated group were fed 9.0, 18.0 and 36.0 g/kg Weining granule, respectively. PCNA: proliferating cell nuclear antigen; PCR: polymerase chain reaction. Values were expressed as mean ± standard deviation. \( P < 0.01 \) vs control group; \( P < 0.01 \) vs gastric cancer group; \( P < 0.01 \) vs control group and vs gastric cancer group; \( P < 0.05 \) vs control group and \( P < 0.01 \) vs gastric cancer group.

![Graph B](image2.png)

PCNA, cyclin D1, and pokemon mRNA expressions were measured by the real time PCR (\( n = 3 \)). Results are expressed relative to \( \beta \)-actin.
when compared with the gastric cancer model group ($P < 0.01$). VEGF protein expression was significantly increased in the group MG when compared with the group CG ($P < 0.01$). After treated with Wein-
ing granule, the VEGF protein expression significantly decreased in a dose-dependent manner (all $P < 0.01$).

**DISCUSSION**

In this study, we investigated the effect of Wein-
ing granule for its chemotherapy activity on a MNNG-in-duced rat model of gastric cancer and its underlying mechanisms, and found that Wein-
ing granule improved the pathological changes of gastric mucosa significantly. Especially, Wein-
ing granule decreased the gene expression of PCNA, pokemon, cyclin D1, Bcl-2, and VEGF, while increased the expression of caspase-3 and PTEN protein and mRNA in the gastric mucosa, respectively. Furthermore, the results provide evidence that Wein-
ing granule prevent MNNG-induced gastric cancer through inhibiting the cell proliferation and angiogenesis, and promoting cell apoptosis. Our previous study had shown a primary data of Wein-
ing granule-induced apoptosis and Wein-
ing granule inhibited metastasis of gastric cancer,\textsuperscript{17,18} but the present study did focus on its molecular mechanism. And we assumed that expect for the induction of apoptosis, the protective eff-
ct of Wein-
ing granule on gastric cancer may be in part due to the blockage of cell cycle. Abnormal or excessive proliferation of cell is consid-
ered to be a hallmark of cancer cells.\textsuperscript{20} A wide spectrum of immunohistochemical methods has been used in re-
cent years to identify proteins that are responsible for the regulation of cell proliferation. Many antibodies

![Graph A]

Figure 2 Effect of Wein-
ing granule on caspase-3, Bcl-2, and PTEN expressions

A: caspase-3, Bcl-2, and PTEN mRNA expressions were measured by the real time PCR ($n = 3$). Results are expressed relative to b-actin. B: relative protein level of caspase-3, Bcl-2, and PTEN ($n = 3$). Bcl-2: B-cell lymphoma-2; PTEN: phosphatase and tensin ho-
molig; PCR: polymerase chain reaction. Values were expressed as mean ± standard deviation. Low-dose Wein-
ing granule treated group, medium-dose Wein-
ing granule treated group, and high-dose Wein-
ing granule treated group were fed 9.0, 18.0 and 36.0 g/kg Wein-
ing granule, respectively. PCNA: proliferating cell nuclear antigen; PCR: polymerase chain reaction. Values were expressed as mean ± standard deviation. $^{a}P < 0.01$ vs control group; $^{b}P < 0.01$ vs control group and $P < 0.05$ vs gastric cancer group; $^{c}P < 0.01$ vs control group and $P < 0.01$ vs gastric cancer group; $^{d}P < 0.01$ vs gastric cancer group.
have been developed to facilitate investigations of the cell cycle as well as cell proliferation such as PCNA, pokemon, cyclin D1, PTEN, Bcl-1, caspase-3, and VEGF. All these markers have been studied in gastric cancer. PCNA expressed in the nuclei of proliferating cells and acts as co-factor of DNA polymerase in DNA synthesis in S phase of the cell cycle. PCNA has a very long half-life, so it is expressed in most cell cycles in proliferating cells. It is reported that PCNA expression is related to prognosis of gastric cancer. Cyclin D1 belong to the family of G1 cyclins, and constitute a critical target for proliferative signals in G1. In normal human tissues, the expression of cyclin D1 protein has been demonstrated to be either low or nil. Overexpression of Cyclin D1 is implicated as driving force in multiple cancers including gastric cancer. High levels of Cyclin D1 expression were reported in gastric cancer tissues. Pokemon has been identified as a POZ and krüppel transcription factor with proto-oncogene. Pokemon enhanced the oncogenesis of colorectal cancer and hepatocellular carcinoma by promoting proliferation and cell cycle progression. However, no studies have evaluated the role of pokemon in gastric cancer. In the current study, as real-time PCR and Western blot analysis showed, PCNA, cyclin D1, and pokemon expression levels were higher in gastric cancer tissues. After treated with Weining granule, the mRNA and protein expression of PCNA, cyclin D1, and pokemon were significantly decreased \( P < 0.01 \). These findings indicated that anti-tumor effect of Weining granule was correlated with inhibiting cell proliferation and/or stimulating cell cycle arrest.

Apoptosis, in connection with cell proliferation, is an important mechanism towards healthy tissues. The balance between cell proliferation and cell apoptosis is of great importance for maintaining gastric mucosal integrity. Abnormal apoptosis contributes to the onset, development, and progression of cancer. Bcl-2 is a crucial regulators of apoptosis that has the ability to block a wide range of apoptotic signals. Research has indicated that Bcl-2 expression is an important factor in biological behavior of gastric cancer. Moreover, Bcl-2 could induce VEGF expression in neovascular endothelial cells and promote tumor growth. Our data showed that Weining granule significantly decreased Bcl-2 expression, which makes gastric cancer cells susceptible to apoptosis. In addition, caspases are central components of the cell death machinery. Of these, caspase-3 is a proteinase and a crucial factor in the apoptotic pathway, which directly cleaves various proteins, resulting in morphological and biochemical changes and then leading to apoptosis. Our data showed that Weining granule increased caspase-3 mRNA and protein expression and activity. The activation of caspase-3 may contribute to DNA fragmentation and nuclear morphologic changes. On the basis of these findings, we hypothesize that Weining granule may affect the open of mitochondrial permeability transition pore, subsequently results in the release of cytochrome c and followed by the increased expression of caspase-3. Moreover, the expression of PTEN mRNA and protein were also detected in the present study. PTEN is a tumor suppressor gene, which encodes a multifunctional phosphatase that regulates cell proliferation, migration invasion, apoptosis, and angiogenesis. Inactivation of PTEN decreased caspase-3 expression, thus interrupted the apoptotic pathway in tumor cells, which in turn accelerate cancer progression.
Our study indicated lower level of caspase-3 and PTEN expression and higher level of Bcl-2 expression were observed in gastric cancer tissues; after treated with Weining granule, the expression of caspase-3 and PTEN significantly increased, while the expression levels of Bcl-2 decreased \( (P < 0.01) \). Together these findings suggested that anti-tumor effect of Weining granule may be correlated with the downregulation of Bcl-2 expression and activation of caspase-3 and PTEN pathways. In addition, Weining granule might restore the balance between cell apoptosis and proliferation.

Another mechanism by which Weining granule to take anti-gastric cancer effects was the suppression of angiogenesis. Angiogenesis, the formation of new capillaries from existing blood vessels, is essential for tumor growth, invasion and metastasis.\(^6\) It is generally recognized that vessel formation around a tumor is stimulated by various angiogenic factors.\(^7\) Among them, VEGF is the key regulator of angiogenesis in gastric cancer.\(^8\)\(^,\)\(^9\)\(^,\)\(^10\) Wang et al.\(^8\) indicated that patients with expressions of VEGF in stomach cancer specimens had greater tumor progression and worse prognoses. Therefore, anti-angiogenic strategies for the treatment of cancer has centered on the VEGF and VEGF signaling pathway. Our study found an increase VEGF both on protein and mRNA levels in the gastric cancer tissues. After treated with Weining granule, the expression of VEGF protein and mRNA were significantly decreased. This result indicated that Weining granule could attenuate tumor angiogenesis.

There are several limitations in this study. Firstly, many pivotal proteins involved in cell cycle progression, survival and apoptosis, such as p53 and Bax were not determined; secondly, Weining granule is a complex Chinese formula and its active ingredients remains unknown. Future studies are warranted to elucidate whether Weining granule in combination with Western Medicine can achieve the additional benefits in the protective effect on gastric cancer.

In conclusion, our study showed that Weining granule was capable of improving pathological changes of gastric cancer induced by MNNG in rat model through suppressing cell proliferation, promoting tumor cell apoptosis, and inhibiting angiogenesis. Our findings provided evidence for the hypothesis that Weining granule may take a chemoprotective effect on MNNG-initiated gastric cancer through inhibition of cell proliferation and angiogenesis, as well as apoptosis induction.

REFERENCES


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