Invest Clin 65(1): 48 - 58, 2024 https://doi.org/10.54817/IC.v65n1a05

The mechanism of folic acid on N-methyl-N'-nitro-N-nitrosoguanidine-induced chronic atrophic gastritis through the PI3K/Akt pathway.

Yun An[1], Weigang Chen[1], Yong Cao[2], Boshen Chen[1], Qiangbin Li[1] , Xia Zhou[1] and Weihan Huang[1]

[1] Department of Digestive Internal Medicine, Panyu District Traditional Chinese Medicine Hospital, Guangzhou,China.
[2] Department of Traditional Chinese Medicine, Jinan University, Guangzhou, China.

Keywords: folic acid; chronic atrophic gastritis; N-methyl-N'-nitro-N-nitrosoguanidine (MNNG); PI3K/Akt.

Abstract. Chronic atrophic gastritis (CAG) is a precancerous atrophic gastritis of the stomach, which generates an urge to develop novel therapeutic schedules. This study aimed to investigate the effect of experimental folic acid administration on N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced CAG through the PI3K/Akt pathway in rats. The rats were divided into a Model Group, a Folic Acid Group and a Blank Group. Rats in the Model Group were induced by MNNG and given 10 mL/kg/d distilled water by gavage, while rats in the Folic Acid Group were induced by MNNG and given 5 mg/kg/d folic acid suspension by gavage. As a control, rats in the Blank Group were given the same amount of distilled water as MNNG and 10 mL/kg/d distilled water by gavage. The levels of gastrin (GAS) and motilin (MTL) in serum were measured by enzyme-linked immunosorbent assay (ELISA), and the mRNA and protein expressions were detected by quantitative polymerase chain reaction (q-PCR) and Western blot. According to hematoxylin and eosin (H&E) pathological analysis, there were inflammatory factors infiltration and derangement of mucosal epithelial cells in the model group, while the gastric tissue injury in the folic acid group was improved. Folic acid could decrease the content of GAS, increase the content of MTL in the serum of the rats, and regulate the expression of PI3K and AKT signal pathways. Folic acid can have a therapeutic effect on CAG by reducing the concentration of GAS in serum and increasing the concentration of MLT in serum. Our study would lay a theoretical foundation for using folic acid to investigate new therapies for CAG in humans.

Corresponding author. Yun An. Department of Digestive Internal Medicine, Panyu District Traditional Chinese Medicine Hospital, Guangzhou, China. Tel: +86 020 85926915. Email: anyun_2022@163.com

Mecanismo de acción del acido fólico en la gastritis atrófica crónica inducida por N-Metil-N'-Nitro-N-Nitrosoguanidina mediante la vía PI3K/Akt.

Invest Clin 2024; 65 (1): 48 – 58

Palabras clave: ácido fólico; gastritis atrófica crónica; N-metil-N'-nitro-N-nitrosoguanidina (MNNG); PI3K/Akt.

Resumen. La gastritis atrófica crónica (CAG) es una condición precancerosa del estómago que refleja la necesidad urgente de desarrollar nuevos regímenes terapéuticos. Este estudio tiene como objetivo investigar el impacto del ácido fólico sobre la CAG inducida en ratas por N-metil-N'-nitro-N-nitrosoguanidina (MNNG) a través de la ruta de señalización PI3K/Akt. Las ratas se dividieron en tres grupos: Grupo Modelo, Grupo de Ácido Fólico y Grupo Control. En el Grupo Modelo, las ratas fueron inducidas por MNNG y recibieron 10 mL/kg/d de agua destilada mediante sonda gástrica, mientras que en el Grupo de Ácido Fólico se indujo con MNNG y se les administró una suspensión de ácido fólico de 5 mg/kg/d mediante sonda gástrica. En el Grupo Control, se administró la misma cantidad de agua destilada que a las ratas en el Grupo Modelo, y se suministró 10 mL/kg/d de agua destilada mediante sonda gástrica. Los niveles séricos de gastrina (GAS) y motilina (MTL) se midieron mediante el ensayo inmunoenzimático (ELISA), y las expresiones de ARNm y proteínas se detectaron mediante reacción en cadena de la polimerasa cuantitativa (q-PCR) y Western blot. Según el análisis patológico con tinción de hematoxilina y eosina (H&E), se observó infiltración de factores inflamatorios y trastorno en las células epiteliales de la mucosa en el Grupo Modelo, mientras que las lesiones del tejido gástrico en el Grupo de Ácido Fólico mostraron una mejora. El ácido fólico podría disminuir el contenido de GAS, incrementar el contenido de MTL en el suero de las ratas, y regular la expresión de las rutas de señalización PI3K y AKT. El ácido fólico puede tener un efecto terapéutico en la CAG al reducir la concentración de GAS en el suero e incrementar la concentración de MTL. Nuestro estudio proporcionaría una base teórica para el uso del ácido fólico en la investigación de nuevas terapias para la CAG en humanos.

Received: 27-06-2023 Accepted: 08-09-2023

INTRODUCTION

Chronic atrophic gastritis (CAG) is a precancerous atrophic gastritis of the stomach, which remains a leading global healthcare problem [1]. In China, the probability of CAG turning into gastric cancer is 1.2%-7.1% [2]. In pathology, CAG develops into gastric carcinoma through a series of processes, such as atrophy of the inherent glands of gastric mucosa, intestinal metaplasia and atypical hyperplasia. Notably, early detection and early treatment of CAG is a critical approach to block the further canceration of the disease and improve the quality of life of patients [3].

Folic acid, also known as vitamin M, plays a crucial role in the synthesis of protein, nucleotide and pantothenic acid in humans. It has been reported that a lack of folic acid can lead to colitis and chronic atrophic gastritis, which increases the risks of gastrointestinal cancer [4]. In addition, Lin *et al.* [5] reported that the natural apoptosis rate of gastric cancer cells was much higher in patients with gastric cancer given a specific concentration of folic acid than in those without intervention. Therefore, the anti-cancer mechanism of folic acid may be to regulate the expression of some cancer cells to accelerate apoptosis.

The PI3K/AKT pathway is a signaling pathway associated with proliferation, differentiation and apoptosis, which regulates the proliferation and survival of tumor cells and plays an essential role in tumor cell migration and adhesion. It has been reported that folic acid could produce positive effects through the PI3K/AKT pathway in different pathological conditions, such as colon cancer, oral squamous cell carcinoma, and so on[6,7]. Furthermore, folic acid could attenuate the hypoxia-induced inflammatory responses of THP-1 cells through inhibiting the PI3K/Akt/HIF-1α pathway [8].

In this study, we aim to investigate the effects of folic acid on chronic atrophic gastritis rats through the PI3K/AKT pathway and lay a preclinical foundation for folic acid in treating CAG.

MATERIALS AND METHODS

Experimental materials

Animals: Specific Pathogen Free (SPF) male Sprague Dawley (SD) rats, weighing 200-300g, purchased from the Guangzhou University of Chinese Medicine Experimental Animal Center.

Reagents: MNNG (Guangzhou Kangming Biotechnology Co., Ltd., batch number: 20210601), N1'-[2-[[5-[(dimethylamino) methyl]-2-furanyl]methylthio]ethyl]-N1-methyl-2-nitroethene-1,1-diamine (Guangdong Hengjian Pharmaceutical Co., Ltd., National Standard Word H44021173), ranitidine (Guangdong Hengjian Pharmaceutical Co., Ltd., H44021173), folic acid tablets (Fuzhou HAIWANGFU Pharmaceutical Co., Ltd., batch number: 21042810).

Materials and equipment: ELISA GAS kit (Cat. MM-21284R1), MTweizL kit (Cat. MM-0491R1), PI3K kit (Cat. MM-0427R1) and Akt kit (Cat.MM-50624H1) were purchased from Jiangsu enzyme-free Industry Co., Ltd. (Yancheng, China), hematoxylin and eosin staining solution (100ML, Beijing Solebo Technology Co., Ltd., Beijing, China). Semi-automatic rotary paraffin sectioning machine (HM340E), Embedding Workstation (Histosta), automatic dyeing machine (Gemini AS), Rapid Tissue Processor (STP120), refrigerated centrifuge (FRESCO 70) and Multiskan SkyHigh Microplate Spectrophotometer (1510) were purchased from Thermo Fisher Inc. (Massachusetts, USA). Electric thermostatic drying oven (Shanghai Jing Hong laboratory Instrument Co.,Ltd., Shanghai, China), electronic analytical balance (UX2200H, Shimatsu Corporation, Tokyo, Japan), and optical microscope (CX-23, Olympus Corporation, Tokyo, Japan).

Modeling and grouping

The model was established, and 30 SPF SD rats were randomly divided into two groups. Ten rats were selected for normal feeding (NC Group), and the remaining 20 rats were used to establish the model. MNNG-induced rats in the Model Group were fed with 180 μg/mL MNNG and 0.003 g/mL ranitidine hydrochloride once every three days. At the same time, the rats in the blank group were given the same amount of distilled water by gavage, and the other feeding conditions were the same. Every six weeks, one rat was selected from the blank group and the model group for dissection, and HE-stained sections were made to observe the histomorphological changes of gastric mucosa and test the effect of its modeling.

After successful modeling, the rats were divided into three groups according to body weight: Model Group (MC Group) and Folic Acid Group (AC Group).

Method of administration

Rats in the Normal Group (NC Group) and Model Group (MC Group) were given 10 mL/kg/d distilled water by gavage, while the Folic Acid Group (AC group) was given 5 mg/kg/d folic acid suspension by gavage.

Specimen collection

After six weeks of treatment, the rats in each group fasted and watered for two days and then were anesthetized with ether, and stomach and blood samples of the inferior vena cava were taken. The blood samples of each rat were centrifuged at 4°C 3500 rpm for 15 min, and the supernatant was extracted and preserved to detect the content of GAS and MTL.

Histopathological examinations

Stomach samples were rinsed with pre-cooled saline three times and fixed in 10% neutral formalin solution. After 24h, specimens were dehydrated in ethanol and xylene, respectively and then embedded in paraffin wax. 5 μm thick serial slices were obtained by microtome and stained with hematoxylin-eosin (H&E). Histopathological changes were determined by photography with light microscopy, and 100×, 200× magnifications were used in the microscopy analysis. Anatomical scoring standards: 0, no damage; 1, mucosal congestion and edema; 2, scattered swelling protrusions on the mucosa; 3, mucosal mass with more protrusions; 4, mucosal mass protruding into patches. Microscopic pathological scoring standards are described in Table 1. An average proportion was calculated from 5 regions in each sample and used for statistical analysis.

Table 1
Microscopic pathological scoring standards.

Pathological changes	0	1	2	3
Neutrophil infiltration	none	mild	moderate	severe
Lymphoid, monocyte, plasma cell infiltration	none	mild	moderate	severe
Gland destruction	none	mild	moderate	severe
Inflammatory infiltration of mucosal muscle layer	none	mild	moderate	severe

Enzyme-linked immunosorbent assays

The concentrations of GAS (96T) and MTL (96T) in rats were determined according to the instructions of the manufacturer in the ELISA kit (Guangzhou Kangming Biotechnology Co., Ltd., Guangzhou, China).

Quantitative PCR for mRNA expression

Tissue total RNA extraction: was extracted with Trizol (Invitrogen). The concentration of total RNA was determined by a UV photometer and dissolved in 20 μL of sterilized double distilled water (DEPC-treated DDH2O) with 1.5% agarose gel at 120 V for 30 min, then RNA electrophoresis was performed to detect the integrity of RNA. mRNA reverse transcription: cDNA synthesis was performed in strict accordance with the instructions of the reverse transcription kit (Guangzhou Ruizhen Biotechnology Co., Ltd., China). The reverse transcription conditions were determined as 94 °C 25 min, 43 °C 5 min, and 50 °C 5 min in one cycle and stored at -200 °C. PCR reaction of reverse transcription products: PCR Kit (Guangzhou Ruizhen Biotechnology Co., Ltd.), primers were synthesized by Shanghai Bioengineering Technology Service Co., Ltd. The primer sequences are described in Table 2. The reac-

tion conditions were as follows: 94 °C 2 min, one cycle, 94 °C 30 sec, 56 °C 30 sec, 72°C 1 min, 35 total cycles; 72 °C Extension 4 min. The internal reference reaction conditions were 94 °C 30 sec, 55 °C 30 sec, 72 °C 1 min, 35 cycles, 72 °C 4 min. Electrophoresis analysis of products: PCR detection products were 1.2%. 5% agarose gel 80 V electrophoresis about 60 min, using a gel image analyzer (produced by BIO-RAD company), using Quantity One analysis software, the ratio of the optical density of the amplified product to that of β-actin was used as a parameter of the expression level.

Western Blotting Assay

The protein was extracted with the high-efficiency RIPA tissue/cell rapid lysate containing 1 mmol/L PMSF (Dexian Xingye Biotechnology Co. LTD, Guangzhou, China) and the phosphatase inhibitor Cocktail III (Guangzhou Kangming Biology Science and Technology Co., LTD , Guangzhou, China). It was quantified with the BCA Protein Assay Kit (Guangzhou Baisheng Biology Science and Technology Co., LTD, Guangzhou, China). SDS-PAGE separated protein bands and then transferred to a polyvinylidene fluoride (PVDF) membrane (Guangzhou Hehua Technology Co., LTD, Guangzhou, China). After being sealed in Tris-buffered saline (TBS) containing 5% bovine serum albumin (BSA) (Guangzhou Xunyi Biology Science and Technology Co., LTD, Guangzhou, China) at room temperature for 1 h, the membranes were incubated with primary antibodies at 4°C overnight (Table 2), washed with TBS-0.1% Tween 20 (TBST), and then incubated with horseradish peroxidase-conjugated second antibody (goat anti-rabbit IgG) at room temperature for 1 h. GAPDH was used as the internal reference, and the protein bands were visualized and quantified by Image-pro Plus 6.0 software.

Statistical methods

The data of each group were analyzed using GraphPad Prism 8.0.2 statistical software, and the specific experimental data were expressed in the form of $\bar{x} \pm SD$. The homogeneity of variance test was performed if the normality test conformed to the normal distribution. Data that did not conform to the normal distribution were presented as median (lower quartile, upper quartile) [M (Q25, Q75)] using the Kruskal-Wallis nonparametric test. $p<0.05$ for the difference was statistically significant. After the variance was homogenized, the LSD test in the one-way variance was used for analysis $p<0.05$ means the difference was statistically significant.

RESULTS

Effect of folic acid on gastric pathology

The anatomical images of rats are shown in Fig. 1A. The gastric mucosa of the model group was elastic, with edema, verrucous process and hyperplasia, and the mucosa was dark and white. Compared with the model group, the gastric mucosa in

Table 2
Antibodies used

Antibodies	Dilution	Manufacturers	Cat. no
Rabbit PI3 kinase	1:500	Affinity	AF6242
Rabbit anti-PI3 kinase p110 beta	1:500	Affinity	AF3242
Rabbit anti-AKT	1:500	Affinity	AF6261
Phospho-AKT (Ser473)	1:500	SAB	11054
GAPDH monoclonal antibody	1:50,000	Proteintech	60004-1-Ig
Goat anti-rabbit IgG (H + L)	1:20,000	ZSGB-BIO	ZB-2301

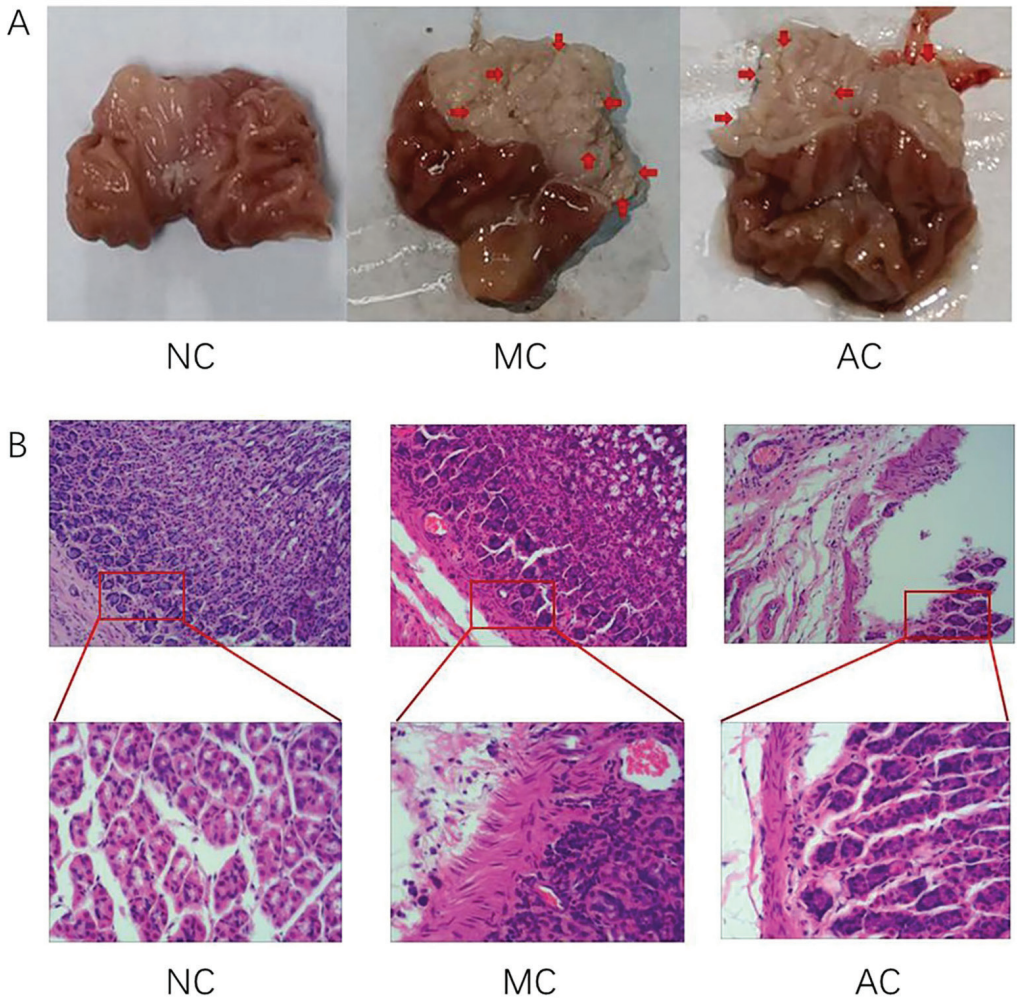

Fig 1. Effect of folic acid on gastric histomorphology of CAG rats. (A) Representative morphology of stomach image of rats. (B) Representative photomicrographs of gastric histopathology in rats. (HE staining, 100×, 200×, Scale bar = 50 μm). N=5. NC, normal group; MC, model group; AC, folic acid group.

the folic acid group was improved, and the gastric mucosa was still ruddy. According to the pathological section of each group of rats (Fig. 1B), the columnar cells in the mucosa of the normal group were intact, the muscular layer of the mucosa was arranged in the inner ring and outer ring, the nuclei distributed orderly, and the mucosal basal layer had no inflammatory infiltration, there was no infiltration of neutrophil and lymphocyte, and inflammatory infiltration was found in the *muscularis* mucosa of the gastric cancer model group, and the number of glands in the *lamina propria* was significantly decreased, in the positive group, the columnar nuclei of the epithelial cells were scattered and not closely arranged, and the tissues were covered with lymphocyte, plasma cells and neutrophil Simple columnar epithelium, the *lamina propria* is slightly reduced, and the stroma is slightly loose with a small amount of lymphocyte, plasma cells, and neutrophil infiltration. Given the lack of alterations in the NC group, the expression of alterations in the other groups studied is significant (Table 3).

Table 3
Effects of folic acid on pathological changes of rat stomach tissue

Group	Gross pathological changes	Pathological changes under a microscope
NC	0.00(0.00,0.00)	0.00(0.00,0.00)
MC	3.00(2.50,3.50)	7.50(7.25,8.50)
AC	1.50(1.25,2.00)*	2.00(0.75,5.25)*

The median and quartile were used to calculate the mean proportions from five regions in each sample, considering that anatomic and pathological section scores were non-normal distributions. NC is the Normal Group, MC is the Model Group, and AC is the Folic Acid Group. Given the lack of alterations in the NC group, the expression of alterations in the other groups studied is significant. * $p<0.05$ vs. MC.

Effects of folic acid on MTL and GAS in the serum of gastric cancer rats induced by MNNG comprehensive method

According to Fig. 2A and 2B, compared with the model group, the content of MTL in the Folic Acid Group was increased ($p<0.05$), while there is no statistical significance in the content of GAS between the Folic Acid Group and the Model Group.

Effect of folic acid on PI3Kand Akt in the serum of gastric cancer rats induced by MNNG comprehensive method

As shown in Fig. 2C, compared with the model group, the content of PI3K of stomach tissue in the Folic Acid Group was decreased (*$p<0.05$), and the content of Akt in both the Normal Group and the Folic Acid Group was decreased (**$p<0.01$).

Effect of folic acid on the mRNA expression of PI3k/Akt/ mTOR2 in gastric cancer rats

According to Fig. 2D, the mRNA expression of PI3K in the Model Group was higher than the Normal Group and Folic Acid Group (**$p<0.01$). In addition, there is no statistical significance in the mRNA expression of Akt between the Folic Acid Group and The Model Group, while the mRNA expression of

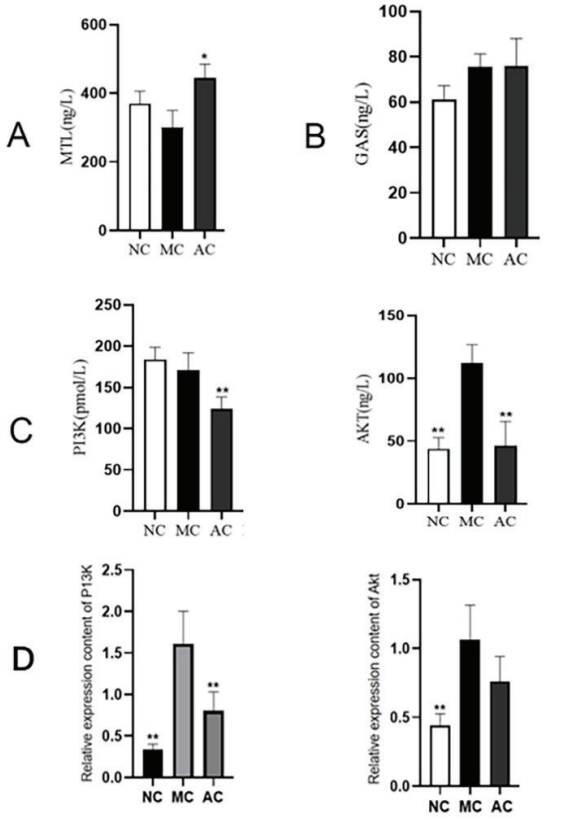

Fig. 2. The difference of GAS, MTL, PI3K and Akt in rat serum and stomach tissue. (A) and (B) Effects of folic acid on serum GAS and MTL in CAG rats induced by MNNG comprehensive method. Levels of gastrin and motilin in rat serum; (C) Levels of PI3K and Akt in rat serum. The mRNA expression of PI3K and Akt in rat serum ; (D) mRNA expression of PI3K and Akt in rat stomach tissue. Data were shown as $\bar{x} \pm SD$. N=5. Data were presented using one-way analysis of variance (ANOVA) followed by the Bonferroni method. ** $p<0.01$ versus model group; * $p<0.05$ versus model group. NC, normal group; MC, model group; AC, folic acid group.

Akt in the Normal Group was lower than in the Model Group (**$p<0.01$).

Effect of folic acid on PI3k/Akt protein expression in gastric cancer rats

As shown in Fig. 3A and 3B, compared with the model group, the expression of

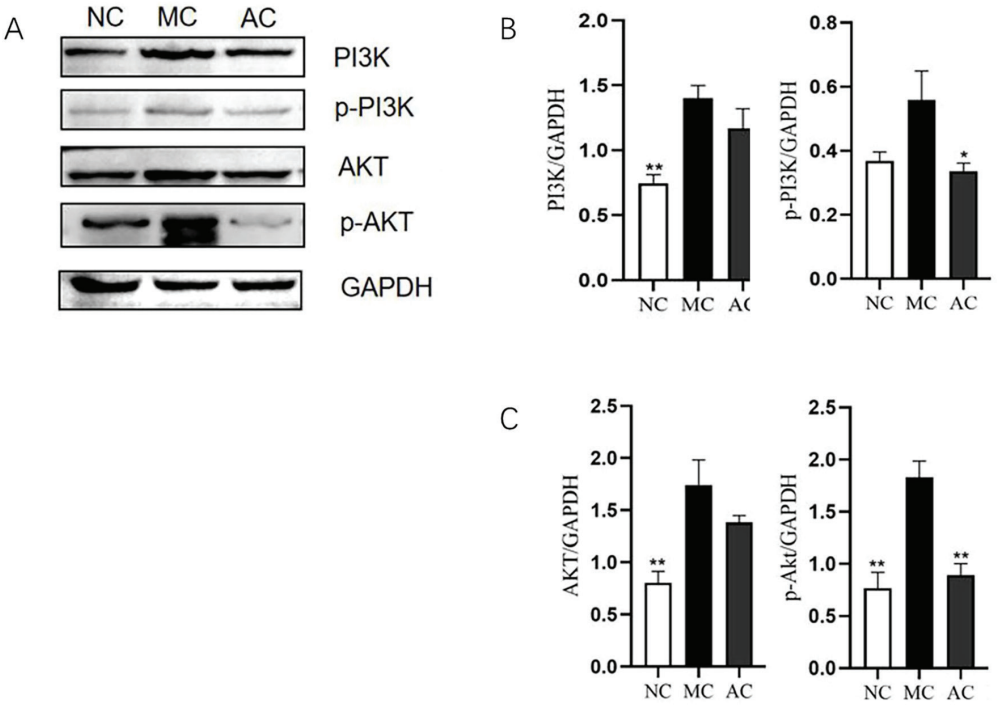

Fig. 3. Effect of folic acid on the protein expression of PI3K and Akt in CAG rats. (A). The WB protein bands of PI3K, p-PI3K, Akt, p-Akt and GAPDH in rat gastric tissue. (B) The protein expression level of PI3K and p-PI3K in rat gastric tissue. (C) The protein expression level of Akt and p-Akt in rat gastric tissue. Data were shown as $\bar{x} \pm SD$. N=5. Data were presented using one-way analysis of variance (ANOVA) followed by the Bonferroni method. ** $p<0.01$ versus model group; * $p<0.05$ versus model group. NC, normal group; MC, model group; AC, folic acid group.

phosphorylated PI3K protein in the gastric tissue of the rats in the folic acid groups was down-regulated (**$p<0.01$). The phosphorylated AKT protein expression in the rats' gastric tissue in the compound medium group was significantly down-regulated (Fig. 3A and 3C, **$p<0.01$).

DISCUSSION

CAG is a precancerous atrophic gastritis of the stomach, but the pathogenesis of CAG in modern medicine is not clear [1]. Therefore, the prevention and treatment of CAG and the development of gastric precancerous lesions are essential issues. At present, there are mainly two ways of treatment: drugs and surgery. Drug therapy mainly includes enhancing gastrointestinal peristalsis, promoting gastric emptying, reducing gastric acid secretion, protecting gastric mucosa, and using antibiotics to treat CAG with positive Helicobacter pylori (Hp). Whereas surgical approaches mainly include endoscopic treatment and general surgical treatment [3,9]. According to clinical management, drug therapy is the routine treatment for CAG, including (Hp) eradication, acid suppression, gastric mucosa protection, gastric motility promotion, anti-anxiety/depression therapy, vitamin, folic acid therapy, and so forth [9].

It has been reported that the changes in MTL and GAS in the treatment process fully reflected the improvement of gastrointestinal function and relief of gastrointestinal symptoms [10]. MTL lowers stomach qi, regulates gastrointestinal transit complex movement during the interdigestive period,

and can increase colon movement [11]. GAS can promote antrum contraction, stimulate the gastric and small intestinal digestive system, promote the growth of gastric mucosa, promote the secretion of water and electrolytes [12], improve the activity of the pyloric pump, and contract gastrointestinal smooth muscle cells, promoting gastric emptying.

Folic acid, also known as vitamin M, plays a crucial role in synthesizing proteins, nucleotides and pantothenic acid in humans. It has been reported that a lack of folic acid can lead to colitis and chronic atrophic gastritis, which increases the risks of gastrointestinal cancer [4]. In addition, Lin *et al.* [5] reported that the natural apoptosis rate of gastric cancer cells was much higher in patients with gastric cancer given a specific concentration of folic acid than in those without intervention. Therefore, the anti-cancer mechanism of folic acid may be to regulate the expression of some cancer cells to accelerate apoptosis. In this study, we investigated the effect of folic acid on gastric cancer model rats by animal experiment. According to Fig. 1, the gastric cancer model rats in the gastric mucosa showed a lot of hyperplasias, verrucous protuberances into pieces, the mucosa white, dark, a little erosion and other phenomena. After treatment, after H&E staining, the pathological sections of the model group were analyzed. The inflammatory factors infiltration and derangement of mucosal epithelial cells were found in the model group, which were improved to some extent after treatment. According to the pathological analysis and score of each group, the gastric tissue injury in the folic acid group improved somewhat compared with the model group. Subsequently, we evaluated the Motilin (MTL) content in each group of rats. Folic acid could increase the MTL content in rats' serum, promote the gastrointestinal movement of rats, and improve the pathological state of rats with gastric cancer. The PI3K/AKT pathway is a signaling pathway associated with proliferation, differentiation and apoptosis, which regulates the proliferation and survival of tumor cells and plays an essential role in tumor cell migration and adhesion. As shown in Fig.2C, the levels of PI3K and Akt in the folic acid group decreased, which may be related to the inhibition of the development of cellular inflammation. As shown in Fig. 2D, the mRNA expression of PI3K was decreased in the folic acid group, which may be related to its promotion of tumor cell apoptosis. As shown in Fig. 3, Folic acid may down-regulate the expression of phosphorylated PI3K and Akt protein, accelerate the apoptosis of cancer cells, and play a role in cancer inhibition.

In summary, folic acid can be therapeutic on CAG, associated with the contents of GAS and MLT in serum and the PI3K/Akt signaling pathway. Our study will lay a theoretical foundation for using Folic acid to investigate new therapies for CAG in humans.

Ethical approval

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part are appropriately investigated and resolved. Experiments were performed under a project license (NO.: 2020041) granted by the Animal Experimentation Ethics Committee of Panyu District Traditional Chinese Medicine Hospital in compliance with national or institutional guidelines for the care and use of animals.

ACKNOWLEDGEMENTS

The Basic and Applied Basic Research Project from Guangzhou Science and Technology Bureau and Panyu District Hospital of Traditional Chinese Medicine, Guangzhou University of Chinese Medicine, supported this work. We thank Prof. Cao Yong for supervision and guidance, Prof. Weigang Chen for guidance in clinical work and case analysis, Prof. Shaozhen Hou and her team for assistance with mouse models and index testing, and Dr. Qiangbin Li and Dr. Boshen Chen for technical support.

Conflict of interest

The authors had no separate personal, financial, commercial, or academic conflicts of interest.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Funding

This work was supported by the Guangzhou Science and Technology Program Project (Project No.: 202102080643) "Study on the Mechanism of Astragalus Guizhi Decoction on Precancerous Lesions in CAG Rats Based on P13k-Akt-mTORC2 Pathway".

ORCID numbers of authors

- Yun An (YA): 0000-0002-9438-2999
- Weigang Chen (WC): 0009-0007-9814-1037
- Yong Cao (YC): 0009-0004-5217-1298
- Boshen Chen (BC): 0009-0005-4367-4456
- Qiangbin Li (QL): 0009-0009-8546-734X
- Xia Zhou (XZ): 0009-0003-3073-3512
- Weihan Huang (WH): 0009-0006-9402-3052

Author contributions

Study conception and design: YA, WC, YC, Data collection: BC, Data analysis and interpretation: QL, WH, Drafting of the article: All authors. Critical revision of the article: All authors.

REFERENCES

1. **Lerch JM, Pai RK, Brown I, Gill AJ, Jain D, Kővári B, Kushima R, Sheahan K, Slavik T, Srivastava A, Lauwers GY, Langner C.** Interobserver agreement of estimating the extent of intestinal metaplasia in patients with chronic atrophic gastritis. Virchows Arch 2022;480(6):1277-1281. *doi: 10.1007/s00428-021-03245-9.*
2. **Deng D.** Progress in clinical treatment of chronic Atrophic gastritis. Elec J Clin Med Literature 2018;5(85):105-106. *doi: 10.3877/j.issn.2095-8242.2018.85.090*
3. **Shah SC, Piazuelo MB, Kuipers EJ, Li D.** AGA Clinical Practice Update on the Diagnosis and Management of Atrophic Gastritis: Expert Review. Gastroenterology. 2021;161(4):1325-1332.e7. *doi: 10.1053/j.gastro.2021.06.078.*
4. **Moazzen S, Dolatkhah R, Tabrizi JS, Shaarbafi J, Alizadeh BZ, de Bock GH, Dastgiri S.** Folic acid intake and folate status and colorectal cancer risk: A systematic review and meta-analysis. Clin Nutr.2018;37(6 Pt A):1926-1934. *doi: 10.1016/j.clnu.2017.10.010.*
5. **Lin Y, Chen Z.** Reversal effect of folic acid and retinoic acid on gastric precancerous lesions and gastric cancer and its mechanism of anti-cancer. Chinese J Clin Gastroenterol 2001;13(01): 41-43. *doi:10.3870/j.issn.1005-541X.2001.01.020.*
6. Ogino S, Liao X, Imamura Y, Yamauchi M, McCleary NJ, Ng K, Niedzwiecki D, Saltz LB, Mayer RJ, Whittom R, Hantel A, Benson AB 3rd, Mowat RB, Spiegelman D, Goldberg RM, Bertagnolli MM, Meyerhardt JA, Fuchs CS; Alliance for Clinical Trials in Oncology. Predictive and prognostic analysis of PIK3CA mutation in stage III colon cancer intergroup trial. J Natl Cancer Inst 2013;105(23):1789-98. *doi: 10.1093/jnci/djt298.*
7. **Qu Y, He Y, Ruan H, Qin L, Han Z.** Abnormal downregulation of 10-formyltetrahydrofolate dehydrogenase promotes the progression of oral squamous cell carcinoma by activating PI3K/Akt/Rb pathway. Cancer Med 2023;12(5):5781-5797. *doi:10.1002/cam4.5327.*

8. **Huang X, He Z, Jiang X, Hou M, Tang Z, Zhen X, Liang Y, Ma J.** Folic acid represses hypoxia-induced inflammation in THP-1 cells through inhibition of the PI3K/Akt/HIF-1α pathway. PLoS One 2016;11(3):e0151553. *doi: 10.1371/journal.pone.0151553.*
9. **Lahner E, Zagari RM, Zullo A, Di Sabatino A, Meggio A, Cesaro P, Lenti MV, Annibale B, Corazza GR.** Chronic atrophic gastritis: Natural history, diagnosis and therapeutic management. A position paper by the Italian Society of Hospital Gastroenterologists and Digestive Endoscopists [AIGO], the Italian Society of Digestive Endoscopy [SIED], the Italian Society of Gastroenterology [SIGE], and the Italian Society of Internal Medicine [SIMI]. Dig Liver Dis 2019;51(12):1621-1632. *doi: 10.1016/j.dld.2019.09.016.*
10. **Zeng Q, Ou L, Wang W, Guo DY.** Gastrin, cholecystokinin, signaling, and biological activities in cellular processes. Front Endocrinol (Lausanne) 2020;11:112. *doi: 10.3389/fendo.2020.00112.*
11. **Kitazawa T, Kaiya H.** Motilin comparative study: structure, distribution, receptors, and gastrointestinal motility. Front Endocrinol (Lausanne) 2021;12:700884. *doi: 10.3389/fendo.2021.700884.*
12. **Kong W, Sun K.** Roles of gastrointestinal hormone in functional disorder of colon in diabetes. World J Chin Digestion 2006;14(5):519-521. *doi: 10.3969/j.issn.1009-3079.2006.05.011.*