Original Article
Protective effects of Kangfuxin liquid (Periplaneta Americana extract) on chronic atrophic gastritis in rats via anti-oxidative stress and inhibition of COX-2

Shaoju Jin¹*, Lei Ma²*, Qing Xu³*, Liucheng Guo⁴*, Liping Ren¹, Zhenjun Shao⁴, Litao Liu⁴, Xiuying Ma⁴, Liming Zhou⁴, Jianguo Wang¹,⁵

¹Department of Pharmacology, Luohe Medical College, Luohe, Henan, China; ²Department of Emergency, General Hospital of Ningxia Medical University, Yinchuan, Ningxia, China; ³Department of Neurosurgery, The First People’s Hospital of Taicang, Taicang, China; ⁴Department of Pharmacology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan, China; ⁵Tumor Occurrence and Prevention Research Innovation Team of Luohe, Luohe, Henan, China. *Co-first authors.

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Abstract: Objective: The purpose of this study is to investigate the protective effects of Kangfuxin liquid (KFX) on experimental chronic atrophic gastritis (CAG) in rats and the mechanism of action. Methods: The chronic atrophic gastritis model rats were induced by synthetic methods for 16 weeks except for the rats in normal group. Then, the rats in normal and model groups were (i.g.) administrated with distilled water, the rats in positive control group were i.g. Sanjiu Weitai (SJWT) 8 g/kg, the rats in the three KFX treated groups were administered 2.0, 1.0, 0.5 ml/100 g body weight (BW), once per day for 4 weeks, respectively. By the end of the experiment, the rats were anesthetized with urethane and their stomachs were resected. Gastric juice was collected from stomach to detect the gastric acidity and pepsin. Serum was stored at -80°C for SOD, MDA and GSH-Px biochemical analysis. The mRNA and protein expression of COX-1 and COX-2 in the gastric tissue were detected by reverse transcription polymerase chain reaction (RT-PCR) and western blot analysis. Results: After KFX (2 ml/100 g, 1 ml/100 g) treatment for 4 weeks, the CAG rats’ gastric acid and pepsin were increased, the gastric histopathological changes were attenuated and closed to normal, the actives of SOD and GSH-Px increased, the concentration of MDA decreased, and the mRNA and protein expression of COX-2 were higher. Conclusion: KFX has the protective effect on chronic atrophic gastritis via anti-oxidative stress and inhibition of COX-2.

Keywords: Chronic atrophic gastritis, kangfuxin, anti-oxidative stress, COX-2

Introduction
Chronic atrophic gastritis (CAG) is a very common disease of digestive system. The world health organization (WHO) listed it as precancerous condition of gastric cancer, which was described as mucosal atrophy, intestinal metaplasia, atypical hyperplasia, inflammatory cell infiltration, gastric acid and reduced pepsin secretion, etc [1, 2]. It is well known that CAG as a premalignant disease with an increasing risk factor in the development of gastric carcinoma, but there are no perfect and effective methods to treat it at present [3]. It is slow, hard to be cured and the disease course is long. Lots of clinical materials indicated that Traditional Chinese Medicine (TCM) has an obvious advantage in prevention and treatment of the CAG [4]. Kangfuxin liquid (KFX) is a kind of pure biologic medicine extracted from the hexapod living fossil Periplaneta American (L) (a subspecies of cockroach), one of the most common animal drugs used in TCM. It contains polyols, epidermal growth factor (EGF), mucopolysaccharide, multiple amino acids, etc. It can promote the cell increment, granulation tissue growth, and angiogenesis, enhance immune function, anti-inflammation, induce cell apoptosis, treat burn wounds, and promote ulcer healing and reduce ulcer replace, etc [5]. Study reports that KFX in treatment of CAG has clinical efficacy, safety and reliability with few adverse reactions [6]. However, its mechanism of action has not been clarified. Therefore, in this study, we aimed to explore the mechanism of KFX on CAG.
In order to study the mechanism of CAG, a variety of animal models have been developed. But synthetic method model was a widely used for induction of CAG in experimental animals [7]. Studies show that from the chronic superficial gastritis, atrophic gastritis, intestinal metaplasia, atypical hyperplasia, early gastric cancer to advance gastric carcinoma, the expression level of COX-2 increasing gradually in gastric tissue, and it’s up and down-stream pro-inflammatory cytokines also played a vital role in CAG [8]. At the same time, the reactive oxygen species (ROS) has also play an important role in CAG [9]. In present study, a rat model of CAG was established successfully and to investigate whether KFX exerts its protective role in the CAG via anti-oxidative stress and inhibition of cyclo-oxygenase-2 (COX-2) pathway.

Materials and methods

Main chemical regents

KFX was provided by Sichuan Good Doctor Pharmaceutical Group (Chengdu, China). Deoxycholic acid sodium salt was bought from Amerso (Ohio, USA). Sanjiu Weitai (SJWT), the positive control, was obtained from China Resources Sanjiu Medical & Pharmaceutical Co., Ltd. (Shenzhen, China). Pepsin, superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) diagnostic agents were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and reagents were of commercial analytic grade.

Experiment animals

Male Sprague-Dawley (SD) rats weighting 180-200 g were supplied by the Experimental Animal Center of Sichuan University. The rats were housed in an air-conditioned room, under hygienic conditions and placed in a controlled environment with at 22±2°C, 55±5% humidity and with a 12/12 hours light/dark cycle. Experimental procedures were performed in accordance with the guidelines of the Experimental Research Institute of Sichuan University.

Modeling and the experiment protocol

The establishment of chronic atrophic gastritis lesions in rats was modified according to Xiang and Lu’s [10, 11] synthetic methods. In brief, 0.05% ammonia solution was used as drinking water everyday ad libitum, intragastric administration 2 mL of 2% salicylic acid ethanol (40%) solution was given twice in fasting per week and intragastric administration of 2 mL of 10 mmol/L deoxycholic acid sodium salt solution without fasting every other day but intragastric administration twice in fasting for 16 weeks. Then, all the survived rats were divided randomly into 5 groups (n=10, each group): (1) model group, i.g. administration distilled water, 1 mL/100 g body weight (BW), (2) positive control group, i.g. SJWT 8 g/kg, (3) low-dose KFX group, i.g. KFX 0.5 mL/100 g BW, (4) median-dose KFX group, i.g. KFX 1.0 mL/100 g BW, (5) high-dose KFX group, i.g. KFX 2.0 mL/100 g BW. All drugs were given once a day. Moreover, a group of normal rats (n=10) were raised as a control group that free access to normal rat chow and water. After 4 weeks, all rats were anesthetized with urethane (1 g/kg BW) by intraperitoneal injection and their stomachs were resected. Gastric juice was collected from stomach to detect the gastric acidity (acid-base titration) and pepsin. Serum was stored at -80°C for SOD, MDA and GSH-Px biochemical analysis (according to manufacturer’s instructions).

Morphological examination

The anterior wall tissue of stomach was excised and fixed in 4% buffered paraformaldehyde solution over 24 hours. Tissues were embedded in paraffin, 5 μm sections, stained with hematoxylin and eosin (H&E). The morphological changes were examined under light microscopy, and photomicrographs taken.

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from the gastric samples by TRIzol regent (Invitrogen, USA) according to the manufacture’s introductions. Total RNA was reverse transcribed with RevertAid™ first stand cDNA Synthesis kits (Ferments, USA) following the manufacturer’s instructions. Primer sequence for rat’s COX-1, COX-2 and β-actin were amplified by polymerase chain reaction (PCR). The sense and antisense primer were 5’-GGCGTCGCTGTCAGATGGCCTAC-3’ and 5’-CACCAATCCGCGGAGGCTCC-3’ for COX-1; 5’-ATCTACCCTCCTCAAGTCCC-3’ and 5’-TACCAG-
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AAGGGCAGGATACAG-3' for COX-2; and 5'-GATTGCTCCTCTGCAGC-3' and 5'-ACTCCTGCTGCTGATCCAC-3' for β-actin. PCR cycle conditions consisted of 35 cycles of 45 s at 94°C, 35 s at 58°C, 55 s at 72°C for COX-1 and COX-2, and 35 cycles of 30 s at 94°C, 20 s at 58°C, 30 s at 72°C for β-actin. β-actin was used as an internal control. The PCR products were electrophoresed in a 1.5% agarose gel stained with ethidium bromide and observed under ultraviolet light.

Western bolt analysis

The gastric tissues were lysed in lysis buffer containing protease inhibitors. The protein extracts were resolved on a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteins were transferred to a poly-vinylidene difluoride membrane (Bio-Rad), and the membranes were blocked with 5% non-fat dry milk for 2 hours at room temperature and incubated with COX-1/COX-2/β-actin antibody (Santa Cruz, USA) overnight at 4°C. Subsequently the membranes were incubated with corresponding horseradish peroxidase-conjugated secondary antibody (Santa Cruz, USA) for 1 hour at room temperature. Expression levels of the proteins were normalized to β-actin.

Statistical analysis

The data were subject to statistical analysis using the SPSS software package version 16.0 (SPSS, Inc., Chicago, USA), parameters were presented as mean ± SD. Differences of data between groups were compared with One-way ANOVA method. P<0.05 was considered to be statistically significant.

Results

Pathological findings

The histopathological changes were observed under microscope. In normal control group, the structure of gastric mucosa was complete, the epitheliums were arranged neatly, and gastric glands shape rules. In CAG model group, the gastric mucosa showed thinning, paleness, erosion and exfoliated, the gastric glands atrophy and arranged irregularly. Compared with the CAG model group, administration of KFX (2 ml/100 g) significant ameliorated the CAG rats' gastric histopathological changes, and closed to normal (Figure 1).

Gastric acid and pepsin

The effects of KFX on gastric acid and pepsin in CAG rats were shown in Table 1. When com-

Table 1. Effects of KFX on gastric acid and pepsin in CAG rats (x±s, n=10)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Gastric acid (mmol/L)</th>
<th>Pepsin (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>--</td>
<td>1.46±0.20</td>
<td>59.96±14.15</td>
</tr>
<tr>
<td>Model control</td>
<td>--</td>
<td>0.91±0.20</td>
<td>34.79±7.16</td>
</tr>
<tr>
<td>SJWT</td>
<td>8 g/kg</td>
<td>1.38±0.15</td>
<td>58.57±18.49</td>
</tr>
<tr>
<td>KFX</td>
<td>2 ml/100 g</td>
<td>1.39±0.11</td>
<td>55.04±12.36</td>
</tr>
<tr>
<td></td>
<td>1 ml/100 g</td>
<td>1.35±0.12</td>
<td>53.43±18.03</td>
</tr>
<tr>
<td></td>
<td>0.5 ml/100 g</td>
<td>1.33±0.12</td>
<td>49.54±13.62</td>
</tr>
</tbody>
</table>

#P<0.01, vs normal control group; ^P<0.05, ^P<0.01, vs model group.

Figure 1. Effects of KFX on histological changes of gastric tissue in CAG rats. A: Normal group. B: CAG model group. C: KFX 2 ml/100 g group (HE 100×).
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pared with the control group, CAG rats showed significant decrease in gastric acid and pepsin \((P<0.01)\), but administration of KFX was not \((P>0.05)\). Compared with the CAG model group, the concentration of gastric acid and pepsin markedly increased in KFX group \((P<0.05, P<0.01)\, \text{Table 1}\).

**Anti-oxidation activity of KFX**

After 4 weeks treatments, when compared with the normal group, the activities of SOD and GSH-Px in CAG group significantly decreased \((P<0.01)\) while MDA obviously increased \((P<0.05)\); Furthermore, administration of KFX \((2 \text{ ml/100 g})\) showed no statistical difference in the levels of SOD and GSH-Px \((P>0.05)\). Compared to the CAG model group, the SOD and GSH-Px activities was significantly increased and MDA concentration was obviously reduced in KFX \((2 \text{ ml/100 g, 1 ml/100 g})\) group \((P<0.05, P<0.01)\). Treatment of SJWT also produced similar effects \(\text{Table 2}\).

**COX-1 and COX-2 expression in CAG rats gastric mucosal**

The mRNA and protein expression of COX-1 or COX-2 were analyzed by RT-PCR and western-blot respectively. The results showed that expression of COX-2 \((\text{mRNA and protein})\) in CAG rat was obviously increased compared with those of the normal group \((P<0.05, P<0.01)\). When compared with the CAG model group, the mRNA and protein expression levels of COX-2 markedly decreased in KFX \((2 \text{ ml/100 g})\) group \((P<0.05, P<0.01)\, \text{Figure 2}\).

**Discussion**

Chronic atrophic gastritis is one of the most common digestive system diseases worldwide, and it is permanent and hard to be cured completely. It has also been found that CAG is closely associated with development and progression of gastric cancer \([12]\). Many factors could induce CAG, such as immunity, bile reflux, infection, wining, smoking and drugs, etc. But the pathogenesis of CAG has not been completely identified \([13-15]\). There are many methods and drugs were used to treating CAG on clinic, but the effect was not ideal \([16]\). Therefore, novel, efficacious and safe agents for CAG are urgently needed. It is well know, the traditional Chinese medicine has a satisfactory effect on CAG \([17-19]\). KFX is a kind of pure biologic medicine extracted from the hexapod living fossil Periplaneta American \((L)\) which is a type of traditional Chinese medicine \([20]\). In present study, we found the KFX has protective effects on CAG. So we designed to evaluate anti-CAG potential of systemically administered KFX in CAG rats.

Oxygen free radicals were middle products of the normal metabolism in body, but the existing of it has two sides \([20-22]\). In the CAG patients, the serum levels of the free radicals were significantly higher than healthy people, and closely related to the degree of gastric mucosal injury \([23]\). MDA was a product of lipid peroxidation, its content can reflect the degree of the lipid peroxide of organism \([24, 25]\). SOD and GSH-Px which were clearance agents to free radical can clear lipid peroxides and was a role in protecting cells and tissue \([26-29]\). Therefore, measuring the levels of SOD, MDA and GSH-Px which were clearance agents to free radical can clear lipid peroxides and was a role in protecting cells and tissue \([26-29]\). Therefore, measuring the levels of SOD, MDA and GSH-Px in CAG patients could reflect the degree of gastric mucosal injury. In present study, we found that KFX \((2 \text{ ml/100 g, 1 ml/100 g})\) could increase the levels of serum SOD and GSH-Px and decrease MDA in the experimental CAG rats.

**Table 2. Effects of KFX on serum SOD, MDA and GSH-Px in CAG rats \((\bar{x} \pm s, n=10)\)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>SOD (U/mL)</th>
<th>MDA (nmol/mL)</th>
<th>GSH-Px (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>--</td>
<td>170.14±9.52</td>
<td>5.27±1.01</td>
<td>289.41±59.89</td>
</tr>
<tr>
<td>Model control</td>
<td>--</td>
<td>141.77±10.32*</td>
<td>11.65±1.79*</td>
<td>173.44±51.51*</td>
</tr>
<tr>
<td>SJWT</td>
<td>8 g/kg</td>
<td>162.96±9.74A</td>
<td>6.50±1.56*</td>
<td>268.95±47.44A</td>
</tr>
<tr>
<td>KFX</td>
<td>2 ml/100 g</td>
<td>163.84±8.56A</td>
<td>7.14±0.95*</td>
<td>264.69±51.65A</td>
</tr>
<tr>
<td></td>
<td>1 ml/100 g</td>
<td>158.86±6.59A*</td>
<td>9.08±1.30*</td>
<td>204.00±32.37*</td>
</tr>
<tr>
<td></td>
<td>0.5 ml/100 g</td>
<td>148.29±9.15*</td>
<td>10.97±1.36*</td>
<td>186.36±43.43A</td>
</tr>
</tbody>
</table>

*P<0.05, *P<0.01, vs normal control group; P<0.05, *P<0.01, vs model group.

**COX-1 and COX-2 expression in CAG rats gastric mucosal**

The mRNA and protein expression of COX-1 or COX-2 were analyzed by RT-PCR and western-blot respectively. The results showed that expression of COX-2 \((\text{mRNA and protein})\) in CAG rat was obviously increased compared with those of the normal group \((P<0.05, P<0.01)\). When compared with the CAG model group, the mRNA and protein expression levels of COX-2 markedly decreased in KFX \((2 \text{ ml/100 g})\) group \((P<0.05, P<0.01)\, \text{Figure 2}\).
within organisms [30]. COX-1 was widespread in many tissues including to blood vessel, stomach, kidney and the platelet in charge of signal transmission and the maintenance of cell function's balance [31]. COX-2 was an inducible enzyme that was associated with damage stimuli such as inflammation, infection and tissue injury [32]. It was closely related with the occurrence and development of CAG. In our study, we found that the expression of COX-2 was rise in CAG rats and the KFX (2 ml/100 g, 1 ml/100 g) could significantly decrease it.

**Conclusion**

In conclusion, it was demonstrated that KFX has the protective action on chronic atrophic gastritis and its mechanism was via anti-oxidative stress and inhibited COX-2.

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**Disclosure of conflict of interest**

None.

**References**


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