Astragalus polysaccharide inhibits apoptosis and inflammation to alleviate chronic atrophic gastritis through NF-κB signaling pathway in rats

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Abstract
Chronic atrophic gastritis (CAG) is a process of inflammation characterized by injured gastric mucosal epithelium leading to reduction of mucosal glands, often accompanied by intestinal or pseudopyloric metaplasia. Astragalus polysaccharide (APS) is reported to improve gastric mucosal damage and inflammation, but its role in CAG remains elusive. Therefore, we intended to analyze the impact of APS on CAG and its potential mechanism to provide novel insight into the treatment for the disorder. After establishment of CAG model using MNNG method, the rats were administered Vitazyme (200 mg/kg), or low, moderate and high concentration of APS (20, 40, 60 mg/kg), respectively, with healthy rats as controls and some CAG rats untreated. After that, the rat gastric tissues were pathologically examined and ELISA, Western blot analysis and staining were carried out to assess the impact of treatment on inflammation and apoptosis. Pathologically, APS treatment alleviated the pathological changes of CAG rats, reducing inflammatory cell infiltration and inflammation. Besides, APS significantly diminished the content of pro-inflammatory factors and decreased the level of gastrointestinal hormones. In the presence of APS, the apoptosis of gastric mucosal cells was inhibited and the p-NF-κB p65 and p-IKKα/β expression was decreased. Collectively, APS could mitigate inflammation and gastric mucosal damage in CAG, as it also suppresses the process of apoptosis and inactivates the NF-κB signaling pathway. In a word, APS treatment improves CAG via NF-κB pathway.

Keywords: Astragalus polysaccharide; NF-κB; apoptosis; inflammation; chronic atrophic gastritis.

Practical Application: Our study demonstrates that APS treatment improves CAG via NF-κB pathway in a rat model. However, whether it exerts similar effect on patients with CAG remains to be further investigated in the future.

1 Introduction
Chronic atrophic gastritis (CAG) is a process of inflammation characterized by injured mucosal epithelium leading to reduction of mucosal glands, often accompanied by intestinal or pseudopyloric metaplasia (Giannakis et al., 2008; Kuipers, 1999). A multicenter prospective epidemiological survey indicated a high prevalence of CAG in China, and Helicobacter pylori (H. pylori) infection, and environmental factors are the main causes (Rautelin & Kosunen, 2004; Ubukata et al., 2011). CAG patients without classic manifestations are often hardly identified in the early stage (Kim et al., 2010). As the disease progresses, thickening of the mucosal muscular layer and intestinal metaplasia often take place with increased cancer risk. Importantly, effective treatment can not only mitigate the patient's clinical symptoms and improve the patient's quality of life (Kim et al., 2010). Despite the improvement in prognosis owing to the treatments, the outcome remains poor and adverse reactions appear (Kim et al., 1997).

Astragalus is the dried root of Astragalus membranaceus, (Lu et al., 2008) which is used in the improvement and treatment of various diseases as medicine and food to invigorate the spleen and replenish qi. Polysaccharides are its main component with protective effect on kidney and liver, and therefore have been frequently studied. The protective effect of Astragalus polysaccharides (APS) has been depicted in human gastric adenocarcinoma cells where APS can also decrease production of TNF-α and other cytokines (Velmurugan et al., 2002a; Velmurugan et al., 2002b). Liu et al. noted that APS can significantly suppress NF-κB pathway in chronic gastritis, and reduce the inflammatory factors IL-8, IL-6 and IL-1β (Li et al., 2011). The NF-κB signaling pathway is a well-known signaling pathway related to inflammation, suggesting that APS may regulate gastric mucosal inflammation (Liu et al., 2011; Yin et al., 2010). In a study by Wu, administration of APS greatly reduced BAX expression and hindered the apoptosis of gastric mucosal cells (Zhou et al., 2011). These findings all suggest that APS has a protective effect on gastric mucosal damage. At present, CAG is not widely applied to clinical practice as its impact has not been fully elucidated (Juan et al., 2011), and there is no report on whether APS can improve CAG. This study aims to explore the effect of APS on CAG. Therefore, attempt of our work is to investigate the efficacy of APS on CAG.
and its potential mechanism to provide novel insight into the treatment for the disorder.

2 Materials and methods

2.1 Animals

Sixty adult male SD rats (230 ~ 280 g) were purchased from Jiangsu Jicui Yaokang Biotechnology Co., Ltd. (Jiangsu, Nanjing), and raised in an environment of 45% ~ 55% humidity and 23 ± 1 °C with free access to food and water for one week. The study was approved by the Animal Care and Use Committee of Shanghai Jiaotong University.

2.2 Animal model

All rats were classified into control group, group II (CAG model), group III (CAG rats treated with Vitazyme group, and group IV-VI (CAG rats treated with APS at 20 mg/kg, 40 mg/kg and 60 mg/kg) (Figure 1). APS (HPLC grade, 98% purity) was obtained from Sichuan Kelun Co., Ltd. Rats in the II-VI group were administrated MNNG (1.7 mg/kg), and fed with water containing MNNG for 12 weeks. After that, the rats in the III-VI group were given 200 mg/kg Vitazyme or APS (20 mg/kg, 40 mg/kg and 60 mg/kg) daily for 5 weeks. The weight and food consumption of the rats was detected every week and their behavior was observed as well. Finally, the blood sample was taken and the gastric sample was placed in 10% formalin, embedded, and stained. The content of gastrointestinal hormones was determined by radioimmunoassay.

2.3 Pathological analysis

Rat stomach tissues were fixed overnight, embedded and sliced into 5 μm sections, deparaffinized and rehydrated. The sections were stained with HE staining using a H&E staining kit (Thermo Fisher Scientific Ltd., USA). Finally, the section was observed under a light microscope.

2.4 ELISA

ELISA kits were used to detect gastrin (GAS), motilin (MTL), TNF-α, IL-6, IL-1β using the ELISA kit (Shanghai Xitang Biotechnology Co., Ltd).

2.5 TUNEL staining

Rat tissues were fixed, dehydrated, embedded, and cut into 5 μm sections. After deparaffinization, cell apoptosis was detected with Roche in situ cell death detection kit (TdT-mediated dUTP Nick-End Labeling), and observed.

2.6 Western blot analysis

Proteins were extracted and quantified with a BCA kit (Beyotime). The protein was separated and transferred to PVDF membrane (Millipore). Upon blocking, the membrane was incubated with primary antibodies and goat anti-mouse IgG (1:10000, Abcam, USA) for 1 h. Finally, the blot was developed with an ECL kit (Millipore) and quantified with ImageJ software (NIH). The primary antibodies used included GAPDH (1:1000; Proteintech), Bcl-2, Bax, caspase-9, caspase-3, cleaved caspase-9, p-NF-κB p65, NF-κB p65 (1:1000, Cell Signaling Technology), IKKa/β, p-IKKα/β, IKB-α (1:1000, Abcam).

2.7 Statistical analysis

Data were processed with Graphpad Prism software and presented as mean ± SEM. One-way ANOVA was used for data analysis to compare between groups. P < 0.05 or p < 0.01 refers to significant difference.

3 Results and discussion

3.1 APS alleviates the histological lesions of gastric tissue in CAG rats

To examine the therapeutic effect of APS, rat gastric tissues were stained with H&E staining (Figure 2). Compared with the control group, the gastric tissue of the model group showed cystic dilatation, irregular arrangement and inflammatory cell infiltration, suggesting successful establishment of the animal model. The above symptoms were alleviated by APS treatment in a concentration-dependent manner, as the infiltration of inflammatory cells was significantly reduced. It is suggested that APS can alleviate the histological lesions of CAG rats.

3.2 APS affects the level of gastrointestinal hormones in the serum of CAG rats

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3.4 APS affects the level of gastrointestinal hormones in the serum of CAG rats

We measured the level of gastrointestinal hormones in the serum of CAG rats. The data indicated the down-regulation of GAS and enhancement of MTL level in the serum of rats in the model group (Figure 3A-3B). After APS treatment, GAS levels were significantly increased, and MTL levels were significantly reduced with high concentration of APS exhibiting the most prominent effect. The above data indicate that APS treatment can restore the balance of gastrointestinal hormones in CAG rats. Inflammatory factors TNF-α, IL-6 and IL-1β are noted to play a crucial role in the pathological process of CAG. Therefore, in this study, we checked up the above cytokines using ELISA. As shown in Figure 3C-3E, the levels of inflammatory factors in CAG rats relative to control group were significantly increased. And the levels of these factors were decreased by APS treatment, indicating that APS mitigates inflammatory response in CAG rats.
Figure 2. APS improves the symptoms of CAG rats. Representative H&E staining imaging of each experimental group (× 100).

Figure 3. APS regulate the levels of gastrointestinal hormones and inflammatory factors in CAG rats (A-B) ELISA of gastrointestinal hormones GAS and MTL. (C) ELISA of inflammatory factors TNF-α, (D) IL-6, (E) IL-1β. ***p < 0.001 vs. Control group; ##p < 0.01, ###p < 0.001 vs. Model group.
3.3 APS inhibits gastric mucosal cell apoptosis in CAG rats

To investigate the function of APS on gastric mucosal cell apoptosis, TUNEL staining was used for analysis. The apoptosis level of gastric mucosal cells in the CAG model group was elevated (Figure 4). Cell apoptosis was dramatically decreased in APS treatment group.

3.4 APS regulates apoptosis-related proteins

As revealed by Western blot, after MNNG treatment combined with an irregular diet, the model group had increased levels of Bcl-2 decreased, while Bax, cleaved caspase-3 and -9 expressions increased (Figure 5). Of note, administration with APS reversed the reduction of Bcl-2 and the enhancement

Figure 4. APS inhibits the apoptosis of gastric mucosal cells in CAG rats. Representative images of TUNEL staining (× 400).

Figure 5. APS affects the expression of apoptosis-related proteins in gastric tissue of CAG rats. Western blot of apoptosis-related protein expression. ***$p < 0.001$ vs. control; $p < 0.05$, ###$p < 0.01$, ####$p < 0.001$ vs. model.
of Bax, Cleave caspase-3 and caspase-9. In a word, APS inhibits the apoptosis in CAG rats.

3.5 APS suppresses the NF-κB signaling pathway in CAG rats

For further identifying the mechanism underlying APS on CAG, we explored potential downstream signaling pathways. Western blot indicated p-P65 and p-IKKα/β expression increased in the model group, and the expression of IκB-α decreased (Figure 6). These abnormalities were restored by APS treatment, as demonstrated with reduced p-NF-κB p65 and p-IKKα/β levels, and increased I-κB-α levels. This result indicates that APS inhibits the NF-κB in CAG rats.

3.6 Discussion

CAG is a common and complicated digestive disease (Giannakis et al., 2008). As the incidence of gastric cancer (GC) has increased and the age of onset decreases, CAG has seriously affected the health and quality of people (Ubukata et al., 2011). The dilemma of therapeutic drugs for CAG has aroused investigators’ interest. In this study, we found that APS treatment attenuated the pathological lesions of CAG rats through inhibition of inflammation and apoptosis of gastric tissue. In conclusion, our data indicate that APS is a potential alternative for CAG treatment.

APS, a class of iridoid glycosides, has been reported to have multiple pharmacological effects, such as neuroprotection, anti-oxidative stress, anti-inflammatory, anti-apoptosis, etc. (Juan et al., 2011; Liu et al., 2010). However, the function of APS on CAG is still elusive. In this study, we found that APS treatment dose-dependently alleviated the histological lesions of CAG rats. GAS is the main nutrient of the gastric mucosa with important nutritional and protective effects on the gastric mucosa. MTL is also an essential gastrointestinal hormone related to gastric motility, which can induce strong gastric contraction, promote pepsin secretion and food digestion (Wang et al., 2010). Clinical data indicated elevation of MTL level in the patients with gastric cancer (Yan et al., 2009). This study suggests that the administration of APS could increase GAS levels and reduce MTL levels, which is consistent with previous studies (Kim et al., 2010). The above evidence indicates that APS has protective effect on CAG.

The release of pro-inflammatory cytokines like TNF-α associates with the development of CAG (Auyeung et al., 2012; Na et al., 2009; Navarrete et al., 2005). It is reported that APS can regulate liver inflammation in type 2 diabetic db/db mice (Navarrete et al., 2005; Sugimura & Fujimura, 1967). APS reduces myocardial injury and apoptosis caused by Coxsackie B3 virus by inhibiting the activation of NLRP3 inflammasomes (Saito et al., 1970). Moreover, administration of APS also could results in a reduction in the levels of IL-6, IL-1β and TNF-α in the myocardium of rats with acute myocardial infarction (Kobori et al., 1976). Consistently, our work elucidates that APS significantly reduced the expression of TNF-α, IL-6 and IL-1β. Based on the above results, we prove that APS can reduce the gastric mucosal damage in CAG by inhibiting inflammation.

Besides, APS can inhibit neuronal apoptosis and eliminate cerebral ischemia/reperfusion injury (Marotta et al., 1990; Rindi et al., 1993). It also suppresses H2O2-induced PC12 cell apoptosis (Sumii et al., 1981). It is believed that APS is able to restrain activation of caspase-3 and -9, and increase Bcl-2 expression (Bortolotti et al., 1987). Notoginsenoside R1 induces the expression of Bcl-2 and reduces Bax expression to improve CAG induced...
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by MNNG combined with irregular diet (Hayashi et al., 1998). Herein, the intervention of APS reduced apoptosis of gastric mucosal cells, when anti-apoptotic genes BCL-2 expression increased, and cleaved-caspase-3 and -9 decreased. A recent study depicts the potential of inhibition of NF-κB signaling pathway in the treatment for CAG (Szaléczy et al., 2000). The traditional Chinese medicine Qinghua Decoction restrains the pathogenesis of CAG by down-regulating the expression of NF-κB signal transduction (Oda et al., 2005). Our work indicated that the expression of p-NF-κB p65 and p-IκKα/β decreased after APS treatment, while the level of IκB-α increased significantly. The above results confirm that APS has a protective effect on MNNG-induced CAG through inhibition of NF-κB pathway.

4 Conclusion

Collectively this study demonstrated that APS can alleviate CAG-induced gastric mucosal damage by inhibiting inflammation, and regulate expression apoptosis-related proteins to decrease apoptosis. In addition, treatment with APS decreases p-NF-κB p65 and p-IκKα/β expression and increases the level of IκB-α in CAG rats. In a word, APS inhibits apoptosis and inflammation through regulating NF-κB signaling pathway, thereby alleviating CAG in rats. These findings provide new ideas about the treatment of CAG, and identify promising potential of APS on CAG management.

Conflict of interest

None.

References


