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A research on the mechanism of NSAID-related gastric ulcer treated by jia wei wu qi san based on the p38mapk signal pathway

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Abstract: This study aims to explore the mechanism of NSAID-related gastric ulcer treated by JIA WEI WU QI SAN. Clean-grade SD rats were randomly divided into four groups. Group A was assigned as the control group. Groups B, C and D were intragastrically administered with 2.5mg/kg of indomethacin solution *QD* after 48 hours. After 15 days of treatment, group B was administered with 0.9% sodium chloride, group C was given rabeprazole (2mg/kg), and group D was administered with JIA WEI WU QI SAN (2g/kg). Abdominal aorta sampling was performed, and gastric tissues were isolated on the 29th day. The protein expression of p-P38MAPK and COX-2 were detected by western blot, while the concentration of PGE2 and IL-1 were determined by ELISA. (1) The expression of IL-1ingroup B dramatically declined in group D (P<0.01). (2)The expression of PGE-2dramatically increased in group D(P<0.01). (3) The expression of COX-2 increased in group D (P<0.05). (4) The expression of p-P38MAPK decreased in group D (P<0.05). JIA WEI WU QI SAN has multiple functions, including the activation of the p-P38MAPK signaling pathway, which promote the activation of COX-2, induce the arachidonic acid to increase the level of PG, and decrease the concentration of IL-1, thereby inducing an inflammatory reaction, and promote gastric mucosa repair.

Keywords: Flavored Wu Qi powder, nonsteroidal anti-inflammatory drugs (NSAIDS), P-38 mapk signaling pathway.

INTRODUCTION

Non-steroid anti-inflammatory drug (NSAID)-related gastropathy mechanism is almost comprised of the cyclooxygenase (COX) inhibition theory; that is, the arachidonic acidin the cell membrane is released under the interaction of phospholipase A2 and gradually generates prostaglandins (PGs) or leukotriene. NSAIDs inhibit COX and thereby inhibits arachidonic acid-generating PGs. Furthermore, PGs inhibit gastric acid secretion and protects the gastric mocusa from strong acid and alcohol damage (Pal *et al*., 2015; Takeeda *et al*., 2003; Takeeda *et al*., 2004). Damage induced by NSAIDs to the gastrointestinal tract have been primarily marked by the mucosa in the stomach, as well as duodenum congestion, edema, erosion, or even bleeding and perforation (Boukthir *et al*., 2010; Laine, 2002). Similarly, NSAIDs have an analgesic effect, which naturally disguises the symptom of the gastrointestinal tract, thereby virtually increasing the incidence of NSAIDs correlated to gastropathy. Previous studies revealed that NSAIDs suppress COX-2, which in turn, decreases PG synthesis and increases gastrointestinal damage (Brzozowski *et al*., 2001). However, COX-2 is an important rate-limiting enzyme, which could be necessarily used in the process of conversion from arachidonic acid to PGs.P38 mitogen-activated protein kinase (p-P38MAPK) is a subclass of mitogen-activated protein kinases (MAPKs) and a group of intracellular signal transduction molecules that can be activated through multiple stimulatory factors such as TNF-α, IL-1 and stress stimulation (heat shock, hypertonic, etc.) (Abdollahi *et al*., 2005; Gong *et al*., 2008; Kim *et al*., 2005; Zhong *et al*., 2012). The aim of this study was to investigate the effects of the p-P38MAPK signal pathway and COX-2, PGE-2 and IL-1 for NSAID-related gastric ulcer and their interaction.

MATERIALS AND METHODS

Animals

A total of 46 Sprague Dawley (SD) clean-grade rats, weighing 200-240g, were selected for the present study. These rats were evenly composed of males and females (supported by the Laboratory Animal Center of Nanjing University of Traditional Chinese Medicine), fasting 48h, drinking freely. Rats were fed on a balanced diet in a quiet place, room temperature 22-26°C.

Preparations

Rats were given indomethacin (25mg/tablet) at a dose of 2.5mg/kg. JIA WEI WU QI SAN: Comprised of WU WEI ZI powder, BAI JI powder, JIU XIANG CHONG powder, SAN QI powder and Calcined sepia bone powder (0.04g/kg). Furthermore, a 0.08/ml solution was prepared by normal saline and preserved in a refrigerator at 4°Cfor use.

Rabeprazole capsules (10mg/tablet) at a dose of 2mg/kg were prepared with 0.28mg/ml of normal saline and preserved at 4°C in a refrigerator for use.

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Main reagents

PGE2 ELISA kit, Abcam (ab133021); IL-1 ELISA kit, Nanjing Xunbei Biotechnology Co. td. (ER008-48); P-P38MAPK (Thr180/Tyr182), CST(4511p); COX-2 antibody, PTG(12375-1-AP); 5×SDS-PAGE loading buffer, KGP101; western blot transfer buffer, PTG (B400020); SDS-PAGE gel quick preparation kit, KGP113K; SDS-PAGE electrophoretic liquid (1×), PTG (B300020); RIPA lysis buffer, PTG(B100020); PVDF Film(0.22um), Millipore (IPVH00010); β-actinantibody, PTG (60008-1-Ig); PBS powder, BOSTER (AR0030).

Detection ofPGE-2 and IL-1 concentrations by ELISA

The kit was firstly removed from the fridge and placed at room temperature. Then, the concentrated detergent was diluted (1:20) by DDW. Add 1mL sample buffer to a reference standard and stand for 15 minutes. After dissolving, mix the sample buffer and reference standard altogether (at a concentration of 4000pg/ml) and dilute them according to the required dose. The standard curve concentrations were as follows: 4,000, 2,000, 1,000, 1,000, 500, 250, 125, 62.5, 31.625 and 0pg/mL. The biotinylated antibody was diluted in accordance with the 1:30 dilution, in order to dilute the concentrated biotinylated antibody into the working concentration. Following the fixed dosage, the concentrated enzyme complex was diluted by the enzyme complex at 1:30, and the plate was repeatedly washed and incubated. Then, add terminal solution 100ul/hole, measure the OD450 values after m.b. The sample concentration was detected through the OD value of the specimen.

Detection of p-P38MAPK and COX-2 protein expression by western blot

(1)One-hundred ?g of total protein per sample was taken, placed in lysate and loading buffer and centrifuge for five minutes;(2) Add the electrophoresis and load the processed sample into the gel sample holes; (3) Conventional electrophoresis was performed; (4) The PVDF film was pretreated;(5)Wet blotted the film;(6)Seal off 5% dried skimmed milk for 2h at 37°C;(7)The antibody was added at 4°Cand preserved overnight; (8) The film was repeatedly washed; (9) Develop and photographic fix the sample.

STATISTICAL ANALYSIS

The outcomes were expressed asmean ± standard deviation (X±SD) and analyzed by SPSS 17.0. Multivariate differences were analyzed by means of variance analysis, in which the differences between each two groups were compared by *q*-test.

RESULTS

Comparison of serum PGE2 and IL-1 levels among the rat groups

Compared with the control group, the level of PEG2 in the model group decreased from 131.65±6.93pg/ml to 97.75±7.68pg/ml (P<0.05), while the level of IL-1 increased from 120.47±8.06pg/ml to 333.38±21.91pg/ml (P<0.01). The level of PEG2 in the rabeprazole group increased to 152.27±6.36pg/ml, which was significantly different from that in the model group (P<0.01); and the level of IL-1 decreased to 222.58±19.22pg/ml (P<0.01) in the model group. The level of PEG2 in the JIA WEI WU QI SAN treatment group increased to 166.03±8.84pg/ml. However, no significant variation was found compared to the model group (P>0.05). Furthermore, IL-1 level decreased to 169.73±10.03pg/ml without significant difference, compared to the model group (P>0.05). These results show that in the process of gastric ulcer therapy, rabeprazole could significantly increase PEG2 levels and reduce IL-1 levels (table 1).

Comparison of the protein expression levels of COX-2 and p-P38MAPK in the gastric mucosa among the groups of rats

Compared with the control group, the level of COX-2 in the model group decreased from 99.4±1.19pg/ml to44.26±3.47pg/ml (P<0.05), while the level of p-P38MAPK increased from 98.59±2.20pg/ml to 180.77±7.89pg/ml (P<0.05). Furthermore, the level of COX-2 in the rabeprazole group was 44.97±7.84pg/ml, and the difference was not statistically significant when compared to the model group (P>0.05). Moreover, the level of p-P38MAPK decreased to 151.77±4.40pg/ml, and the difference was statistically significant when compared with the model group (P<0.05). The level of COX-2 in the JIA WEI WU QI SAN treatment group increased to

Table 1: Comparison of serum PGE2 and IL-1 levels among the rat groups

Groups	PGE2	IL-1
Control group	131.65±6.93[a]	120.47±8.06[#]
Model group	97.75±7.68[b]	333.38±21.91*
Rabeprazole group	152.27±6.36[c]	222.58±19.22[##]
JIA WEI WU QI SAN treatment group	166.03±8.84[d]	169.73±10.03[**]

Note: Compared the model group with control group: [a]p<0.05, [#]p<0.05; Compared the rabeprazole group with model group: [b]p > 0.05, [*]p<0.05; compared JIA WEI WU QI SAN treatment group with model group: [d]p<0.05, [**]p<0.01; Compared JIA WEI WU QI SAN treatment group with Rabepeazole group: [c] p > 0.05, [##]p > 0.05.

Table 2: Comparison of the protein expression levels of COX-2 and p-P38MAPK in the gastric mucosa among the groups of rats

Groups	COX-2	P-P38MAPK
Control group	99.4±1.19[a]	98.59±2.20[#]
Model group	44.26±3.47[b]	180.77±7.89*
Rabeprazole group	44.97±7.84[c]	151.77±4.40[##]
JIA WEI WU QI SAN treatment group	79.16±4.19[d]	121.39±4.90[**]

Note: Compared the model group with control group: [a]p<0.05, [#]p<0.05; Compared the rabeprazole group with model group: [b]p > 0.05, [*]p<0.05; compared JIA WEI WU QI SAN treatment group with model group: [d]p<0.05, [**]p<0.01; Compared JIA WEI WU QI SAN treatment group with Rabepeazole group: [c] p > 0.05, [##]p > 0.05.

79.16±4.19pg/ml and the difference was statistically significant when compared with the model group (*P*<0.05).In addition, p-P38MAPK level decreased to121.39±4.90pg/ml, and the difference was statistically significant when compared with the model group (*P*<0.01). These results revealed that in the process of gastric ulcer therapy, JIA WEI WU QI SAN could significantly increase COX-2 level and reduce p-P38MAPK level (table 2).

The protein expression of COX-2

The expression of COX-2 in the gastric mucosa of rats treated with indomethacin was significantly lower than that in the model group (a: *P*<0.05), indicating that indomethacin inhibited the expression of COX-2. After treatment with rabeprazole, COX-2 expression increased, indicating that rabeprazole can restore COX-2 activity (b: *P*>0.05). Furthermore, COX-2 expression was higher (d: *P*<0.05) after treatment with JIA WEI WU QI SAN (fig. 1).

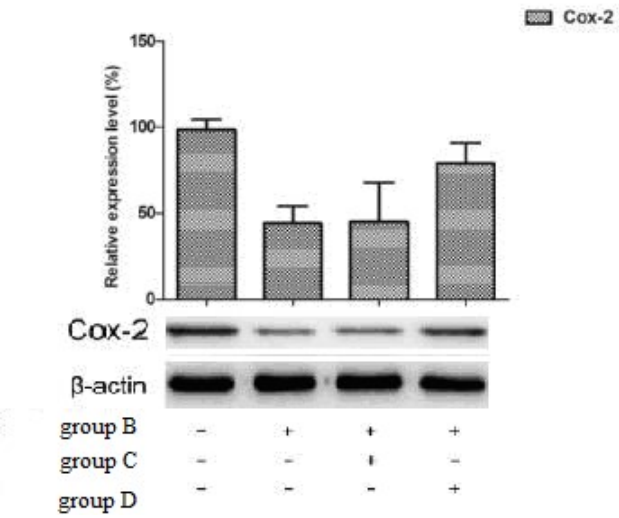

Fig. 1: The protein expression of COX-2

P-P38MAPK protein expression

The expression of p-P38MAPK significantly increased in the gastric mucosa of rats in the indomethacin group (*P*<0.05). However, this expression decreased after treatment with rabeprazole (**P*<0.05).Furthermore, p-P38MAPK expression was lower (*P*<0.01) and the expression of p-P38MAPK was lower than that of the levofloxacin group (*P*<0.05) after treatment with JIA WEI WU QI SAN (fig. 2).

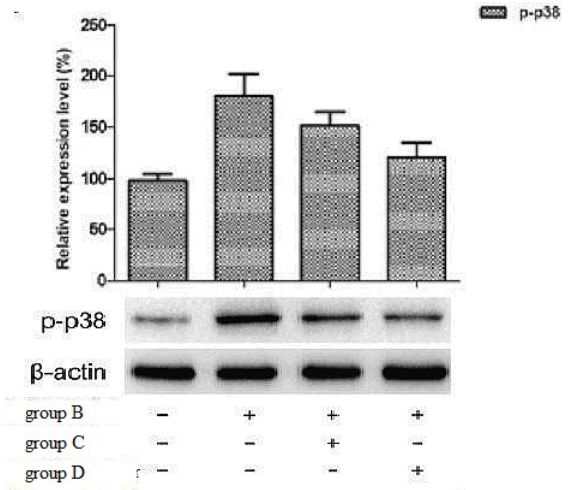

Fig. 2: P-P38MAPK protein expression

DISCUSSION

JIA WEI Wu QI SAN is one of the clinical experiences of Professor Zheng, which mainly comprises of WU WEI ZI powder, SANQI powder, BAI JI powder, JIU XIANG CHONG powder, and calcined squid bone powder; and has been mainly used for the treatment of NSAID-related stomach conditions. The prescription was 100 mesh superfine powder, which should be taken accompany with lotus root powder. Strong adhesion can be attached to the ulcer surface, repair damaged wounds, better promote the healing of ulcers, and reduce the recurrence rate.

Indomethacin is a non-steroidal anti-inflammatory drug that belongs to COX inhibitors (Tonby *et al*., 2016). After modeling, rats exhibited varying degrees of loss of appetite, yellow hair color, weight loss and so on. This study revealed that COX-2 expression was down regulated in the model group, which is consistent with the theory of indomethacin that belongs to the COX inhibitor. After treatment, the expression of both the traditional Chinese medicine group and Western medicine group were upregulated. Rats were also faster than others and

their appetite was good and active. PG is the product of COX-2, which can induce smooth muscle contraction and inhibit gastric acid secretion. PGE2 decreased in the model group and had different degrees of increase in the traditional Chinese medicine group and Western medicine group, indicating that the JIA WEI WU QI SAN may be promoting the increase inCOX-2, inducing the production of PGE2 to increase, and achieving the purpose of protecting the gastric mucosa.

P38MAPK can be activated by stress stimuli, inflammatory factors (TNF-α, IL-1, etc.) and cell wall components of LPS and Gram-positive bacteria, which play an important role in inflammatory response and stress response (Qiu *et al*., 2016; Yousif *et al*., 2018). The study conducted by Fan *et al*. (2001) revealed that COX-2 inhibitor (PD98059, SB203580) could not only inhibit the protein expression of COX-2, but also significantly upregulate the protein expression of p-P38MAPK in cells. Furthermore, TNF-α can induce the expression of p-P38 MAPK in human pulmonary vascular endothelial cells (HPVEC) (Hashimoto *et al*., 1999). Pathogenic factors, such as endotoxin and cytokines, acted on vascular endothelial cells and induce to become dysfunction, shedding and necrosis, or even apoptosis by p38MAPK regulation (Norata and Catapano). Oxidative stress can also lead to the activation of p38MAPK, which result in increased intracellular calcium, and thereby leads to the increased permeability of endothelial cells and increased inflammatory response. Thus it could reduce endothelial cell damage or reduce apoptosis by blocking the expression of p-P38MAPK or inhibiting at the level of the signaling pathway (Cai *et al*., 2016; Wang *et al*., 2017). At the present study, the protein expression of p-P38MAPK in the model group was higher, and the expression of COX-2 decreased in the model group. In this study, the expression of p-P38MAPK protein in the model group was higher than that in the blank group and the expression of COX-2 was decreased in the model group. The expression of P-P38MAPK was decreased in different flavors and rabbits Increased, while the inflammatory factor IL-1 in the model group were increased, the treatment group were reduced.

In summary, JIA WEI WU QISAN can regulate the P38MAPK signaling pathway, reduce inflammatory factor IL-1 content and promote downstream product COX-2 activation, which induces arachidonic acid-generated PG to increase and in turn, reduces inflammatory response, in order to protect the gastric mucosa. This indicates that JIA WEI WU QISAN can promote gastric mucosa repair and enhance the gastric mucosa resistance to the inflammatory factors, which effect equaled to the rabeprazole therapy for NSAIDs related gastric ulcer. This finding suggests that the occurrence of NSAID-associated gastric ulcer is closely correlated to the role of COX and is achieved by influencing the P38MAPK signaling pathway. The conclusion of the present study provides an in-depth theoretical basis for the treatment of NSAID-related gastric ulcer, and much more positively instructive effect on clinical practice.

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