Effects of Coptidis Rhizoma Herbal Acupuncture Extract on the Acute Gastric Mucosal Lesion Progression Induced by Compound 48/80 in Rats

Jong-Cheng Mou#, Sena Lee, Myung-Gyou Kim, Il-Bok Seo, Kang-Hyun Leem*

College of Oriental Medicine, Semyung University, Jechon 390–711, Korea

ABSTRACT

Objectives: Coptidis Rhizoma has been used for stomach disease. However, its property is so cold that it might be avoided to prescribe for the elderly and the infirm having indigestion or diarrhea. Accordingly, the present study was designed to investigate the protective effects of Coptidis Rhizoma herbal acupuncture extract against acute gastric mucosal lesions induced by compound 48/80 in rats.

Methods: The Coptidis Rhizoma herbal acupuncture (CRHA) was injected in Choksamni and Chungwan 1 h before compound 48/80 treatment. The animals were sacrificed under anesthesia 3 h after compound 48/80 treatment. The stomachs were removed and the amount of gastric adherent mucus, gastric mucosal hexosamine, SOD, XO, TBARS and histological examination were performed.

Results: The decline of gastric adherent mucus, gastric mucosal hexosamine and the histological defects of gastric mucus were significantly protected by CRHA treatment. Gastric adherent mucus in control group was reduced to 38.2 ± 5.0%, CRHA groups significantly protected the loss of mucus to 77.5 ± 4.9%. Mucosal hexosamine content showed similar patterns. Mucosal hexosamine content in control group was reduced to 45.2 ± 6.2%. CRHA groups significantly protected the loss of mucus to 83.0 ± 7.0%. The changes of gastric mucosal SOD and TBARS were recovered by CRHA treatment as well.

Conclusions: CRHA showed the protective effects on the acute gastric mucosal lesions induced by compound 48/80 in rats. These results suggest that CRHA may have protective effects on the gastritis.

Key words: Coptidis Rhizoma herbal acupuncture, Choksamni (ST36), Chungwan (CV12), Acute gastric mucosal lesion, Compound 48/80

Introduction

Coptidis Rhizoma is a root of *Coptidis japonica* Makino, *C. chinensis* Franch, *C. deltoidea* C.Y. Cheng et Hsiao, and *C. teeta* Wall1). It has been used to clear heat, drain dampness, and stop bleeding caused by hot blood1,2). It has been used for the treatment of damp-heat in the stomach. However, its property is so cold that it might be avoided to prescribe for the elderly and the infirm having indigestion or diarrhea. Accordingly, the herbal acupuncture therapy could be considered to bypass the gastrointestinal tract.

Recently developed drugs for gastritis are mainly histamine receptor (H2) blockers. Histamine plays an important role in the production of stomach acid. Histamine is made by special cells called enterochromaffin-like cells in the stomach in response to signals from the nervous system. When histamine binds to H2 receptors in the stomach, it stimulates acid secretion by cells called parietal cells3).

There are many gastritis animal models. The H2 receptor blocking effects could be evaluated by using acute gastritis model induced by compound 48/80. Compound 48/80 is known to cause degranulation of...
connective tissue mast cells, but not mucosal mast cells, with release of serotonin and histamine from the cells\textsuperscript{4,5}. However, many studies revealed that a treatment of compound 48/80 in rats could produce the development of gastric mucosal lesions\textsuperscript{6-10}. Accordingly, the protective effects of Coptidis Rhizoma herbal acupuncture against acute gastric mucosal lesion progression induced by compound 48/80 were evaluated. Famotidine (H2 blocker) was used as a positive control.

It is well known that gastric mucin interacts with ROS, especially hydroxyl radical in vitro\textsuperscript{19}. The gastric mucosal superoxide dismutase (SOD, an enzyme to scavenge O2 to H2O2 and O2\textsuperscript{•−}), xanthine oxidase (XO), and thiobarbituric acid reactive substances (TBARS, an index of lipid peroxidation) were measured as well.

**Materials and Methods**

1. **Sample preparation**

Coptidis Rhizoma was purchased from Omniherb (Daegu, Korea). Coptidis Rhizoma herbal acupuncture extract (CRHA) was prepared as follow, 100 g of CRHA in 2,000 ml distilled water was heated in a heating extractor for 3 hours. The extract was filtered and concentrated by using the rotary evaporator. The extract was lyophilized by using freeze dryer (15,1 g). The lyophilized extract was dissolved in normal saline solution (20 mg/ml) and filtered three times through microfilter paper (Whatman no. 2, 0.45-0.2 µm). It was placed in a disinfected vial and sealed for further study.

2. **Reagents**

Sodium chloride was purchased from Duksan (Korea). Saccharose and perchloric acid were purchased from Dae Jung (Korea). Alcian blue was purchased from BHD laboratory supplies (USA). Sodium phosphate monobasic and sodium phosphate dibasic were purchased from Jin chemical (Japan). All other reagents were purchased from Sigma-Aldrich (USA).

3. **Animals**

Male Wistar rats, aged six weeks (225 - 235 g), were purchased from Samtaco Co. (Korea). The animals were housed in a ventilated animal room with controlled temperature \((23 ± 2^\circ C)\) and relative humidity \((55 ± 5\%)\) with 12 h of light (7:00 to 19:00). The animals were maintained with free access to rat chow (Samtaco Co., Korea) and tap water ad libitum for one week. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee.

4. **Induction of gastric mucosal lesion**

Compound 48/80 (0.75 mg/kg body weight), dissolved in normal saline, was intraperitoneally injected to 7-week-old rats, which had been fasted for 24 h. The normal rats received an intraperitoneal (i.p.) injection of an equal volume of normal saline. All animals were maintained with free access to water and food during the experiment. They were fasted overnight one day before experiment. The CRHA and normal saline was injected in Choksamni (ST36) in left and right legs and Chungwan (CV12) on abdomen 1 h before compound 48/80 treatment in CRHA and control group respectively\textsuperscript{12,13}.

Famotidine is a recently developed drug for gastric ulcer. Accordingly, famotidine was used as a positive control in this experiment. The positive control group was administrated orally with famotidine (4 mg/kg). The number of animals in each group was eight. The animals were sacrificed under ether anesthesia 3 h after compound 48/80 injection. The stomachs were removed, inflated with 10 ml of 0.9% NaCl, and put into 10% formalin for 10 min. The stomachs were then opened along the greater curvature.

5. **Determinations of gastric mucosal SOD, XO, TBARS, hexosamine and adherent mucus**

For the assay of these enzymes, gastric mucosal tissues were homogenized in 9 vol of ice-cold 0.05 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 4\textdegree C (10,000 x g, 20 min); and the resultant supernatant was dialyzed against 100 vol of the same buffer at 4\textdegree C for 24 h. Gastric mucosal SOD was assayed by the method of manufacturer’ s protocol (SOD assay kit, Dojindo, Japan). XO activity was assessed by measuring the increase in absorbance at 292 nm following the formation of uric acid at 3 0\textdegree C. One unit (U) of this enzyme is defined as the amount of enzyme forming 1 µmol uric acid per min as the method of manufacturer’ s protocol (Cayman chemical, USA). Gastric mucosal TBARS was spectrophotometrically determined by the manufacturer’ s protocol (TBARS assay kit, Zeptometrix, USA). Hexosamine obtained from the hydrolyzed mucin was assayed using acetylacetone and Ehrlich’ s reagent. Gastric adherent mucus was assayed by the method of Kitagawa et al\textsuperscript{14}, as follows: the removed stomach was cut open along the
greater curvature and rinsed with 10 mL of ice-cold 0.25 M sucrose. Then, 50 mm² (approx. 8 mm in diameter) of the glandular portion of the stomach was excised with a scalpel and the excised part was weighed. The excised stomach was soaked in 2 mL of 0.1% alcian blue, which was dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate (pH 5.8), for 2 h. Uncomplexed dye was removed by two successive washes in 2 mL of 0.25 M sucrose for 15 and 45 min, and then the dye complex with mucus was extracted with 30% dioctyl sodium sulfosuccinate (DSS) for 2 h. After centrifugation (3,000 rpm for 10 min), the optical density of the solution of alcian blue extracted with DSS was read at 620 nm and the concentration of the extracted alcian blue was calculated in comparison with a calibration curve obtained with alcian blue solutions of known concentrations. The concentration of gastric mucosal adherent mucus is expressed as that of alcian blue adhered to the gastric mucosal surface (µg/g tissue).

6. Histological examination

Stomach samples were excised and transferred to 10% fresh formalin and later processed by routine techniques before embedding in paraffin. Sections (5 µm thick) were mounted on glass slides and stained with alcian blue. Coded slides were examined by an experienced pathologist blinded to the treatment using a light microscope (BX60, Olympus, Japan).

7. Statistical analysis

The results were expressed as means ± standard error of the mean (SEM). Significances of changes were evaluated using the one-way ANOVA and Dunnett’s post hoc test (SPSS ver. 10.0). Values of p < 0.05 were considered significant.

Results

1. Effect of CRHA on gastric adherent mucus and gastric mucosal hexosamine contents

In order to evaluate the effects of CRHA on gastric adherent mucus, CRHA was injected in Choksamni (ST36) and Chungwan (CV12). The normal saline was injected in ST36 and CV12 in control group.

Gastric adherent mucus in control group was reduced to 38.2 ± 5.0%. Both positive control and CRHA groups significantly protected the loss of mucus to 68.5 ± 12.3% and 77.5 ± 4.9%, respectively (Fig. 1). Mucosal hexosamine content showed similar patterns. Mucosal hexosamine content in control group was reduced to 45.2 ± 6.2%. Both positive control and CRHA groups significantly protected the loss of mucus to 73.6 ± 11.9% and 83.0 ± 7.0%, respectively (Fig. 2).

2. Effect of CRHA on gastric mucosal lesion

As shown in Figure 3, gastric mucosal lesions appeared 3 h after treatment with compound 48/80 (Fig. 3B). The surface mucous cell layer was stained with alcian blue to dark blue (upper layer in figure). The mucous cell layer was damaged after compound 48/80 treatment. Both famotidine and CRHA treatments protected the damage of mucous cell layer (Fig. 3C and D),
3. Effect of CRHA on gastric mucosal SOD activity

To evaluate the amount of antioxidative activity, superoxide dismutase (SOD), xanthine oxidase (XO) activity, and thiobarbituric acid reactive substances (TBARS) was quantitatively measured.

SOD in control group showed 38.6 ± 2.3%. Both positive control and CRHA groups significantly increased the SOD activities to 76.7 ± 13.1%, and 102.8 ± 28.1% compared with normal group, respectively (Fig. 4).

4. Effect of CRHA on gastric mucosal XO activity

XO activity in control group was increased to 340.3 ± 111.7 µU/protein from 148.9 ± 24.9 µU/protein (normal group). The XO activities of positive control group and CRHA group reduced to 251.6 ± 18.7 µU/protein and 314.2 ± 42.5 µU/protein compared with control group, respectively (Fig. 5). However, there was no statistical significance.

5. Effect of CRHA on gastric mucosal TBARS content

TBARS in control group was significantly increased to 4.0 ± 0.8 µM/mg protein compared to normal group (1.1 ± 0.3 µM/mg protein). Both positive control and CRHA groups significantly reduced the elevation of TBARS content to 1.5 ± 0.3 µM/mg protein and 1.4 ± 0.6 µM/mg protein, respectively (Fig. 6).

Discussion

Coptidis Rhizoma is a root of *Coptidis japonica* Makino, *C. chinensis* Franch, *C. deltoidea* C.Y. Cheng et Hsiao, and *C. teeta* Wall. It has been used to clear heat and drain dampness. The heat and dampness in stomach or intestines cause diarrhea or
dysenteric disorder. Those symptoms are vomiting and/or acid regurgitation. It has also been used for high fever, irritability, disorientation, delirium, red tongue, and a rapid and full pulse.1,2,3

In recent years, several studies have attempted to find and explore the scientific evidence of the effects of Coptidis Rhizoma, Wang showed that ethanol extract from a Coptidis Rhizomaincluding herbal formula, “Zuojin Pill”, inhibited the expression of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 mouse macrophages. Other studies showed the effects on gastritis. However, there is no study on the herbal acupuncture treatment of Coptidis Rhizoma for the treatment of gastritis.

Histamine is a chemical which is made from an amino acid (histidine). It is a chemical which is made by the body and has a number of roles in the human body. Medications that interfere with histamine can be used to treat allergies as well as acid reflux. This molecule is able to affect the immune, digestive and nervous systems by affecting cells in many different parts of the body. Once histamine binds to one of its receptors, a chemical signal is generated within the cell that has the histamine receptor. Consequently, the effects of histamine depend on the type of receptor involved. When histamine binds to the H1 receptor, it causes activation of a protein called phospholipase C. That protein then makes a chemical called cAMP, which catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. This enzyme plays an important role in the catalysis of purines in some species, including humans. Compound 48/80 treatment increased the XO activity and famotidine and CRHA treatment increased the reduction of SOD activities. XO is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. This enzyme plays an important role in the catalysis of purines in some species, including humans. Compound 48/80 treatment increased the XO activity and famotidine and CRHA treatment showed the reducing tendency the elevated XO activity. Concentrations of TBARS are an index of lipid peroxidation and oxidative stress. In this study, TBARS was increased in compound 48/80 treated control group and both famotidine and CRHA treatment reduced the elevation of TBARS content.

In conclusion, CRHA showed the protective effects on acute gastritis induced by compound 48/80. The effects could be partially explained via antioxidative effects of CRHA. Present results suggest that CRHA may have potential activities as an anti-gastritis treatment. Further studies about the mechanisms or more effective acupoints should be needed.
References

23. Nowak JZ, Sek B, D'Souza T, Dryer SE. Does histamine stimulate cyclic AMP formation in the avian pineal gland via a novel (non-H1, non-H2, non-H3) histamine receptor subtype, Neurochem