Journal of Inflammation Research

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Study on the Effect of Antiplatelet and Gastric Mucosal Protection of Traditional Chinese Medicine Invigorating Qi and Hemostasis

Chen-Hao Zhang[1,*]
Xiao-Lan Su[2,*]
Yi-Ying Liu[1]
Long Lin[1]
Yin-Chun Wang[1]
Xiao-Dong Li[1]
Jin-Ze Zhang[1]
Wei Wei[2]

[1]Department of Emergency, Wangjing Hospital of China Academy of Chinese Medical Sciences, Beijing, 100102, People's Republic of China; [2]Department of Gastroenterology, Wangjing Hospital of China Academy of Chinese Medical Sciences, Beijing, 100102, People's Republic of China

*These authors contributed equally to this work

Background: To investigate the effects of Chinese herbal medicine in tonifying qi and attaining hemostasis caused by the metabolism of the drug clopidogrel and as a result of platelet and gastric mucosa injury in an ischemia-reperfusion rat model.

Methods: A pharmacokinetic model was established to record the drug metabolism parameters of clopidogrel metabolites. Then, absorption of the drug was compared with approaches using the traditional Chinese medicine (TCM) approach of tonifying qi and establishing hemostasis, to using the drug pantoprazole and applying these approaches in combination with clopidogrel. Intragastric administration was performed, and all indicators were tested.

Results: The area under the curve (AUC; 0–T, 300.342 ± 35.832 mg/L* h; AUC 0–∞, 320.462 ± 40.213 mg/L* h), the plasma peak concentration (30.622 ± 9.917 mg/L*), and the peak time and half-life (7.954 ± 1.121 h) in the clopidogrel and the TCM groups were higher than those in the clopidogrel and pantoprazole groups. In terms of antiplatelet aggregation, compared with model group, the platelet aggregation rate induced by arachidonic acid (AA) and adenosine diphosphate (ADP) was significantly decreased by the TCM approach of tonifying qi and stopping bleeding ($p < 0.05$). The ADP, thromboxane A2, GPII B/Pa-A, CD62P and platelet factor 4 content in the TCM yiqi decoction and hemostasis approach were significantly decreased ($p < 0.01$). Compared with the clopidogrel group, the gastrin and motilin in the serum, the cyclooxygenase (COX)-1 and prostaglandin E2 in gastric tissue, and expression of vascular endothelial growth factor messenger ribonucleic acid in the serum were all significantly increased using TCM approach to protect against gastric mucosal injury ($p < 0.05$).

Conclusion: TCM invigorating qi and hemostasis has an inhibitory effect on platelet activation. It can reduce the local inflammatory reaction at the same time as protecting gastric mucosa.

Keywords: supplementing qi and hemostasis herbs, proton pump inhibition, antiplatelet effect, gastric mucosal protection, traditional Chinese medicine

Correspondence: Chen-Hao Zhang
Department of Emergency, Wangjing Hospital of China Academy of Chinese Medical Sciences, Beijing, 100102, People's Republic of China
Tel +86 136 7139 3316
Email zhangch_dr@163.com

Wei Wei
Department of Gastroenterology, Wangjing Hospital of China Academy of Chinese Medical Sciences, No. 6 of Wangjing Zhonghuan South Street, Chaoyang District, Beijing, 100102, People's Republic of China
Tel +86 13601233816
Email weiweigrt@163.com

Introduction

Aspirin combined with clopidogrel for at least 12 months as an intensive dual antiplatelet therapy (DAPT) is the standard protocol for antiplatelet treatment following percutaneous coronary intervention (PCI).[1] The DAPT approach carries the potential risk of gastrointestinal bleeding because of its antiplatelet action. Research indicated the incidence of gastrointestinal bleeding following PCI as being 2.3%, while the incidence of bleeding in high-risk groups was as high as

Received: 21 April 2021
Accepted: 16 June 2021
Published: 7 July 2021

12%.[2] Hemorrhaging is related to the use of DAPT, and aspirin can directly damage the epithelial cells of gastrointestinal mucosa.[3] Furthermore, clopidogrel may delay the healing of gastric mucosal injuries.[4]

Following a hemorrhage, the withdrawal of antiplatelet medications may increase the risk of stent clots. Digestive-tract bleeding after PCI is a common postoperative adverse event that affects the ultimate impact of PCI and the long-term prognosis of patients.[5] To reduce the incidence of gastric mucosal injury and gastrointestinal bleeding during DAPT, the use of a proton pump inhibitor (PPI) is extremely common,[6] particularly for people at high risk of bleeding. Studies have shown, however, that the application of PPI significantly increases the incidence of cardiovascular events in patients following PCI.[6] The mechanism of PPI primarily involves the competitive inhibition of PPI on the main metabolic link of clopidogrel, the cytochrome P450 (CYP450) enzyme system for hepatic drug metabolism, which seriously affects the generation of effective clopidogrel metabolites and weakens the drug's antiplatelet effect, while also increasing the incidence of thrombotic events.[7] In this regard, the safety of using a PPI to prevent gastrointestinal bleeding after PCI has been questioned and challenged.

In recent years, the multiple effects of medicinal plants, eg, their antiplatelet and gastric mucosa protective characteristics, have attracted increased attention.[8,9] Gastrointestinal bleeding falls into the blood syndrome category in traditional Chinese medicine (TCM). According to TCM theory, "qi" is an extremely subtle substance in the human body and one of the prime movers of human life (New 1). Based on a TCM approach, qi has the function of directing blood; therefore, a qi deficiency will impair the controlling function of blood, which manifests as blood leakage outside of the blood vessels, ie, "bleeding". Qi deficiency syndrome and blood stasis are primary TCM disorders of gastrointestinal bleeding following PCI (New 2). The TCM prescription of tonifying qi and achieving hemostasis has been established for this pathogenesis and primarily comprises *Astragalus membranaceus, Radix Pseudostellaria heterophylla* root, rhubarb charcoal, Baihe powder, cuttlefish bone, and *Panax notoginseng*. A randomized controlled study[10] found that the Chinese herbal ingredients of *yiqi* and hemostasis had some preventive effect on gastrointestinal bleeding after PCI, while the results of a thromboelastic diagram suggested that intervention using Chinese herbal medicine in this context as a result of clopidogrel's antiplatelet effect was less likely compared with using pantoprazole. Pharmacological studies[8,9] suggest that the main components of Chinese herbal medicine for replenishing qi and achieving hemostasis can interfere with gastric mucosal injury, and concurrently, effect a degree of influence on platelet activation.

The main components of *Astragalus membranaceus* are water-soluble extracts, such as Astragalus polysaccharide, Astragalus IV and IV, and flavonoids. These components play important roles in the protection of gastrointestinal mucosa, in the regulation of immunity, and also have anti-inflammatory properties.[3,4] The active ingredients of *Pseudostellaria* root include squalene, mecanin, and *Robinia pseudoacacia*, which can fight myocardial ischemia, reduce inflammation, promote angiogenesis following myocardial infarction, reduce the risk of intestinal disease, restore the villus structure of the small intestine, and enhance the body's antioxidant levels.[5,6] As the main active ingredient of *Panax notoginseng*, the herb's total saponin levels have specific antiplatelet adhesion and aggregation effects, which can enhance the antiplatelet effect of aspirin and reduce the mucosal injury caused by the drug.[7,8]

Given the incongruity that arises between the antithrombotic effects and the bleeding caused by the combination of a PPI and clopidogrel, we believe that the TCM approach of enriching qi and stopping bleeding may play a dual role, ie, by providing both antithrombotic and gastric mucosa protection, thereby increasing the clinical benefits for patients experiencing bleeding following PCI. However, the exact mechanism of the antiplatelet and gastric mucosal protective effects of yiqi decoction as part of TCM has not been clarified, which hinders the further clinical application of this prescription. Therefore, the purpose of this study was to explore the effect of hemostasis achieved using *yiqi* decoction on the metabolism of clopidogrel, and this TCM treatment's possible mechanism for inhibiting platelet aggregation and protecting gastric mucosa to provide a basis for clinical application.

Materials and Methods

Experimental Materials

Clopidogrel hydrogen sulfate tablets (75mg; Sanofi), and pantoprazole sodium enteric capsules (40 mg, Hangzhou Zhongmei Huadong Pharmaceutical Co., Ltd) were used in this study. Chinese medicine decoction ingredients

including *Astragalus membranaceus, Panax notoginseng*, cuttlefish bone, *Bletilla striata* root, and rhubarb charcoal were purchased from the Herbal Medicine Room of Wangjing Hospital, at the China Academy of Chinese Medicine. Platelet factor 4 (PF-4) (Hy-2564), adenosine diphosphate (ADP) (Hy-D0046), thromboxane A2 (TXA2; Hy-D0046), a TXA2 immunoassay kit (Hy-D0046), a TXA2 immunoassay kit (Hy-D0046), a TXA2 immunoassay kit (Hy-D0046), a TXA2 (HY-10257) kit, GPII B/Ab (HY-2502), P-selectin (CD62P) (BS-0561R), tumor necrosis factor-alpha (TNF-α) (HY-10116), interleukin (IL)-6 (HY-10105), IL-8 (HY-10106), IL-10 (HY-10107), motilin (MTL) (HY-098), gastrin (GAS) (HY-10167), and prostaglandin E2 (PGE2) (HY-10047) immunoassay kits were provided by the Beijing Huaying Institute of Biotechnology. Cyclooxygenase (COX)-1 (polyclonal antibody, rabbit anti-mouse) (SC-19998), and COX-2 (polyclonal antibody, rabbit anti-mouse; SC-376861) antibodies were purchased from Santa Cruz. Vascular endothelial growth factor antibody (VEGF; mouse vs mouse, monoclonal antibody; AB1316) was provided by ABeam, Inc.

The content related to the experimental research program of this project was discussed in earnest by the Laboratory Animal Use and Management Committee of the Wangjing Hospital of the China Academy of Chinese Medical Sciences. It was considered to meet the necessary ethical requirements and was approved by vote (No. IACUC-AMSS-20190215-02).

Detection of the Clopidogrel Plasma Concentration

Wistar male rats (SPF grade, 200 ± 20 g) were fed adaptively for one week, fasted for 12 h before administration, and blank whole blood samples were collected at the start of the experiment. Following administration, 0.5 mL of blood was extracted from the caudal vein of rats and placed in heparinized Eppendorf (EP) tubes at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 36 h. The blood plasma was separated after centrifugation for 5 min at 3000 r/min, and then frozen at –80°C to await determination. A plasma sample of 200 μL was placed in a 1.5 mL EP tube to be precisely absorbed and centrifuged at 13,000 r/min for 5 min. The supernatant (100 μL) was then removed, and 400 mg/L of moxifloxacin (10 μL) was added as the internal standard working solution (SR26334). Next, 70% perchloric acid (10 μL) was added, swirled, and mixed for 30 s. Centrifugation was continued at 13,000 r/min for 5 min, and then 50 μL of the supernatant was placed in an automatic sample injector bottle, and 5 μL was set for injection detection.

The chromatographic and liquid phase conditions were as follows. An Agilent 1290 Infinity System chromatograph (Agilent Technology) was used with a Zorbax SB-C18 (2.1 mm × 50 mm, 3.5 μm) chromatographic column, a mobile acetonitrile-to-water phase-ratio, and 0.1% trifluoroacetic acid (TFA; = 24:56:20 (V/V/V). Test wavelengths of 230nm (0–4.5 min), and 280nm (4.5–6 min) were applied. The flow rate was 1.0 mL/min, and the column temperature was 40°C.

A 100 μL blank plasma sample was taken, and 20 μL of the SR26334 standard working solution at different concentrations was added successively to prepare plasma samples measuring 0.5, 1, 2.5, 5, 10, 25, and 50 mg/L. The plasma samples were then processed as described above.

Establishment of an Ischemia-Reperfusion Model

Wistar male rats (SPF, 200 ± 20 g) were fed adaptively for one week. To narcotize the rats, 60 mg/kg of intraperitoneal pentobarbital sodium was administered; the degree of anesthesia used was determined by the plantar reflex. The fur of rats was shaved from the neck to the chest; then, medical alcohol was used to wipe and disinfect the skin. The rats were laid supine on a thermostatic electric blanket heated to 37°C, and a sterile surgical towel was placed on the body to reduce contamination. Hemostatic forceps were used to carry out a thoracotomy on the left fourth intercostal area of the rats, and a thoracic retractor was used to expand the ribs and expose the left heart ventricle. The epicardium was opened up to find the left anterior descending (LAD) artery, and the blood vessel was ligated proximal to the LAD using a 6/0 ene suture to avoid the external placement of the heart. If the distal myocardium at the suture site was pale due to ischemia, it was concluded that it had been ligatured successfully. Once the ST segment of the electrocardiogram limb lead had been arched upward for more than 30 min, the establishment of the infarction model was considered successful. The heart was then reset, the ribs were closed with a 2/0 intermittent polypropylene suture, and the skin was closed with a 6/0 continuous polypropylene suture. Normal saline (5 mL) was injected intraperitoneally, and penicillin sodium (60,000 U/d) was injected intramuscularly for three consecutive days to prevent

infection. The rats were divided into model, clopidogrel (CLOP), clopidogrel + pantoprazole (CLOP + Pant), invigorating qi and hemostasis (YQZX), clopidogrel + invigorating Qi and hemostasis (YQZX), and mock-surgery groups; in the latter, the surgical procedures were the same as for the operation group, except without vascular ligation. Each group included six rats.

Dosage Regimen

An equivalent dose was given to the experimental animals by gavage by forcing the ratio of the object surface area via intragastric administration. The CLOP group was given 6.25 mg/kg of clopidogrel by gavage, and the CLOP + Pant group was given 6.25 mg/kg of clopidogrel plus 1.3 mg/kg of pantoprazole. For the YQZX group, the intragastric administration included 6.25 mg/kg of clopidogrel and 8.32 mg/kg of pantoprazole, while for the CLOP + YQZX group, the intragastric administration included 6.25 mg/kg of clopidogrel and 8.32 mg/kg of TCM invigorating qi and established hemostasis. Western medicine was administered at 9:00 AM through intragastric and oral administration. Chinese medicine was administered orally twice a day at 9:00 and 18:00 for 14 consecutive days (see Table 1)

The dosage regimen for clopidogrel–plasma concentration detection was established by dividing 24 healthy male SD rats into four groups with six rats in each group. Each group was given one of the following: 6.25 mg/kg/d of clopidogrel, 6.25 mg/kg of clopidogrel plus 1.3 mg/kg of pantoprazole, 6.25 mg/kg of clopidogrel + 8.32 mg/kg of TCM invigorating qi and established hemostasis.

Detecting the Degree of Platelet Activation

The enzyme-linked immunosorbent assay (ELISA) method was adopted to determine the concentrations of PF-4, ADP, and TXA2. After the rats had been anesthetized, blood (5 mL) was collected from the abdominal aorta and placed in a red biochemical procoagulant tube. After standing for 30 min, the serum was centrifuged at 3000 r/min and 4°C for 5 min. The serum was collected and divided into different parts and then stored in a refrigerator at –20°C for later use. Standard substances and samples (100 μL) were absorbed into the enzyme plate and incubated at 37°C for 30 min; the loading buffer was then absorbed and discarded. After washing, 50 μL of a horseradish peroxidase-conjugated reagent was added to each well, then incubated at 37°C for 30 min. The conjugate reagent was then absorbed and discarded. After washing, 50 μL of chromo-developing solutions A and B were added to each well, and coloration was developed at 37°C for 10 min away from light. The reaction was stopped by adding 50 μL of stop solution per well, at which point the color changed from blue to yellow. The blank hole was set to zero, and the absorbance (optical density, [OD]) value of each hole was measured at a 450 nm wavelength. The concentration of the standard substance was taken as the abscissa and the OD value was adopted as the ordinate for drawing a standard curve, for calculating the linear regression equation for the standard curve, and substituting it into the equation according to the OD value of the sample. The sample concentration was then calculated and multiplied by the dilution factor to obtain the actual concentration of the sample.

The platelet membrane glycoprotein GPIIb/III and the platelet surface CD62P are classic markers of platelet activation; platelet membrane GPIIb/IIIa was detected using the ELISA method, and the CD62P content was detected using PF-4.

Table 1 Grouping and Administration

Groups	N	Dose
Sham group	6	Isopyknic normal saline
Model group	6	Isopyknic normal saline
Clop group	6	Clopidogrel 6.25mg/kg, 1 time /d
Clop+Pant group	6	Clopidogrel 6.25mg/kg + pantoprazole 1.3mg/kg, 1 time /d
YQZX group	6	TCM invigorating Qi and hemostasis 8.32mg/kg, 2 times /d
Clop+YQZX group	6	Clopidogrel 6.25mg/kg+ invigorating Qi and hemostasis 8.32mg/kg, 1 time /d

Platelet Aggregation Rate Measured by Turbidimetry

After the rats had been anesthetized, 5 mL of blood was collected from the abdominal aorta and placed in a 3.8% sodium citrate anticoagulant tube for full mixing. The blood-to-anticoagulant volume ratio was 9:1. After centrifugation at 1000 r/min for 10 min, the upper platelet-rich plasma (PRP) was extracted. The remaining blood was centrifuged at 3000 r/min for 20 min, and the upper platelet-poor plasma (PPP) was extracted. The PRP and PPP were added to the two turbidimetric tubes, and the transmittance of PRP and PPP was adjusted in an LBY-NJ4A platelet aggregator (Beijing Telekangxin Company). The turbidimetry tube was incubated at 37°C for 3 min, and AA and ADP (the final concentration of both was 20

μmol·L⁻) were added to the PRP as inductive agents. The aggregation wave patterns were recorded for no less than 5 min, and the instrument automatically delineated the aggregation curve and calculated the results, ie, the maximum aggregation rate of platelets.

Detecting the Degree of Gastric Mucosal Injury

The GAS, MTL, and PGE2 content of the rat serum were determined by ELISA (refer to 2.5 for the detailed process).

The content of the inflammatory cytokines, TNF-α, and ILs-6, 8, and 10 of the rat serum was also determined using the ELISA method (refer to 2.5 for the detailed process).

Detecting the Messenger Ribonucleic Acid Expression of Cyclooxygenase-1 and 2, Tumor Necrosis Factor-Alpha, and Vascular Endothelial Growth Factor in the Rat Gastric Tissue

Real-time quantitative polymerase chain reaction (PCR; CFX384 Touch Real-time PCR Detection System, Bio-RAD) was used to evaluate the mRNA expression of COX-1 and 2, TNF-α, and VEGF in gastric mucosa. Here, β-actin mRNA was used as the unchanged control to calculate the relative amount of mRNA.

(1) Gastric mucosal specimens were taken and placed in a mortar, and liquid nitrogen was added to grind them; an appropriate amount of Trizol was also added. After shaking, the specimens were left standing for 5 min. Next, 0.2 mL chloroform was added to the specimens, which were then shaken violently for 5 min, and left standing for 5 min. The specimens were then centrifuged at 12,000 r/min and 4°C for 15 min. The aqueous phase was transferred to a new EP tube; 0.5 mL of isopropanol was added to the tube, which was then shaken and mixed, and thereafter left to stand for 10 min. The tube was then centrifuged at 12,000r/min and 4°C for 15 min. The supernatant was discarded, and 1 mL of 75% anhydrous ethanol (stored at 4°C) was added, oscillated, mixed, and centrifuged at 4°C for 5 min at 7500 r/min. The supernatant was again discarded and placed at 37°C for 20 min (the EP tube was placed upside down on the filter paper) to precipitate and dry the RNA. Then, 20 μL of diethyl pyrocarbonate (DEPC) water was added to the EP tube and incubated at 50°C for 3–5 min to fully dissolve the RNA. An RNA template of 0.5–2 μg was taken, 1 μL of Oligo(dT) primer was added, and the DEPC water was replenished to 10 μL. This mixture was blended gently and briefly centrifuged, after which the reaction solution was placed on ice at 65°C for 5 min for 1 min, and then promptly centrifuged.

(2) We continued to add the following:

5 × buffer4 μL

200 ul ribonuclease inhibitor0.5 μL

10 mm dNTP mix1 μL

reverse transcriptase1 μL

RNase-free water3.5 μL

The above mixtures were blended, immediately centrifuged, placed at 37°C for 60 min, heated to 75°C for 15 min, and then the reaction was terminated.

(3) The PCR comprised a 25 μL reaction system operated on ice, and instantaneous centrifugation:

template1 μL

primer 11 μL

primer 21 μL

10*Taq buffer5 μL

dNTPs (10 mM)1 μL

Taq enzyme1 μL

DdH_2Osupplemented to 25 μL

(4) The PCR loop is given as follows:

94°C 5 min 94°C 45 s Tm + 4°C 45 s 72°C 45 s 72°C 10 min 4°C.

(5) The β-actin was taken as an internal reference for reading the data; the 2 delta-delta CT method was used to represent the mRNA expression level.

Table 2 shows the primer sequence.

Data Statistics

The SPSS Statistics 17.0 software program was used to conduct the statistical analysis of the experimental data,

Table 2 Primer Sequence

Name	Sequence (5′ −3′)	Product Length (bp)
β-actin	F: GAAGTGTGACGTTGACATCCG R: GCCTAGAAGCATTTGCGGTG	282
COX-1	F: GAGTGGTTTTGTCTGGCGTCT R: TCAACTGCCTCTGCCTTCC	160
COX-2	F: CACGGACTTGCTCACTTTGTT R: GGAGAAGCGTTTGCGGTA	165
TNF-α	F: CGAGTGACAAGCCCGTAGCC R: GGATGAACACGCCAGTCGCC	255
VEGF	F: ACTGGACCCTGGCTTTACTGC R: TTGGTGAGGTTTGATCCGCATG	310

and the measurement data were expressed as the mean ± the standard deviation. One-way analysis of variance (ANOVA) was used for comparison between multiple experimental groups. Tukey's range test was used for the analysis of those with equal variances, and Games-Howell was used for analysis of those with different variances. The data of the small sample sizes were analyzed using the Kruskal–Wallis test of nonparametric one-way ANOVA; $P < 0.05$ was considered statistically significant.

Experimental Results

Pharmacokinetics Results

Chromatography Conditions

Under the above chromatographic conditions, SR26334 and the moxifloxacin internal standard working solution demonstrated a clean peak, good peak shape, complete separation, and no interference from the impurity peak. The SR26334 retention time was 4.252 min, and the internal standard retention time was 4.987 min.

Standard Curve

The peak area ratio of the SR26334 to the internal standard peak area (ratio area [R]) was used to perform linear regression on the concentration of the corresponding points, based on the results of the plasma standard curve. Then, the regression equation (SR26334) was obtained as $R = 0.0212C + 0.0058$ ($r^2 = 0.9999$).

Precision and Recovery Rate

Precision includes intra-day precision and inter-day precision. The SR26334 plasma standard solution was prepared in 1, 10, and 50 mg/L samples (five for each concentration), treated as described in Detection of the Clopidogrel Plasma Concentration, and measured the same day. The detection amount was calculated according to the daily standard curve, and the intra-day precision was calculated. The same process was repeated for five days and the intra-day precision was then calculated. The results showed that the intra- and inter-day precision were both below 5%. The relative and absolute recoveries of SR26334 were measured as 100.833% (± 0.692%) and 85.034% (± 0.006%) respectively.

The Stability of SR26334 Under Different Storage Conditions

The plasma samples were stored in a refrigerator at –80°C and were stable for seven days; the plasma samples were stable at room temperature for 24 h and remained stable after freezing/thawing twice.

Pharmacokinetic Parameters

(1) Drug-time curve: The SR26334 plasma drug concentration was measured by high-performance liquid chromatography at different times, and a drug-time curve was drawn (see Figure 1).

(2) The SR26334 pharmacokinetic parameters: After the SR26334 plasma concentrations were treated using the DAS system, the pharmacokinetic parameters were estimated using a non-atrioventricular model. The area under the plasma concentration-time curve (AUC), plasma peak concentration (Cmax), peak time (Tmax), and the half-life (T1/2Z) were also established. The SR26334, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and Cmax were processed by logarithmic transformation. The DAS system was used to deriveCLz/F and T1/2Z statistical data. Table 3 provides the statistical results.

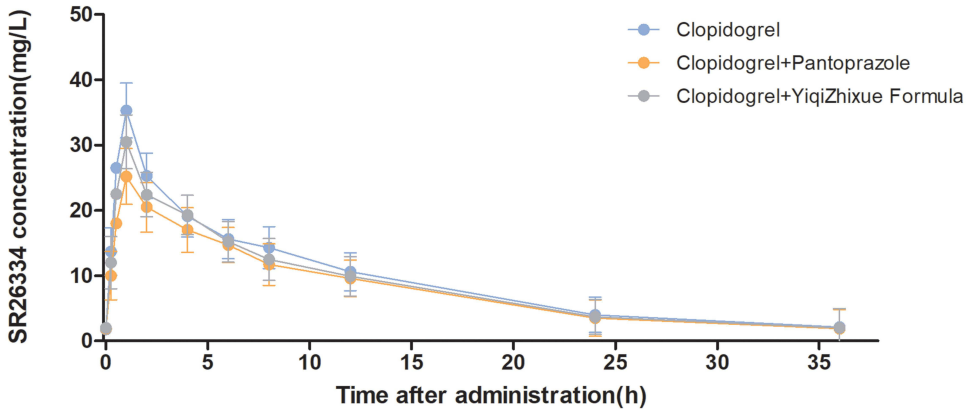

Figure 1 SR26334 mean plasma concentration - time curve.

Table 3 Pharmacokinetic Parameters of SR26334

Pharmacokinetic Parameters	Clopidogrel	Pantoprazole + Clopidogrel	Clopidogrel + TCM Invigorating Qi and Hemostasis
$AUC_{(0—t)}$ (mg /L* h)	323.425±39.263	282.134±30.231	300.342±35.832
$AUC_{(0—\infty)}$ (mg /L* h)	340.227±40.461	299.163±36.634	320.462±40.213
C_{max} (mg /L* h)	35.441±9.256	25.274±8.034	30.622±9.917
$t_{1/2z}$ (h)	8.052±0.852	7.836±1.142	7.954±1.121
T_{max} (h)	1	1	1

Traditional Chinese Medicine Invigorating Qi and Hemostasis – the Effect on Thrombocyte Release in Rat Serum

The concentrations of ADP and TXA2 were determined and the platelet release function was evaluated by ELISA. Figure 2 shows that, compared with the mock-surgery group, the content of ADP and TXA2 in the model group were significantly increased ($P < 0.01$). Compared with the model group, the levels of ADP and TXA2 in the clopidogrel, clopidogrel + pantoprazole, invigorating qi and hemostasis, and clopidogrel + invigorating qi and hemostasis groups were significantly lowered ($P < 0.01$).

Traditional Chinese Medicine Invigorating Qi and Hemostasis – the Effect on Thrombocyte Activation Markers in Rat Serum

Platelet factor 4 is a specific protein secreted by a platelet alpha (α) granule and is the specific indicator of platelet activation. Platelet membrane GPIIb/IIIa and platelet surface CD62P are classic markers of platelet activation. The results in Figure 3 show that, compared with the mock-surgery group, GPIIb/IIIa, PF-4, and CD62P levels in the model group were significantly increased ($P < 0.01$). Compared with the model group, the PF-4, GPIIb/IIIa, and CD62P levels in the clopidogrel, clopidogrel + pantoprazole, invigorating qi and hemostasis, and clopidogrel + invigorating qi and hemostasis groups were significantly lowered ($P < 0.01$).

Traditional Chinese Medicine Invigorating Qi and Hemostasis – the Effect on the Platelet Aggregation Rate Induced by Arachidonic Acid and Adenosine Diphosphate

Figure 4 shows that, compared with the mock-surgery group, the platelet aggregation rate induced by AA and ADP in the model group was significantly reduced ($P < 0.01$). Compared with the model group, the platelet

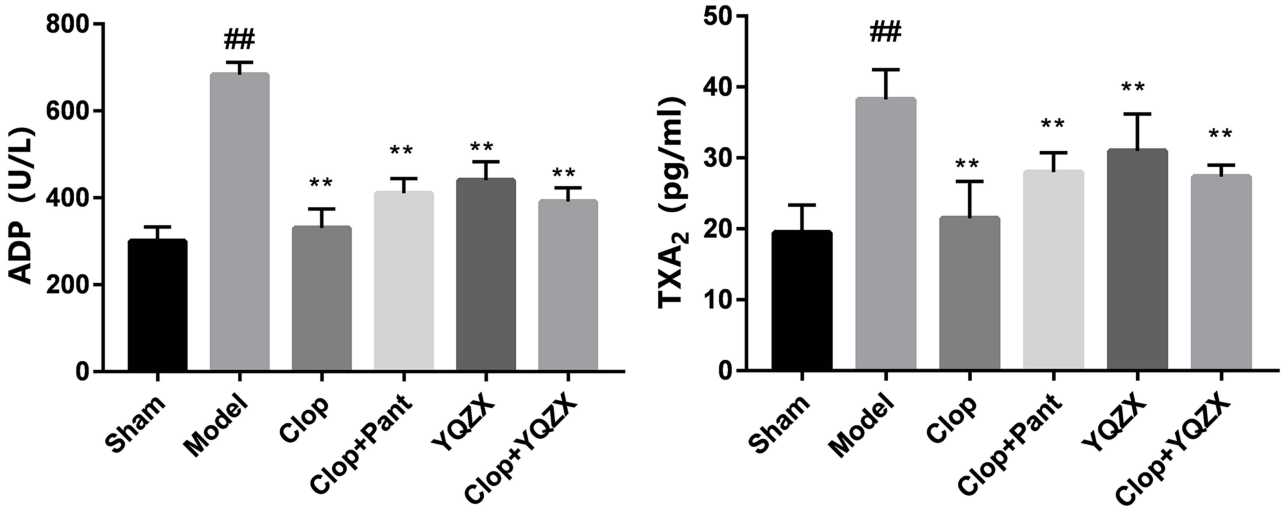

Figure 2 Platelet activation and release function ADP, TXA_2.
Note: Compared with Sham group, $P^{\#\#}<0.01$; compared with Model group, $P^{**}<0.01$.

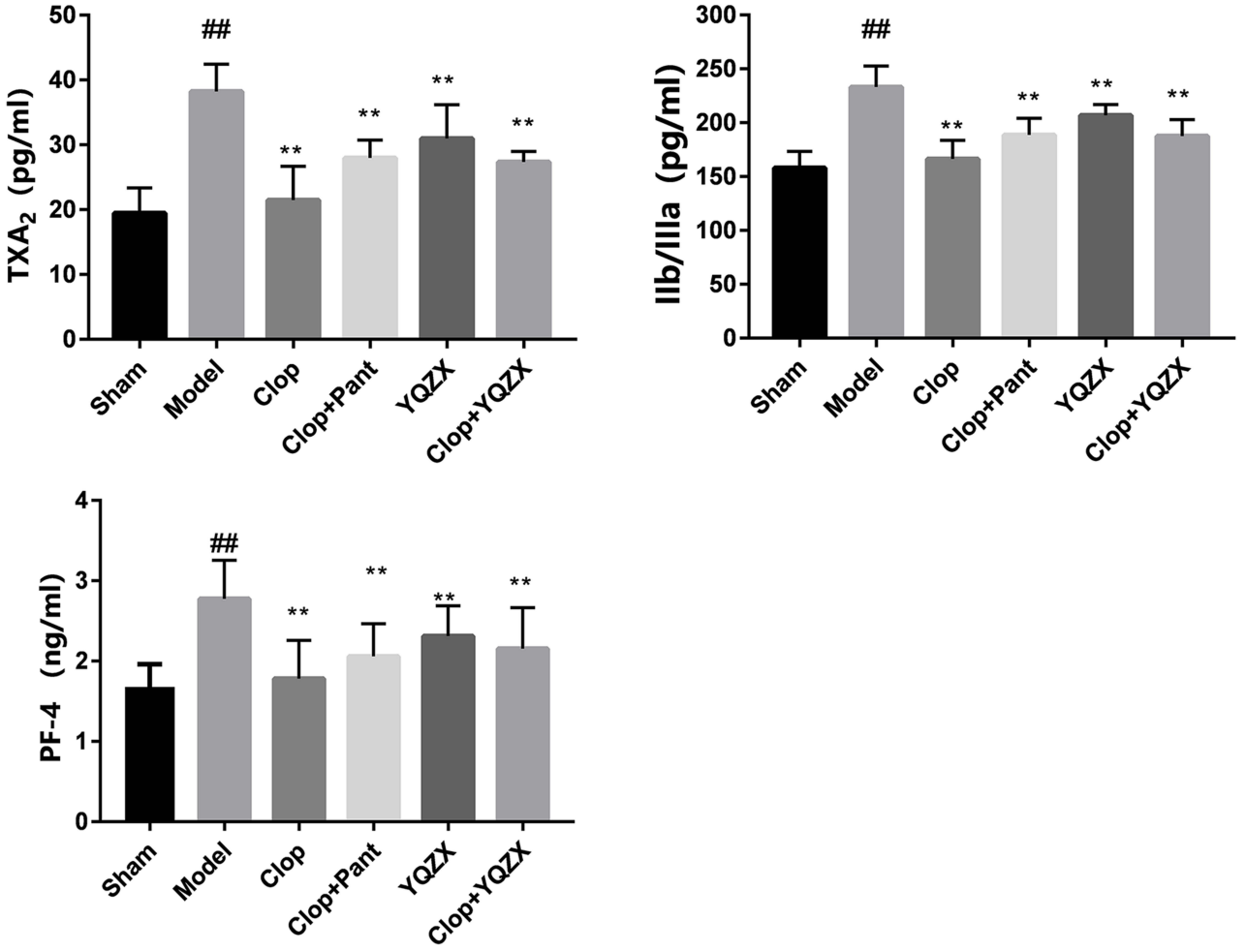

Figure 3 Platelet activation markers.
Note: Compared with Sham group, P##<0.01; compared with Model group, P**<0.01.

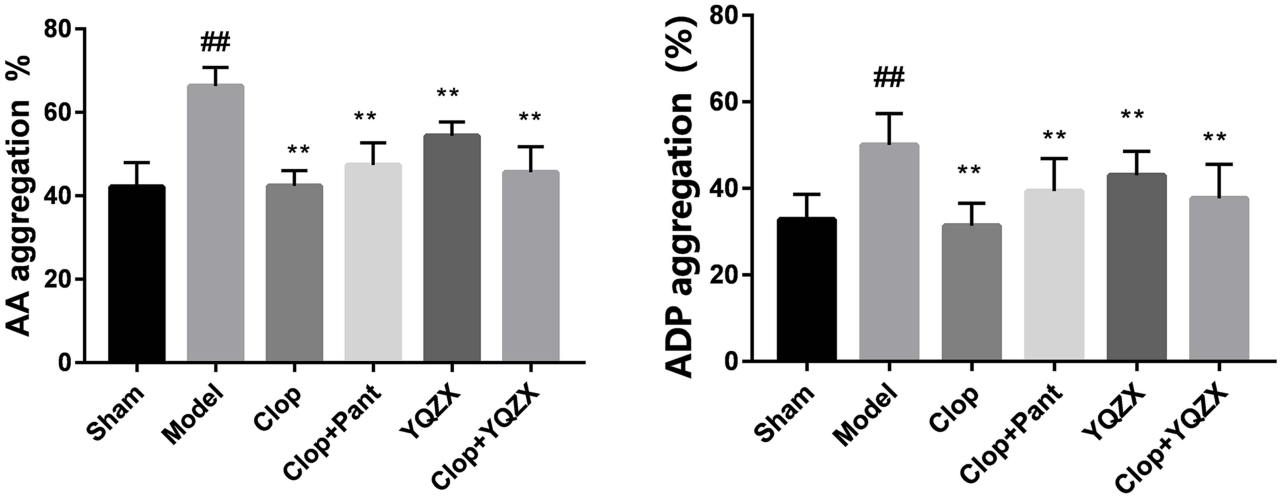

Figure 4 AA, ADP platelet aggregation rate.
Note: Compared with Sham group, P##<0.01; compared with Model group, P**<0.01.

aggregation rate of the clopidogrel, clopidogrel + pantoprazole, invigorating qi and hemostasis, and clopidogrel + invigorating qi and hemostasis groups was significantly lowered ($P < 0.05$).

Traditional Chinese Medicine Invigorating Qi and Hemostasis – the Effect on the Gastrin, Motilin, and Prostaglandin E2 Content in Rat Serum

Figure 5 shows that, compared with the model group, the GAS, MTL, and PGE2 content in the clopidogrel group were significantly decreased ($P < 0.05$). Compared with the clopidogrel group, the GAS, MTL, and PGE2 content in the clopidogrel + pantoprazole, invigorating qi and hemostasis, and clopidogrel + invigorating qi and hemostasis groups showed a remarkable increase ($P < 0.05$).

Traditional Chinese Medicine Invigorating Qi and Hemostasis – the Effect on the Content of Inflammatory Factor Cytokines, Tumor Necrosis Factor-Alpha, and Interleukins 6, 8, and 10 in Rat Serum

According to Figure 6, compared with the model group, the TNF-α and ILs-6, 8, and 10 levels in the clopidogrel group were significantly increased ($P < 0.01$). Compared with the clopidogrel group, the TNF-α and ILs-6, 8, and 10 levels in the clopidogrel + pantoprazole, invigorating qi and hemostasis, and clopidogrel group + invigorating qi and hemostasis groups were significantly lowered ($P < 0.01$). Compared with the clopidogrel + pantoprazole group, the content of TNF-α and ILs-6, 8, and 10 in the invigorating qi and hemostasis and clopidogrel + invigorating qi and hemostasis groups were significantly reduced ($P < 0.05$).

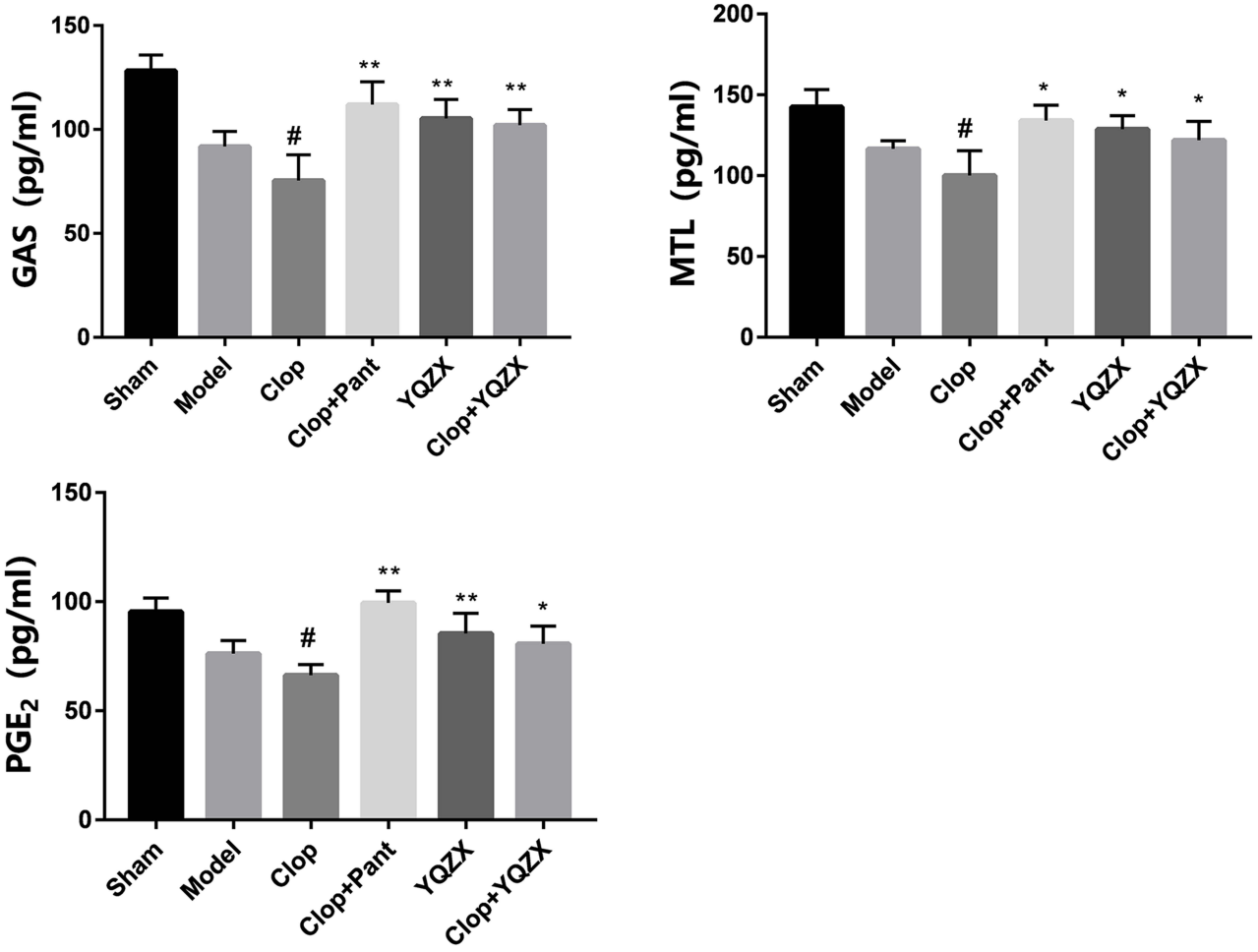

Figure 5 The influence of the content GAS, MTL, and PGE_2.
Note: Compared with Model group, $P^{\#}<0.05$; compared with Clop group, $P^{*}<0.05$, $P^{**}<0.01$.

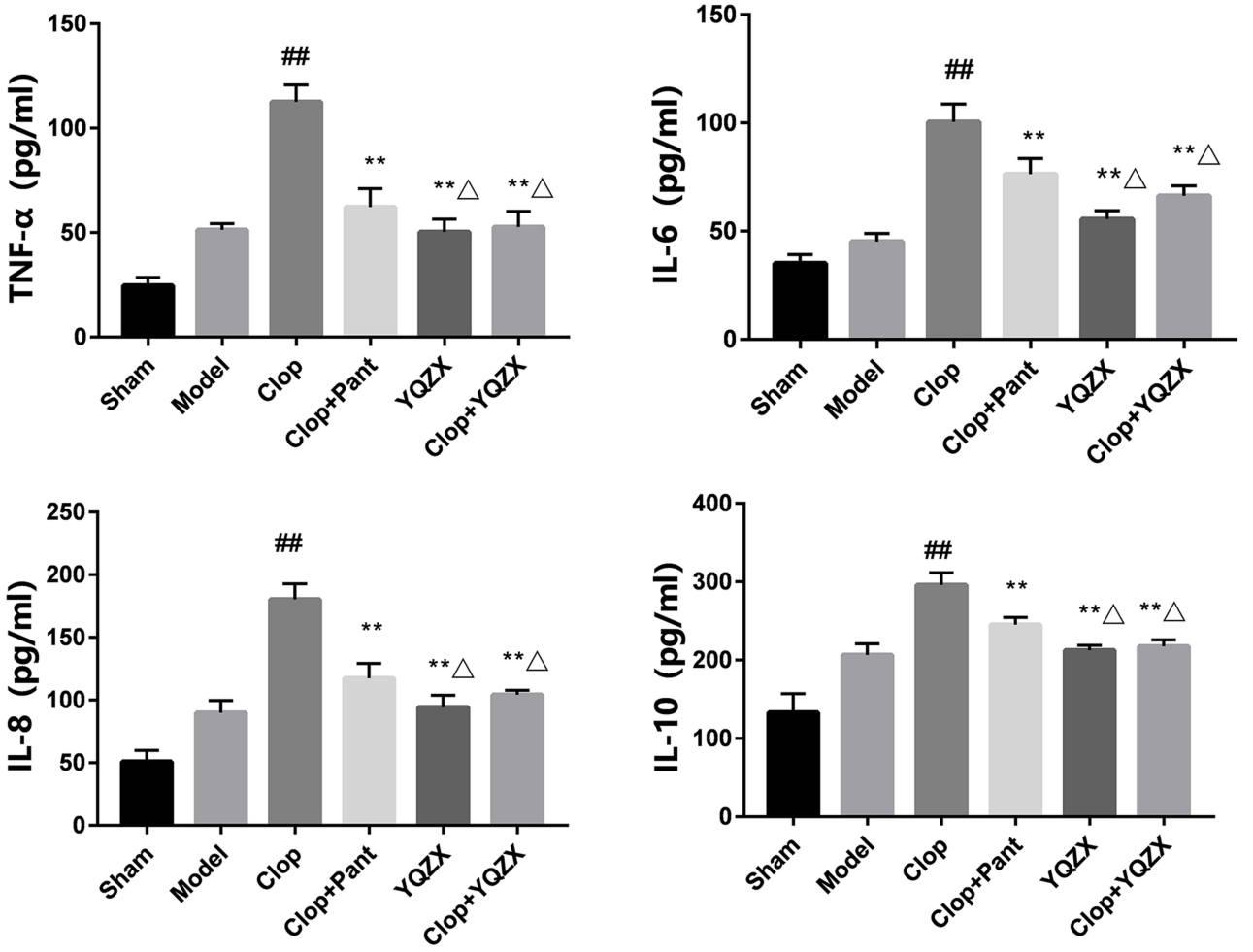

Figure 6 Content of TNF-α, IL-6, IL-8, and IL-10.
Note: Compared with Model group, $P^{\#\#}<0.01$; compared with Clop group, P**<0.01; compared with Clop+Pant group, $P^{\Delta}< 0.05$.

Traditional Chinese Medicine Invigorating Qi and Hemostasis – the Effect on the Messenger Ribonucleic Acid Expression of Cyclooxygenase 1 and 2, Tumor Necrosis Factor-Alpha, and Vascular Endothelial Growth Factor in Rat Gastric Tissue

The results set out in Figure 7 indicate that compared with the model group, the mRNA expressions of COX-1 and VEGF in the clopidogrel group were significantly decreased ($P < 0.05$), while the mRNA expressions of COX-2 and TNF-α were significantly increased ($P < 0.01$). Compared with the clopidogrel group, the mRNA expressions of COX-1 and VEGF in the clopidogrel + pantoprazole, invigorating qi and hemostasis, and clopidogrel + invigorating qi and hemostasis groups were significantly increased ($P < 0.01$), while the mRNA expressions of COX-2 and TNF-α showed a significant decrease ($P < 0.01$).

Discussion

As the main regulatory enzyme in the cytochrome P450 isozyme system, CYP2C19 plays a key role in the metabolism of clopidogrel. The PPI metabolic process is also involved through the CYP2C19 enzyme, with no competitive inhibitory relationship between the two metabolic pathways. Li et al[11] conducted in vitro tests and found that five types of PPIs (including pantoprazole) could competitively inhibit CYP2C19. Clinically, the competitive inhibition of the PPI and clopidogrel in the hepatic drug enzyme CYP2C19 weakened clopidogrel's antiplatelet effect. In the PRINCIPLE-TIMI 44 study[12], 201 patients enrolled after PCI were randomly divided into

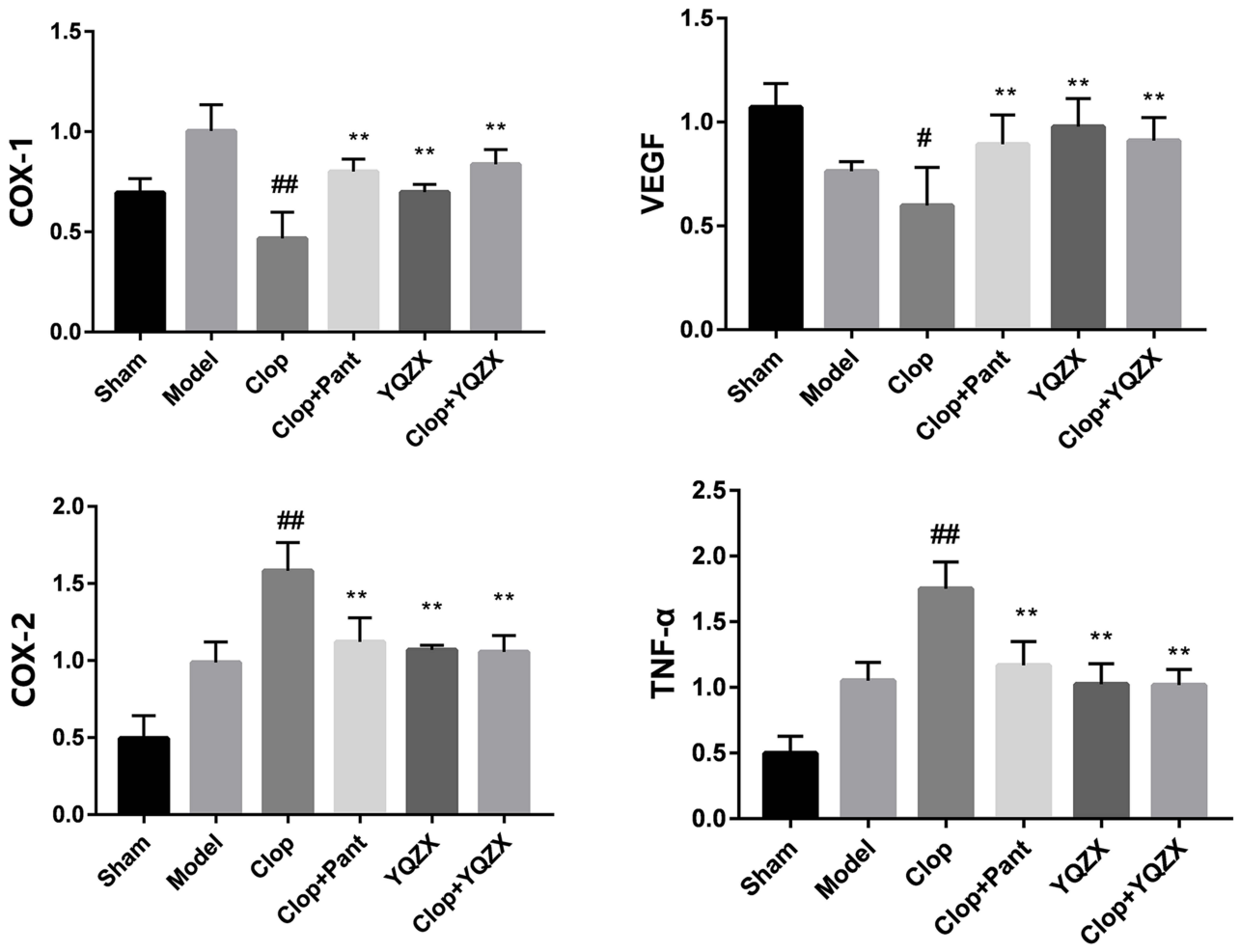

Figure 7 COX-1, COX-2, TNF-α, and VEGF mRNA expressions.
Note: Compared with Model group, $P^{\#}<0.05$, $P^{\#\#}<0.01$; compared with Clop group, $P^{**}<0.01$.

high-dose clopidogrel (99 cases) and prasugrel (102 cases) groups. The results showed that the mean inhibition rate of platelet aggregation in patients who took a PPI six hours after a clopidogrel-loading dose of 600 mg was significantly higher than in patients who took a PPI over a long period. The combination of clopidogrel and pantoprazole to some extent increased the risk of cardiac events in patients after PCI. However, the combination of TCM invigorating qi and hemostasis with clopidogrel may present a greater benefit to patients. Despite this, the present research found that TCM invigorating qi and hemostasis still had only a minor effect on clopidogrel, which we speculate may have been related to the interference of the two drugs in CYP3A4. The CYP3A4 enzyme is another important substance in the metabolism of clopidogrel. At present, pharmacological studies consider that *Panax notoginseng*, the main component of TCM invigorating qi and hemostasis, demonstrates a specific effect on the CYP3A4 enzyme.[13] Accordingly, it is speculated that the effect of TCM invigorating qi and hemostasis on clopidogrel metabolism may be related to this; however, the specific mechanism requires further study.

Inhibition of platelet activation is the main effect of DAPT following PCI.[14] Additionally, GPIIb/IIIa are markers of platelet activation; in all types of platelet activators, to activate the signal generated by the final common pathway, GPIIb/IIIa content produces a degree of platelet activation.[15] Platelet factor 4 is a specific protein secreted by the platelet α granule, which can slow the inactivation process of thrombin, and is a specific indicator of platelet activation.[16] P-selectin is a glycoprotein of the platelet α granulosa membrane that is not expressed in platelets at rest or only expressed in activated platelets and can indirectly reflect the activation state of platelets.[17] Results show

that the content of GPIIb/IIIa, CD62P, and PF-4 in the TCM invigorating qi and hemostasis, and in the clopidogrel + invigorating qi and hemostasis groups were significantly reduced. Adenosine diphosphate and TXA2 are important substances that are released during platelet activation and play a critical role in this process.[18]

The mechanism of TCM invigorating qi and hemostasis concerning platelets still requires additional research and discussion. Presently, in a pharmacological context, the study of the antiplatelet effect of the main components of TCM invigorating qi and hemostasis provides some suggestions in this regard. For example, rhabdomyolysis, which is found in rhubarb, can inhibit platelet aggregation induced by ADP, and the effect is stronger compared with antiplatelet drugs used separately.[8] Furthermore, ginsentriol saponins can directly inhibit platelet aggregation induced by various agonists. Concurrently, the Ca^{+}/mitogen-activated protein kinase level in the process of platelet aggregation is affected, and subsequently, the downstream ERK2/P38 pathway is also affected.[19] These pathways may be among the potential antiplatelet effects for TCM invigorating qi and hemostasis.

Existing studies found that TCM invigorating qi and hemostasis helped to reduce the incidence of gastrointestinal bleeding in high-risk groups while inhibiting platelet aggregation.[10] The gastric mucosal protection effect of TCM invigorating qi and hemostasis may be related to the regulation of gastric mucosal protective hormones. Additionally, MTL and GAS are crucial gastrointestinal hormones. The latter is secreted by the gastric antrum and duodenal mucosa open G cells and plays a role in protecting gastrointestinal mucosa, thereby improving protective factors and promoting secretions in the gastrointestinal tract.[20,21] Traditional Chinese medicine invigorating qi and hemostasis can enhance the GAS and MTL content in increased gastric mucosal blood flow to protect the gastric mucosal cells. The current research found that TCM invigorating qi and hemostasis could increase the COX-1 and PGE2 content, as well as the VEGF mRNA expression in gastric tissue. The metabolite formed by structural COX-1 is a regulator of gastric mucosa, which can inhibit gastric acid secretion, promote mucus secretion, and improve gastric microcirculation. Moreover, PGE2 can increase gastric mucosal blood flow, protect epithelial cells, and maintain the integrity of gastric mucosa. Furthermore, VEGF can protect gastric mucosal cells, promote the proliferation of the gastrointestinal mucosal epithelial cells, and maintain the integrity of the gastrointestinal tract, and plays an important role in defense. Furthermore, TCM invigorating qi and hemostasis can play a role in gastric mucosal protection by adjusting the protection factors noted above.

Tumor necrosis factor-alpha is the earliest and most important inflammatory mediator in the process of inflammation. It can increase the permeability of vascular endothelial cells, and drive the synthesis and release of other cytokines, such as ILs-6, 8, and 10.[22] Inducible COX-2, which is involved in the chronic inflammation of gastric mucosa, is secreted by cells under the stimulus of inflammation. It is highly expressed during any injury to the gastric mucosa.[23] The combination of TCM invigorating qi and hemostasis with clopidogrel can reduce inflammation, promote and inhibit gastric acid secretion, influence gastrointestinal movement, and maintain the integrity and protection of gastric mucosa.

In this research, a PPI was combined with clopidogrel as a control. The gastric mucosal protective effect of TCM invigorating qi and hemostasis was found to be comparable with PPIs. A PPI is a classic gastric acid inhibitor and gastric mucosal protectant. The acid inhibition of a PPI is related to the inactivation of the H^{+}, K^{+}-ATP enzyme (proton pump) in parietal cells. However, the effect of a PPI in regulating the expression of inflammatory mediators is unclear. The results of this study show that TCM invigorating qi and hemostasis was superior to using a PPI in terms of inhibiting the inflammatory factors of gastric mucosal injury, which is different from the results produced by a PPI. A pharmacological study confirmed that the main components of TCM invigorating qi and hemostasis, such as Astragalus, can reduce the degree of gastric mucosal injury and inhibit the inflammatory myeloperoxidase level of TNF-α, as well as the levels of IL, inhibit neutrophil infiltration, and reduce the expression of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), transcription factor p65, TNF receptor-associated factor 2, and inflammatory cytokines.[24] In this regard, the inhibitory expression of inflammatory factors, and the regulation of inflammatory pathways in gastric tissue (such as the TNF-α/NF-κB signaling pathway) may be a crucial mechanism in gastric mucosal protection by TCM invigorating qi and hemostasis.

In this research, only the dose for the TCM invigorating qi and hemostasis group (following conventional-dose conversion) was designed; high, medium, and low-dose groups were not designed. As a result, we could not further evaluate the dose-effect relationship of TCM invigorating

qi and hemostasis. This will be conducted in future studies. In terms of platelet activation, the scope of our focus was limited. We will conduct additional research on the antiplatelet function of TCM invigorating qi and hemostasis in future experiments.

In terms of the metabolic issues related to the TCM compounds and clopidogrel, some scholars have tried to explore the effects of different types of blood-activating drugs on the metabolism of double antigens. Research such as that conducted by Xiao[9] indicated that kudzu root and *Angelica sinensis* can lead to pharmacokinetic diaminopyridine (DAP) changes in rCyp2c11, and also had a significant inhibition on the activity of carboxylesterase. Accordingly, this study posits that kudzu vine root, *Angelica sinensis*, and *Ligusticum wallichii* root may offset the anticoagulant activity of DAPT, but pose no bleeding risk in the presence of gastric mucosa damage. Therefore, the overall pharmacodynamic results are not harmful; however, further validation of the clinical effects is needed. It also suggests that different drug combinations have different effects on the metabolism of double antibodies and that some groups of blood-activating drugs may have negative effects on the efficacy of double antibodies. The present study did not establish the effect of the entire drug group on the metabolism of double antibodies; accordingly, further analysis of the specific group cooperation of two/three drugs should be considered.

The present study found that, compared with pantoprazole, the main components of TCM for qi tonifying and hemostasis had a lower influence on clopidogrel metabolism, which was conducive to clopidogrel's antiplatelet effect. The main components of TCM tonifying qi and hemostasis had some antiplatelet effect, mainly through the inhibition of platelet activation, ie, inhibiting the platelet-related activation pathway induced by ADP and AA.

Traditional Chinese medicine aimed at replenishing qi and hemostasis plays a protective role for gastric mucosa by increasing its protective factors and alleviating local inflammatory reactions by reducing inflammatory factors. The results of our study help understand how TCM can enrich qi and achieve hemostasis, thereby serving a protective role for gastric mucosa and alleviating gastrointestinal bleeding, and, concurrently, assisting clopidogrel in antiplatelet treatment. Based on the experiment conducted herein, the approach of using TCM to enrich qi and effect hemostasis can be used as a potential alternative method for screening patients at risk of bleeding after PCI.

The current study had some limitations. Only the dose of the TCM tonifying qi and hemostasis group (following conventional-dose conversion) was designed. High, medium, and low-dose groups were not created and, as such, the dose-effect relationship of the TCM approach of tonifying qi and hemostasis could not be further evaluated. We aim to address these aspects in future studies. Additionally, in the platelet activation section of the study, the scope of our attention was limited. Future studies will aim to address the antiplatelet function of TCM aimed at replenishing qi and achieving hemostasis.

Conclusion

This research found that TCM aimed at invigorating qi and effecting hemostasis demonstrated a lower effect on clopidogrel metabolism than using a PPI, which was beneficial in terms of clopidogrel exerting an antiplatelet effect. In addition, the effects of TCM aimed at invigorating qi and achieving hemostasis on platelet release activity, platelet activation markers, gastrointestinal hormones, gastric mucosal protection factors, and gastric mucosal inflammatory factors were evaluated, and a preliminary explanation of the antiplatelet effect and the pharmacological mechanism of the gastric mucosal protection of TCM aimed at invigorating qi and achieving hemostasis was presented. This research contributes to an understanding of how we can use TCM aimed at invigorating qi and establishing hemostasis to assist clopidogrel in effecting an antiplatelet effect, protect the gastric mucosa, and relieve alimentary tract hemorrhage.

Ethics Approval

All experiments were evaluated and approved by the ethics Committee of the Wangjing Hospital of the China Academy of Chinese Medical Sciences (No. IACUC-AMSS-20190215-02) and complied with the National Institutes of Health Guides for the Care and Use of Laboratory Animal Use.

Acknowledgments

We are particularly grateful to all the people who have given us help on our article.

Funding

This study was funded by the "Capital Featured Projects" of Beijing Municipal Science and Technology Commission (No.Z151100004015085). The funding body had no role in the design of the study and collection,

analysis, and interpretation of data and in writing the manuscript.

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Yan MY, Wang YT. Objectization of conceptual and state of affairs of spatiotemporal sequence by qi's reference in traditional Chinese medicine. *J Beijing Univ Trad Chin Med*. 2018;41(09):717–725. Article in China.
2. Zhang C, Huang C, Wang M, Kong X, Liu G, Wang J. Correlation analysis between traditional chinese medicine syndromes and gastrointestinal bleeding after percutaneous coronary intervention. *Evid Based Complement Alternat Med*. 2018;2018:7356546.
3. Zhang W, Ma W, Zhang J, Song X, Sun W, Fan Y. The immunoregulatory activities of astragalus polysaccharide liposome on macrophages and dendritic cells. *Int J Biol Macromol*. 2017;105(Pt 1):852–861. doi:10.1016/j.ijbiomac.2017.07.108
4. Fu J, Wang Z, Huang L, et al. Review of the botanical characteristics, phytochemistry, and pharmacology of Astragalus membranaceus (Huangqi). *Phytother Res*. 2014;28(9):1275–1283. doi:10.1002/ptr.5188
5. Kong YT, He H, An FP, et al. Intestinal probiotic function and antioxidant capacity-enhancing effect of an ethanolic extract of radix pseudostellariae. *Mod Food Sci Tech*. 2021; 37(05):9–16. Article in China.
6. Teng LQ, Zhou T, Wang X, et al. Analysis of the mechanism of Pseudostellariae Radix against myocardial ischemia based on network pharmacology and molecular docking. *Shandong Sci*. 2021; 34(02):24–33. Article in China.
7. Wang MM, Xue M, Xu YG, et al. Panax notoginseng saponin is superior to aspirin in inhibiting platelet adhesion to injured endothelial cells through COX pathway in vitro. *Thromb Res*. 2016;141:146–152. doi:10.1016/j.thromres.2016.03.022
8. Wang WT, Xue M, Yang L, et al. Experimental research on the protective effect of panax notoginseng saponins on the gastric mucosa and the anti-platelet effect of aspirin based on arachidonic acid metabolic pathway. *Chin J Integr Med Cardio Cerebrovasc Dis*. 2019; 17(09):1315–1320. Article in China.
9. Xiao M, Qian C, Luo X, et al. Impact of the Chinese herbal medicines on dual antiplatelet therapy with clopidogrel and aspirin: pharmacokinetics and pharmacodynamics outcomes and related mechanisms in rats. *J Ethnopharmacol*. 2019;235:100–110. doi:10.1016/j.jep.2019.01.040
10. Zhang C, Huang C, Kong X, et al. A randomized double-blind placebo-controlled trial to evaluate prophylactic effect of traditional chinese medicine supplementing qi and hemostasis formula on gastrointestinal bleeding after percutaneous coronary intervention in patients at high risks. *Evid Based Complement Alternat Med*. 2018;2018:3852196. doi:10.1155/2018/3852196
11. Li XQ, Andersson TB, Ahlström M, Weidolf L. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos*. 2004;32(8):821–827. doi:10.1124/dmd.32.8.821
12. O'Donoghue ML, Braunwald E, Antman EM, et al. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. *Lancet*. 2009;374(9694):989–997. doi:10.1016/S0140-6736(09)61525-7
13. Shi J, Chen AJ, Zhang F, et al. Effect of total saponins of Panax notoginseng on CYP450 and prediction of drug interactions. *Chin J Pharmacoepidemiol*. 2008; 17(05):281–284. Article in China.
14. Flannery L, Liu R, Elmariah S. Dual antiplatelet therapy: how long is long enough? *Curr Treat Options Cardiovasc Med*. 2019;21(4):17. doi:10.1007/s11936-019-0721-8
15. Lefkovits J, Plow EF, Topol EJ. Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. *N Engl J Med*. 1995;332(23):1553–1559. doi:10.1056/NEJM199506083322306
16. Vaage J, Gjesdal K. Proceedings: release of platelet factor 4 (PF 4) during repeatedly induced platelet aggregation in vivo. *Thromb Diath Haemorrh*. 1975;34(2):575.
17. Au AE, Josefsson EC. Regulation of platelet membrane protein shedding in health and disease. *Platelets*. 2017;28(4):342–353. doi:10.1080/09537104.2016.1203401
18. Cho MJ, Pestina TI, Steward SA, Jackson CW, Kent Gartner T. The roles of LAT in platelet signaling induced by collagen, TxA2, or ADP. *Biochem Biophys Res Commun*. 2002;292(4):916–921. doi:10.1006/bbrc.2002.6738
19. Qi H, Huang Y, Yang Y, et al. Anti-platelet activity of panaxatriol saponins is mediated by suppression of intracellular calcium mobilization and ERK2/p38 activation. *BMC Complement Altern Med*. 2016;16(1):174. doi:10.1186/s12906-016-1160-7
20. Rehfeld JF, Friis-Hansen L, Goetze JP, Hansen TV. The biology of cholecystokinin and gastrin peptides. *Curr Top Med Chem*. 2007;7(12):1154–1165. doi:10.2174/156802607780960483
21. Xu L, Gao S, Guo F, Sun X. Effect of motilin on gastric distension sensitive neurons in arcuate nucleus and gastric motility in rat. *Neurogastroenterol Motil*. 2011;23(3):265–e121. doi:10.1111/j.1365-2982.2010.01661.x
22. Zhang J, Wang YR, Zhang QZ. Effects of astragaloside IV on serum IL-6, IL-8 and TNF-α in rats with gastric mucosal injury induced by clopidogrel. *J Hebei North Univ Nat Sci*. 2018;34(06):12–14+18. Article in China.
23. Wu HP, Jiang J, Wu AL, et al. Effect of total flavonoids of licorice on the expression of COX-1 and COX-2 in gastric mucosa of patients with chronic superficial gastritis. *Prod Mod Biomed*. 2015; 15(27):5302–5305. Article in China.
24. Qin S, Huang K, Fang Z, Yin J, Dai R. The effect of Astragaloside IV on ethanol-induced gastric mucosal injury in rats: involvement of inflammation. *Int Immunopharmacol*. 2017;52:211–217. doi:10.1016/j.intimp.2017.09.011