



Panax Quinquefolius Saponins Combined with Dual Antiplatelet Drug Therapy Alleviate Gastric Mucosal Injury and Thrombogenesis through the COX/PG Pathway in a Rat Model of Acute Myocardial Infarction

DOI

<https://dx.doi.org/10.17504/protocols.io.mmhc436>

Na Kou¹, Da-Zhuo Shi¹

¹Xiyuan Hospital



Na Kou

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account



DOI: <https://dx.doi.org/10.17504/protocols.io.mmhc436>

Protocol Citation: Na Kou, Da-Zhuo Shi . Panax Quinquefolius Saponins Combined with Dual Antiplatelet Drug Therapy Alleviate Gastric Mucosal Injury and Thrombogenesis through the COX/PG Pathway in a Rat Model of Acute Myocardial Infarction.

protocols.io <https://dx.doi.org/10.17504/protocols.io.mmhc436>

Manuscript citation:

- [1] Michelson AD. Advances in antiplatelet therapy. Hematology Am Soc Hematol Educ Program. 2011;2011(1): 62-69.
- [2] Steinhubl SR, Berger PB, Mann JT 3rd, Fry ET, DeLago A, Wilmer C, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA 2002;288(19): 2411-2420.
- [3] Bhatt DL, Fox KA, Hacke W, Berger PB, Black HR, Boden WE, et al. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. N Engl J Med. 2006;354(16): 1706-1717.
- [4] Gladding P, Webster M, Ormiston J, Olsen S, White H. Antiplatelet drug nonresponsiveness. Am Heart J. 2008;155(4): 591-599.
- [5] Patrono C. The multifaceted clinical readouts of platelet inhibition by low-dose aspirin. J Am Coll Cardiol. 2015;66(1): 74-85. doi: 10.1016/j.jacc.2015.05.012
- [6] Mitchell JA, Warner TD. COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nat Rev Drug Discov. 2006;5(1): 75-86.
- [7] Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD. et al. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: A report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol. 2008;52(18): 1502-1517.
- [8] Lin Y. Identification of ginsenosides from *Panax quinquefolius* in Xinyue Capsula by LC-ESI-MSn.[J]. Chinese Traditional & Herbal Drugs, 2010, 41(12):1942-1947.
- [9] Guo M, Zi MJ, Xi RX, Yang QN, Bai RN, Zhang YS, et al. Effect of Xinyue Capsules on patients with coronary heart disease after percutaneous coronary intervention: study protocol for a randomized controlled trial. Trial 2016;17: 412. doi:10.1186/s13063-016-1531-x.
- [10] Zhang QX, Liu JG, Zhang L, Shi DZ. Reparative effects of *Panax quinquefolius* saponin on gastric mucosal lesions induced by dual antiplatelet drugs in AMI model r

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: January 14, 2018

Last Modified: February 22, 2018

Protocol Integer ID: 9609

Keywords: Acute myocardial infarction; Antithrombosis; Dual antiplatelet therapy; Gastric mucosal injury; *Panax quinquefolium* saponins; American ginseng , dual antiplatelet drug therapy alleviate gastric mucosal injury, thrombogenesis through the cox, addition of panax quinquefolius saponin, panax quinquefolius saponin, acute myocardial infarction, antiplatelet effects in ami rat, dual antiplatelet therapy, acute myocardial infarction objective, thrombogenesis, myocardial infarct size, antithrombotic effect, myocardial necrosis, antiplatelet effect, reducing gastric mucosal injury, myocardial infarct area, coronary artery, clopidogrel, aspirin, damage to the gastric mucosa, combined drug therapy, thromboxane b2, gastric mucosal injury, plasminogen activator inhibitor

Abstract

Objectives: Previous studies have found that *Panax quinquefolius* saponins (PQS) combined with dual antiplatelet therapy (DAPT) of aspirin and clopidogrel enhances antithrombotic effects while reducing gastric mucosal injury induced by DAPT. We investigated the effects of the combined drug therapy (PQS+DAPT) through the COX/PG pathways.

Methods: Acute myocardial infarction (AMI) was induced in Wistar rats by ligation of the left anterior descending (LAD) coronary artery, and the animals were randomly divided into Model, DAPT, and PQS+DAPT groups. Rats in the sham group did not undergo artery ligation. They were intragastrically treated for 14 days. Myocardial infarct size; myocardial pathology; platelet aggregation rate, CD62p activation, concentrations of thromboxane B₂ (TXB₂), 6-keto-PGF1 α , tissue plasminogen activator (t-PA), and plasminogen activator inhibitor (PAI), the TXB₂/6-keto-PGF1 α ratio were measured. The ultrastructure of the gastric mucosa was observed by scanning electron microscopy. The expression of PGE₂ and 6-keto-PGF1 α in gastric mucosa was measured by radioimmunoassay, and levels of COX-1, COX-2, and VEGF in gastric mucosa were assessed using immunohistochemistry.

Results: The addition of *Panax quinquefolius* saponins (PQS+DAPT) to standard DAPT therapy significantly decreased the myocardial infarct area, degree of myocardial lesions, TXB₂ and PAI levels, and the TXB₂/6-keto-PGF1 α ratio, while increasing 6-keto-PGF1 α and t-PA levels and reducing the degree of gastric mucosal injury. Expression of PGE₂, 6-keto-PGF1 α , COX-2, and VEGF in the gastric mucosa was upregulated in the PQS+DAPT group compared with the standard DAPT group.

Conclusion: PQS increases the degree of DAPT inhibition of myocardial necrosis and antiplatelet effects in AMI rats, as well as reducing damage to the gastric mucosa caused by DAPT. The mechanism may be related to inhibition of TXB₂ and PAI activity and elevation of 6-keto-PGF1 α and t-PA levels in blood, and may be associated with upregulated expression of COX-2, PGE₂, PGI₂, and VEGF in gastric tissue.

Attachments



Revised Manuscript w...

274KB

TTC staining

- 1 a stock solution of TTC reagent was prepared in 1% phosphate-buffered saline (PBS) at 25–30°C in the dark. Rat hearts were frozen at –20°C for 20 min, and transected into 5 slices approximately 2-mm thick perpendicular to the long axis. The sections were incubated in prepared TTC solution for 30 min at 37°C in the dark, and fixed with 4% paraformaldehyde for 5 min. The normal myocardium was red while the infarction area was white. The area of each region was calculated with Image-Pro Plus 6.0 (IPP 6.0; Roper Industries, Sarasota, FL, USA) on the scanned image, and the data are presented as the proportion of infarction area to the whole area of the heart.

[M] 4 Mass Percent

 05:00:00

Hematoxylin and eosin staining

- 2 Cardiac tissue specimens were placed in prepared 10% formaldehyde fixative; after dehydration, paraffin embedding, sectioning, coating, and dewaxing, the tissues were stained with hematoxylin and eosin, dehydrated, and sealed. Myocardium structure was observed under a microscope.

Measurement of platelet aggregation

- 3 Blood was collected from the abdominal aorta and anticoagulated with sodium citrate (3.8%; 1 part anticoagulant to 9 parts blood). Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 1,000 rpm for 8 min; blood was centrifuged at 3,000 rpm for 15 min to prepare platelet-poor plasma (PPP). The platelet concentration was adjusted to 1.8×10^9 /mL for PPP. 0.3 mL PRP was placed in a cuvette and stirred with a rotor at 37°C for 5 min, after which 6 μ L of ADP was added. Aggregation was measured with a platelet aggregometer (LBY-NJ4, Pulisheng Instrument Co. Ltd., Shanghai, China). Results were recorded as turbidimetry at maximal aggregation after the addition of an aggregating agent. Data are expressed as percentage of maximal aggregation.

Measurement of CD62p expression

- 4 Two milliliters of abdominal aorta blood were taken and added to 3.8% sodium citrate for anticoagulation, shaken, and left to settle for 20 min. Five-microliter aliquots were then added to 5 μ L of CD61FITC and 5 μ L of CD62p PE, and incubated at 25–30°C for 20 min. PBS was added for a final volume of 1 mL. The solution was analyzed with a flow cytometer (NAVIOS, Beckman Coulter, Brea, CA, USA). Five microliters of PE-labeled immunoglobulin (IgG, CD62p negative) served as the control.

Measurement of TXB₂ and 6-keto-PGF1 α levels in plasma and t-PA and PAI levels in serum

- Concentrations of TXB₂ (nonenzymatic metabolite of TXA₂) and 6-keto-PGF1 α (nonenzymatic metabolite of PGI₂) in plasma, and t-PA and PAI in serum were measured using a radioimmunoassay (RIA). Abdominal aorta blood was drawn and centrifuged at 3,500 rpm for 10 min. Plasma and serum were collected and measured by RIA on an automatic RIA counter (XH-6020, Xi'an Nuclear Instrument Factory, Xi'an, China) according to the manufacturer's instructions. The data are presented as pg/mL.

Observation of gastric mucosa ultrastructure

- Rat gastric antrum, 0.5 cm $\times$ 0.5 cm in size, was rinsed in saline and then rapidly removed to 2.5% glutaraldehyde (PBS preparation) for fixation. Tissue samples were placed in 1% osmic acid, and then underwent gradual dehydration with alcohol, isoamyl acetate replacement, critical-point drying, and metal spraying. A scanning electron microscope (S-520, Hitachi Ltd., Tokyo, Japan) was used to observe gastric mucosa ultrastructure. Observed the gastric mucosa ultrastructure changes by pathologists, on the principle of a double blindness.

Measurement of PGE₂ and 6-keto-PGF1 α in gastric mucosa

- The concentrations of PGE₂ and 6-keto-PGF1 α in gastric tissue were measured by RIA. The data are presented as pg/mL.

Measurement of COX-1, COX-2, and VEGF in gastric mucosa

- Rat gastric antrum tissue was extracted and prepared in paraffin sections. The paraffin sections were baked, deparaffinized, and hydrated in an ethanol gradient. This was followed by antigen retrieval and incubation in 3% H₂O₂ at 25–30°C. Primary antibodies were added for COX-1 (1:100), COX-2 (1:25), and VEGF (1:100). The samples were placed in a humidified box for incubation overnight at 4°C. Secondary antibodies were added dropwise the next day, followed by DAB color development, counterstaining, differentiation, dehydration, and section sealing. PBS served as the negative control for all antibodies, and a known positive tissue section served as a positive control. The sections were observed under an optical microscope, and five areas (four corners and one central area) were selected for imaging by a Motic MED 6.0 digital medical image analysis system (Beijing Mike Audi Image Technology Co. Ltd., Beijing, China) under 200 $\times$ magnification. All images were analyzed by IPP 6.0. The expression of COX-1, COX-2, and VEGF was quantitatively analyzed with integrated optical density (IOD) as a testing index.

For western blotting, equal amounts of protein were loaded onto SDS-PAGE gels, and proteins were then transferred to nitrocellulose membranes (Millipore, Billerica, MA,



USA). The membranes were blocked and incubated overnight at 4°C with the following antibodies: COX-1 (1:1,000), COX-2 (1:500), and GAPDH (1:500). The membranes were then incubated with secondary antibodies as follows: COX-1 (1:2,000), COX-2 (1:100), and GAPDH (1:500), for 1 h. Finally, the membranes were exposed and scanned, and the optical density of the bands (measured in arbitrary densitometry units) was determined using IPP 6.0. Results are expressed as a relative density ratio, normalized to the mean value of the Sham group. Two pathologists completed test using blind principle after each sample numbered.