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Network pharmacology-based identification of protective mechanism of Panax Notoginseng Saponins on aspirin induced gastrointestinal injury

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ABSTRACT

Background & Aims: Aspirin is the first line therapy for cardiovascular and cerebrovascular diseases and is widely used. However aspirin-induced gastrointestinal injury is one of its most common side effect which limits long-term use. Panax Notoginseng Saponins(PNS) which is also used to prevent thrombus may alleviate this side effect according to previous clinical evidences. Owing to the complexity of drug combination, the protective mechanism of PNS on aspirin-induced gastrointestinal injury remains unclear. Therefore, a network pharmacology-based strategy was proposed in this study to address this problem.

Methods: A network pharmacology approach comprising multiple components, candidate targets of each component, known therapeutic targets, network analysis has been used in this study. Also, we establish aspirin-induced gastrointestinal injury model by the oral administration of aspirin (0.5 g/kg body weight) to verify the predicted targets from network pharmacology. All rats was randomly allocated to control groups (n = 6),aspirin groups (n = 6)and aspirin + PNS groups (n = 6) and conducted H&E staining and ELISA for VEGFA.

Results: The comprehensive systematic approach was successfully to identify 5 compounds and 154 candidate targets in PNS and 479 candidate targets in aspirin. After network establishment and analysis, 27 potential targets hit by PNS, aspirin and 6 kind of gastrointestinal diseases were found. The experiments results indicated that aspirin group has visible inflammation and lesions while aspirin + PNS group have not. The higher expression of VEGFA in aspirin + PNS group verified the predicted potential protective targets of PNS.

Conclusions: PNS may have protective function for aspirin-induced gastrointestinal injury through increasing VEGFA expression. Network pharmacology strategy may provide a forceful tool for exploring the mechanism of herb medicine and discovering novel bioactive ingredients.

1. Introduction

Aspirin serving as a non-steroidal anti-inflammatory drugs (NSAIDs) has an irreplaceable and important therapeutical effect on acute myocardic infarction(AMI), acute coronary syndrome(ACS), ischemic stroke, transient ischemic attack(TIA), percutaneous coronary intervention(PCI) and peripheral artery diseases [1]. The incidence of main events in patients with cardiovascnlar and cerebrovascular diseases has reduced by 25% due to the application of aspirin [2]. Statistically, incidence have reduced by 1/3 in AMI,1/4 in ischemic stroke and 1/6 in separately and mortality have reduced by 1/6 [3]. Therefore nowadays aspirin is the first line therapy for cardiovascular and cerebrovascular diseases and one of the most widely prescribed oral anti-platelet medications which should be long-term use. It can effectively inhibit platelet aggregation and prevent blood coagulation and thrombosis [4]. However, the side effects especially gastrointestinal mucosal injury and bleeding happen more and more frequently in patients with long-term use or with origin gastrointestinal diseases [5]. Even more, Peptic ulcer incidence in old people with long-term use of aspirin increased year by year [6].10%–25% long-term user get peptic ulcer in spite of using enteric coated aspirin [7].Due to the gastrointestinal side effects caused by aspirin, the long-term use has been limited to a certain extent. This limitation poses a dilemma for the regular use of aspirin, and increases the risk of cardiovascular events.

Herb medicine like Panax Notoginseng(PN) has a long-term using history as a complementary and alternative treatment for cardiovascular diseases in China and worldwide [8,9]. Panax Notoginseng Saponins (PNS) is the main mixture of effective compounds in PN. It

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contains several saponins like ginsenoside Rb1 and ginsenoside Rg1.In the long history of Traditional Chinese Medicine(TCM) practice, PNS has been already shown to have multiple pharmacological activities. PNS has multiple function of improving hemodynamics, hemorheology, antithrombosis, and microcirculation [10,11]. Many preparations of PNS are widely used to prevent and treat cerebrovascular and cardiovascular diseases along with aspirin in China [12,13]. Good results in clinical practice have been achieved when the two medicines were taken together [14]. Interestingly, on one hand, PNS could enhance aspirin' s efficacy like inhibit platelet aggregation and prevent blood coagulation and thrombosis, also it could decrease side effect like aspirin-induced gastrointestinal injury [15]. Therefore, more and more clinicians are exploring combination rationality of PNS and aspirin to achieve effect of synergy and attenuation [16]. But it is still lack of support from clinical trial results about how to correctly combine PN and aspirin. Meanwhile, pharmacological mechanism of PNS on treatment for aspirin induced gastrointestinal injury is still unclear. There are several scientific questions like the interaction between aspirin and PN that need to be solved.

Network pharmacology is a new discipline based on the basic theories of systems biology. It conducts a comprehensive analysis of biological systems and further find the specific node with multiple targets. The network analysis based on widely existing databases allows us to form an initial understanding of the action mechanisms of medicine.In the present work, we develop a comprehensive network pharmacology-based approach to identify protective mechanism of PNS on aspirin induced gastrointestinal injury and verify the result by in vivo experiment.

2. Materials and methods

2.1. Candidate targets of drugs

The identities of the chemical ingredients in PNS were compiled in the TCMSP Databases (http://ibts.hkbu.edu.hk/LSP/tcmsp.php). This database is a unique systems pharmacology platform of Chinese herbal medicines that captures the relationships between drugs, targets and diseases. A total of 5 chemicals was collected. The targets of aspirin and PNS were obtained from Genecards Databases (http://www.genecards.org/). 746 of aspirin, 10 of notoginsenoside R1, 43 of ginsenoside Rg1, 140 of ginsenoside Rb1, 32 of ginsenoside Rd, 87 of ginsenoside Re were obtained. After screening targets (relevance score ≥ 1)and removing duplicate value, a total of 478 candidate targets of aspirin and 149 of PNS were collected.

2.2. Known therapeutic targets of drugs in the treatment of gastrointestinal injury

The known therapeutic targets of drugs used in the treatment of gastrointestinal injury were acquired from DisGeNET database (http://www.disgenet.org/). We searched 6 diseases related to gastrointestinal injury in the database.In total, 96 known therapeutic targets for treating gastric ulcer, 88 for treating gastritis, 68 for treating gastrointestinal Hemorrhage, 79 for treating duodenal ulcer, 43 for treating enteritis, 279 for treating inflammatory bowel diseases were chosen. After screening targets (relevance score ≥ 0.001) and removing duplicate value, a total of 473 targets related to gastrointestinal injury were collected and used for data analysis.

2.3. Network construction

The network construction was made by cytoscape software (http://www.cytoscape.org/). The procedure for network construction was as following: (1) the candidate compound-candidate target network(cC-cT network) was constructed by linking the compounds to their candidate targets. (2) potential compound-potential target network(pC-pT network) was established by connecting the potential compounds with their validated potential targets which are related to gastrointestinal injury.

2.4. Pharmacological verification on network analysis

2.4.1. Materials

PNS were obtained from Yunnan Baiyao Group Co., Ltd. PNS contents were determined as:Notoginsenoside R1 (NGR1), 6.9%; GinsenosideRg1 (GRg1), 28.0%;Ginsenoside Rb1 (GRb1), 29.7%; Ginsenoside Re (GRe), 3.8%;Ginsenoside Rd (GRd), 7.3%. Raw material medicine of aspirin were purchased from Xi'an Yue Lai Medicine Technology Co., Ltd. VEGFA Elisa kit was purchased from LiuHe Biological Technology (Wuhan, China LOT20170519003), BCA Protein Assay Kit (Keygen Biotech, Jiangsu, China, LOT 20171012).

2.4.2. Animals

Male Sprague-Dawley (SD) rats (200–220 g, 6–7 weeks old)were supplied by Vital River Laboratory Animal Technology Co. Ltd. (Beijing, People's Republic of China). Animals were housed in the specific-pathogen-free (SPF) facility at Beijing University of Chinese Medicine Laboratory. All the animal studies were performed under the Guidelines for the Care and Use of Laboratory animals and the experimental protocols were approved by the institutional animal experimentation committee of Beijing University of Chinese Medicine (BUCM-4-2017061327-2027).

2.4.3. Animal treatment and tissue preparation

Rats were acclimatized for 4 days before experiment. They were randomly allocated to control groups (n = 6),aspirin groups (n = 6)and aspirin + PNS groups (n = 6). Aspirin group underwent aspirin-induced gastrointestinal injury model by the oral administration of aspirin (0.5 g/kg body weight [17,18]) for 7 days. Aspirin + PNS groups were administrate orally with 31.25 mg/kg PNS and aspirin (0.5 g/kg body weight) for 7 days. Control group were administrate orally with water at the same time. Aspirin and PNS suspension were prepared by dissolving crude drugs and 0.3% sodium carboxymethylcellulose into purified water. The suspension was freshly prepared every day to avoid aspirin's natural degradation. After 7 days' administration, rats were executed, stomach and jejunum were taken.

2.4.4. Hematoxylin and Erosin (H&E) staining

Stomach and jejunum were taken, gently rinsed to remove contents. Segments of stomach and jejunum were fixed on a 4% paraformaldehyde solution for 48 h and embedded in paraffin. Then the samples were cut, deparaffinized, hydrated and stained with H&E. The histology of the stomach and jejunum was evaluated using a microscope (BX53; Olympus, Tokyo, Japan).

2.4.5. Enzyme-Linked Immunosorbent Assays (ELISA)

VEGF activity of stomach and small intestine was measured according to manufacturer recommendations of ELISA kit. 3 pieces of stomach(1 cm × 1 cm each, taken from different part of stomach) and 20 cm of small intestine(including 5 cm of duodenum, 10 cm of jejunum and 5 cm of ileum) were taken and homogenized respectively in RIPA solution(with 0.1 mM PMSF in it).The supernatant was taken after 15 min 1000xg centrifugation. Protein concentrations were determined. 20 μg of protein were used for ELISA measurement. Each tissue was added in 2 wells,take average for statistical analysis.

2.4.6. Statistics

Statistical analysis was completed using SPSS 20.0 (IBM). The VEGF activity of each group were compared using ANOVA with statistical significance set at $p < 0.05$.