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Synergistic effects of *chuanxiong-chishao* herb-pair on promoting angiogenesis at network pharmacological and pharmacodynamic levels

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Objective: The objective of the study is to investigate the synergistic effects of Chuanxiong-Chishao herb-pair (CCHP) on promoting angiogenesis *in silico* and *in vivo.*

Methods: The mechanisms of action of a herb-pair, Chuanxiong-Chishao, were investigated using the network pharmacological and pharmacodynamic strategies involving computational drug target prediction and network analysis, and experimental validation. A set of network pharmacology methods were created to study the herbs in the context of targets and diseases networks, including prediction of target profiles and pharmacological actions of main active compounds in Chuanxiong and Chishao. Furthermore, the therapeutic effects and putative molecular mechanisms of Chuanxiong-Chishao actions were experimentally validated in a chemical-induced vascular insufficiency model of transgenic zebrafish *in vivo*. The mRNA expression of the predicted targets was further analyzed by real-time polymerase chain reaction (RT-PCR).

Results: The computational prediction results found that the compounds in Chuanxiong have antithrombotic, antihypertensive, antiarrhythmic, and antiatherosclerotic activities, for hypoxic-ischemic encephalopathy, ischemic stroke, myocardial infarction and heart failure.In addition, compounds in Chishao were found to participate in anti-inflammatory effect and analgesics. Particularly, estrogen receptor α (ESRα) and hypoxia-inducible factor 1-α (HIF-1α) were the most important potential protein targets in the predicted results. In vivo experimental validation showed that post-treatment of tetramethylpyrazine hydrochloride (TMP•HCl) and paeoniflorin (PF) promoted the regeneration of new blood vessels in zebrafish involving up-regulating ESRα mRNA expression. Co-treatment of TMP•HCl and PF could enhance the vessel sprouting in chemical-induced vascular insufficiency zebrafish at the optimal compatibility proportion of PF 10 μmol/L with TMP•HCl 1 μmol/L.

Conclusions: The network pharmacological strategies combining drug target prediction and network analysis identified some putative targets of CCHP. Moreover, the transgenic zebrafish experiments demonstrated that the Chuanxiong-Chishao combination synergistically promoted angiogenic activity, probably involving ESRα signaling pathway.

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