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Full paper

Systems pharmacology dissection of the anti-stroke mechanism for the Chinese traditional medicine Xing-Nao-Jing

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ABSTRACT

Xing-Nao-Jing (XNJ) is a well-known injection that has been extensively applied in clinical treatment of stroke in China. However, the underlying mechanism of clinical administration of XNJ in stroke remains unclear. In this study, a systems pharmacology strategy based on pharmacokinetic and pharmacodynamics data was applied to analyze the pharmacological effect of XNJ on stroke. Sixteen active compounds were filtered from XNJ through Drug-likeness (DL) and Brain-blood-barrier (BBB) evaluations. Ninety-four potential targets of these active components were identified by SysDT and SEA. Biological process and pathway enrichment analyses of these targets demonstrated that XNJ exerted anti-stroke effects by biological processes and pathways, such as the response to oxidative stress, regulation of blood pressure, calcium signaling pathway, and apoptosis. Integrating the compound-target network and stroke-related PPI network, we found that Akt1, HIF-1α and ITGB2 may play key roles in the treatment of stroke. The experiments demonstrated that oxycurcumenol may prevent PC12 cells from oxidative stress-induced cell damage. Our study indicates that XNJ has an effect on stroke by protecting neuro cells from oxidative stress-induced cell damage via HIF1α, and the research strategy at the systems pharmacology level is feasible to reveal the mechanisms of novel lead compounds from natural products.

1. Introduction

According to the World Health Organization, a stroke, also called a cerebrovascular accident, is a sudden ischemic or hemorrhagic interruption in the blood flow supplying oxygen and nutrients to the brain tissue.[1] This event leads to brain cell death and, consequently, partial loss of neurological function.[2] As a debilitating neurological impairment, the incidence of stroke has been progressively increased. Currently, stroke is a major cause of death in adults, and, annually involves approximately 22 million people worldwide.[3] Additionally, based on the epidemiologic data, approximately 60% of patients who survive after stroke will struggle with mental and physical disability.[4–6] Therefore, the most stroke patients develop long-term disabilities, leading to serious social and economic impacts. Additionally, well-founded therapy options to tackle stroke are urgently needed.

A significant shift from chemically synthesized drugs to naturally active compounds is being witnessed. Therefore, natural

Abbreviations: XNJ, Xing-Nao-Jing; DL, Drug-likeness; BBB, Brain-blood-barrier; PPI network, Protein–protein interaction network; siRNA, Small interfering RNA; HIF, Hypoxia-inducible factor; AKT, RAC-alpha serine/threonine-protein kinase; ITGB, Integrin beta; CNS, Central nervous system; NSPC, Neural stem and progenitor cells; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; SEA, Similarity ensemble approach; rtPA, recombinant tissue plasminogen activator; KEGG, Kyoto Encyclopedia of Genes and Genomes; IL 6, Interleukin-6; CXCL2, C–X–C motif chemokine 2; SH2, Src-homology 2-like; HCS, high content screening.

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products become the major sources of chemical diversity for starting materials while driving pharmaceutical discovery.[7] Interestingly, Xing-Nao-Jing (XNJ), a Chinese injection, has been widely used in clinical practice for stroke treatment.[8] Despite the attractiveness of natural products, technologies that convert them into drugs are still limited, awaiting the discovery of methods sufficient to increase their understanding. As such, there is a pressing need to explore, at a molecular level, the targets, pathways, networks, and mechanisms of XNJ that prevent the damage and death of CNS tissue following an ischemic brain event.

To achieve specific mechanism-based discovery of drugs, the tools of systems pharmacology will be integrated using pharmacodynamics and pharmacokinetics.[9] Success of this effort assumes that a systems network that consists of drug-induced perturbations of physiological functions can be characterized.[10] In this work, based on systems pharmacology, we first constructed the compound database of XNJ and filtered the active molecules through pharmacokinetics evaluation; subsequently, we predicted targets for active molecules using SysDT[11] and the similarity ensemble approach (SEA)[12]; next, to screen the key targets of stroke, we mapped the targets of XNJ to the newly built stroke's gene–gene network; finally, the targets closely related to stroke are validated experimentally to demonstrate the potential of network analysis. This process would contribute to developing drugs from natural products and clarifying their molecular mechanisms in clinical treatment.

2. Materials and methods

2.1. Materials

PC12 cells (ATCC, CRL-1721), CellTiter 96 one solution cell proliferation assay (Promega, G5430), the BrdU and Ki-67 Cell Proliferation Reagent Kit (Thermo Scientific, USA), HIF1α-siRNA (Santa Cruz, sc-45919), HIF1α (Santa Cruz, USA), 1,7-diphenyl-3-acetoxy-6(E)-hepten, oxycurcumenol and beta-sitosterol were generously donated by Professor Xuejun. Li (Peking University, China).

2.2. Construction of a chemical database of XNJ

All the ingredients of XNJ are extracted from the literature and the Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP, available online: http://lsp.nwsuaf.edu.cn/tcmsp.php).[13]

2.3. Pharmacokinetic evaluation

The drug-likeness (DL) index is computed for every ingredient in XNJ using the Tanimoto coefficient.[14] This equation has been successfully applied to evaluate the drug likeness of compounds from natural products.[15,16] The permeation of compounds in XNJ across the blood brain barrier permeability (BBB) is examined by a partial least squares discriminant analysis.[17] The threshold values of the DL and BBB models for bioactive compounds screening are, respectively, DL $\geq$ 0.18[18] and BBB $\geq$ 0.3.[19] Additionally, molecules that simultaneously meet the above two conditions are nominated as bioactive compounds for further analysis.

2.4. Target identification

To identify the target proteins of bioactive compounds in XNJ, we applied a combinatorial approach that integrates the SEA and SysDT methods, which have been successfully applied to the target prediction of Reduning injection[20] and Xinnaoxin pill [21]. To normalize the targets obtained from different sources, we further mapped them to the UniProt database (http://www.uniprot.org/).

2.5. Stroke-related network construction and analysis

We constructed the "stroke-related protein–protein interaction (PPI) network" and analyzed it. The details are as follows[1]: the human PPI data from BIND, BioGRID, DIP, HPRD, IntAct, MINT, MIPS, PDZBase, Reactome Databases and STRING Database were collected to build a comprehensive background network[2]; stroke-related proteins were exploited from the TCMSP database and DisGeNET[3]; stroke-related proteins were mapped to the human PPIs to construct stroke-related PPI network[4]; we clustered the stroke-related PPI network using the Markov Clustering (MCL) algorithm, which is implemented by Cytoscape[22] plugins of cluster Maker 2 (http://apps.cytoscape.org/apps/clustermaker2)[5]; for each cluster, the biological functions were annotated utilizing biological processes of gene ontology (GO) enrichment analysis in the Database for Annotation, Visualization and Integrated Discovery (DAVID)[6,23]; To investigate the relativity of the XNJ's targets for the stroke-related proteins, we defined the nearness between XNJ's targets p and stroke-related proteins p' based on the PPI network by the expression:

Where pi represents the herbal medicine target, p'j is the 'Basic Depression Pathway' related protein, and Dpip'j is the shortest distance between pi and p'j on the PPI network. n and m, respectively, represents the number of XNJ targets p and stroke-related proteins p', which can be mapped on the PPI network. If two proteins are unconnected on the PPI network, the Dpip'j is defined as $+\infty$.

2.6. Cell culture and reagents

PC12 cells were cultured in DMEM containing special supplements as previously described. The cells were pretreated with 1,7-diphenyl-3-acetoxy-6(E)-hepten (1, 5, 10 μmol/L), oxycurcumenol (1, 5, 10 μmol/L) and beta-sitosterol (0.01, 0.05, 0.1 μmol/L) to represent XNJ,[14] supplemented with 2% FBS for 24 h, after which the medium was removed and replaced with medium containing 2% FBS and 200 μmol/L H_2O_2. After an additional 4 h of incubation, the cells were assessed as described subsequently.

2.7. Cell viability assay

Cell viability was determined by the colorimetric assay with MTS. Briefly, while being cultured on 96-well culture plates, cells were pretreated with various concentrations of 1,7-diphenyl-3-acetoxy-6(E)-hepten, oxycurcumenol and beta-sitosterol for 24 h, followed by treatment with H_2O_2. To measure cell viability, 10 μL of MTS assay solution was added to 100 mL of DMEM medium per well, the plates were incubated for 3 h, and then the optical densities of the wells were read on a microplate reader (Molecular Device). The percentage of living cells was calculated by the ratio of the optical density of the experimental wells to that of the normal wells.

2.8. Multiparametric proliferation assay by high content screening (HCS)

The BrdU and Ki-67 Cell Proliferation Reagent Kit was used for simultaneous quantification of four fundamental parameters associated with cell proliferation: cell number, DNA content, BrdU incorporation and Ki-67 expression in the same cell. The cell number and DNA content are quantified with DAPI staining. BrdU and Ki-67 are determined with primary antibodies toward them

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