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Fusion of core pathways reveals a horizontal synergistic mechanism underlying combination therapy

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ABSTRACT

Combination therapies have recently been shown to be more effective than monotherapies that may provide synergistic effects in the treatment of stroke, but its selective mechanism still remains unclear. Based on the median-effect method, the combination therapy of jasminoidin and ursodeoxycholic acid had a synergic effect on reducing the infarct volume. The numbers of up- or down-regulated genes by at least 1.5-fold in the vehicle, jasminoidin, ursodeoxycholic acid, and the combination of jasminoidin and ursodeoxycholic acid treatment groups were 228, 95, 136, and 101, respectively. According to clustering and principal component analysis, the pattern of gene expression in the combination group was similar to that of jasminoidin group rather than ursodeoxycholic acid group. Based on these nine top sequences in the combination group excluding four overlapping pathways (MAPK-ERK, Kitlg, Icam1-Ap1, and prolactin), the jasminoidin group had four (PRLR-STAT1, AcvR2-AcvR1B, ACVR1/2A-SMAD1, GHR-NF-κB) contributing pathways, and the ursodeoxycholic acid group had one (IL-6) contributing pathway. Based on the multiple-pathway-dependent comparison analysis (MPDCA), it may lead to the conclusion that jasminoidin possibly contributes more important pharmacological effect in the combined treatment as jasminoidin regulated 80% of the pathways that the combination group mediated. The study reveals a horizontal synergistic effect by optimizing the fusion of more pathways from the compounds with more contribution to the combination therapy. Rather than selecting compounds only based on experience in the past, this study would give a new insight into the systematic strategies for designing synergistic combination therapies.

1. Introduction

Stroke remains a major cause of death and disability worldwide. The pathophysiology of stroke is highly complex. The molecular events that mediate ischemic brain damage, including glutamate accumulation, aberrant calcium fluxes (Stanika et al., 2010), free radical formation (Allen and Bayraktutan, 2009), and lipid peroxidation (Gaur and Kumar, 2009), are logical targets for pharmacological intervention. However, disappointing outcomes highlight the limitations of the single-target drug paradigm (Hiroaki, 2007). Combination therapies have recently been shown to be more effective than monotherapies that may provide synergistic effects in the treatment of cardiovascular diseases (Sleight et al., 2006). Analysis of the 117 drug combinations identified general and specific modes of action (Jia et al., 2009); developing a strategy for determining the most promising combinations and prioritizing their evaluation is crucial and remains a major challenge (Black and Sang, 2005).

It seems unlikely that a single pathway is sufficiently critical to define an outcome on its own. One promising alternative is to use combination therapy to provide neuroprotection in cerebrovascular surgery (Zhang et al., 2006). The discovery of new neuroprotective agents has spurred efforts to understand the intracellular signaling pathways that mediate the cellular response to stroke and to identify the mechanism to this response. It is thought that such a mechanism would fit within the concept that several brain injury pathways must be inhibited to optimize therapeutic efficacy (Zanelli et al., 2005). The simultaneous or sequential action on targets of different related pathways represents horizontal synergistic and additive interactions, such as those of fludioxonil with FK506 (Chen et al., 2006) and ondansetron with droperidol (Chan et al., 2006). Vertical synergistic and additive interactions are represented by interactions between terbinafine with azoles (Perea et al., 2002) and ampicillin with

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imipenem (Fuda et al., 2004). Although several tools for visualizing these interactions graphically and for analyzing the biological data have been developed, the numbers of core pathways that are affected simultaneously in a single-compound intervention and the variations in combination therapy responsible for a global phenotype change remain unclear.

Jasminoidin and ursodeoxycholic acid, the chemical structures of which are shown in Fig. 1, are two active ingredients in Qingkailing which is a herbal formula clinically used to treat stroke in China. In PubMed from January 1, 2000 to January 1, 2010, a total of 133 articles on jasminoidin and 1536 articles on ursodeoxycholic acid have been published. Based on the number of articles, the top four pharmacological actions of jasminoidin are presented as neuroprotective agents, cholagogues, enzyme inhibitors, and anti-inflammatory agents. And the top four pharmacological actions of ursodeoxycholic acid are presented as cholagogues, immunosuppressive agents, gastrointestinal agents, and anti-inflammatory agents. Jasminoidin has been shown to have a preventive effect against ischemic stroke by promoting the expression of brain-derived neurotrophic factor (BDNF) and inhibiting the expression of caspase-3 (Zhang et al., 2006). Jasminoidin is also a newly identified agonist of glucagon-like peptide-1 (GLP-1) receptor, which protects PC12 cells from oxidative damage via the mitogen-activated protein kinase (MAPK) pathway (Liu et al., 2007). Ursodeoxycholic acid has been reported to have cytoprotective and antioxidative properties (Brito et al., 2008; Yasukawa et al., 2009). In this study, we used the multiple-pathway-dependent comparison analysis (MPDCA) to explore the various potential core pathways in ischemic mouse hippocampal cells treated with jasminoidin, ursodeoxycholic acid, or the combination of both.

2. Material and methods

2.1. Animal model

Animal experiments were performed in accordance with the Prevention of Cruelty to Animals Act 1986 and the National Institutes of Health guidelines for the care and use of laboratory animals for experimental procedures, and were approved after review by a local committee. One hundred seventy adult male mice (3 months old, 38–48 g, Kunming strain, China) were divided into 5 groups of 34 mice each. Focal cerebral ischemia–reperfusion model was induced in mice anesthetized with 2% pentobarbital (4 mg/kg, i.p.) by occluding the left middle cerebral artery with an intraluminal filament as described (Hara et al., 1996). The middle cerebral artery was exposed and ligated for 1.5 h using an intraluminal filament and then reperfused for 24 h. The sham-operated mice underwent identical procedures without middle cerebral artery occlusion.

2.2. Drug administration

Experimental animals were randomly divided into 5 groups: sham-operated mice; ischemic mice receiving one of the three herbal preparations at a dose of 2 ml/kg [25 mg/ml jasminoidin, 7 mg/ml ursodeoxycholic acid, or the combination of jasminoidin and ursodeoxycholic acid with a ratio of 1:1)]; or vehicle-treated mice (0.9% NaCl, 2 ml/kg). The herbal preparation or vehicle was given by intravenous injection in the tail vein once a day. The herbal preparations were a chemically standardized product from China Natural Institute for the Control of Pharmaceutical and Biological Product or Beijing University of Traditional Chinese Medicine, and its composition was validated using fingerprint chromatographic methodologies. These preparations were dissolved in 0.9% NaCl just before use. Jasminoidin and ursodeoxycholic acid at concentrations of 1, 2, 4, 8, and 16 mg/ml were combined (1:1) in equal volumes to analyze the synergistic effect using CompuSyn software (ComboSyn, Inc. USA). The combination index (CI), a quantitative measure based on the mass-action law of the degree of drug interaction in terms of synergism and antagonism for a given endpoint of the effect measurement (Chou and Talalay, 1981), was calculated.

2.3. Histological analysis

After 24 h reperfusion, 6 animals from each group were anesthetized with chloral hydrate (400 mg/kg). The brain was perfused immediately with 37 °C saline through cannulation of the aorta. The blood was washed out, and the brain was then perfused with cold 4% formaldehyde for 30 min to induce polymerization. The brain was removed and post-fixed in 4% formaldehyde for at least 24 h, embedded in mineral wax, sectioned coronally into 5-μm slices, and stained with thionine. And the hippocampal CA1 region was selected for observation.

2.4. 2, 3, 5-Triphenyltetrazolium chloride (TTC) staining

The infarct ratio after the 24 h reperfusion was calculated in another 13 mice from each group. In brief, the cerebrum was removed and cut into five slices in the coronal plane 1, 3, 5, and 7 mm from the prefrontal cortex. The slices were transferred to 4% TTC solution and incubated for 30 min at 37 °C in darkness and then transferred into 10% formalin. Images of the slices were captured using a digital camera (Color CCD camera TP-6001A, Topica Inc., Japan). The area of the infarct region was calculated using a Pathology Image Analysis System (Topica Inc.), and the ratio of the infarct volume to the total slice was calculated.

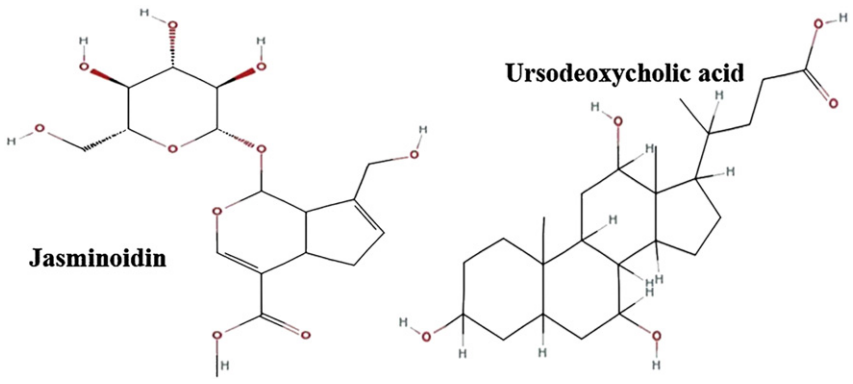

Fig. 1. The chemical structure of jasminoidin and ursodeoxycholic acid.