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The multi-target capabilities of the compounds in a TCM used to treat sepsis and their *in silico* pharmacology

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Natural products;
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Summary
Objective: This study aims to explain the mechanisms at the molecular level of a traditional Chinese medicine (TCM) for the treatment of sepsis.
Methods: We first identified 16 targets involved in the sepsis disease network; then we constructed a molecular ligand database and investigated the effects between the ligand database and the sepsis targets using computational biology methods. The results of the calculation were validated with *in vitro* biological testing against bovine thrombin.
Results and conclusion: We found that multiple active compounds contained in the TCM interact with multiple sepsis-related targets. We predicted several promising compounds for sepsis treatment, and the first 10 compounds were characterised. Among those tested, rosmarinic acid displayed the strongest biological activity in the *in vitro* activity test with a half-maximal inhibitory concentration (IC_{50}) of 85 μM. This study demonstrates a novel way of identifying naturally occurring chemical entities as new leads for sepsis treatment.

Great progress has been made in the development of antimicrobial therapeutics, but sepsis is still one of the leading causes of death worldwide, and sepsis-associated mortality rates remain unacceptably high.[1–3] Despite extensive basic research and clinical studies, the pathogenesis and pathophysiology of sepsis are still poorly understood because of its complications.[4] There is no specific remedy for the disease despite the wide choice of effective antibiotic prophylaxis, immunisations or other treatments for infection and immune system abnormalities, and sepsis still remains the leading cause of morbidity and mortality for patients admitted to an intensive care unit (ICU).

Sepsis treatments are widely perceived as an unmet medical need[2,3] and the TCM (trade name Xuebijing, China drug

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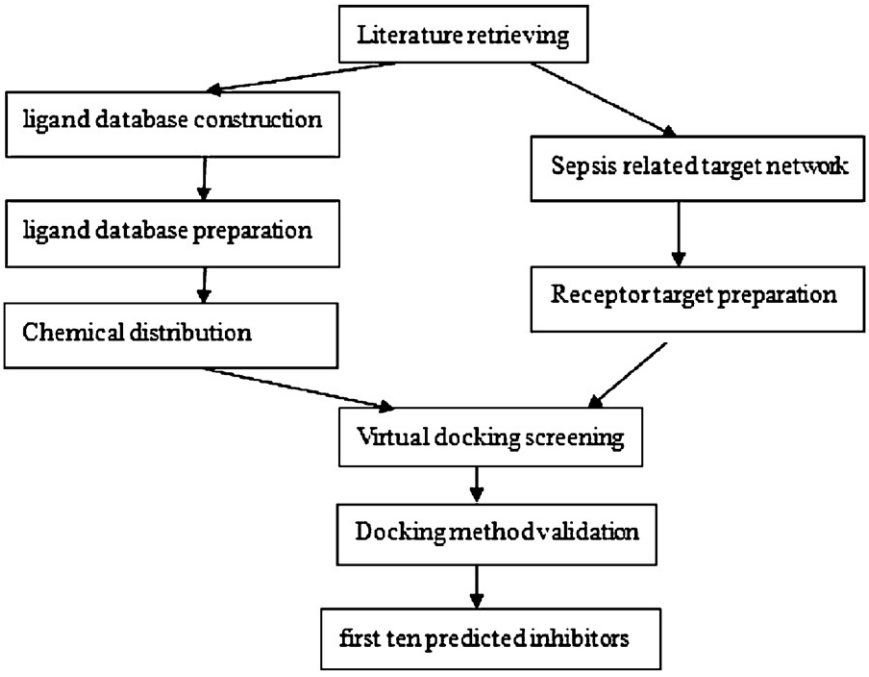

Figure 1 Schematic workflow of the procedure.

No. Z20040033) from plant sources is effective at relieving complicated disease when used singly[5] or in combination with drugs such as ulinastatin[6] and methylprednisolone.[7] Because the bioactive compounds in the TCM may target multiple protein receptors[8] in the sepsis biological network, the disease network would attain a new equilibrium to reduce the harmful impacts. The TCM is prepared as a mixture of Chinese herbs consisting of Flos Carthami (from Chinese honghua, FC for short), Radix Salviae (from Chinese danshen, RS for short), *Paeonia anomala* L. (from Chinese chishao, PA for short), Rhizoma Ligustici chuanxiong (from Chinese chuanxiong, RL for short), and Radix Angelicae sinensis (from Chinese danggui, RA for short). This TCM is widely used in China for the treatment of sepsis and has shown satisfactory anti-endotoxin and anti-inflammatory effects in clinical trials (50—100 ml, intravenous drip twice a day for 7 days). It has improved the quality of life of sepsis patients and reduced the morbidity and mortality rates.[5–7,9] As for the major constituents, approximately 21 compounds, including several amino acids, flavonoid glycosides, phenolic acids, terpene glycosides and phthalides, were identified or tentatively characterised,[10] among which the concentration of hydroxysafflor yellow A, paeoniflorin, ferulic acid, benzoic acid and danshensu had been determined by high-performance liquid chromatography (HPLC).[11]

Most research efforts have focussed on the therapeutic effects of individual TCM components on a single factor, such as interleukin-1 (IL-1). Meanwhile, there has been limited study on the entire sepsis network, and the modes of action of the TCM have not been clearly defined. To date, no reports have identified all of the constituents of the disease network due to difficulties in lab work and clinical trials. *In silico* pharmacology is a computing method which predicts the preferred orientation of a molecule to a certain target receptor (just like a key to a lock). It deals with the quick search of large libraries of chemical structures in order to identify those structures which are most likely to bind to a drug target. The method is thought to have the potential to speed up the rate of discovery while reducing the need for expensive lab work and clinical trials by producing and screening drug candidates more effectively.

In a previous study,[12] we constructed the three-dimensional (3D) structure of 5-lipoxygenase(5LOX) using homology modelling and found that several molecules contained in the TCM inhibited inflammation targets such as cyclooxygenase 2(COX-2) and 5LOX. The aim of this present study was to investigated the *in silico* pharmacology of all the components and the sepsis target network. Biological tests were performed to validate the computational models. This novel method could be a powerful tool for combinatorial drug discovery and the development of multi-targeted drugs.

Methods

Construction and preparation of the compound ligand database

The workflow is shown in Fig. 1. First, 343 compounds were identified in the five herbs FC, RS, PA, RL and RA using the chemistry database of the Chinese Academy of Sciences (http://202.127.145.134/scdb), Dr. Duke's Phytochemical and Ethnobotanical Database (http://sun.ars-grin.gov:8080/npgspub/xsql/duke/super.xsql) and the recent literature[10,13–17] (from 2004 to 2012) about these five herbs. Our database contains the most compounds found in the TCM to date.

Schrodinger maestro (version 8.5) was employed to add hydrogen atoms and adjust the bond directions, and the