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A novel systems pharmacology platform to dissect action mechanisms of traditional Chinese medicines for bovine viral diarrhea disease

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ABSTRACT

Due to the large direct and indirect productivity losses in the livestock industry caused by bovine viral diarrhea (BVD) and the lack of effective pharmacological therapies, developing an efficient treatment is extremely urgent. Traditional Chinese medicines (TCMs) that simultaneously address multiple targets have been proven to be effective therapies for BVD. However, the potential molecular action mechanisms of TCMs have not yet been systematically explored. In this work, take the example of a herbal remedy Huangqin Zhizi (HQZZ) for BVD treatment in China, a systems pharmacology approach combining with the pharmacokinetics and pharmacodynamics evaluation was developed to screen out the active ingredients, predict the targets and analyze the networks and pathways. Results show that 212 active compounds were identified. Utilizing these lead compounds as probes, we predicted 122 BVD related-targets. And in vitro experiments were conducted to evaluate the reliability of some vital active compounds and targets. Network and pathway analysis displayed that HQZZ was effective in the treatment of BVD by inhibiting inflammation, enhancing immune responses in hosts toward virus infection. In summary, the analysis of the complete profile of the pharmacological activities, as well as the elucidation of targets, networks and pathways can further elucidate the underlying anti-inflammatory, antiviral and immune regulation mechanisms of HQZZ against BVD.

1. Introduction

Bovine viral diarrhea (BVD), caused by bovine viral diarrhea virus (BVDV) (Carman et al., 1998), is an acute, highly contagious disease usually along with the occurrence of fever, diarrhea, pneumonia, mucosal lesions, or even sudden death. The prevalence of BVDV infection is not only determined in calves but also in other ruminant species such as pigs and sheep, which poses a serious impact on livestock (Carman et al., 1998). Western medicine has studied BVD for many years since it was originally described in 1946 (Houe, 1995), however, people continue to face challenges in treating BVD. Although vaccination (modified-live or killed) programs can provide some protection from BVD and the development of persistently infected fetuses, it alone cannot control or eliminate BVDV (Brock, 2003). Antiviral therapy, such as the administration of ribavirin, is effective to control BVDV (Buckwold et al., 2003; Ouzounov et al., 2002), but this therapy yields significantly higher sustained virologic responses (approximately 40%) (Gutfreund and Bain, 2000). In recent years, a large number of therapies have been investigated in attempt to fight BVD, however, it's still difficult to achieve the most ideal treatment effect.

Traditional Chinese medicines (TCMs) are effective to relieve complicated diseases in a multi-target/multi-component manner, which makes them unique among all traditional medicines (Qiu, 2015). And TCMs have been applied to the livestock industry for at least 1000 years (Stogdale, 2008). For instance, a series of formulas like wei-cang-san, jian-pi-san, sheng-yang-yi-wei tang, have been used for the treatment of different types of chronic diarrhea in horses (Xie et al., 1997). Note that Huangqin Zhizi (HQZZ) formula, a widely used herbal formula in Chinese medicine for BVD treatment, is applied in

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this work. This formula is consisted of 10 different herbs, *Radix Scutellariae* (RS., Huang-qin), *Cortex Fraxini* (CF., Qin-pi), *Cape Jasmine* (CJ., Zhi-zi), *Rheum Officinale* (RO., Da-huang), *Cortex Moutan* (CM., Mu-dan-pi), *Raw Rehmanniae Radix* (RRR., Sheng-di-huang), *Terminalia Chebula Retz* (TCR., He-zi), *Pomegranate Rind* (PR., Shi-liu-pi), *Cortex Magnoliae Officinalis* (CMO., Hou-po) and *Fructus Aurantii* (FA., Zhi-qiao). Previous research has shown that the effective rate of HQZZ for cow diarrhea is 95% and cure rate is 75% in vivo (Wang et al., 2010). Meanwhile, HQZZ also plays a vital role in fighting against BVD by directly inhibiting BVDV in vitro (Yang et al., 2009). Although HQZZ has been proven to be dramatically efficient in curing BVD, the fundamental molecular action mechanisms are still not systematically explored. The bioactive compounds, the potential targets and the related pathways of HQZZ remain unknown. The advancement of analytical tools including systems biology (Kitano, 2002), network biology (Barabasi and Oltvai, 2004) and network pharmacology (Hopkins, 2007, 2008) potentially offer an attractive way to elucidate the intricate and holistic mechanisms of Chinese herbal formula in treating BVD.

Herein, combining with pharmacokinetic (the absorption, distribution, metabolism, excretion (ADME) properties of drugs) evaluation, as well as pathway and network analysis (Huang et al., 2013), systems pharmacology offers a platform for identifying multiple mechanisms of action of herbal veterinary medicines. In our previous work, we have constructed a systems-pharmacology-based method which is specially designed for drug discovery and therapeutic insight for herbal medicines (Li et al., 2014; Wang et al., 2013). Thus, in order to resolve the underlying action mechanisms of herbal medicines in the treatment of livestock diseases, we urgently need to introduce the method of systems pharmacology.

In this study, we employed a modified systems-pharmacology method to probe the anti-BVDV mechanisms of HQZZ. Firstly, we filtered active compounds from the constructed HQZZ molecular database by calculating pharmacokinetic properties and evaluating their drug-likeness. Then, the potential targets were predicted by our newly constructed cross-species drug–target interaction assessment model (CSDT). And the obtained targets were validated by Gene Ontology enrichment analysis and target-disease interactions analysis. Finally, the acquired pharmacological data were further integrated into compound-target and target–pathway network. The systems pharmacology approach framework for the present work is shown in Fig. 1.

2. Materials and methods

2.1. Molecular database construction

We manually collected all molecules of HQZZ from our in house database TCMSP: Traditional Chinese Medicines for Systems Pharmacology Database and Analysis Platform (http://lsp.nwsuaf.edu.cn/tcmsp.php) (Ru et al., 2014). Finally, a total of 237 molecules with 32 in RS., 7 in CF., 35 in CJ., 22 in RO., 34 in CM., 24 in RRR., 21 in TCR., 20 in PR., 41 in CMO., and 57 in FA. were obtained in this study. Besides, because glycosides are usually hydrolyzed to liberate aglycone which is then absorbed at intestinal mucosa (Németh et al., 2003), the corresponding 74 aglycone chemicals of glycosides in herbs were also added into the molecular database for HQZZ. The information of the total 311 compounds are shown in Table S1.

2.2. Drug-likeness evaluation

Drug-likeness (DL) is a qualitative concept used in drug design for an estimate on how "drug-like" a prospective compound is, which helps to optimize pharmacokinetic and pharmaceutical properties, such as solubility and chemical stability (Vistoli et al., 2008). In this work, Tanimoto Similarity (TS) is used to select out compounds which are considered to be chemically suitable for drugs (Yamanishi et al., 2010) between herbal ingredients and the average molecular properties of all veterinary drugs in FDA (Wishart et al., 2006). The TS index is defined as following:

$$T(A,B)=\frac{A\cdot B}{\|A\|^2+\|B\|^2-A\cdot B} \quad (1)$$

where A represents the molecular descriptors of herbal compounds, B represents the average drug-likeness index of all veterinary drugs in FDA. In this study, compounds with DL ≥ 0.15 were selected as the candidate bioactive compounds, because the mean DL value for all veterinary drugs in FDA is 0.15.

2.3. Comparison of active compounds and randomly selected compounds based on chemicals

The "Lipinski's rule" for DL defines five simple physicochemical parameters: molecular weight (MW), octanol–water partition coefficient (Mlog P), H-bond donors (nHDon), H-bond acceptors (nHAcc), and number of rotatable bonds (RBN), which serves as a very efficient guideline for orally bioavailable small-molecule drug discovery. In order to validate the efficiency of the potential active compounds, herbal ingredients comparison based on the above chemical properties between the screened active compounds in Section 2.2 and the randomly selected equal number TCMSP compounds (Table S2) that do not overlap with the active compounds were performed. These five important pharmacology-related parameters were calculated by the DRAGON software (version 5.6) (Todeschini et al., 2003).

2.4. Target fishing and analysis

2.4.1. Drug-targeting

Firstly, an in silico CSDT model was applied in this work to derive the target information of active ingredients (Zheng et al., 2016). The details of the CSDT model were provided in Supplementary methods. Then, taking the obtained candidate targets as queries, targets from bovine were preserved by searching Uniprot (http://www.uniprot.org) database. Finally, the targets were mapped to Therapeutic Target Database (TTD, http://database.idrb.cqu.edu.cn/TTD/), Comparative Toxicogenomics Database (CTD, http://ctdbase.org/), Pharmacogenomics Knowledgebase (PharmGKB, https://www.pharmgkb.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp/) to obtain their corresponding diseases, providing a well-defined target-disease network.

2.4.2. GOBP enrichment analysis for targets

In order to further probe the meaningful functional annotation of our achieved targets, in this work, Gene Ontology (GO) enrichment analysis was performed by linking the targets to DAVID (The Database for Annotation, Visualization and Integrated Discovery, http://david.abcc.ncifcrf.gov). The controlled vocabularies with GO can describe genes and gene products in living organisms (Shah et al., 2003). The terms from "Biological Process" (GOBP), which is one of the three broad GO categories (the other two being "Molecular Function" and "Cellular Component"), were utilized to symbol gene function. Only GO terms with p-value ≤ 0.05 were selected. FDR (the false discovery rate) was introduced to perform a multiple-hypothesis testing error measure of p-values by using the web tool DAVID, we used a 0.05 FDR criterion as a significance cutoff in our analysis.

2.4.3. Experimental validation

In order to validate the accuracy and efficiency of the CSDT model, we constructed in vitro experiments to further validate the inhibitory effects of compounds on their predicted targets. 2 key and commercially available targets were selected. The inhibitory effects of targets PDE (Cyclic Nucleotide Phosphodiesterase), and SDHA (flavoprotein subunit of complex II) were assayed using Cyclic Nucleotide Phosphodiesterase