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Strategy of integrated evaluation on treatment of traditional Chinese medicine as 'interaction of system to system' and establishment of novel fuzzy target contribution recognition with herb-pairs, a case study on Astragali Radix-Fructus Corni

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ABSTRACT

To date, in the struggle against diseases and the development of TCM, what we lack is wisdom rather than knowledge. Studies on pharmacology of traditional Chinese medicine are facing critical challenges on how to select the proper parameters or targets to represent the pharmacological evaluation system. With seven steps of optimized modules established by ourselves, we can re-evaluate TCM in a panorama view with a proper pharmacological evaluation system. In this article, with the treatment of TCM as 'interaction of system to system', a novel and generally applicable approach called fuzzy target contribution recognition was established and agents from Astragali Radix-Fructus Corni in resisting diabetic nephropathy were successfully discovered for the first time. CG_6, a promising agent from this herb-pair on the treatment of diabetic nephropathy, was finally acquired and its possible molecular mechanism was explored through a nuclear factor erythroid 2-Like 2 (NFE2L2) activation-dependent pathway.

1. Introduction

Diabetic nephropathy (DN), a severe renal disease arisen from renal capillary damage, is an important and common complication of both type 1 and type 2 diabetes leading to end-stage renal disease (ESRD), which has resulted in a high morbidity and mortality both in teenagers and elderly persons throughout the world irrespective of underlying causes (Kato and Natarajan, 2014; Brenner et al., 2001; Friedman, 2006). It is important to note that DN has become a growing global threat since the rapid increase of patients with diabetes in both developed and developing countries. Apparently, DN has been usually characterized by polydipsia, polyuria, polyphagia, and weight reduction, which has typically been referred to "a little as three more district" symptom in China. Several studies have shown that oxidative stress, the result of excessive production of reactive oxygen species (ROS) playing a

Abbreviations: TCM, traditional Chinese medicine; DN, diabetic nephropathy; ESRD, end-stage renal disease; ROS, reactive oxygen species; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; UAER, urinary albumin excretion rate; GBM, glomerular basement membrane; Keap1, Kelch-like ECH associating protein 1; SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; FBG, Fasting blood glucose; TC, total cholesterol; TG, triglyceride; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; Ucr, urinary creatinine; Scr, serum creatinine; BUN, blood urea nitrogen; Ccr, creatinine clearance; UAER, urinary albumin excretion rate; WT1, Wilm's Tumor 1 protein; ILK, integrin-linked kinase.

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crucial role in the development and progression of DN, can induce mitochondrial dysfunction, decline adenosine triphosphate, and eventually lead to DN (Goldfine, 2008). Furthermore, oxidative stress is a major factor of diabetic glomerular injury by increasing insulin resistance or impairing insulin secretion and contributes to the diabetic complications including nephropathy (Annuk et al., 2001; Oberg et al., 2004). More importantly, accumulating evidences in recent years have revealed that inflammation plays a vital role in the progression of microvascular diabetic complications such as DN (Impellizzeri et al., 2014; Chawla et al., 2007). Consistently, a large number of clinical and animal studies have shown the increased macrophage infiltration and leukocyte adhesion molecules in DN. It is worth to note that interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) are all important inflammatory biomarkers involving in the development of DN. Furthermore, over the past decade, there have been important advances in understanding the pathogenesis of DN, with particular focus on hyperglycemia and hyperlipidemia, which are common in DN patients (Wiseman et al., 1985; Danaei et al., 2011). Numerous reports have shown that good blood glucose and serum lipid control can prevent DN. Additionally, the renal functional parameters are closely related with the structural changes, such as 24-h urinary protein and 24-h urinary albumin excretion rate (UAER), which make a definite diagnosis to DN. In particular, podocytes having a central role in maintaining the structure and function of the glomerular filtration barrier are terminally differentiated cells reside on the outer surface of the glomerular basement membrane (GBM). In recent years, a growing number of people have agreed that DN podocyte injury greatly contributes to the deterioration of renal function (Yu et al., 2013; Petermann et al., 2003; Reddy et al., 2008).

Currently, there are no ideal therapies of DN available around the world, which make it essential to discover anti-DN agents. Our previous investigations found that the pathogenesis of DN is extremely complex and piles of evidences showed that multiple pathogenic factors, including “a little as three more district” symptom, oxidative stress, inflammation, hyperglycemia, hyperlipidemia, renal functional parameter, and podocyte injury, contribute to the progression of renal damage in DN and ultimately cause glomerular and tubular mesangial matrix expansion and glomerulosclerosis as well as interstitial fibrosis in mesangium and interstitium. As is well-known, traditional Chinese medicine (TCM) has played a pivotal role in the treatment of numerous complex diseases with its specific advantages and characteristics of multi-component and multi-target or a single, highly connected target. Nevertheless, it has been reported that the pharmacological research on DN is still immature and is facing challenges on the ambiguous ‘one drug, one target’ drug discovery paradigm or just ‘one drug with two or three solitary targets’. It is quite difficult to investigate the pharmacology of a certain TCM on just one or two targets individually since a great amount of targets were involved in the pathogenesis of DN. Thus, the development of a rational approach that could be utilized in evaluation of the pharmacology of a certain TCM on DN becomes necessary.

In present study, our purpose is to develop and establish a novel generally applicable approach and methodology for pharmacological research of extremely complex diseases. In this article, we choose DN, an extremely complicated disease, as a case to evaluate the pharmacology of a certain TCM by a novel approach called fuzzy target contribution recognition strategy (Fig. 1). However, due to the lack of rational pharmacological evaluation system, current pharmacological research on TCM is mainly restricted to one or a few solitary targets. We investigated on multiple targets involved in the pathogenesis of DN and calculated the total contributions of these fuzzy targets rather than a specific target. Additionally, compatibility is another pith of TCM and we plan to explore the best proportion from TCM to cure a certain disease. Based on this idea, a novel strategy was developed in this study involving preparation of each proportion by column chromatography, classification of multiple targets on the pathogenesis of DN, different techniques to acquire various parameters, and quantification and standardization of various parameters to acquire multiple data. With the acquirement of multiple data related to DN, PLS-DA was applied to evaluate on the fuzzy target contribution and quantify this contribution based on distance calculation. In this study, the specific target was not determined and we adopt eight targets consisting of a DN pathogenesis network to represent the pharmacological evaluation system on the orthogonal experimental design for herbs A and B. Subsequently, the best proportion of compatibility with shortest distance to the NG group was determined and Western Blot analysis was employed to investigate its molecular mechanism.

Such a novel approach was successfully applied to Astragali Radix (AR, Huangqi in Chinese)-Fructus Corni (FC, Shanzhuyu in Chinese) herb-pair when we planned to discover a novel agent, which is a best proportion of multi-subchemome compatibility to target a specific DN disease from this herb-pair (our previous studies predicted that there should have a corresponding and explicit best proportion of multi-subchemome compatibility targeted to a specific disease inside the herb-pair). AR is derived from the dried root of *Astragalus membranaceus* (Fisch.) Bge. (family Leguminosae), and first recorded in Shen Nong Ben Cao Jing. FC is derived from the dry ripe sarcocarp of *Cornus officinalis Sieb. et Zucc*, which has drawn increasing attention due to the fact of its most cherish herbal medicines in clinic. AR-FC herb-pair was recorded in detail in Zhang-Xi-Chun-Dui-Yao (ZXCDY), a well-known traditional Chinese medical book sorting out Zhang’s medication experience and has been used as a kind of herb-pair remedy for metrorrhagia and DN for many centuries. Until now, despite sporadic reports of AR and its active extracts have been stated to promote renal protective effect in DN patients, very little was known regarding the mechanisms of AR-FC herb-pair or the therapeutic material basis in the treatment of DN, and the systematic and proper pharmacological evaluation methods are still lacked. Therefore, by using the presently developed and established methodology in this study, the different proportions from AR-FC herb-pair were investigated on the treatment of DN, the best proportion of multi-subchemome compatibility was explored, and this pharmacological evaluation strategy to find its wide utilization and potential applicability to any other complex diseases in the research of TCM was further illuminated. Moreover, NFE2L2, a transcription factor inducing a battery of cytoprotective genes, plays a vital role in response to oxidative stress (Kin et al., 2015). It is well-known that NFE2L2 is bound to its repressor, Kelch-like ECH associating protein 1 (Keap1), in the cytoplasm under physiological conditions. However, in response to numerous stimuli, particularly oxidative stress, NFE2L2 is released from Keap1 to translocate into the nucleus and promotes the transcription of its downstream genes in order to trigger cytoprotection. In this paper, we also provided a novel insight into a molecular mechanism of acquired best agent in the prevention and intervention of DN, which could be triggered by NFE2L2 activation. This pathway may further warrant the rational designs of new therapeutic approaches for DN.

2. Materials and methods

2.1. Preparation of polysaccharides, flavonoids, and saponins from AR and iridoid glycosides from FC

AR and FC were purchased individually from the local markets