SCIENCE CHINA
Chemistry

• ARTICLES •

November 2010 Vol.53 No.11: 2337–2342
doi: 10.1007/s11426-010-4082-0

Computational network pharmacological research of Chinese medicinal plants for chronic kidney disease

ZHU Wei[1], QIU XiaoHui[1], XU XiaoJie[1,2*] & LU ChuanJian[1*]

[1]*Guangdong Hospital of Traditional Chinese Medicine, Guangzhou 510006, China*
[2]*College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China*

Received April 26, 2010; accepted May 12, 2010

The interaction between drug molecules and target proteins is the basis of pharmacological action. The pharmacodynamic mechanism of Chinese medicinal plants for chronic kidney disease (CKD) was studied by molecular docking and complex network analysis. It was found that the interaction network of components-proteins of Chinese medicinal plants is different from the interaction network of components-proteins of drugs. The action mechanism of Chinese medicinal plants is different from that of drugs. We also found the interaction network of components-proteins of tonifying herbs is different from the interaction network of components-proteins of evil expelling herbs using complex network research approach. It illuminates the ancient classification theory of Chinese medicinal plants. This computational approach could identify the pivotal components of Chinese medicinal plants and their key target proteins rapidly. The results provide data for development of multi-component Chinese medicine.

chronic kidney disease, traditional Chinese medicine (TCM), molecular docking, complex network

1 Introduction

CKD is a progressive loss of renal function over a period of months or years. CKD leads to end-stage renal disease and is a growing epidemic throughout the world. A report determined that CKD prevalence was 13.1% among U.S. adults surveyed from 1999 to 2004 [1]. Because multiple pathogenetic factors are implicated in CKD, the hitting-one-target therapeutic strategy available has been demonstrated to be inefficient to CKD. Traditional Chinese medicine (TCM) has been used for over 4000 years to treat CKD in China and has gained its popularity recently because of its efficacy and low cost [2]. However, only a few drugs derived from Chinese medicinal plants for CKD, such as tripterygium glycosides and rhein, were discovered by random clinical practice until now [3, 4]. It apparently cannot meet the requirement of clinical medicine. The creation of target-special "magic bullets" has been a therapeutic goal for 30 years. Single-component drugs (magic bullet) may have a magic effect that can be targeted against a disease caused by a single factor. Along with the progress of system biology, several lines of evidence now suggest that an ideal drug may be one whose efficacy is based not on the inhibition of a single target, but rather on the rebalancing of several proteins or events, that contribute to the etiology, pathogeneses, and progression of diseases. Therefore, promiscuous drugs and multicomponent drugs will be promising for complex diseases and the polypharmacologic approaches can be used for the treatment of complex diseases. Recent experimental and clinical studies showed that a combination of drugs is superior to either drug alone in the treatment of various diseases [4–8]. The foundation of traditional Chinese medicine's clinical effect is precisely the "multi-component, multi-target". Thousands of prescriptions are valuable sources of drug discovery [9, 10]. Con-

*Corresponding author (email: xiaojxu@pku.edu.cn; luchuanjian888@vip.sina.com)

struction of complex biological networks and development of analytic techniques provide a new means to study the interaction between components of TCM and their targets. This article attempts to use molecular docking, construction and analysis of complex networks to study components of TCM in treating CKD and their targets. We hope to identify some lead compounds for further development of new drugs and illuminate the ancient TCM theory from the complex network angle.

2 Methods

2.1 Molecular datasets

62 Chinese medicinal plants for CKD are listed in the *Pharmacopoeia of People's Republic of China* [11]. These Chinese medicinal plants were classified into tonifying herbs (22 herbs) and evil expelling herbs (40 herbs) according to TCM's theory. 2215 components identified in Chinese medicinal plants were collected from *Chinese Herbal Drug Database*, Beilstein data (http://www.mdl.com) and other reports [12]. All molecular structures were optimized by molecular mechanics optimization method under MMFF94 force field, and the stop condition was set as the RMS of potential energy smaller than 4.184 J $Å^{-1}$ mol^{-1}. For those flexible components, the most stable conformations were chosen from standard conformational analysis. In addition, 99 drug components, which were referred to CKD target enzymes, were collected from Drugbank (http://www.drugbank.ca), and optimized by the same method. The respective molecular datasets were saved as SD files named tonifying herbs (836 components), evil expelling herbs (1379 components) and drugs (99 components).

2.2 Molecular descriptors and principal component analysis (PCA) calculation

Thirty eight molecular descriptors of all components were calculated. In order to perceive reasonable and intuitionistic information from multiple descriptor values, PCA was performed to map these multiple descriptor values into a 3D plane.

2.3 Molecular cluster analysis

Five molecular descriptors (ALogP, number of hydrogen donors, number of hydrogen acceptors, molecular volume, number of rotatable bonds) were used as cluster criteria. All components were clustered in the program Cluster Ligand. The network relationship between the cluster and cluster center was mapped in Pajek (http://vlado.fmf.uni-lj.si).

2.4 Molecular docking and score

The therapeutic target proteins of CKD were collected from Therapeutic Target database (http://bidd.nus.edu.sg/group/cjttd/TTD_HOME.asp). The X-ray crystal structures of nineteen significant CKD relational targets (Table 1) were downloaded from Protein Data Bank (http://www.rcsb.org). The structure was preprocessed. Hydrogen was added to the model, and its orientation was optimized using the CHARMm force field energy minimization while all non-hydrogen atoms were not allowed to move. The ligand position in target proteins was used to define the active site cavity. Docking protocol was performed to show the interaction with CKD target enzymes using LigandFit. The dockscore of the original protein complex was used as the cutoff value in this protocol. The whole work was conducted using commercial software Discovery Studio 2.5 (http://www.accelrys.com).

2.5 Molecule-target interaction network

According to the cutoff value, the components of higher dockscore were obtained, and the corresponding component and target could be regarded as nodes. The interactions between

Table 1 Nineteen significant targets related to CKD

Target protein	PDB code	Target protein	PDB code
Adenosine A2α receptor	3EML	monoamine oxidase B	1OJ9
Angiotensin converting enzyme	1O86	lymphocyte function-associated antigen	1CQP
β_2 adrenergic receptor	3D4S	peroxisome proliferator-activated receptor	1K74
Vasopressin V1α receptor	1YTV	C–C chemokine receptor type 1	1Y5D
Hypoxia-inducible factor 1α	1H2K	macrophage metalloelastase	1UTT
Placeta growth factor	1FZV	protein-glutamine γ-glutamyltransferase	2Q3Z
RAC-α serine/threonine kinase	1AO2	Raf kinase	1C1Y
C–C motif chemokine-2	2BDN	mitogen-activated protein kinase 1	1PME
Nuclear factor NF-κB	1NFI	plasminogen activator inhibitor-1	10CO
Soluble epoxide hydrolase	1ZD3		

the component and the target could be further regarded as edges. Thus, if the protein is a known target of the component, the molecule-target network could be mapped by cytoscape, which is a software environment for integrated models of biomolecular interaction networks (http://www.cytoscape.org). A comprehensive set of topological parameters including the number of nodes, edges, centralization, heterogeneity, the characteristic path length, and distributions of node degrees, neighborhood connectivities, and shortest path lengths, were computed and displayed by the versatile Cytoscape plugin Network Analyzer [13].

3 Results and discussion

Every Chinese medicinal plant has its own specific characteristics according to TCM theory. In TCM, the different characteristics of Chinese medicinal plants are used to treat diseases, rectify the hyperactivity or hypo-activity of Yin & Yang, and help the body restore its normal physiological functions and thus health. Chinese medicinal plants are divided into tonifying herbs and evil expelling herbs according to their characteristics. Tonifying Herbs nourish and replenish the qi, blood, yin and yang of the human body when they are deficient or weak. Evil expelling herbs are used to eliminate pathogenic factors inside the body. This simple classification is not accurate. It is of great theoretical significance to study TCM theory by modern science and technology. Information visualization technology can be used for data analysis to display the complex relationship between information. It helps us make better use of information resources. Data are divided into meaningful clusters in clustering analysis which is an unsupervised classification. Data of the same cluster share similar characteristics while data of different clusters have significant difference. Our results (Figure 1) show the distribution of components of Chinese medicinal plants and drugs has apparent regional characteristics. The components of tonifying herbs and drugs are more concentrated in the center while the components of evil expelling herbs are relatively dispersed. This suggests that components of tonifying herbs and drugs share similar physiological activities and components of evil expelling herbs have wider biological activities due to their different properties in chemical space.

Currently, many TCM studies follow the conventional strategy: Separation, structure identification, combined with pharmacological experiments to determine the principal active components. The procedure of operation is complex, time-consuming, blind and costly. Computational pharmacology is a new research method integrating computer chemistry, medicine, biological informatics to predict the pharmacological characteristics of chemical molecules. Our previous work showed that using the available information by computational pharmacology to explore the mechanism of TCM is an effective new way of TCM modernization [14–19].

Pharmacological activities of Chinese medicinal plants are based on the interaction of components and target proteins of the human body. They belong to the complex network area. From the 20th century, the complex network gradually became a new research field and was even referred to as “the network's new science.” It has attracted many scientists’ attention for the past 10 years [20]. Jeong *et al*. [21] used the complex network theory to study the metabolic network topology characteristics. It provided a new research strategy to study biological networks at the system level. We put docking results into cytoscape software to construct the interaction network between components and target proteins. The results are shown in Figure 2.

Each network has its unique topology features. Some features have a significant impact on the function of the network. Different networks can be compared and analyzed by quantitatively comparing different topology features of complex networks. Table 2 shows the topology features of the component-target protein interaction network of tonifying herbs, evil expelling herbs and drugs are quite different except network heterogeneity. The hierarchical structure of the network can be measured by clustering coefficients and correlation of degrees. In many real networks, when degree k increases, the aggregate coefficient $C(k)$ generally decreases accordingly. This shows that the network has an apparent hierarchical structure. In other words, the nodes with a low degree have low probability of connection with adjacent nodes. The network centralization of the component-target interaction network of Chinese medicinal plants is significantly higher than that of drugs, which may result in the characteristic path length and shortest paths of the component-target interaction network of Chinese medicinal plants being longer than those of drugs. It suggested that the hierarchical structure of the component-target interaction network of drugs is poor. Another significant parameter of

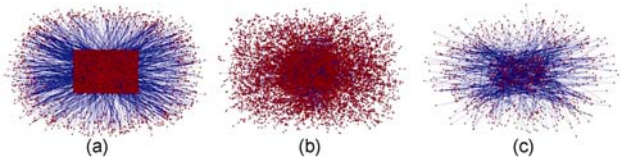

Figure 1 Cluster image of components from tonifying herbs (a), evil expelling herbs (b) and drugs (c).

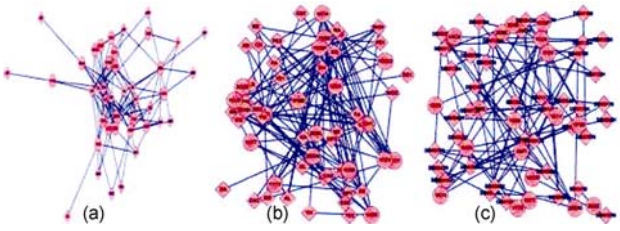

Figure 2 Component-target protein interaction network of tonifying herbs (a), evil expelling herbs (b) and drugs (c). In this network, the shape of nodes corresponds to the type of nodes. △: compound; ○: protein.

Table 2 Some network parameters of component-target interaction networks

Parameter	Tonifying herbs	Evil expelling herbs	Drugs
Number of nodes	42	60	62
Number of edges	111	115	42
Network density	0.129	0.065	0.022
Network heterogeneity	0.602	0.710	0.637
Average number of neighbors	5.286	3.833	1.355
Characteristic path length	2.535	3.050	1.475
Shortest paths	1722 (100%)	3092 (87%)	160 (4%)
Network centralization	0.172	0.161	0.062

real networks is the average number of neighbors. It is used to measure the correlation between a node's degree and that of its adjacent nodes. The average number of neighbors of the component-target interaction network of Chinese medicinal plants is higher than that of drugs. It showed that drugs are more target-specific than components of Chinese medicinal plants.

We constructed the component-component interaction network using cytoscape software to explore the similarities and differences of components of tonifying herbs and expelling herbs. Our results (Figure 3) show the components of tonifying herbs have more target proteins than that of evil expelling herbs.

We also constructed the target protein-target protein interaction network to explore the similarities and differences of target proteins of tonifying herbs and evil expelling herbs. The results (Figure 4) show that target proteins of tonifying herbs are quite different from that of evil expelling herbs. There is only one common target protein, C–C motif chemokine-2, of tonifying herbs and evil expelling herbs.

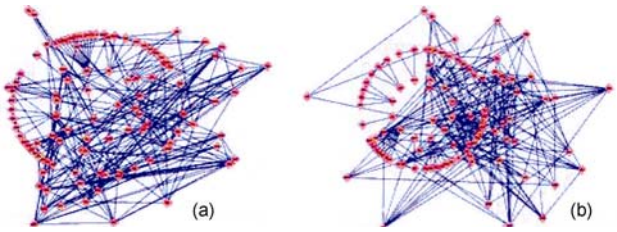

Figure 3 Component-component interaction network of tonifying herbs (a) and evil expelling herbs (b).

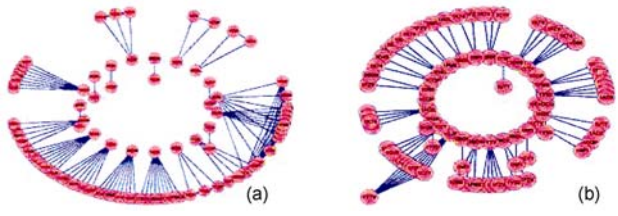

Figure 4 Target protein-target protein interaction network of tonifying herbs (a) and evil expelling herbs (b).

Chemokines are a family of secreted proteins with 70 to 100 amino acids. The major role of chemokines is to act as a chemoattractant to guide the migration of cells. They are involved in a wide variety of processes including acute and chronic types of inflammation, infectious diseases, and cancer [22]. They are an essential part of the defense system and promote wound repair. However, the dramatic increase in the secretion of chemokines during some diseases, such as chronic nephritis and asthma, results in tissue injure. There is growing evidence from studies that the neutralization of chemokine activity may have therapeutic value. Chemokine or chemokine-receptor antagonists may inhibit autoimmune, allergic, and septic processes. C-C motif chemokine-2 receptor inhibitor (Spiropiperidines) does not get FDA approval until today because of unknown reasons [23]. On the contrary, the clinical widespread use of Chinese medicinal plants shows that components of Chinese medicinal plants may have a weak interaction with C-C motif chemokine-2.

Our results (Table 3) also indicate that most of the components or target proteins of the interaction network are not very significant because they are only involved in one or two interactions. Only a few components and target proteins are critical to the network structure. Identifying those key components and target proteins is very significant for understanding the molecular mechanism of Chinese medicinal plants. The degree distribution is one of the most basic quantitative properties of a network. The degree measures the overall network activity of an individual node which can reflect nodes' significance in the network. Pivotal nodes are those whose removal would cause the greatest decrease in interactions between components and target proteins. We used the degree value as the criterion to identify those pivotal nodes which result in 10 promising lead components of Chinese medicinal plants. Not all components of Chinese medicinal plants have pharmacological activities. Some of them are even toxic. In view of this, some scholars put forward the concept of "multi-components of Chinese medicinal plants" [24]. They hoped to develop effective modern Chinese medicines which only contain active components. The data we obtained here will guide the further research.

Table 3 Pivotal nodes in component-target protein interaction networks

	Target protein	Degree	Component	Degree
Tonifying herbs	1CQP	12	Quercetin	10
	3EML	11	Glycyrrhizic acid	10
	2BDN	9	Danshensuan B	9
	1OJ9	9	Glycyhetinic acid	9
	1UTT	8	Ginsenoside Rg1	9
Evil expelling herbs	1NFI	13	Corilagin	9
	1K74	11	Berberine	6
	1CQP	10	Kaempferol	5
	1Y5D	7	Fumalic acid	5
	2BDN	7	Paeoniflorin	5

4 Conclusions

Recent results from system biology research showed many marked drugs have off-target pharmacological activities. Research in this area will discover new applications of these drugs efficiently [25–27]. However, the component-target protein interaction system is too complex to study using conventional methods. Many scholars claimed that network construction and analysis can solve this problem [28–31].

The average number of neighbors, characteristic path lengths and shortest paths are three of the most significant and frequently-invoked characteristics of a network. Given a specific network, it can be of considerable interest to know how these three indices compare with the efficiency of interactions for networks. Our results suggest that components of Chinese medicinal plants only have partial efficacy to the targets relative to drugs. It is meaningful to understand the pharmacological activities of components of Chinese medicinal plants. There are three main differences between component-target protein interaction networks of tonifying herbs and evil expelling herbs: (1) the network degree, (2) the average number of neighbors, and (3) characteristic path lengths and shortest paths. They may be attributed to differences in the classification of Chinese medicinal plants.

Although many problems still need further research, this study reveals the similarities and differences of components of tonifying herbs, evil expelling herbs and drugs through docking and complex network analysis techniques. It also gives a new insight into the TCM theory.

This work was supported by the Natural Science Foundation of Guangdong Province (9151063201000050), Foundation of Administration of Traditional Chinese Medicine of Guangdong Province (2008334) and Major Program of Science and Technique Project of Guangdong Province (2009A030100006).

1 Coresh J, Selvin E, Stenvens LA, Manzi J, Kusek JW, Eggers P, Lente FV, Levey AS. Prevalence of chronic kidney disease in the United States. *JAMA*, 2007, 298: 2038–2047
2 Ye RG. *Integration of Chinese and Western Medicine: Nephrology*. Beijing: People's Health Press, 2004. 25
3 Zhu W, Wang XM, Zhang L, Li XY, Wang BX. Pharmacokinetic of rhein in healthy male volunteers following oral and retention enema administration of rhubarb extract: a single dose study. *Am J Chin Med*, 2005, 33: 839–850
4 Jia J, Zhu F, Ma XH, Cao ZW, Li YX, Chen YZ. Mechanisms of drug combinations: interaction and network perspectives. *Nat Rev Drug Discov*, 2009, 8: 111–128
5 Zimmermann GR, Lehár J, Keith CT. Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discov Today*, 2007, 12: 34–42
6 Hippisley CJ, Coupland C. Effect of combinations of two drugs on all cause in patients with ischaemic heart disease: Nested case-control analysis. *Brit Med J*, 2005, 330: 1059–1063
7 Hennekens CH, Sacks FM, Tonkin A, Jukema JW, Byington RP, Pitt B, Berry DA, Berry SM, Ford NF, Walker AJ, Natarajan K, Chen SL, Fiedorek FT, Belder R. Additive benefits of pravastain and aspirin to decrease risks of cardiovascular disease. *Arch Intern Med*, 2003, 164: 40–44
8 The Indian Polycap Study (TIPS). Effects of a polypill (Polycap) on risk factors in middle-aged individuals without cardiovascular disease (TIPS): A phase II, double-blind, randomised trial. *Lancet*, 2009, 373: 1341–1351
9 Zhu W, Ruan XM, Chen KJ. A new insight into application of evidence-based medicine in TCM. *J Trad Chin Med*, 2006, 47: 333–335
10 Wang L, Zhou GB, Liu P, Song JH, Liang Y, Yan XJ, Xu F, Wang BS, Mao JH, Shen ZX, Chen SJ, Chen Z. Dissection of mechanisms of Chinese medicinal formula Realgar-*Indigo naturalis* as an effective treatment for promyelocytic leukemia. *Proc Natl Acad Sci USA*, 2008, 105, 4826
11 Editorial Committee of the Pharmacopoeia of People's Republic of China. *The Pharmacopoeia of People's Republic of China (part 1)*. Beijing: Chemical Industry Press, 2005
12 Zhou JJ, Xie GR, Yan JX. *Handbook of Chemical Composition in Chinese Herbal Original Plants*. Beijing: Chemical Industry Press, 2004
13 Assenov Y, Ramírez F, Schelhorn SE, Albrecht M. Computing topological parameters of biological networks. *Bioinformatics*, 2008, 24: 282–284
14 Xu XJ. Study on computer simulation for Chinese traditional compound medicine. *Prog Chem*, 11: 202–204
15 Xu XJ. New concepts and approaches for drug discovery based on traditional Chinese medicine. *Drug Discov Today*, 2006, 3: 247–253
16 Huang Q, Qiao XB, Xu XJ. Potential synergism and inhibitors to multiple target enzymes of Xuefu Zhuyu decoction in cardiac disease therapeutics: A computational approach. *Bioorg Med Chem Lett*, 2007, 17: 1779–1783
17 Li XD, Huang Q, Xu XJ. Target-ligand space distribution of the compound sets from XuefuZhuyu decoction, a traditional Chinese medicinal recipe. *Acta Phys-Chim Sin*, 2008, 24: 547–551
18 Wu DH, Xu XJ. Computational simulation of benefiting Qi and activating blood mechanism of traditional Chinese medicine. *Acta Phys-Chim Sin*, 2009, 25: 446–450
19 Gu JY, Yuan G, Zhu YH, Xu XJ. Computational pharmacological studies on cardiovascular disease by Qishen Yiqi Diwan. *Sci China Ser B-Chem*, 2009, 52: 1879–1884
20 Watts DJ. The "new" science of networks. *Annu Rev Sociol*, 2004, 30: 243–270
21 Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL. The large-scale organization of metabolic networks. *Nature*, 2000, 407: 651–654
22 Yoshie O, Imai T, Nomiyama H. Chemokines in immunity. *Adv Immunol*, 2001, 78: 57–110
23 Rousset F, Garcia E, Defrance T, Peronne C, Vezzio N, Hsu DH, Kastelein R, Moore KW, Banchereau J. Interleukin 10 is a potent

growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci USA*, 1992, 89: 1890–1893
24 Feng JT, Xu Q, Xue XY, Zhang FF, Xiao YS, Liang XM. Preparation of standard multi-component of traditional Chinese medicine and construction for its library. *World Science and Technology-Modernization of Traditional Chinese Medicine and Materia Medica*, 2006, 8: 1–7
25 Campillos M, Kuhn M, Gavin AC, Jensen LJ, Bork P. Drug target identification using side-effect similarity. *Science*, 2008, 321: 263–266
26 Günther S, Kuhn M, Dunkel M, Campillos M, Senger C, Petsalaki E, Ahmed J, Garcia Urdiales E, Gewiess A, Jensen LJ, Schneider R, Skoblo R, Russell RB, Bourne PE, Bork P, Preissner R. SuperTarget and Matador: resources for exploring drug-target relationships. *Nucl Acids Res*, 2008, 36: 919–922
27 Kuhn M, Szklarczyk D, Franceschini A, Campillos M, Mering CV, Jensen LJ, Beyer A, Bork P. STITCH 2: An interaction network database for small molecules and proteins. *Nucl Acids Res*, 2010, 38: 552–556
28 Keith CT, Borisy AA, Stckwell BR. Multicomponent therapentics for networked system. *Nat Rev Drug Discov*, 2005, 4: 1–8
29 Imming P, Sinning C, Meyer A. Drug, their targets and the nature and number of drug targets. *Nat Rev Drug Discov*, 2006, 5: 821–834
30 Kitano H. A robustness-based approach to systems-oriented drug design. *Nat Rev Drug Discov*, 2007, 6: 202–210
31 Watts DJ, Strogatz SH. Collective dynamics of “small-world” networks. *Nature*, 1998, 393: 440–442