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Discovery of molecular mechanism of a clinical herbal formula upregulating serum HDL-c levels in treatment of metabolic syndrome by in vivo and computational studies

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ABSTRACT

Decreased HDL cholesterol (HDL-c) is considered as an independent risk factor of cardiovascular disease in metabolic syndrome (Mets). Wendan decoction (WDD), a famous clinical traditional Chinese medicine formula in Mets in China, which can obviously up-regulate serum HDL-c levels in Mets. However, till now, the molecular mechanism of up-regulation still remained unclear. In this study, an integrated approach that combined serum ABCA1 in vivo assay, QSAR modeling and molecular docking was developed to explore the molecular mechanism and chemical substance basis of WDD upregulating HDL-c levels. Compared with Mets model group, serum ABCA1 and HDL-c levels intervened by two different doses of WDD for two weeks were significantly up-regulated. Then, kohonen and LDA were applied to develop QSAR models for ABCA1 up-regulators based flavonoids. The derived QSAR model produced the overall accuracy of 100%, a very powerful tool for screening ABCA1 up-regulators. The QSAR model prediction revealed 67 flavonoids in WDD were ABCA1 up-regulators. Finally, they were subjected to the molecular docking to understand their roles in up-regulating ABCA1 expression, which led to discovery of 23 ABCA1 up-regulators targeting LXR beta. Overall, QSAR modeling and docking studies well accounted for the observed in vivo activities of ABCA1 affected by WDD.

Metabolic syndrome (Mets) is defined as a set of interrelated risk factors of diabetes and cardiovascular diseases, which has become a worldwide problem.[1] Epidemiological studies firmly confirmed that decreased HDL cholesterol (HDL-c) was an independent risk factor of cardiovascular disease in Mets.[2] Thus, new therapies for raising HDL-c levels have been the focus of significant efforts by the cardiovascular medicine community.[3] Wendan decoction (WDD), a famous traditional Chinese medicine formula widely applied in Mets in clinics in China, which can obviously up-regulate serum HDL-c levels in Mets.[4] However, till now, the molecular mechanism of up-regulation HDL-c of WDD involved in Mets still remained unclear. It is important to decipher the action mechanism of WDD affecting the HDL-c levels, which can help elucidate therapeutic effects of WDD against Mets as well find new lead compounds for cardiovascular diseases from herbs. The ATP-binding cassette transporters A1 (ABCA1) is a membrane-associated protein involved in reverse cholesterol transport, which mediates the rate-controlling step in the formation of HDL particle, and hence affect whole body cholesterol and HDL-c metabolism.[5,6] Moreover, either the transcriptional upregulation of ABCA1 expression or inhibition of ABCA1 protein degradation can promote HDL-c biosynthesis and exhibit anti-atherogenesis.[7,8] More recent studies showed that the reduced or impaired ABCA1 activity could also cause type 2 diabetes.[9] Thereby, we attempt to elucidate the potential mechanism of WDD affecting the HDL-c from the expression of ABCA1.

In this study, an integrated approach that combined serum ABCA1 in vivo assay, quantitative structure activity relationship (QSAR) modeling and molecular docking was applied to explore the molecular mechanism and chemical substance basis of WDD upregulating HDL-c level. First, ABCA1 in vivo detection in forty Wistar rats including normal control group, Mets model group, herb treated group and low-dose herb treated group was directly performed to confirm whether the effect of WDD up-regulating

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HDL-c was related to the up-regulation of ABCA1 expression. Then, QSAR methods were introduced to establish highly predictive QSAR classification models to screen highly potent ABCA1 up-regulators from WDD chemical ingredients, which predict the bioactivity of compounds by a mathematical model between physicochemical properties and bioactivity and have been very extensively applied in many fields for predicting compound properties,[10–12] including biological activity prediction, physical property prediction and toxicity prediction. Finally, molecular docking was applied to investigate the action mechanism by which WDD altered ABCA1 expression, which predicts the binding-conformation and binding affinity of ligands to the appropriate target binding site by the search algorithm and the scoring function and has been successfully in identifying experimentally active compounds from large chemical libraries and revealing the action mechanism of compounds.[13,14]

All animal experiments were approved by the Animal Care and Use Committee, Fujian University of Traditional Chinese Medicine, Fuzhou, P. R. China, and all experiments were performed in accordance with relevant guidelines and regulations. Forty Wistar male rat serum samples of normal control group, Mets model group, herb treated group and low-dose herb treated group were taken from our lab.[15,16] Ten rats in the normal control group were fed with normal diet for 17 weeks, and thirty rats in the Mets model group were fed with fifteen-week's high-sugar-fat-diet and two-week's high-fat emulsion. The specific information of making Mets animal model was listed in Support information. After Mets modeling successfully, herb treated group and low-dose herb treated group were conducted by two weeks' WDD treatment of ten randomized Mets model rats by intragastric at a dose of 10 ml/kg/d and 2.5 ml/kg/d, respectively.[17] The dose of WDD employed in the rat experiments was within the human therapeutic range according to the Guidance of FDA-CDER and Chinese pharmacopoeia.[17,18] Then, all rats were sacrificed and blood samples were isolated by centrifugation at 2,500 rpm at 4 °C. And, serum samples were separated into 200 μL sub-aliquots and stored at −80 °C until analysis.

The serum concentrations of ABCA1 and HDL-c were measured using an ABCA1 ELISA kit and HDL-c ELISA kit according to manufacturer's instructions (Nanjing jiancheng bioengineering institute, Co., LTD, Nanjing, China) by using Tecan Infinite F200/M200 microplate reader (Tecan, Switzerlant), respectively. The one-way ANOVA and least significant difference methods were followed to compare the serum ABCA1 and HDL-c levels between four groups in a statistical Package for social science package 20.0 version.

In the QSAR analysis, forty-one strong and weak flavonoids-based ABCA1 up-regulators were taken from Hu et al.[19] All 2D structures of compounds in Table S1 (see support information) were sketched in ChemDraw software and were converted into 3D structures using energy minimization module embedded in Molecular Operating Environment software (MOE2008.10, Chemical Computing Group Inc., Montreal, Canada). Then, their conformer structures were optimized by stochastic conformational search and followed to generate 327 diverse descriptors by utilizing the QSAR module of MOE.[20] The redundant information among descriptors was conducted by deleting constant or almost constant values for all molecules and removing one of intercorrelated descriptors (a pairwise correlation coefficient greater than 0.95).[21] To obtain reliable QSAR models, the studied chemicals were firstly separated into a training set and a test set using a kohonen's self-organizing map (5 × 5 neurons, 500 epochs), which ensured the training set spanned the whole descriptor space and kept a balance distribution of the chemicals in two data sets.[22] Additionally, a stepwise method combined with linear discriminant analysis (LDA) was employed to construct the QSAR models.[23] Then, the widely applied internal and external validations, such as leave-one-out (LOO) cross-validation and the external test set validation were followed to evaluate the predictive ability and reliability of QSAR models.[24] Then, the performance parameters of QSAR models including train accuracy, test accuracy, overall prediction accuracy, sensitivity (SE), and specificity (SP) were also calculated.[25] All algorithms were accomplished using the default sets in MATLAB 8.0.

ABCA1 up-regulator QSAR models were applied to decipher the chemical substance basis of WDD upregulating the serum HDL-c levels. First, chemical ingredients from herbs in WDD were collected from the Beilstein/Gmelin CrossFire Chemical database, Chinese Herbal Drug Database and the Handbook of the Constituents in Chinese Herb Original Plants.[26] After removing duplicates, a total of 618 compounds were retained and subjected to conformational optimization and descriptor calculation by using MOE2008. Considering that our ABCA1 up-regulator QSAR models were constructed based on flavonoids, only compounds with flavonoid skeletons in WDD can be well-predicted with the derived QSAR model.[27] And then, all fit compounds of WDD were picked out for further QSAR analysis.

Molecular docking was further performed to explore the mechanism of WDD up-regulating the serum ABCA1 expression. It is well-known that liver X receptor (LXR) beta activation markedly induced the transcription of ABCA1.[28] Additionally, potent ABCA1 up-regulators, such as 1-(4-amino-2-hydroxyphenyl)-3-(5-methylfuran-2-yl)prop-2-en-1-one and 1-(4-amino-2-hydroxyphenyl)-3-(naphthalen-2-yl)prop-2-en-1-one preferentially activated LXR beta.[19] Therefore, ABCA1 up-regulators screened from WDD were further docked into the LXR beta active site to better understand the binding modes and important interactions in MOE2008.10.

The docking simulation was carried out as follows.[29] First, the three dimension crystal structure of LXR beta-T0901317 complex from the RSCB protein databank (PDB: 1PQC) was protonated using AMBER99 force field and minimized with a RMSD gradient of 0.05 kcal/mol Å.[30] In addition, the binding site and docking placement were using the ligand atom mode and triangle matcher algorithm, respectively. Finally, two rescoring methods including London dG and Affinity dG, together with a force field were adopted to compute the interactions.

As shown in Figs. S1 and S2 (support information), compared with the normal control group, the abdominal perimeters, serum levels of insulin and HOMA-IR of rats in Mets model group were significantly increased, whereas the serum HDL-c levels were significantly decreased ($P < .05$) before WDD interventions. After two weeks treatment, by comparison of Mets model group, either full dose or low dose of WDD interventions resulted in significant increase in the serum HDL-c levels ($P < .05$), while full dose performed better than low dose on abdominal perimeters ($P < .05$).

The serum levels of ABCA1 were calculated using our ABCA1 standard curve (shown in Fig. 1), and listed in Table 1. Compared

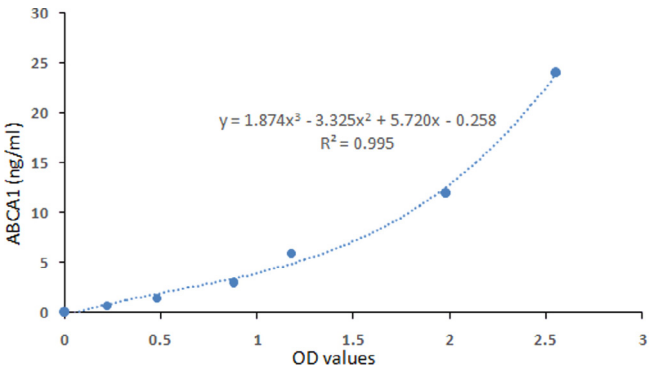

Fig. 1. The standard curve between serum ABCA1 levels and absorbing values at 450 nm.