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Using neural networks to determine the contribution of danshensu to its multiple cardiovascular activities in acute myocardial infarction rats

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ABSTRACT

Ethnopharmacological relevance: Danshensu is an active water-soluble component from *Salvia Miltiorrhiza*, which has been demonstrated holding multiple mechanisms for the regulation of cardiovascular system. However, the relative contribution of danshensu to its multiple cardiovascular activities remains largely unknown.
Aim of the study: To develop an artificial neural network (NN) model simultaneously characterizing danshensu pharmacokinetics and multiple cardiovascular activities in acute myocardial infarction (AMI) rats. The relationship between danshensu pharmacokinetics (PK) and pharmacodynamics (PD) were evaluated using contribution values.
Materials and methods: Danshensu was intraperitoneally injected at a single dose of 20 mg/kg to AMI rats induced by coronary artery ligation. Plasma levels of danshensu, cardiac troponin T (cTnT), total homocysteine (Hcy) and reduced glutathione (GSH) were quantified. A back-propagation NN model was developed to characterize the PK and PD profiles of danshensu, in which the input variables contained time, area under plasma concentration–time curve (AUC) of danshensu and rat weights (covariate). Relative contribution of input variable to the output neurons was evaluated using neuron connection weights according to Garson's algorithm. The kinetics of contribution values was also compared and was validated using bootstrap resampling method.
Results: Danshensu exerted significant cTnT-lowering, Hcy- and GSH-elevating effect, and these marker profiles were well captured by the trained NN model. The calculation of relative contributions revealed that the effect of danshensu on the PD marker could be ranked as cTnT > GSH > Hcy, while the effect of AMI disease on the PD marker could be ranked in the following order: cTnT > Hcy > GSH. The activity of transsulfuration pathway was quite obvious under the AMI state.
Conclusion: NN is a powerful tool linking PK and PD profiles of danshensu with multiple cardioprotective mechanisms, it provides a simple method for identifying and ranking relative contribution to the multiple therapeutic effects of the drug.

1. Introduction

Integration of PK/PD concepts is a potential tool to reduce the cost and enhance the efficiency of decision making process in the drug development (Meibohm and Derendorf, 2002). PK/PD models characterize time course of effect intensity resulted from certain dosing regimen, thus supporting identification and evaluation of drug–response determinants (Dahl et al., 2010). Traditionally, PK/PD models are constructed by using explicit or ordinary differential equations, with the goal reflecting the pharmacologic properties of the drug and the physiologic characteristics of biologic

Abbreviations: AMI, acute myocardial infarction; AUC, area under plasma concentration–time curve; BP, back-propagation; *C*, contribution value; CAL, coronary artery ligation; *Contr*, global contribution value; cTnT, cardiac troponin T; DSS, danshensu; GSH, reduced glutathione; Hcy, homocysteine; ME, mean prediction error; NA, naive averaging; NN, neural networks; PD, pharmacodynamics; PK, pharmacokinetics; RMSE, root mean square error; SEM, standard error of the mean.

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system (Mager et al., 2003). However, for the traditional Chinese medicines containing multiple components and therapeutic targets (Wagner, 2006; Zimmermann et al., 2007), the process of developing such structural models can represent a formidable task and can be fraught with methodologic difficulties (Bellissant et al., 1998). In this case, other approaches to the structural PK/PD modeling are necessary, among which, artificial neural networks (NN) is given the most consideration.

Artificial neural networks is a versatile soft computing tool that can perform simulations and estimations for problems with poorly understood or multiple complex inputs such as predictions, classification, noise filtration, data compression (Zou et al., 2008). For prediction purpose, the most widely used type is back-propagation (BP) NN for its simplicity and predictive power (Goh, 1995). BP NN generally consist of an input and output layer fully interconnected through hidden layers, the number of which is dictated by the complexity and nonlinearity of the problem. Hence, it allows the signals to travel through the network in parallel, as well as serially. It has been shown that BP NN could approximate the PK and PD profiles generated from simulations of several structural PK/PD models (Gobburu and Chen, 1996). Furthermore, BP NN has been successfully applied in the bioequivalence study (Opara et al., 1999), population pharmacokinetics (Chow et al., 1997), clinical pharmacology (Brier and Aronoff, 1996; Urquidi-Macdonald et al., 2004; Mager et al., 2005) and PK/PD modeling (Veng-Pedersen and Modi, 1993; Haidar et al., 2002).

Ischemic heart disease has been regarded as a complex disorder associated with numerous risk factors such as hypertension, diabetes and atherosclerosis (Ferdinandy et al., 2007). The prediction and classification of cardiovascular disease raised extensive attention, in which, NN was found to have significant potential (Baxt and Skora, 1996; Eggers et al., 2007; Colak et al., 2008). It has been shown that many drugs could alleviate the ischemic myocardial injury through multiple pathways (Chen et al., 2007; Yue et al., 2008). However, there are still no studies using NN technique to evaluate the contribution of each cardioprotective mechanism to the total cardiovascular activity of the drug.

Increasingly evidences have shown that danshensu (DSS), a mainly active ingredient from *Salvia Miltiorrhiza*, hold multiple mechanisms for the regulation of cardiovascular disturbances such as acute ischemic myocardial injury (Wu et al., 2007), endothelial dysfunction (Chan et al., 2004), venular thrombosis (Wang et al., 2009) and oxidative stress (Zhao et al., 2008). This implies that danshensu is a promising multi-target agent to treat cardiovascular disease. Our previous work showed that danshensu could promote the transsulfuration pathway in methionine-loading rats, exhibiting the homocysteine-lowering and glutathione-elevating effect (Cao et al., 2009). Meanwhile, the bidirectional effect of danshensu on plasma homocysteine (Hcy) was described using a PK/PD model (Chen et al., 2009). Hcy is regarded as an independent risk factor in the cardiovascular diseases such as arteriosclerosis and arterial thrombosis (Nygard et al., 1997; Wald et al., 2002). Via the transsulfuration pathway, Hcy could convert to cysteine indirectly. The latter is an important determinant for the synthesis of GSH, which in turn acts a first line of defence against oxidative stress (Mosharov et al., 2000).

The purpose of this study was to evaluate the relative contribution of danshensu and disease state on the Hcy and GSH after ischemic myocardial injury. As cTnT was a highly specific and sensitive diagnostic biomarker of myocardial cell damage (Gaze and Collinson, 2008), the relative contribution to cTnT was also evaluated. First, the regulatory effects of danshensu on the PD markers were evaluated in the AMI rats. Then a BP NN model was developed to characterize the danshensu PK and PD profiles, and neuron connection weights were used to calculate the relative contribution of input variable to each PD marker. The kinetics of contribution values were also evaluated and finally were validated using bootstrap resampling method.

2. Materials and methods

2.1. Chemicals and animals

Danshensu (3-(3,4-dihydroxy-phenyl) lactic acid, purity 99%) was obtained from QingZe Co. Ltd. (Nanjing, China). Chloramphenicol was supplied by Chiatai Qingchunbao Pharmaceutical Co. Ltd. (Hangzhou, China). Methanol and formic acid (Merck Corporation, Darmstadt, Germany) were of HPLC grade. D,L-Homocysteine, dithiothreitol (DTT), 5,5′-dithio-bis-nitrobenzoic acid (DTNB) and sulfosalicylic acid were purchased from Sigma Company (USA). Other chemicals and solvents were of analytical grade and purchased from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China).

Male Sprague–Dawley rats weighing 220–265 g were obtained from Laboratory Animal Center of Nantong University in China. The rats were housed at $22 \pm 5\,^{\circ}C$ with a 12-h dark/light cycle, and were provided with water and standard chow *ad libitum* for 1 week before experiment. The experiments were performed under a license granted by Jiangsu Science and Technology Office (China), with approval from the Animal Ethics Committee of China Pharmaceutical University. Each effort was made to minimize stress to rats.

2.2. Animal studies

A myocardial infarction model was produced by the ligation of left coronary artery based on a previous study (Stanton et al., 2000) with minor modifications. Briefly, rats were anesthetized with chloral hydrate (0.3 g/kg, *i.p.*), and then underwent left thoracotomy. After tracheal intubation, rats were ventilated by a respirator (HX-300S, Taimeng Co. Ltd., Chengdu, China) using room air. The heart was exteriorized, and ligated at the proximal left coronary artery 2–3 mm from its origin between the pulmonary artery conus and the left atrium. Then the heart returned to its normal position, and the left thorax closed rapidly. Time began when the left coronary artery was ligated completely. The sham-operated rats underwent identical thoracotomy and cardiac exposure without coronary artery ligation (CAL). Electrocardiogram was recorded before and after the surgical procedure. ST segment elevation could be observed after successful induction of AMI. In addition, rat weight was recorded just before the surgery.

Surviving rats were randomly assigned into three groups: Group I were sham operated rats ($n = 7$), Group II were AMI control rats receiving normal saline (*i.p.*) 4 h after CAL ($n = 6$), Group III were AMI rats administered with 20 mg/kg danshensu intraperitoneally 4 h after CAL ($n = 10$). Preliminary experiment showed that the absolute availability of danshensu exceed 95% through peritoneal injection, therefore we chose it as the administration route in this study. For the PK study, blood samples were collected after 0.083, 0.25, 0.5, 1, 1.5 and 2 h of danshensu administration. For the PD study, blood samples were collected before and after 2, 4, 8, 12, 24, 36 and 48 h following CAL. Our preliminary study demonstrated that such PK and PD sampling time could capture the variation of plasma level of danshensu and PD indices quite well. To minimize blood-sampling injury, Group III rats (AMI + DSS) were divided into two subgroups ($n = 5$ each), sparse sampling scenario was used in the PK study: blood samples in Group III-(A) were collected at 0.083, 0.5, 1 and 2 h post-dosing, while the blood samples in Group III-(B) were collected at 0.25, 1, 1.5 and 2 h post-dosing.

At each time point, blood was taken from orbital sinus after light ethyl ether anesthesia. Approximate 200 μL or 400 μL blood was collected in the PK or PD study, respectively. The samples were