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SHORT COMMUNICATION

Pharmacokinetic parameters of morroniside in iridoid glycosides of *Fructus corni* processing based on back-propagation neural network

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Abstract

Context: *Fructus Corni* is derived from the dry ripe sarcocarp of *Cornus officinalis* Sieb. et Zucc. (Cornaceae). Morroniside is an active constituent of *Fructus Corni* used in many traditional Chinese medicines (TCMs). This article describes a sensitive and specific assay for the quantitation of morroniside in rat plasma after oral administration of iridoid glycosides from *Fructus Corni*.

Materials and methods: In this article, back-propagation (BP) neural network method was first developed for the prediction of pharmacokinetic (PK) parameters of morroniside in *Fructus Corni*.

Results: The results show that mean square error (MSE) of neural network model with 11 hidden neurons and 90% training data is 0.092.

Discussion and conclusion: This article provides a new method to calculate PK data, one do not need to figure out all the compartment parameters to acquire PK data of morroniside. Therefore, the BP neural network method would be useful for guiding the holistic PK study in consistence with the intrinsic theory and characteristics of TCM.

Keywords: Pharmacokinetic, morroniside, iridoid glycosides, *Fructus Corni*, BP neural network

Introduction

Traditional Chinese medicine (TCM) has played an important role in the prevention and treatment of diseases in china for thousands of years (Wu et al., 2007; Wen et al., 2007; Qian et al., 2008). *Fructus Corni*, a well-known TCM herbal medicine with the Chinese name “Shanzhuyu,” was prepared from the dry ripe sarcocarp of *Cornus officinalis* Sieb. et Zucc. (Cornaceae) (Ding et al., 2008). Morroniside is the main active components in *Fructus Corni*. Pharmacological studies on morroniside show antioxidation and protective effects on rat mesangial cell proliferation (Wang et al., 2009). In this article, high-performance liquid chromatography (HPLC) method has been successfully applied to a pharmacokinetic (PK) study of the morroniside after oral

administration of iridoid glycosides from *Fructus Corni* to rats.

Artificial neural networks (ANNs) are versatile computational tools that are well suited to recognize complex nonlinear relationships (Hunt & Deller, 2005; Bernardos & Vosniakos, 2007). Neural networks could be envisaged as a model-independent approach to PK and pharmacodynamic (PD) modeling, in that ANNs do not attempt to capture the intricacies of the *in vivo* characteristics but endeavor to generalize the association of the observed concentrations of active substance(s) in biofluid(s) with the detected PD effect as a function of time (and other variables where applicable). Previously, ANNs were successfully applied to a variety of interesting areas (Gevrey et al., 2003; Olden

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et al., 2004; Zou et al., 2008). In this article, the application of the back-propagation (BP) neural network in the prediction of pharmacokinetic parameters of morroniside in *Fructus Corni* is discussed in depth, and a comparison with the normal prediction methods is made.

Materials and methods

Materials and standards

The *Fructus Corni* was collected from Henan suppliers. Morroniside was prepared in our own laboratory, and its identity was verified by LC-MS, ^1H NMR and ^{13}C NMR. The purity of each standard compound was >98% as shown by HPLC analysis. Analytical grade acetonitrile was purchased from Merck (Darmstadt, Germany) and used without further purification. Deionized water was purified using the Milli-Q system (Millipore, Bedford, MA). Solvents of HPLC grade were used for all preparations. All other chemicals were of analytical grade and commercially available.

Blood sample processing

A volume of 0.3 ml blood sample was collected into heparinized tubes (final concentration about 20 IU/ml) at each time point from rat carotid catheter after administration. Blood samples were centrifuged for 5 min at 4500g under 4°C . The plasma were harvested and transferred into tubes in a 96-well plate format and frozen at -20°C until thawed for bioanalysis. The rat plasma samples were thawed to room temperature. The rat plasma (25 μl) were transferred into a microcentrifuge tube (1.7 ml) to precipitate the plasma protein by the acetonitrile. The upper layer was transferred to a clean tube after centrifugation at 5000 rpm for 10 min. Acetonitrile was evaporated to dryness under a gentle stream of nitrogen gas at room temperature. The obtained residue was redissolved in 100 μl acetonitrile and 20 μl aliquots of the supernatants were injected into the HPLC system for analysis.

HPLC analysis

Analyses were performed using an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA) with a diode array detector. The monitoring wavelength was set at 218 nm for gallic acid. An Agilent Zorbax Extend C_{18} column ($250 \times 4.6 \text{ mm}$, $5 \mu\text{m}$) was used with a flow rate of 1.0 ml/min. Mobile phase was composed of (A) aqueous phosphoric acid (0.1%, v/v) and (B) acetonitrile phosphoric acid (0.1%, v/v) using a gradient elution of 2% B at 0–10 min, 2–5% B at 10–15 min, 5–15% B at 15–45 min, 15–25% B at 45–55 min, 25–90% B at 55–70 min, 90% B at 80 min. All samples were centrifuged at 15,000g for 10 min. The column temperature was maintained at 30°C .

PK study

To evaluate the suitability of the assay for PK studies, serial blood samples were collected at pro-dose, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240 and 300 min after oral uptake of 20, 40 and 80 mg/kg body mass dose of iridoid

glycosides from *Fructus Corni*. Each sample was immediately transferred to a heparinized glass tube and centrifuged at 1500g for 15 min at $8\text{--}10^\circ\text{C}$. The plasma was then transferred to another glass tube and stored at -20°C until analyzed.

For the absorbing area studies under ether anaesthesia, five rats were ligated the end of stomach sphincter, the passage at pylorus that opens into duodenum to prevent drug solution from entering the duodenum. Animals were oral administrated with iridoid glycosides 20, 40 and 80 mg/kg when they regained consciousness. Blood samples were obtained 15 min after gavaging. Plasma samples were separated and stored at -20°C until analyzed.

BP neural network modelling

The BP neural network is also called an error BP network, which is composed of input, hidden and output layers. BP neural network, one of the multilayered feed forward structures, is the most commonly used and investigated neural network (Goh, 1995; Ren & Gao, 2004; Xu & Yu, 2010). Each layer has several neurons. In this study, the input layer contains two neurons: dose and weight; while the output layer contains five neurons, which represent five PK parameters of morroniside in *Fructus Corni*: $t_{1/2}$, C_{max} , T_{max} , $\text{AUC}_{0\text{--}t}$ and $\text{AUC}_{0\text{--}\infty}$. The BP neural network structure is shown in Figure 1. The interconnected BP neurons receive signals from input neurons performing a simple processing and then pass signals to other neurons. The input neurons are each connected to the hidden nodes, which are in turn connected to the output neurons. The number of hidden neurons was selected using heuristics combined with a trial and error approach. Another important aspect of the networks is variable transformation. The *tansig* function was used as the input neuron transfer, while the *purelin* function

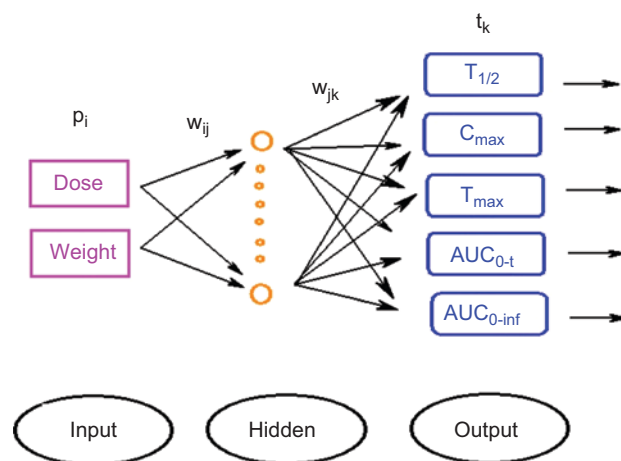


Figure 1. Structure of BP network. The arrows indicate the transfer of neuron excitations of information flow. w_{ij} represents the connection weight between p_i and the i th hidden node, while w_{jk} denotes the connection weight between the i th hidden node and t_k .

was used as the output neuron transfer. Transformed variables were employed to calculate the MSE (mean square error) in separate experiments. The entire data set was scaled to lie in the range $(-1,1)$, so that sensitivity can be adjusted to at the same level. The output of the network was calculated each time, and difference between the predicted data and the observed data was propagated back to adjust the strengths of the connection weights.

Calculations and statistics

The plasma concentrations of morroniside were evaluated using the equation from the standard curves that were run with each batch of samples. Compartmental and non-compartment PK analysis of plasma concentrations-time data were carried out using the Drug and Statistics 2.0 software package to determine PK parameters. The observed value of C_{\max} was obtained from the observed data and the observed value of AUC_{0-t} was calculated using the trapezoidal rule. The total AUC of these four active constituents was calculated by summing each AUC value. ANN analysis was performed using the software of MATLAB 7.1 (Mathworks Inc., Natick, MA).

Results and discussion

Optimization of chromatographic conditions

Different mobile phase compositions were tested: (i) water-methanol; (ii) water-acetonitrile; (iii) aqueous phosphoric acid (0.1%, v/v)-acetonitrile phosphoric acid (0.1%, v/v); (iv) aqueous ammonium acetate (0.5%, v/v)-acetonitrile. As a result, the combination of aqueous phosphoric acid (0.1%, v/v)-acetonitrile phosphoric acid (0.1%, v/v) for the mobile phase gave the best separation. Furthermore, other chromatographic variables were also optimized, including the analytical column (Hanbon Hedera ODS-2, Hanbon Lichrospher C_{18} and Agilent-Zorbax Extend C_{18}), the column temperature (20, 25 and 30°C) and the flow rate (0.8 ml/min and 1.0 ml/min). The optimal separation was achieved on an Agilent Zorbax Extend C_{18} column (250 × 4.6 mm, 5 μ l) at a column temperature of 30°C with a flow rate of 1.0 ml/min.

Application to PKs

The method was used to determine the plasma concentration of morroniside in rats after oral administration of 20, 40 and 80 mg/kg iridoid glycosides. The dosages for C_{\max} were 0.7642 ± 0.0845 , 1.300 ± 0.3439 , and 200.77 ± 32.12 mg/l, respectively, for $AUC_{(0-t)}$ they were 109.0716 ± 14.6384 , 166.014 ± 30.3033 , and 182.6015 ± 40.2990 mg/h/l, respectively. The PK parameters are listed in Table 1 and the mean plasma concentration-time profile is illustrated in Figure 2. Results of the study are consistent with the works by Li et al. (2007) and with other previously published studies.

Application of the BP network model to PK parameters prediction of morroniside

Determined by heuristics combined with a trial and error approach, with the test range of neurons from 3 to 12, the results show that the number was 11, when the least MSE 0.092 was approached. The final results with their respective coefficient of regression (R) values were shown in Figure 3. These relationships appear to show significant correlation (R ranges from 0.521 to 0.951) and some of them have good predictive performance. Further investigation shows that dose emerged as the primary determinant of the variability in $AUC_{0-\infty}$, C_{\max} and AUC_{0-t} , while weight was also an important parameter during prediction. Three major conclusions were drawn from above analysis: (i) Area under curve and maximum concentration are highly co-related with dose and weight. PK parameters prediction of simple neural networks containing certain architecture was acceptable. (ii) Half life and time to maximal plasma concentration were not related with

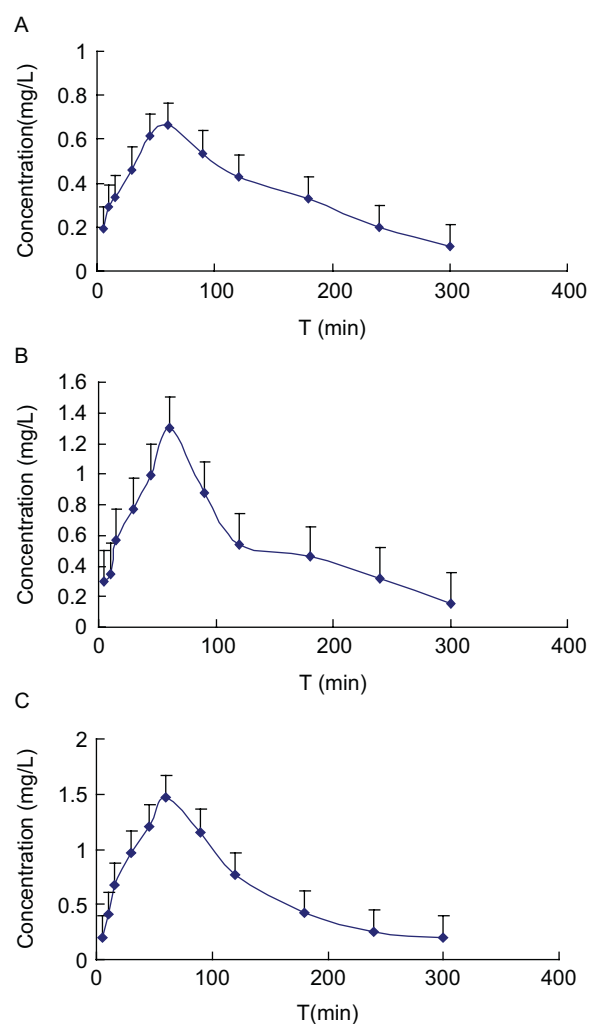


Figure 2. Mean plasma concentration of morroniside after Oral administration iridoid glycosides of Fructus Corni. (A) Oral administration of 20 mg/kg; (B) Oral administration of 40 mg/kg; (C) Oral administration of 80 mg/kg. Each point and bar represent the mean \pm SD ($n=5$)

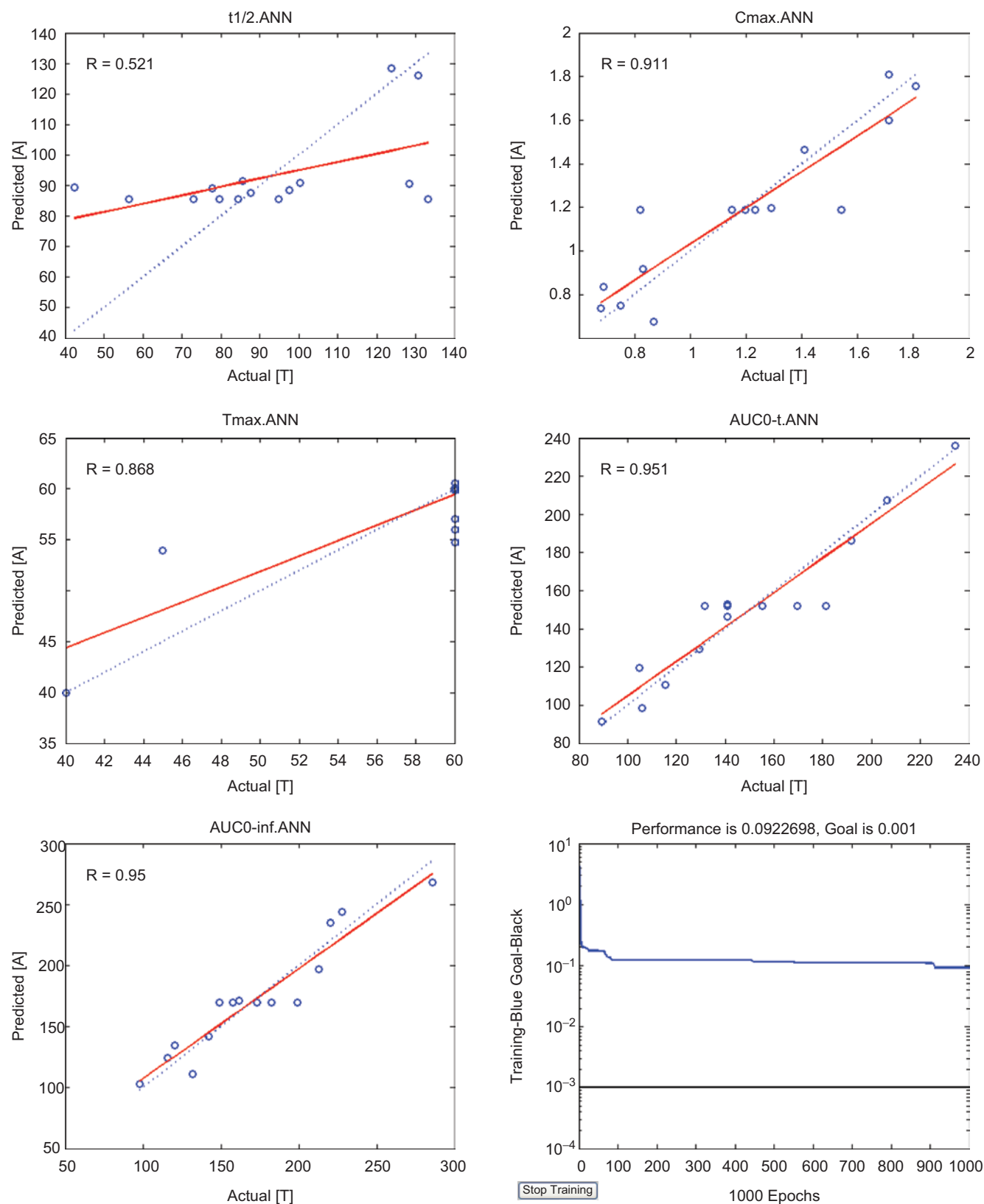


Figure 3. Predicted vs actual PK parameters: (A) $t_{1/2}$ (min); (B) C_{max} (mg/l); (C) T_{max} (min); (D) AUC_{0-t} and (E) AUC_{0-inf} ; (F) MSE of neural network model with 11 hidden neurons and 90% training data is 0.092.

Table 1. Pharmacokinetic parameters of oral administration iridoid glycosides of *Fructus Corni*. (mean \pm SD, $n=5$).

Pharmacokinetic parameters	Oral administration		
	20 mg/kg	40 mg/kg	80 mg/kg
K_e (min^{-1})	0.0097 ± 0.0015	0.0066 ± 0.0008	0.0074 ± 0.0014
$t_{1/2}$ (min)	72.6346 ± 11.3283	106.2873 ± 12.2475	95.9570 ± 18.6468
C_{\max} (mg/L)	0.7642 ± 0.0844	$1.3.0000 \pm 0.2645$	1.4734 ± 0.2782
T_{\max} (min)	53.0000 ± 9.7467	58.9974 ± 5.4680	58.7683 ± 10.2643
AUC_{0-t}	109.0716 ± 14.6384	166.0140 ± 30.3033	182.6015 ± 40.2990
$AUC_{t-\infty}$	11.8985 ± 6.1306	23.99332 ± 8.9463	31.92453 ± 10.3409
$AUC_{0-\infty}$	120.9701 ± 15.1934	190.0073 ± 30.8816	204.526 ± 53.4153

dose and weight; they were determined by the properties of themselves. (iii) It provides a new method to calculate PK data, one do not need to figure out all the compartment parameters to acquire pk data of morroniside.

Conclusion

A simple, rapid and specific HPLC method has been established for investigating PKs of morroniside after oral administration of iridoid glycosides from *Fructus Corni* to rats. Furthermore, this article demonstrates the BP neural network has been successfully applied to predict PK parameters of morroniside. This study will help in rationalizing the design of clinical trails and reducing considerable cost of preclinical experiments. Besides, it provides a promising approach to investigate and explain the PK data of TCM in a new way. In addition, it proves that ANN is a valuable modelling tool that can be used to explain the relationships between the dosing regimen and PK parameters, which may also be helpful in drug discovery and development. This study would be useful for guiding the holistic PK study in consistence with the intrinsic theory and characteristics of TCM.

Declaration of interest

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