Pharmacological properties of galenical preparations (XIX)[1] Pharmacokinetic study of 6,7-dimethylesculetin in rats

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Abstract

The main compound absorbed into the blood of rats after oral administration of Inchinko-to and 6,7-dimethylesculetin (6,7-DME) was analyzed by three dimensional high performance liquid chromatography (3D-HPLC). In blood samples, 6,7-DME and capillarisin were identified after oral administration of Inchinko-to. A high performance liquid chromatographic method was developed to determine the concentration of 6,7-DME in rat plasma and a pharmacokinetic study was performed in rats at the same time. Quantitative analysis with high reproducibility was achieved for 6,7-DME over the concentration range of 0.01-10 μg/ml. After bolus intravenous administration at a dose of 2 and 5 mg/kg, the plasma concentration-time curve was described by a one compartment open model. 6, 7-DME was cleared from plasma with a half-life of 11 min and a total body clearance of 44 ml/min/kg.

Key words 6,7-dimethylesculetin, Inchinko-to, *Artemisia capillalis*, pharmacokinetics, determination, HPLC.

Abbreviations 3D-HPLC, three dimensional high performance liquid chromatography ; 6,7-DME, 6, 7-dimethylesculetin.

Introduction

The Capillalis flower (flower of *Artemisia capllaris* THUNBERG) is one of the oldest Chinese medicines[2] and is used as an antiinflammatory, antipyretic, choleretic and diuretic agent in the case of liver disorder and yellow disease.[3,4] In traditional medicine, it has been called "Inchinko (茵蔯蒿)". Many pharmacological experiments proved that the main coumarin derivatives in Capllaris flower, 6,7-dimethylesculetin (6,7-DME) and capillalisin, showed antiinflammatory, analgesic and choleretic properties.[5] But whether 6,7-DME existed in crude drugs, and prescriptions such as Inchinko-to can be absorbed in its original form into blood has been unknown and what is more, little has been known about the pharmacokinetics of the compound. Therefore it is essential to analyze the blood sample after oral administration of 6,7-DME in plasma samples after administration and evaluate the pharmacokinetic properties of the drug to clarify the clinical effects of the traditional medicine. The purpose of the present study was to solve these problems.

Material and Methods

Materials : Capillalis flower, Gardenia fruit and Rhubarb were commercially obtained in Osaka in 1992. 6,7-DME[6] and capillalisin[6] were isolated from Capillalis flower. Albumin, Bovine (Sigma Chemical Co. Ltd.) was purchased from Wako Pure Chemical Industries Ltd..

Animals : Male Wistar/ST rats 6 weeks old (150-180 g body weight, Shizuoka Laboratory Animal Center) were housed under following conditions for 3 days

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acclimation. The animals were bred in a breeding room with a temperature of 24±1°C, humidity of 50± 5 %, and 12 hours dark-light cycle. They were given tap water and fed normal foods *ad libitum*, then were fasted for about 24 hours before the experiment.

Preparation of Extract : Inchinko-to : According to the literature,[7] a daily dosage of the prescription consists of Capillalis flower 6.0 g, Gardenia fruit 1.4 g and Rhubarb 2.0 g. First of all, 6 g Capillalis flower was added to 480 ml of water, then the mixture was heated and kept at a boil until the volume of the solution evaporated to about 240 ml, at which time Rhubarb and Gardenia fruit was added to the solution. Boiling continued for 10 min more, and then it was filtrated through a 5-layer gauze. The filtrate solution was then freeze-dried and stored at 4°C.

Drug administration : Oral administration : Inchinko-to or 6,7-DME was suspended with 2 % Tween 80. The administration volume was 10 ml/kg. Intravenous administration : The administration liquid was prepared by adding 150 μl of a 6,7-DME ethanol solution to 800 μl of a 5 % albumin aqueous solution, then adding a further 5 % albumin aqueous solution to obtain 1 ml. The solution was injected into a rat through the tail vein. The administration volume was set at 4 ml/kg.

Determination of blood sample : To 1.0 ml of plasma, 6 ml of MeOH was added and vortexed for 30 s. The mixture was centrifuged at 3000 rpm. for 10 min at room temperature and the supernatant obtained was evaporated to dryness *in vacuo*. The residue was reconstituted in 200 μl methanol and filtrated through 0.45 μm membrane filter (Millipore Co. Ltd, Tokyo) and an aliquot (20 μl) of the filtrated solution was injected into HPLC.

HPLC condition for Determination : A Waters 510 LC pump equipped with a Waters 991J photodiode-array detector was used. Samples were chromotographed with an analytical column of Inertsil ODS-2 (GL Sciences, 5 μm, 4.6 I.D.×250 mm) at 40°C. The mobile phase was MeCN/Water (35/65) with a flow rate of 1.0 ml/min. The eluate was monitored by UV spectrophotometric detector at 345 nm.

Calibration graph : To blank plasma were added known amounts of 6,7-DME in the final concentration range of 0.01-10.0 μg/ml. These plasma samples were treated according to the above assay procedure. The calibration curve was constructed by using the peak area of the drug and the corresponding concentration.

Reproducibility : Blood samples were obtained from the rat at appropriate times after the administration of 6,7-DME. Aliquots (1.0 ml) of plasma sample were repeatedly analyzed according to the above procedure. Ten repeated analyses were carried out.

Accuracy : To the plasma samples which were obtained from the rats administered with 6,7-DME were added known amounts of 6,7-DME and then the compound in the plasma was determined before and after the addition. The recovery of the compound was calculated by comparing the experimental value with the corresponding theoretical value.

Pharmacokinetics study : 6,7-DME was administered to one group of 10 rats. After a designated time period, blood was collected from the carotid artery under ether anesthesia. The blood samples were immediately kept in an ice-box. After being centrifuged at 3000 rpm. for 10 min at room temperature, the plasma were immediately separated and kept frozen until the analysis. Plasma concentration-time data after administration of 6, 7-DME was analyzed by a non-linear least squares regression program, MULTI.[8] The area under the drug plasma concentration-time curve (AUC) was calculated by the trapezoidal method from a graph for up 3h.

Results

Identification of 6.7-dimethylesculetin and capillalisin in blood

After oral administration of Inchinko-to, the blood sample was analyzed by three dimensional HPLC. (Fig. 1) Based on the retention time and UV spectrum, we compared the results with that of 6,7-DME and capillalisin pure compound in blood samples, and it was clear that 6, 7-DME and capillalisin existing in Inchinko-to was absorbed into blood in the original form as the main component.

Pharmacokinetics study

Typical chromatograms resulting from the analysis of blank and plasma samples obtained from rats which were given 6,7-DME was well separated from

the peak which seemed to be derived from endogenous materials in the rat plasma. The retention time 6,7-DME was about 8.4 min. Calibration curve for 6,7-DME was generated by least-square linear regression analysis, satisfactory linearity was observed in the concentration range of 0.01-10.0 μg/ml of the drug. The regression equation was $Y = 14206.2X + 22.8$, $r = 0.9999$, in the equation, Y is the peak area, X is the concentration (μg/ml) of the drug in plasma and r is the coefficient of correlation.

Table I Precision and accuracy on the determination of 6,7-dimethylesculetin in rat plasma.

Reproducibility[a]		Recovery[b]		
Mean (μg/ml)	CV (%)	Added (μg/ml)	Found (μg/ml)	Mean (%)
0.13	4.7	None	1.21	-
1.21	3.8	1.00	2.24	103.2
5.17	2.5	5.00	6.19	99.5

a) Based on 10 determination.
b) Based on 5 determination.

Reproducibility was evaluated by ten replicate assays at each of three different concentrations of the drug in plasma sample. The coefficients of variation (C.V.) range from 2.5-4.7 % as shown in Table I.

Table I also shows the recovery data for the determination. The data was obtained when the drug was added to the rat plasma sample of three different concentrations. The average recovery at both concentrations was nearly 100 %.

After the bolus i.v. administration of 2 or 5 mg/kg of 6, 7-DME, the plasma level of the drug declined with time in a monophasic pattern as shown in Fig. 2. A one compartment open model was found to describe the data most adequately. The corresponding pharmacokinetic parameters which were estimated by the analysis of the data obtained from the individual animal at different times are given in Table II. 6. 7-DME was cleared from plasma with a half-life of 11 min and a total body clearance of 44 ml/min/kg.

Table II Pharmacokinetic parameters for 6,7-dimetylesculetin after administration to rats.

Sample	Dose	$T_{1/2}$[a] (min)	Vd[a] (l/kg)	AUC_{0-3} (μg · min/ml)	Bioavailability (%)
i. v.					
6,7-DME	2.0 mg/kg	11.6	0.74	45.0	–
6,7-DME	5.0 mg/kg	11.0	0.71	112.6	–
p. o.					
6,7-DME	5.0 mg/kg	–	–	72.3	64.2
Inchinko-to (6,7-DME 5 mg/kg)	2 g/kg	–	–	25.8	22.9
Inchinko-to (6,7-DME 15 mg/kg)	6 g/kg	–	–	20.4	6.0

a) Estimated by program MULTI.

Discussion

In order to elucidate the clinical effects of the traditional Chinese medicinal prescriptions, it is important to investigate their pharmacokinetics. Based on the fact that certain compounds existed in the crude drug or that prescriptions must be absorbed into the body in order to show the pharmacological activities, we analyzed the blood after oral administration of Inchinko-to. And by further analyzing the chromatograms of the plasma, we found the main compound absorbed into blood is the original form of 6, 7 - DME as shown in Fig. 1. Capillarisin can be detected to one-two hundredths in blood. Even though capillarisine, another compound[9, 10] found in the crude drug, has a stronger choleretic effect than 6,7-DME. 6,7-DME can be considered as the main effective compound.

Because of the absence of the method for determining 6,7-DME in biological fluids, we first developed a very simple and precise method for the determination of the drug in plasma by HPLC. The pretreatment procedure in the present method consisted of only two processes, the deproteinization and concentration, without any solvent extraction. There-

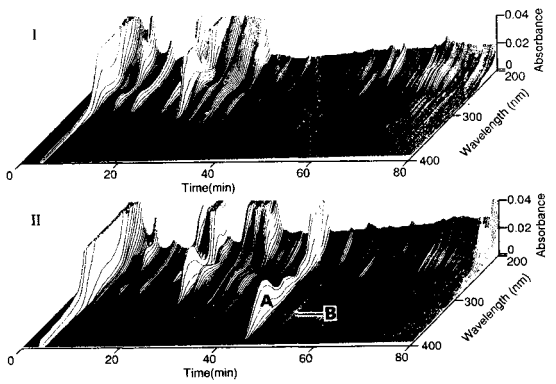

Fig. 1 Three dimensional HPLC profile of plasma in rat. I : control plasma, II : sample plasma (after oral administration of Inchinko-to 6.0 g/kg). Analytical conditions : A Waters 510 gradient system equipped with a Waters 991J photodiode-arry detctor. Column ; Inertsil ODS-2 (5 μm, 4.6 I.D.×250 mm, GL Sciences). Column temperature ; 40 °C. Detection ; 200～400 nm. Mobile phase ; water (A)-acetonitril (B) gradient, (A)/(B)=100/0→0/100, for 120 min. Flow rate ; 1 ml/min. A : 6,7-dimetylesculetin, B : capillarisin.

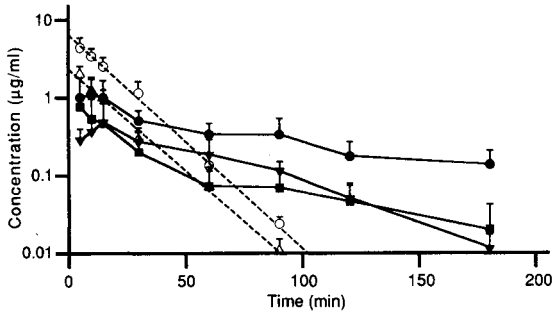

Fig. 2 Time course of concentration in plasma after intravenous and oral administration of 6,7-dimethylesculetin to rats (n=10). Each point and vertical bar represents the mean and S.D..
i.v. ; ○ : 6,7-DME (5 mg/kg), △ : 6,7-DME (2 mg/kg),
p.o. ; ● : 6,7-DME (5 mg/kg), ■ : Inchinko-to (6 g/kg),
▼ : Inchinko-to (2 g/kg)

fore, nothing was employed as the internal standard. The data listed in Table I indicates the satisfactory precision and accuracy of the determination method.

Using the method we performed the pharmacokinetic study of 6,7-DME after administration. The plasma concentration was analyzed at 8 different times after administration, beyond 180 min it was impossible to monitor the plasma level of 6,7-DME, since the concentrations were quite close to the limit of sensitivity. From the pharmacokinetic parameters it was found 6,7-DME has a little shorter half-life (11 min) ; it was cleared rapidly from plasma. But, the plasma level was retained after oral administration. It has been reported that the choleretic action was maintained until 3 h. after oral administration at a dose of 25 mg/kg.[11] Thus, it is possible to consider that 6,7-DME takes part in the choleretic action. After oral administration of Inchinko-to 2 and 6 g/kg, the AUC showed about equal value. The result was considered to be one cause which was not a correlation between dose and effect in pharmacological experiments. Further detailed work is necessary to clarify the specific disposition and metaboliates of the drug is now in progress.

和文抄録

茵蔯蒿湯あるいは 6,7-dimethylesculetin (6,7-DME) 経口投与後のラット血中に吸収する主要成分を三次元高速液体クロマトグラフ装置により分析した。血液試料において茵蔯蒿湯経口投与後に 6,7-DME と capillarisin を確認した。血中成分の高速液体クロマトグラフによる定量法を開発し，同時にラットにおける生物薬剤学的研究を行った。0.01-10 μg/ml の濃度範囲の 6,7-DME のための高い再現性を持つ定量法を開発した。2 あるいは 5 mg/kg 急速静注後の血中濃度時間曲線は one compartment open model に従って消失した。6,7-DME の血中からの消失は 11 min，全身クリアランスは 44 ml/min/kg であった。

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