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Pharmacological properties of galenical preparations (XVIII)[1] : Pharmacokinetics of active compounds of Polygalae Radix

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Abstract

We studied the pharmacokinetic properties of 3,4,5-trimethoxycinnamic acid (TMCA), methyl 3,4,5-trimethoxycinnamate (M-TMCA) and p-methoxycinnamic acid (PMCA), which are the bioactive substances derived from Polygalae Radix, and studied the time course of plasma concentration of TMCA and M-TMCA in rats after oral administration of the water extract of Polygalae Radix. We analyzed the plasma samples by a three dimensional high performance liquid chromatography (3D-HPLC), and successfully established the method to determine the concentration of TMCA and its metabolite M-TMCA at the same time and PMCA in rat plasma. The favorable linear relationship with 0.9999, 0.9997 and 0.9999 of correlation coefficient were obtained in the range of 0.5-32.0 μg/ml of TMCA, 0.25-4.0 μg/ml of M-TMCA and 1.0-30.0 μg/ml of PMCA, respectively. The recovery in plasma was 80.4-103.4 % of TMCA, 85.8-106.0 % of M-TMCA and 85.1-103.6 % of PMCA, respectively. The coefficients of variation were less than 5.0 %. The studies on pharmacokinetic properties of TMCA and PMCA were performed on rats after intravenous (i.v.) administration of TMCA at a dose of 10 mg/kg and PMCA at 5 mg/kg. The plasma concentration-time curves of TMCA and PMCA were described by a one-compartment open model. TMCA and PMCA were cleared from plasma with the half-life time ($T_{1/2}$) of 14.0 min and 17.4 min, respectively. The volume of distribution (Vd) of TMCA and PMCA were 252 ml/kg and 127 ml/kg. The total body clearance (CL_T) of TMCA and PMCA were 0.747 L/hr/kg and 0.304 L/hr/kg. TMCA and M-TMCA could be changed forms with each other in rat body. We also studied the time course of the concentration of TMCA and M-TMCA in rat plasma after oral administration of the water extract of Polygalae Radix. We were very interested in the results that TMCA and M-TMCA in rat plasma were kept in a constant concentration from 15 min to 3 hr after oral administration of the water extract of Polygalae Radix. It was suggested that TMCA and M-TMCA detected in the blood were derived from prodrugs contained in Polygalae Radix, such as onjisaponin E, F,G, sucrose derivatives and others, which contain a 3,4,5-trimethoxycinnamoyl moiety within their chemical structures.

Key words *Polygala tenuifolia,* methyl 3,4,5-trimethoxycinnamate, 3,4,5-trimethoxycinnamic acid, p-methoxycinnamic acid, absorption, metabolite, pharmacokinetics.

Abbreviations 3D-HPLC, three dimensional high performance liquid chromatography ; M-TMCA, methyl 3,4,5-trimethoxycinnamate ; TMCA, 3,4,5-trimethoxycinnamic acid; PMCA, p-methoxycinnamic acid.

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Introduction

Onji (遠志), Polygalae Radix, *Polygala tenuifolia* WILLDENOW (Yuan zhi in China), is a well known Chinese traditional medicine used as a sedative, expectorant and tonic agent. It has been reported that Polygalae Radix has various pharmacological effects, e.g., inhibitory effects on cyclic adenosine monophosphate (c-AMP) phosphodiesterase[2] and prolongation effects on hexobarbital sleeping time in mice,[2] which are principally based on onjisaponins, and the sedative activity of the derivatives of TMCA.[3]

In our previous study,[1] we made a trial search for the bioactive substances in blood and bile samples of rats after oral administration of the extracts of Polygalae Radix, and finally found TMCA, M-TMCA and PMCA which induced the prolongation of hexobarbital sleeping time in mice. In order to elucidate the contribution of TMCA, M-TMCA and PMCA to the pharmacological activity of Polygalae Radix, it is important to investigate their pharmacokinetic properties. However, little has been known about the pharmacokinetics of these compounds. The purpose of this paper is to develop a simple and sensitive method for determination of TMCA, M-TMCA and PMCA in rat plasma by 3D-HPLC and to clarify the pharmacokinetic properties of these compounds after i.v. administration to rats. We also wish to report an interesting result in the time course of plasma concentration of TMCA and M-TMCA after oral administration of the water extract of Polygalae Radix.

Materials and Methods

Crude drug : Polygalae Radix, the dried root of *Polygala tenuifolia* WILLD., was commercially obtained from the Japanese market, Mikuni Co., Ltd. in Osaka. Polygalae Radix was cut into small pieces and used for this experiment.

Chemicals : 3,4,5-trimethoxycinnamic acid (TMCA), p-methoxycinnamic acid (PMCA), sodium isomytal and albumin bovine (Sigma Chemical Co., Ltd.) were purchased from Wako Pure Chemical Industries Ltd. in Japan. Methyl 3,4,5-trimethoxycinnamate (M-TMCA) was isolated from the bile of rats after administration of TMCA. The details for the methods of isolation and identification have been reported in a previous report.[1]

Animals : Male Wistar/ST rats, weighing 190-210 g, used in this experiment were purchased from Nihon SLC Co., Ltd. in Hamamatsu, Japan. They were housed under conditions of 24±1°C and 12 hr light (from 6 a.m. to 6 p.m.) and fed a commercial diet (MF, Oriental Yeast Co., Tokyo) and allowed tap water *ad libitum* before the experiments.

Preparation of water extract of Polygalae Radix : 250.0 g of Polygalae Radix was mixed with 5000 ml of distilled water, and boiled until the volume was reduced to 2500 ml. The filtered decoction was freeze-dried and the powder obtained (1.0 g corresponds to 4.0 g crude drug) was kept in a refrigerator. The water extract of Polygalae Radix was dissolved and / or suspended in water just before oral administration to rats.

Pharmacokinetic study of TMCA, M-TMCA and PMCA

Quantitative analysis of TMCA, M-TMCA and PMCA in rat plasma by 3D-HPLC : 1) Calibration curve : Standard samples were made by the addition of known amounts of TMCA, M-TMCA and PMCA to blank plasma of rat in the final concentration range of 0.5-30.0 μg/ml of TMCA, 0.25-4.0 μg/ml of M-TMCA and 1.0-30.0 μg/ml of PMCA, respectively. These standard samples were treated as follows : 6 ml of methanol was added to 1.0 ml of standard sample, the mixture was centrifuged at 3000 rpm for 10 min at room temperature and the supernatant was evaporated to dryness below 40°C *in vacuo*. The residue was extracted with 1.0 ml of chloroform : methanol (4 : 1), and evaporated again. The residue was dissolved in 200 μl of acetonitrile : water (4 : 1), and filtrated through a 0.45 μm membrane filter (Tokyo Kagaku Sangyo Co. Ltd., Tokyo). 20 μl of the solution was injected for 3D-HPLC analysis. The plasma sample for PMCA was treated with methanol by the same way with that of TMCA but the residue was extracted with 1.0 ml of acetonitrile : methanol (4 : 1) and evaporated. The residue was dissolved in 400 μl of acetonitrile : water (4 : 1) and filtrated. 10 μl of the solutions was injected for 3D-HPLC analysis. The calibration curves were constructed by using the peak

areas of the standard samples and the corresponding concentration. 2) Accuracy : According to the results of the above standard samples, we calculated the recovery of standard compounds in plasma by comparing the volume found with the added volume. The results were based on three determinations. 3) Reproducibility : Blood samples were taken from rat vena portae at appropriate times after i.v. administration of TMCA and PMCA, and the plasma samples were analyzed repeatedly by the above procedure. 4) 3D-HPLC condition for quantitative analysis : Pump ; Waters 600 multisolvent delivery system. Detector ; Waters 991J Photodiode-array detector. Column ; Inertsil ODS-2 (4.6×250 mm, GL Science Inc.). Mobile phase ; acetonitrile (0.1 % acetic acid) : water (0.1 % acetic acid)=30 : 70 for TMCA and M-TMCA, and 40 : 60 for PMCA. Flow rate ; 1.0 ml/min. Temperature of the column ; 40°C. Detection wavelength ; 300 nm for TMCA and M-TMCA, and 310 nm for PMCA. Injection volume ; 10 and 20 μl.

Pharmacokinetics of TMCA and PMCA : 1) Drug administration : TMCA and PMCA were dissolved in the least amount of ethanol possible, then the solutions were uniformly dispersed in a 5 % albumin solution. The solutions were intravenously administered to rats at the dose of 10 mg/kg of the TMCA solution and 5 mg/kg of the PMCA solution, respectively. 2) Collection and analysis of blood sample : After administration of the drugs, the blood samples were collected from a portal vein at the designated time periods (1 min, 15 min, 30 min, 1 hr, 1.5 hr, 2 hr) and immediately kept in an ice-box. After being centrifuged at 3000 rpm for 10 min below 4°C, the plasma samples were obtained. The plasma samples were treated according to the above quantitative analysis procedure. 3) Pharmacokinetic analysis : Plasma concentration-time data after i.v. administration of TMCA and PMCA were analyzed by a non-linear least squares regression program MULTI.[4] The pharmacokinetic parameters were estimated by convention equations.

Plasma concentration-time course of TMCA and M-TMCA after administration of M-TMCA

The solution of M-TMCA was made by the same method as that of TMCA. The rats were given the drugs at the dose of 10 mg/kg. The blood samples were collected from a portal vein at 1 min, 15 min, 30 min, 1 hr and 2 hr after i.v. administration and at 0-1 min after injection into a small intestine, The treatment and determination of the blood samples were carried out by the same method as that of TMCA.

Plasma concentration-time course of TMCA and M-TMCA after oral administration of TMCA and the water extract of Polygalae Radix

Quantitative analysis of TMCA and M-TMCA in rat plasma by 3D-HPLC : Standard solution for a calibration curve was made by the addition of known amounts of TMCA and M-TMCA to blank plasma in the final concentration range of 0.0625-4.0 μg/ml of TMCA and 0.125-2.0 μg/ml of M-TMCA. These standard plasma samples were treated as follows : 12 ml of methanol was added to 2.0 ml of plasma, the mixture was centrifuged at 3000 rpm for 10 min at room temperature and the supernatant was evaporated to dryness below 40°C *in vacuo*. One ml of methanol was added to the residue and the mixture was centrifuged. The supernatant was evaporated and the residue was extracted with 1.0 ml of chloroform : methanol (4 : 1), and evaporated again. The residue was dissolved in 200 μl of acetonitrile : water (4 : 1), and filtrated through a 0.45 μm membrane filter. 50.0 μl of the solution was injected for 3D-HPLC analysis. 3D-HPLC conditions were the same as the above. The calibration curves were constructed by using the peak areas of standard compounds and the corresponding concentrations. Accuracy and reproducibility were made by the same method with the above.

Time course of TMCA and M-TMCA after oral administration of TMCA and the water extract of Polygalae Radix : TMCA and the water extract of Polygalae Radix were dissolved in distilled water to make the solutions of TMCA in 0.50 mg/ml and the water extract in 0.40 g/ml. The drugs were orally administered to rats, at a dose of 5 mg/kg of TMCA and 5.0 g/kg of the water extract of Polygalae Radix. The blood samples were collected from a portal vein at 15 min, 30 min, 1 hr, 1.5 hr, 2 hr and 3 hr after oral administration of drugs. The treatment and determination of the blood samples were carried out in the same method recorded above.

Results

Pharmacokinetic studies of TMCA, M-TMCA and PMCA

Quantitative analysis of TMCA, M-TMCA and PMCA in rat plasma by 3D-HPLC

1. Calibration curves : Fig. 1 and 2 show representative chromatograms for one example of blank plasma and sample plasma obtained from the rats which were given water, TMCA and PMCA, respectively. The peaks for TMCA, M-TMCA and PMCA in the plasma were well separated from the peaks which seemed to be derived from endogenous materials in the rat plasma. The retention time of TMCA, M-TMCA and PMCA was 10.9 min, 35.3 min and 7.5 min, respectively. According to the peak areas of the designated concentration which were obtained from the HPLC chart of standard solution, we got the equations for the calibration curve by the least squares method. The favorable linear relationship with 0.9999 of correlation coefficient was obtained in

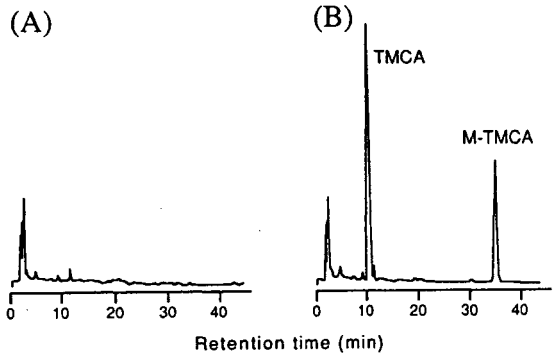

Fig. 1 Chromatograms of (A) a plasma blank and (B) a plasma sample obtained after intravenous administration of TMCA to a rat.

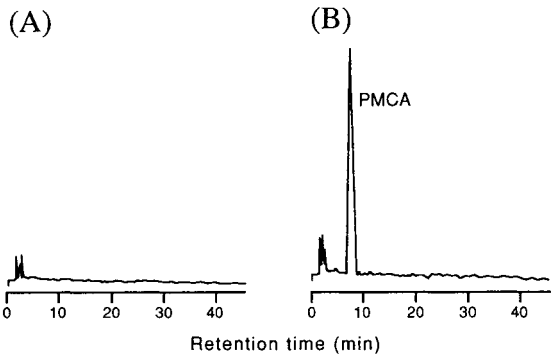

Fig. 2 Chromatograms of (A) a plasma blank and (B) a plasma sample obtained after intravenous administration of PMCA to a rat.

Table I Precision on the determination of TMCA, M-TMCA and PMCA in rat plasma.

Drugs	Mean (μg/ml)	S.D.	n	C. V. (%)
TMCA[a)]	2.07	0.05	6	2.3
	15.8	0.68	6	4.3
M-TMCA[b)]	0.48	0.02	6	4.8
	2.03	0.10	6	4.8
PMCA[c)]	3.61	0.07	4	1.9
	34.4	1.21	4	3.5

a) 3,4,5-trimethoxycinnamic acid. b) methyl 3,4,5-trimethoxycinnamate. c) p-methoxycinnamic acid.

Table II Accuracy on the determination of TMCA, M-TMCA and PMCA.

TMCA[a)]			M-TMCA[b)]			PMCA[c)]		
Added (μg/ml)	Found (μg/ml)	Recovery (%)	Added (μg/ml)	Found (μg/ml)	Recovery (%)	Added (μg/ml)	Found (μg/ml)	Recovery (%)
0.05	0.04	80.0	0.25	0.21	85.8	1.00	0.85	85.1
1.00	0.98	98.0	0.50	0.48	95.5	2.00	1.92	96.1
2.00	2.07	103.4	1.00	1.06	106.0	4.00	4.08	101.9
4.00	4.09	102.3	2.00	2.02	100.9	8.00	8.29	103.6
8.00	8.18	102.3	4.00	3.98	99.5	16.0	15.9	99.3
16.0	15.7	98.1				32.0	32.0	99.9
32.0	32.1	100.3						
	(r=0.9999)			(r=0.9997)			(r=0.9999)	

a) 3,4,5-trimethoxycinnamic acid. b) methyl 3,4,5-trimethoxycinnamate. c) p-methoxycinnamic acid. The data were based on 3 determinations.

the range of 0.5-32.0 μg/ml of TMCA, the regression equation was $y_1 = 216.9x_1 - 0.12$ (x_1 : peak area of TMCA, y_1 : concentration of TMCA in plasma). 0.9997 of correlation coefficient was obtained in 0.25-4.0 μg/ml of M-TMCA, the regression equation was $y_2 = 163.3\ x_2 - 0.05$ (x_2 : peak area of M-TMCA, y_2 : concentration of M-TMCA in plasma) and 0.9999 of correlation coefficient in 1.0-30.0 μg/ml of PMCA, the regression equation was $y_3 = 486.8x_3 - 0.47$ (x_3 : peak area of PMCA, y_3 : concentration of PMCA in plasma).

2. Accuracy and reproducibility : The results are shown in Table I and II. The accuracy of the quantitative analysis of TMCA, M-TMCA and PMCA was 80.0-103.4 %, 85.8-106.0 % and 96.7-111.3 %, respectively. The reproducibilities which were shown by the coefficients of variation (C.V., %) of TMCA, M-TMCA and PMCA in the range of determination were less than 5.0 %.

The pharmacokinetic parameters of TMCA and PMCA

The plasma concentration-time curves of TMCA, M-TMCA and PMCA after i.v. administration of TMCA (10 mg/kg) and PMCA (5 mg/kg) are shown in Fig. 3 and 4. The one compartment open model was used to describe the elimination of TMCA and PMCA in rat body. The pharmacokinetic parameters which were estimated by the data obtained from the individual animal (n=6) at different time are shown in Table III. The elimination half-life time ($T_{1/2}$) of TMCA and

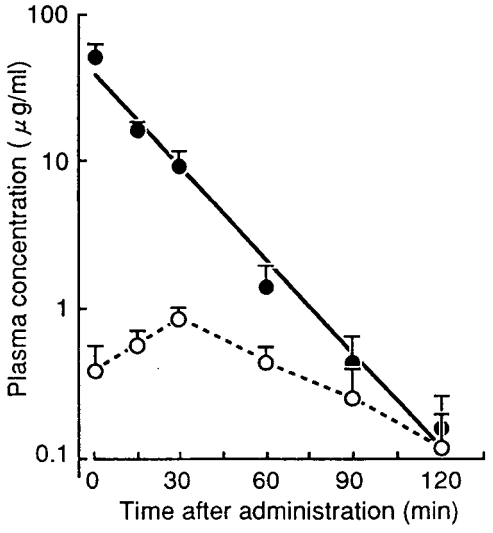

Fig. 3 Plasma concentration-time profile of TMCA and M-TMCA after intravenous administration of TMCA (10 mg/kg) to rats.
●—● : TMCA. ○---○ : M-TMCA.
Each point and vertical bar represent the mean and S.D. of 6 rats.

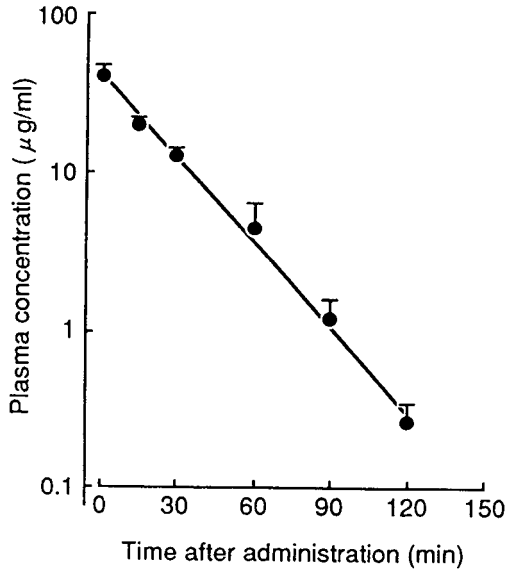

Fig. 4 Plasma concentration-time profile of PMCA after intravenous administration (5 mg/kg) to rats.
Each point and vertical bar represent the mean and S.D. of 6 rats.

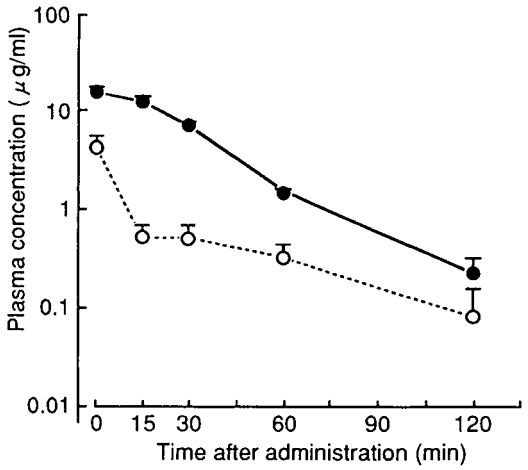

Fig. 5 Plasma concentration-time profile of M-TMCA and TMCA after intravenous administration of M-TMCA (10 mg/kg) to rats.
●—● : TMCA. ○---○ : M-TMCA.
Each point and vertical bar represent the mean and S.D. of 7 rats.

Table III Pharmacokinetics parameters for TMCA and PMCA after intravenous administration of TMCA and PMCA to rats.

Parameter[a]		TMCA[b]	PMCA[c]
Co	(mg/L)	39.7	39.2
Ke	(1/hr)	2.97	2.39
$T_{1/2}$	(min)	14.0	17.4
Vd	(L/kg)	0.252	0.127
CL_T	(L/kg/hr)	0.747	0.304
AUC	(mg/hr/L)	13.4	17.4

a) : estimations based on the one-compartment open model by program MULTI [Weight=1/Cp(1)]
b) : i. v. administration at 10 mg/kg of TMCA, based on the data of 6 rats.
c) : i. v. administration at 5 mg/kg of PMCA, based on the data of 6 rats.

PMCA was 14.0 min and 17.4 min, respectively.

Plasma concentration-time course of M-TMCA

The plasma concentration-time course of TMCA and M-TMCA after i.v. administration of M-TMCA (10.0 mg/kg) was shown in Fig. 5. The results showed that M-TMCA was rapidly changed into TMCA after i.v. administration of M-TMCA to rat. TMCA and M-TMCA in the plasma were eliminated from 30 min to 2 hr at the same rate as in the case of i.v. administration of TMCA.

Plasma concentration-time course of TMCA and M-TMCA after oral administration of TMCA and the water extract of Polygalae Radix

1. Calibration curves, accuracy and reproducibility : According to the peak areas of HPLC which were obtained from the standard solution, the equations for the calibration curve was calculated by the least squares method. The favorable linear relationship with 0.9998 of correlation coefficient was obtained in the concentration range of 0.0625-4.0 μg/ml of TMCA, the equation was $y_1=40.6x_1-0.01$ (x_1 : peak area of TMCA, y_1 : concentration of TMCA in plasma). 0.9998 of correlation coefficient was also obtained in 0.125-2.0 μg/ml of M-TMCA, the equation was $y_2=38.7x_2-0.03$ (x_2 : peak area of M-TMCA, y_2 : concentration of M-TMCA in plasma). The accuracy of the quantitative analysis of TMCA and M-TMCA was 93.2-108.8 % and 86.4-103.8 %, respectively. The reproducibility which were shown by the coefficients of variation (C.V., %) of TMCA and M-TMCA in the concentration range of determination were less than 5.0 %. The results are shown in Table IV and V.

2. Time course of plasma concentration : The time courses of plasma concentration of TMCA and

Table IV Precision on the determination of TMCA and M-TMCA in rat plasma after oral administration of the water extract of Polygalae Radix.

Drugs	Mean (μg/ml)	S.D.	n	C. V. (%)
TMCA[a]	0.123	0.005	5	4.1
	1.046	0.044	5	4.2
M-TMCA[b]	0.108	0.005	5	4.6
	1.002	0.025	5	2.5

a) 3,4,5-trimethoxycinnamic acid.
b) methyl 3,4,5-trimethoxycinnamate.

Table V Accuracy on the determination of TMCA and M-TMCA in rat plasma after oral administration of the water extract of Polygalae Radix.

TMCA[a]			M-TMCA[b]		
Added (μg/ml)	Found (μg/ml)	Recovery (%)	Added (μg/ml)	Found (μg/ml)	Recovery (%)
0.0625	0.058	93.2	0.125	0.108	86.4
0.125	0.136	108.8	0.250	0.251	100.4
0.250	0.242	96.8	0.500	0.519	103.8
0.500	0.455	91.0	1.000	1.002	100.2
1.000	1.042	104.2	2.000	1.998	99.9
2.000	2.017	100.9			
4.000	3.987	99.7			
	(r=0.9998)			(r=0.9998)	

a) 3,4,5-trimethoxycinnamic acid. b)methyl 3,4,5-trimethoxycinnamate.
The data were based on 4-5 determinations.

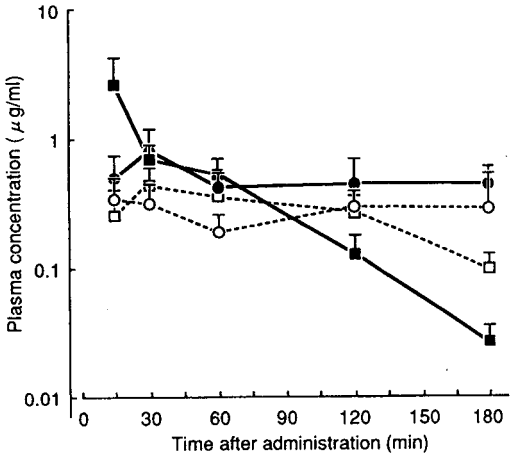

Fig. 6 Time course of plasma concentration of TMCA and M-TMCA after oral administration of water extract of Polygalae Radix (5 g/kg) and TMCA (10 mg/kg).
●—●, TMCA ; ○----○, M-TMCA : Plasma concentration after administration of water extract.
■—■, TMCA ; □----□, M-TMCA : Plasma concentration after administration of TMCA.
Each point and vertical bar represent the mean and S.D. of 4 rats.

M-TMCA after oral administration of TMCA and the water extract of Polygalae Radix were shown in Fig. 6. The result showed that the peak of concentration of TMCA in plasma was at 15 min after oral administration of TMCA, and that of its metabolite M-TMCA was at 30 min ; and then they were eliminated at a certain rate, the plasma concentrations of TMCA and M-TMCA at 3 hr were in a very low level. On the other hand, TMCA and M-TMCA in rat plasma after oral administration of the water extract of Polygalae Radix did not show the elimination course from 15 min to 3 hr, namely, they could be kept in a constant plasma concentration.

Discussion

In the studies on pharmacological properties of galenical preparations, we have found the bioactive compounds, TMCA, M-TMCA and PMCA ascribed to Polygalae Radix, in rat blood and bile after oral administration of the water extract of Polygalae Radix.[1] In order to elucidate and evaluate the clinical effects of the traditional Chinese medicines, it is nesessary and important to study the pharmacokinetic properties of the bioactive compounds included in them. Thus, we selected TMCA, M-TMCA and PMCA, as model bioactive compounds of Polygalae Radix and investigated the pharmacokinetic properties of these compounds in rats.

In the first place, sensitive and selective methods for the determination of these compounds in biological fluids were required to investigate the pharmacokinetic properties of these compounds. We developed the simple and sensitive methods for determinations of these compounds in rat plasma and bile by 3D-HPLC. The data listed in Table I and II indicated that the present methods had the satisfactory precision and accuracy in spite of the absence of an internal standard.

Using the present determination methods we performed pharmacokinetic studies on these compounds after i.v. administration. Our experimental results of the absorption and metabolism of TMCA and M-TMCA showed that a part of TMCA could be metabolized in rat liver and excreted into the small intestine with bile in the form of M-TMCA. T. Meyer and R. R. Scheline[5] have reported that the major metabolite of TMCA in rat bile was 3-hydroxy-4,5-dimethoxycinnamic acid ; but our result was M-TMCA as mentioned above. It may be caused by the difference of the strain of rats used in the respective experiments. The results at 1 min after i.v. administration of M-TMCA showed that M-TMCA in the blood could be changed into TMCA rapidly. M-TMCA excreted with bile into the small intestine[1] could be reabsorbed into blood in forms of TMCA and M-TMCA ; and then will undergo the enterohepatic cycle. These results will provide a part of the reasons for it being kept in a constant plasma concentration of TMCA and M-TMCA from 15 min to 3 hr after oral administration of the water extract of Polygalae Radix. From the present pharmacokinetic analysis, it was found that TMCA and PMCA were rapidly cleared from rat plasma and the half life time was 14.0 min and 17.4 min, respectively.

We were very interested in the results which showed that after oral administration of the water extract of Polygalae Radix, the plasma concentration of TMCA and M-TMCA were kept at a constant level

for about three hours. It was suggested that there were some prodrugs except TMCA contained in the water extract of Polygalae Radix. This suggestion was confirmed by the use of the butanol extract of Polygalae Radix which scarcely contained any TMCA. We detected TMCA in the plasma at two hours after oral administration of the butanol extract of Polygalae Radix. It was found that TMCA in the plasma must be derived from prodrugs in the butanol extract. Before some prodrugs are absorbed into a gastrointestinal tract, they may be hydrolyzed or metabolized at a different time ; and then will be absorbed into blood in the form of TMCA and/or M-TMCA. It was suggested that the prodrugs were onjisaponin E, F, G,[6,7] sucrose derivatives[8] and others which contain 3,4,5-trimethoxycinnamoyl moiety within their chemical structures. Further detailed study on the origin of TMCA and M-TMCA in the plasma is necessary to clarify the contribution of the prodrugs to the pharmacokinetic properties of the water extract of Polygalae Radix and is now in progress.

Acknowledgement

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和文抄録

遠志に由来する生物活性物質である 3,4,5-trimethoxycinnamic acid (TMCA), methyl 3,4,5-trimethoxycinnamate (M-TMCA), p-methoxycinnamic acid (PMCA) の薬物動態学的特性をラットを用いて研究した。また，遠志水エキスの経口投与後，ラット血漿中での TMCA と M-TMCA の経時的濃度変化を調査した。三次元高速液体クロマトグラフ装置を用いて血漿試料を分析し，TMCA とその代謝物である M-TMCA の同時定量法,また PMCA の定量法を確立した。検量線の作成においては，TMCA では 0.5-32.0 μg/ml, M-TMCA では 0.25-4.0 μg/ml, PMCA では 1.0-30.0 μg/ml の濃度範囲でそれぞれ 0.9999, 0.9997, 0.9999 の良好な直線関係を示す相関係数を得た。添加回収率は TMCA, M-TMCA, PMCA でそれぞれ 80.4-103.4 %, 85.8-106.0 %, 85.1-103.6 % であった。変動係数は 5.0 % 以下であった。薬物動態学的特性はそれぞれ TMCA 10 mg/kg と PMCA 5 mg/kg の用量をラット静注後に調査した。TMCA と PMCA の血中消失曲線は 1-コンパートメント・モデルで表された。TMCA と PMCA は，それぞれ 14.0 分, 17.4 分の半減期で消失した。分布容積は TMCA が 252 ml/kg, PMCA が 127 ml/kg であった。全身クリアランスは TMCA が 0.747 L/hr/kg, PMCA は 0.304 L/hr/kg であった。TMCA と M-TMCA はラット体内で相互変換されうる。またさらに，遠志水エキスの経口投与後におけるラット血漿中 TMCA と M-TMCA の経時的濃度変化について調べた。大変興味深い実験結果として，遠志水エキス経口投与後 15 分から 3 時間までラット血漿中 TMCA と M-TMCA が一定濃度に維持されることが分かった。血液中で検出された TMCA と M-TMCA は，化学構造式内に 3,4,5-trimethoxycinnamoyl の部分構造を有する onjisaponin E, F, G, sucrose 誘導体，その他化合物のような遠志が含有するプロドラッグに由来することが示唆された。

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