Pharmacokinetics of Caffeine and Its Metabolites in Horses after Intravenous, Intramuscular or Oral Administration

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The pharmacokinetics of caffeine (CAF) and its metabolites, dimethylxanthines, were examined in horses administered 2.5 mg/kg of CAF intravenously (i.v.), intramuscularly (i.m.), or orally (p.o.). The plasma samples were extracted by Extrelut[®] and the concentrations of CAF and metabolites were determined by high performance liquid chromatography (HPLC) with a short column. The pharmacokinetics of CAF after bolus i.v. injection were described by the assumption of a two-compartment model, and those of CAF after i.m. or p.o. administration were done by the assumption of a one-compartment model. The biologic half lives of CAF were 15.5, 18.6, and 16.4 h after administering i.v., i.m. and p.o., respectively. The extent of the bioavailability of the p.o. administration was determined as 1.04 times the dose. The differences in pharmacokinetic parameters were not statistically significant among administration routes. A straight correlation existed between the logarithms of body weights of different species of animals and those of their biologic half lives of CAF. Therefore, the biologic half life of CAF in an animal might be predictable as a function of its body weight.

Keywords caffeine; pharmacokinetics; dimethylxanthine; horse; metabolite

Introduction

Caffeine (CAF), which is not only consumed as a drug but also contained in many beverages, has pharmacological effects as a central nervous stimulant, cardiostimulant and diuretic. CAF is utilized in horses in the clinical treatment of colic, heat stroke, disturbances of circulation and fatigue. In addition, CAF has been used as a doping drug in races. Although the use of this drug in horse races is prohibited by law, due to its widespread usage, it has been detected in doping tests at our laboratory.

The pharmacokinetics of CAF were reported in men,¹⁾ monkeys,²⁾ rabbits,³⁾ rats,^{1a,4)} mice,⁵⁾ dogs,⁶⁾ and Chinese hamsters,⁷⁾ and are known to be variable in these species. Greene *et al.* reported the pharmacokinetics of CAF in horses,⁸⁾ but not those of its metabolites. CAF and its metabolites have been determined in biological fluid.⁹⁾ CAF has eighteen metabolites,¹⁰⁾ in which we extracted CAF and theophylline (TPL), theobromine (TBM) and paraxanthine (PXT) of dimethylxanthines in horse plasma, using Extrelut[®]. CAF and dimethylxanthines extracted were separated in a short time by high performance liquid chromatography (HPLC) with a short column.

In this paper the pharmacokinetics of CAF and dimethylxanthines in horses after intravenous (i.v.), intramuscular (i.m.) or oral (p.o.) administration were investigated.

Experimental

Materials Twenty percentage CAF-sodium benzoate for injection, CAF, TPL and TBM were obtained from Fuso Yakuhin Kogyo Inc., Sanko Seiyaku Kogyo Inc., Wako Pure Chemical Industries Ltd. and Tokyo Kasei Kogyo Co., Ltd., respectively. PXT and β -hydroxyethyltheophylline as internal standard (I.S.) were from Sigma Chemical Co. The reagents obtained from Nacalai Tesque Inc. were of HPLC grade and special grade. Extrelut[®] was from Merck.

The mixture solutions of CAF, TPL, TBM and PXT (0.2–200 μ g/ml in methanol) and the I.S. solution (100 μ g/ml in methanol) were stored at –20°C.

Methods of Experiments with Animals Healthy riding horses which had free access to water and were fed three times a day were used and animal parameters in the present study are shown in Table I. CAF at a dose of 2.5 mg/kg was administered i.v., i.m. and p.o. Each group consisted of 4

or 5 horses. The 20% CAF-sodium benzoate was injected *via* the jugular vein or the jugular muscle at a dose of 2.5 mg/kg of CAF when converted to CAF. In the case of p.o. administration, 2.5 mg/kg of CAF was challenged with 2 l of water by a catheter. All administrations were performed 3 h after the morning feeding. The horses were kept in a stall for one day after the treatments and were used for riding purposes the day after. Each administration was carried out at an interval longer than four weeks. Blood samples were collected into heparinized tubes from the jugular vein and the plasma separated immediately. The plasma samples were stored at –20°C until assay.

Methods of Assay Two ml of plasma and 4 ml of 0.4 M phosphate buffer (pH 5.3) were added to the test tubes containing 2 μ g of I.S. Three ml of the mixture was put onto an Extrelut column which was a glass column (12 mm i.d.) packed with one quarter of a package of 20 ml Extrelut. After 10 min, the column was eluted with 25 ml of chloroform, and then the elute was evaporated at 40°C. The residue was dissolved in 200 μ l of a mobile phase of HPLC and a 20 μ l of aliquot was injected into HPLC.

The Hitachi 655 HPLC system with a pre-column filter of 2 μ m mesh and Cosmosil 3C₁₈ packed column (50 $\times$ 4.6 mm i.d.; Nacalai Tesque Co., Ltd.) was used. The mobile phase was 1% triethylamine solution (adjusted

TABLE I. Animal Parameters

Horse No.	Strain	Sex	Age (year)	Body weight (kg)
1	HB	F	7	501
2	HB	F	6	554
3	AA	G	8	530
4	TB	F	4	450
5	TB	G	9	550
6	AA	F	4	488
7	AA	F	4	448
8	TB	F	4	460

HB, half-bred; AA, anglo-arab; TB, thoroughbred; F, female; G, gelding.

Fig. 1. Two Compartment Open Model

K_1, K_2, K_3 : Rate constant of disposition. V_1, V_2 : Volume of each compartment.

to pH 5.3 with phosphoric acid) and tetrahydrofuran (98.75:1.25 v/v). The flow rate of the mobile phase was 1.0 ml/min at 40 °C. The ultraviolet (UV) absorbance (Hitachi 638 UV detector) was monitored at 274 nm.

Pharmacokinetic Analysis of Data The pharmacokinetic parameters are as follows. CL_{tot} : Total body clearance. D : Dose of CAF. F : Extent of bioavailability of CAF. K_a : Absorption rate constant of CAF. K_e : Elimination rate constant of CAF. V_d : Distribution volume of CAF. Cl_m : Metabolic clearance of CAF. K_{em} : Elimination rate constant of metabolite. V_m : Distribution volume of metabolite.

A schematic representation of the two compartment open model is shown in Fig. 1. After bolus i.v. injection, the plasma concentration of CAF in systemic circulation is described by Eq. 1 based on the assumption of a two-compartment model.

$$B(t) = \{D/V_1(\alpha - \beta)\} \{(\alpha - K_2) \cdot \exp(-\alpha t) + (K_2 - \beta) \cdot \exp(-\beta t)\} \quad (1)$$

After i.m. or p.o. administration, the plasma concentration of CAF is described by Eq. 2 based on the assumption of the one compartment model.

$$B(t) = \{K_a D F / V_d (K_a - K_e)\} \{ \exp(-K_e t) - \exp(-K_a t) \} \quad (2)$$

The plasma concentrations of metabolites are described by Eq. 3.

$$B_m(t) = \int_0^t B(t-\theta) (Cl_m/V_m) \cdot \exp(-K_{em}\theta) d\theta \quad (3)$$

In this equation, Cl_m and V_m can't be calculated separately. Cl_m/V_m is replaced with K , and then Eq. 3 can be Eq. 4.

$$B_m(t) = \int_0^t B(t-\theta) K \cdot \exp(-K_{em}\theta) d\theta \quad (4)$$

The experimental values measured in this study were put into Eqs. 1 and 2 and the parameters, i.e., V_1 , α , β , K_2 , V_d , K_a , K_e , for the pharmacokinetics of CAF were computed from the equations using the non-linear least square method. K and K_{em} for CAF metabolites were calculated by the same method as the pharmacokinetic analysis of CAF, applying the parameters for $B(t)$ which were obtained from Eqs. 1 and 2 to Eq. 4.

Results and Discussion

Determination of CAF and its Metabolites The recoveries of TPL and PXT which were extracted from Extrelut decreased at pH 8 or above, whereas those of CAF, TBM, and I. S. were independent of the pH at the range of pH

Fig. 2. HPLC Chromatograms of Blank Plasma (A) and CAF-Administered Plasma (B)

TBM (1), PXT (2), TPL (3), I.S. (4) and CAF (5).

4.3–9.0. Therefore, the plasma samples were extracted at pH 5.3. The recoveries of CAF, dimethylxanthines and I.S. (0.1 and 1.0 $\mu\text{g/ml}$ plasma) were 90.3–99.7%, and the coefficients of variations in one day were 0.5–3.9%. Other metabolites such as monomethylxanthines and uric acids were not extracted by the present method. The minimal detectable amount of CAF and its metabolites was 0.01 $\mu\text{g/ml}$. The calibration curves of CAF and its metabolites were linear at the range of 0.01–5.0 $\mu\text{g/ml}$.

CAF and dimethylxanthines were separated in 8 min by

Fig. 3. The Time Courses of Plasma Concentration of CAF and Its Metabolites after Bolus i.v. Administration of CAF

CAF-sodium benzoate was administered at the dose of 2.5 mg/kg of CAF when converted to CAF. The left figures show the results of horse No. 4 and the right figure represents the means $\pm$ S.D. of four horses (Nos. 1, 2, 3 and 4). The lines were produced by Eqs. 1 and 4, in the text. CAF ($\circ$ — $\circ$), TPL ($\bullet$ — $\bullet$), TBM ($\triangle$ — $\triangle$) and PXT ($\blacktriangle$ — $\blacktriangle$).

Fig. 4. The Time Courses of Plasma Concentration of CAF and Its Metabolites after i.m. Administration of CAF

CAF-sodium benzoate was administered at the dose of 2.5 mg/kg of CAF when converted to CAF. The left figures show the results of horse No. 4 and the right figure represents the means $\pm$ S.D. of five horses (Nos. 3, 4, 5, 6 and 7). See also legend to Fig. 3.

a short column as shown in Fig. 2.

Pharmacokinetics of CAF in Horses Figures 3, 4 and 5 show the time courses of the plasma concentrations of CAF and its metabolites after i.v., i.m. or p.o. administration of 2.5 mg/kg of CAF. Symbol marks in the figures represent experimental values, and lines are produced by Eqs. 1, 2 and 4. The simulated curves fitted to the experimentally observed values. These results demonstrate that the pharmacokinetics of CAF and its metabolites can be expressed by these equations, and that no rate limited procedure was found in the absorption of CAF, the production of metabolites or the elimination of CAF and metabolites at this dose.

The pharmacokinetic parameters of CAF and its metabolites in horses, which were calculated from the data shown in Figs. 3, 4 and 5 and the equations, are shown in Tables II, III and IV. The differences in the parameters were not statistically significant except V_d between i.m. and p.o. administrations. It is likely that the difference in V_d was due to apparent values brought by the calculation since the

Fig. 5. The Time Courses of Plasma Concentration of CAF and Its Metabolites after p.o. Administration of CAF

CAF at the dose of 2.5 mg/kg was administered. The left figures show the results of horse No. 6 and the right figure represents the means $\pm$ S.D. of four horses (Nos. 2, 5, 6, and 8). See also legend to Fig. 3.

Cl_{tot} of the i.m. administration was not different from that of the p.o. administration, although V_d equals Cl_{tot}/K_e . The present study was not carried out at a cross-over and V_d was affected by small variations of the measured values. The difference of V_d may result from the variations of V_d in different horses.

The ratio of average area under the blood concentration curve (AUC) of CAF after p.o. to that after i.v. was 1.04, hence the extent of bioavailability was estimated to be approximately 1. Consequently, CAF was considered to be almost completely absorbed and transported into systemic circulation. This hypothesis was confirmed by the following results. The differences in K and the ratio of (AUC of metabolite)/(AUC of CAF) in all of the three determined metabolites were not statistically significant between i.v. and p.o. administrations. Even after p.o. administration, most of the metabolites of CAF detected in the plasma were considered to be from CAF in systemic circulation and the first pass effect may be negligible.

The results presented in this study reveal that the pharmacokinetic behavior of CAF in the horse is only slightly influenced by the route of administration.

Figure 6 shows a straight correlation between the logarithms of the body weights and of the biologic half lives of CAF in mammalian species. In spite of the different species, as shown in this figure, biologic half life $T_{1/2}$ might

TABLE II. Pharmacokinetic Parameters of CAF and Its Metabolites after i.v. Administration of CAF

Compd.	Parameter	Horse No.				Mean $\pm$ S.D.
		1	2	3	4	
CAF	α (h^{-1})	2.50	2.13	1.68	2.26	2.14 ± 0.30
	$T_{1/2\alpha}$ (h)	0.28	0.33	0.42	0.31	0.34 ± 0.05
	β (h^{-1})	0.0549	0.0497	0.0398	0.0382	0.0457 ± 0.0069
	$T_{1/2\beta}$ (h)	12.6	13.9	17.4	18.1	15.5 ± 2.3
	K_2 (h^{-1})	1.202	0.984	1.005	1.149	1.085 ± 0.093
	V_1 (l/kg)	0.413	0.415	0.526	0.460	0.454 ± 0.046
	V_d (l/kg)	0.862	0.898	0.881	0.905	0.887 ± 0.017
	AUC ($\mu g \cdot h/ml$)	52.8	56.0	71.3	72.3	63.1 ± 8.8
TPL	Cl_{tot} (ml/h $\cdot$ kg)	47.3	44.6	35.1	34.6	40.4 ± 5.6
	K (h^{-1})	0.0104	0.0095	0.0068	0.0076	0.0086 ± 0.0014
	K_{em} (h^{-1})	0.0481	0.0414	0.0425	0.0398	0.0430 ± 0.0031
	K (h^{-1})	0.0043	0.0036	0.0034	0.0038	0.0038 ± 0.0004
TBM	K_{em} (h^{-1})	0.0410	0.0332	0.0314	0.0297	0.0338 ± 0.0043
	K (h^{-1})	0.0089	0.0077	0.0083	0.0065	0.0079 ± 0.0009
	K_{em} (h^{-1})	0.278	0.222	0.219	0.213	0.233 ± 0.026

TABLE III. Pharmacokinetic Parameters of CAF and Its Metabolites after i.m. Administration of CAF

Compound	Parameter	Horse No.					Mean $\pm$ S.D.
		3	4	5	6	7	
CAF	K_a (h^{-1})	2.06	1.56	3.26	2.60	1.04	2.10 ± 0.78
	K_e (h^{-1})	0.0344	0.0355	0.0419	0.0322	0.0467	0.0381 ± 0.0054
	$T_{1/2}$ (h)	20.2	19.6	16.5	21.6	14.9	18.6 ± 2.5
	V_d (l/kg)	1.003	1.015	0.936	0.955	0.915	0.965 ± 0.038
	AUC ($\mu g \cdot h/ml$)	72.6	69.5	63.8	81.4	58.6	69.2 ± 7.8
	Cl_{tot} (ml/h $\cdot$ kg)	34.4	36.0	39.2	30.7	42.7	36.6 ± 4.1
TPL	K (h^{-1})	0.0076	0.0097	0.0096	0.0064	0.0114	0.0090 ± 0.0017
	K_{em} (h^{-1})	0.0381	0.0419	0.0472	0.0294	0.0480	0.0409 ± 0.0068
TBM	K (h^{-1})	0.0034	0.0033	0.0038	0.0021	0.0046	0.0034 ± 0.0008
	K_{em} (h^{-1})	0.0268	0.0255	0.0291	0.0186	0.0404	0.0281 ± 0.0071
PXT	K (h^{-1})	0.0088	0.0071	0.0086	0.0057	0.0073	0.0075 ± 0.0011
	K_{em} (h^{-1})	0.190	0.218	0.183	0.354	0.142	0.217 ± 0.073

TABLE IV. Pharmacokinetic Parameters of CAF and Its Metabolites after *p.o.* Administration of CAF

Compd.	Parameter	Horse No.				Mean $\pm$ S.D.
		3	5	6	8	
CAF	K_a (h^{-1})	5 <	4.51	2.86	4.38	$3.92 \pm 0.75^{a)}$
	K_e (h^{-1})	0.0457	0.0468	0.0322	0.0500	0.0437 ± 0.0068
	$T_{1/2}$ (h)	15.2	14.8	21.5	13.9	16.4 ± 3.0
	V_d (l/kg)	0.861	0.845	0.883	0.933	0.881 ± 0.033
	AUC ($\mu g \cdot h/ml$)	63.5	63.2	82.8	53.6	65.8 ± 10.6
	Cl_{tot} (ml/h $\cdot$ kg)	39.4	39.6	30.3	46.6	39.0 ± 6.7
TPL	K (h^{-1})	0.0085	0.0103	0.0082	0.0125	0.0099 ± 0.0017
	K_{em} (h^{-1})	0.0422	0.0478	0.0384	0.0527	0.0453 ± 0.0054
TBM	K (h^{-1})	0.0037	0.0040	0.0025	0.0043	0.0036 ± 0.0007
	K_{em} (h^{-1})	0.0287	0.0323	0.0253	0.0402	0.0316 ± 0.0055
PXT	K (h^{-1})	0.0079	0.0086	0.0046	0.0082	0.0073 ± 0.0016
	K_{em} (h^{-1})	0.182	0.233	0.209	0.233	0.212 ± 0.019

a) Mean $\pm$ S.D. of three horses (Nos. 5, 6, and 8).

Fig. 6. Biologic Half Life of CAF in Mammalian Species as a Function of Body Weight

Rat (A), rabbit (B), monkey (C), human (D) and horse (E). A, B, C and D are referred to Bonati, *et al.*¹⁰⁾be describable with Eq. 5 as a function of body weight W .

$$T_{1/2} = AW^B \quad (5)$$

In this equation, A and B are constants. When the unit of

$T_{1/2}$ is h and that of W is kg, the values of A and B calculated by the least square method were 1.315 and 0.369, respectively and the correlation coefficient was 0.969. It is suggested that the biologic half life of CAF can be calculated by the body weight of an animal.

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