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PHARMACOKINETICS OF KHELLIN IN RABBITS: ORAL, INTRAVENOUS, AND INTRAMUSCULAR ADMINISTRATIONS

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ABSTRACT

This study was undertaken to investigate the pharmacokinetics of khellin in rabbits following oral, intravenous, and intramuscular administrations. Analysis of khellin in the plasma samples was performed according to a previously developed HPLC method. The data obtained from the rapid intravenous administration experiments fitted the two-compartment open model with β, α, total body clearance (TBC), and volume of central compartment (V_c) of 0.0306 hr^{-1}, 1.93 hr^{-1}, 573 $ml.hr^{-1}.kg^{-1}$, and 2.1 $liter.kg^{-1}$, respectively. The concentration-time profiles acquired following the administration of sugar-coated tablets of khellin were typical of sustained release formulations with time to peak concentration (t_{max}) and dose-normalized peak

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plasma concentration (C_{max}) of 21 hr and 23 $ng.ml^{-1}.mg^{-1}.kg$, respectively. With the exception of one animal, rapid absorption was obtained following the intramuscular or oral suspension administration with (t_{max}) ranging from 0.083 to 4 hr. A complete absorption was obtained with intramuscular injection, whereas, the fraction of dose absorbed following oral suspension administration was 38%.

INTRODUCTION

Khellin, I, is an active component of Ammi visnaga with vasodilating and smooth muscle relaxing actions. Because of these activities, this drug has been used in the treatment of variety of diseases including angina and bronchial asthma (1); however, its main use has been in the treatment of renal colic. Recently, a renewed interest has been displayed in I following reports of its marked effectiveness in the treatment of vitiligo in conjunction with phototherapy (2,3).

Although khellin has been in use for over 35 years, a little or no information is available on its pharmacokinetics. This is partially due to lack of a reliable method for its analysis in biological fluids. Recently, a rapid and sensitive high performance liquid chromatographic (HPLC) method for the analysis of I in plasma and urine was developed, and its blood profiles in two human volunteers were reported (4). Some preliminary data on its pharmacokinetics in rats were recently reported (5).

This study was undertaken to examine the pharmacokinetics of I using the rabbit as an in vivo model. The pharmacokinetics was studied following different modes of administration viz. oral suspension, rapid intravenous injection, and intramuscular injection. The plasma levels of I were determined by HPLC, and estimates of various pharmacokinetic parameters for I in rabbits were calculated.

MATERIALS AND METHODS

Khellin injectable solution (50 mg/2 ml) and sugar-coated tablets (50 mg) (Delalande Laboratories, Paris, France) were obtained commercially. The oral suspension was prepared by pulverizing a few of the above tablets and suspending an accurately weighed aliquot of the powder equivalent to 2.75 mg/kg dose of I in 5 ml of water prior to each oral suspension administration experiment.

Animals

Nine mature female New Zealand albino rabbits weighing 2.1-6.3 kg and maintained on commercial rabbit food (Rabbit Diet TR2, Birmingham, UK) and tap water were employed for this study. The animals were fasted over night prior to each oral administration experiment. Water was offered ad libitum for the duration of the experiments.

Preparation of Animal Prior to Drug Administration

Blood collection and intravenous administration of I were performed via an intravenous catheter. The

catheterization procedure was described earlier (6). Blank sample of 6-7 ml of blood for standard curve preparation was collected prior to each experiment. The blood was immediately centrifuged at 2800 rpm and the plasma was harvested and frozen at -20° until analysis.

Drug Administration

Administration of the oral suspension of I was performed by intubation using a 19 cm long, 4.5 mm I.D. endotracheal tube (National Catheter Co., Argyle, New York). A small aliquot of water (1 ml) was then injected through the tube to insure that it is not in the air passageway. If no rejection was observed, the liquid (equivalent to 2.75 mg/kg dose of I) was well stirred and injected into the tube, and 5 ml of water followed. The rapid intravenous administration was performed by injecting a dose of 2.75 mg/kg of I in injectable solution into the intravenous catheter over a period of 1 min. For the intramuscular administration, the rabbit was firmly restrained and one of its legs was gently pulled. A dose of 2.75 mg/kg of I in injectable solution was then administered rapidly into the thigh muscles after insuring that the needle is in an extravascular site.

Blood Collection

Blood samples (1.1 ml) were collected in heparinized tubes via the intravenous catheter at various intervals. The tube was immediately spun for 10 min at 2800 rpm, and 0.5 ml of plasma was transferred to

a clean conical centrifuge tube and frozen at -20° until analysis.

Analysis of Khellin in Plasma

The concentrations of I in the plasma samples were analysed according to a reverse-phase HPLC method developed previously (4). The only modification in the assay was that instead of a stainless steel C_{18} column, a C_{18} cartridge in conjunction with a radial compression module (Waters Associates, Milford, Massachusetts) was employed. This allowed the analysis to be carried out at higher flow rate. Also, the plasma volume was reduced from 1 to 0.5 ml. The assay was revalidated by examining the linearity, accuracy, and reproducibility of the modified procedure. No appreciable differences were found in these features between the original and modified version.

Data Analysis

The concentration-time data obtained from the intravenous administration experiments were fitted to the two-compartment open model (7) using the BMDPAR computer program (non-linear least-square regression analysis) (8) and the various parameters were generated.

RESULTS

Oral Suspension

The normalized area-under-the-curve obtained in 30 hr ($AUC_{30\ hr}$), peak concentration time (t_{max}), and normalized peak plasma concentration (C_{max}) obtained for the three animals which received oral suspension are

Table 1

Pharmacokinetic Data Obtained Following Oral Administration of Khellin Suspension.

	Animal No.				
	OS1	OS2	OS3	Mean	S.D.
Weight, kg	4.75	5.51	2.45	4.24	1.59
Dose, mg/kg	10.53	9.08	6.4	8.67	2.1
AUC, $ng.hr.ml^{-1}.mg^{-1}.kg$	550	509	1346	802	472
t_{max}, hr	.25	.25	12.0	4.17	6.78
C_{max}, $ng.ml^{-1}.mg^{-1}.kg$	511	40.6	74.3	209	262
TBC/F, $ml.hr^{-1}.kg^{-1}$	1820	1965	816	1534	626

presented in Table 1. The normalization of the area-under-the-curve and peak plasma concentration was achieved by dividing the value obtained for each of these parameters by the dose/weight.

The TBC/F listed in this table is the total body clearance (TBC) divided by the fraction of dose absorbed (F), and was estimated according to the following equation:

$$\frac{TBC}{F} = \frac{D}{AUC} \qquad \text{(Eq. 1)}$$

where D is the dose and AUC is the normalized area-under-the-curve from $0 \longrightarrow \infty$. The AUC was determined by adding the area-under-the-curve up to the last concentration determined (C_f), AUC_f, to the

area-under-the-tail (AUC_{tail}). The AUC_f was estimated according to the trapezoidal rule, whereas, the AUC_{tail} was calculated according to the following equation:

$$AUC_{tail} = -\frac{C_f}{S} \qquad \text{(Eq. 2)}$$

where S is the slope of the terminal linear segment of the ln concentration-time curve. Because of the very rapid absorption observed with these experiments (vis. t_{max} for two of these rabbits was 0.25 hr which coincided with the time of the first sample collected), the data could not be fitted to the two-compartment model with first-order absorption. Irrespective of the initial values employed, the apparent first-order rate constant for absorption was exceedingly large (varied with initial estimates), and the data were treated by the BMDP computer program as if they were intravenous data. Similar fitting patterns were observed with the data obtained from intramuscular administration experiments.

Intramuscular Administration

The pharmacokinetic parameters acquired from the intramuscular administration experiments are presented in Table 2. A plasma concentration-time profile of I obtained in one of these experiments is depicted in Fig. 1.

Rapid Intravenous Administration

Fig. 1 shows a plasma concentration versus time plot obtained for one of the three rabbits used for

Table 2

Data Obtained Following Intramuscular Administration of 2.75 mg/kg of Khellin.

	Animal No.				
	IM1	IM2	IM3	Mean	S.D.
Weight, kg	4.78	2.3	2.57	3.22	1.36
AUC, $ng.hr.ml^{-1}.mg^{-1}.kg$	2451	2805	3721	2992	655
t_{max}, hr	0.083	2.0	4.0	2.03	1.96
C_{max}, $ng.ml^{-1}.mg^{-1}.kg$	501	1028	867	799	270
TBC/F, $ml.hr^{-1}.kg^{-1}$	408	357	269	344	70.5

Table 3

Pharmacokinetic Parameters Obtained Following Intravenous Administration of 2.75 mg/kg of Khellin.

	Animal No.				
	IV1	IV2	IV3	Mean	S.D.
Weight, kg	2.79	2.09	3.24	2.71	.58
AUC, $ng.hr.ml^{-1}.mg^{-1}.kg$	2676	1746	1982	2135	483
TBC, $ml.hr^{-1}.kg^{-1}$	441	464	814	573	209
V_c, ml/kg	1571	2856	1883	2103	670
V_β, liter/kg	24.9	26.2	14.5	21.8	6.4
α, hr^{-1}	2.37	2.23	1.2	1.93	.637
β, hr^{-1}	.0177	.0177	.0563	.0306	.0223
k_{12}, hr^{-1}	1.96	1.84	.67	1.489	.712
k_{21}, hr^{-1}	.15	.243	.157	.183	.0521
k_{10}, hr^{-1}	.281	.163	.432	.292	.1353

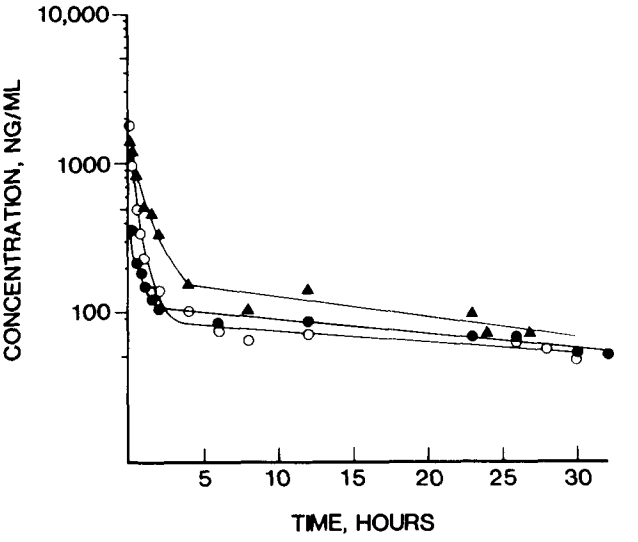

FIGURE 1

Representative concentration-time plots for khellin obtained following the administration of oral suspension (●), and intravenous (o) and intramuscular (▲) injectable solutions of this drug to rabbits.

these experiments. Pharmacokinetic parameters acquired from fitting the data to the two-compartment model as well as the AUC estimated as described above are presented in Table 3.

DISCUSSION

An extremely rapid absorption was observed with two animals following the administration of oral suspension. Indeed, the t_{max} in these two rabbits was 0.25 hr (Table 1) which coincided with the time of the first sample collected. The absolute bioavailability of I from oral suspension was estimated to be 38%.

With rapid intravenous administration, the plasma concentration-time data acquired displayed the characteristics of the two-compartment open model (7) with mean post-distributive phase rate constant (β) of 0.0306 hr^{-1} and mean distributive phase rate constant (α) of 1.93 hr^{-1}. The mean values for the rate constants k_{12}, k_{21}, and k_{10} were 1.49, 0.183, 0.292 hr^{-1}, respectively. The mean of the total body clearance (TBC) was 573 $ml.hr^{-1}.kg^{-1}$, whereas the volume distribution of the central compartment (V_c) was 2.1 liter/kg. These values, except for k_{12}, k_{21}, and k_{10}, are not in agreement with values reported by Said in rats (5). This may attributed to interspecies differences, or to fundamental differences in experimental design between the two studies. Indeed, much higher doses were used by the above worker, and sampling was performed by decapitation of a group of rats at each interval and was stopped only 7 hr following the administration of I. Also, an unpublished gas chromatographic method was used for the analysis of I. The ratio k_{12}/k_{21} was 8.1 indicating that I has a great affinity to the peripheral compartment which may include the skin. This is in agreement with recent reports demonstrating a marked effectiveness of I in the treatment of vitiligo when the skin lesions are subjected to phototherapy.

The absorption of I following intramuscular administration was complete, and extremely rapid in one animal where the peak concentration occurred 5 min after the administration, whereas it was not as rapid with the

other two animals (Table 2). The t_{max} was 2 hr for rabbit no. IM2 and 4 hr for rabbit no. IM3. The mean normalized peak plasma concentration (799 $ng.ml^{-1}.mg^{-1}.kg$) was much higher than that obtained after the administration of oral suspension. For rabbits IM2 or IM3 there appeared to be a minor secondary peak which suggests a possible existence of an "enterohepatic recycling" process with a lag time (9). A similar secondary peak was observed with an oral suspension administration experiment. Studies are underway to examine the biliary excretion of this drug in rabbits, and to compare its plasma concentration-time profile pre- and post- bile duct cannulation.

In conclusions, this study characterizes the pharmacokinetics of khellin following different modes of administration using the rabbit as in vivo model. In light of the fact that it is the first comprehensive study which adequately investigates the pharmacokinetics of khellin, it is hoped that it can answer some of the questions regarding this vital aspect of khellin. A similar study in human is already planned and underway.

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