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Pharmacokinetics and tissue distribution of piperine in animals after i.v. bolus administration

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A reverse phase HPLC method to determine piperine, a pungent constituent of black pepper, in rabbit serum and various tissues of the rat was developed. A pharmacokinetic study was performed in rabbits and tissue distribution studies were carried out in rats. High reproducibility was achieved in quantitative analysis over the concentration range of 0.2–20 μg/ml serum. After bolus intravenous administration of piperine at a dose of 10 mg/kg, the serum concentration – time curve fitted the two-compartment open model. The tissue distribution pattern of piperine in rats also supports the two-compartment open model.

1. Introduction

The powder of dried seeds of black pepper (*Piper nigrum* Lin, Piperaceae) has been used not only as a seasoning spice but also as a useful drug in Indian medicine [1]. In folk medicine, the vapors of pepper species are used in treating epilepsy, ordinary cold, headach [2] and pepper also has been used as abortificent in few areas of India [3–5]. There have been studies on the antimalarial activity [6], antifertility [7] and neurogenic effect [8] of piperine. Piperine has also been shown to enhance the bioavailability of many drugs by inhibiting their metabolism [9–13]. The pharmacology and clinical use of piperine and its derivatives in the treatment of epilepsy has been reviewed [14]. Piperine is reported to inhibit aflatoxin B_1-induced cytotoxicity [15] and modulate the carcinogen induced oxidative stress [16]. Piperine exhibited analgesic activity against tail-clip pressure and anti-inflammatory activity against carrageenin-induced edema in rats [17]. The antifungal [18] and anti-amoebic activity [19] of piperine have been reported. Regular oral feeding of piperine did not show any adverse effect in weanling rats [20] and caused increased food uptake in rats and an increase in liver weight mainly due to higher total and neutral lipid contents [21]. Acute, sub-chronic and chronic toxicity studies have shown that at pharmacologically effective doses, piperine did not cause any abnormality in the general growth pattern, body to organ weight ratio and clinical pathology [22].

On the other hand, nothing has been reported on the pharmacokinetics of piperine. Ganesh Bhat and Chandrasekhara studied the tissue distribution of piperine in rats with TLC and densitometric methods for the estimation of piperine in urine and tissues [23]. Hozel and Spiteller detected various metabolites of piperine in human urine [24]. As piperine is used as a dietary ingredient and in medicine, the need for pharmacokinetic data seems apparent. The purpose of the present study is to develop a HPLC method for the estimation of piperine in serum and tissues, and to investigate the pharmacokinetics of piperine in rabbits and tissue distribution in rats.

2. Investigations and results

2.1. Pharmacokinetic study

Piperine was administered to the rabbits and blood samples (about 1.5 ml) were withdrawn from the marginal ear vein at 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0 and 8.0 h after administration. Sampling times were fixed after a preliminary study. The blood was allowed to clot, the tubes were centrifuged, and the serum was immediately separated and kept frozen until the analysis. Serum concentration data after i.v. bolus administration of piperine was analyzed by a two compartment open model.

2.2. Tissue distribution study

Piperine was administered to the rats via their tail vein and the rats were sacrificed 0.5, 1.0, 3.0, 6.0 and 10 h later. Six rats were sacrificed at each time period. Blood and various tissues viz. brain, liver, spleen and kidneys were collected. Weighed quantities of tissues were homogenized in a tissue homogenizer in isotonic saline and the final volumes of homogenate were noted. Piperine was extracted from serum and tissue homogenates. The amount of piperine per gram of tissue is used for the evaluation of tissue distribution of piperine.

Fig. 1 shows representative chromatograms for serum blank and serum sample obtained from a rabbit treated with piperine. The peak for piperine was well separated from the peaks that seem to be derived from endogenous materials in the rabbit serum. The retention times for rifampicin and piperine were about 5.6 and 6.9 min respectively. Fig. 2 shows the representative chromatograms for tissue samples obtained from a rat, treated with piperine. A linear calibration graph was seen over the concentration range 0.2–20 μg/ml. The regression equation was $y = 1255\ x + 200$, $r = 1.000$, where y is the peak-area of

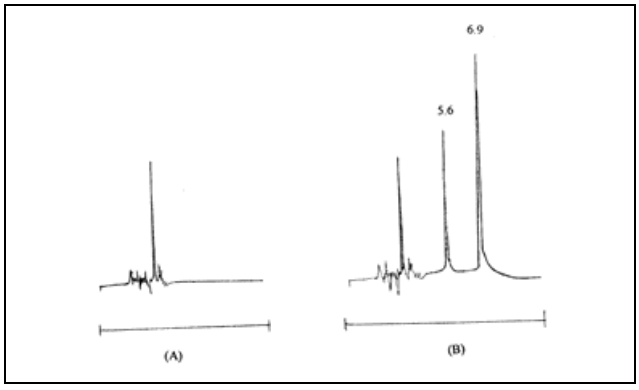

Fig. 1: Representative chromatograms for serum blank (A) and serum sample obtained from the rabbit, which was given piperine (B). Retention times for rifampicin and piperine were 5.6 min and 6.9 min respectively.

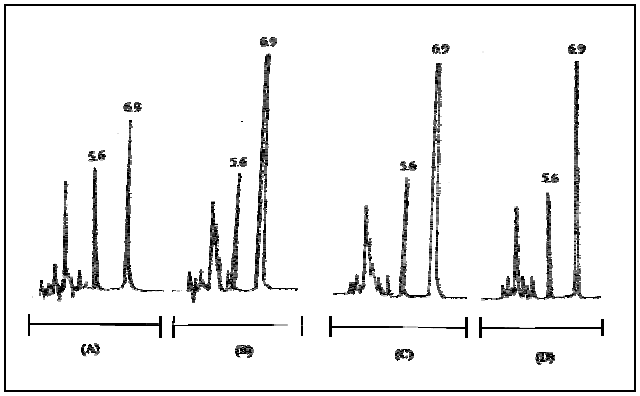

Fig. 2: Representative chromatograms for tissue samples (A. Brain, B. liver, C. kidney and D. spleen) obtained from the rat, which were given piperine. Retention times for rifampicin and piperine were 5.6 min and 6.9 min respectively.

the drug, x is the serum concentration (μg/ml) of the piperine and r is the correlation coefficient. The coefficients of variation (CV) for the peak-area at piperine concentrations tested were within 6.2%. Similar results were obtained when tissue homogenates were used instead of serum. Table 1 shows the reproducibility and recovery of data for the determination of piperine in serum. The precision was examined by performing six replicate analyses at three different concentrations of the drug in serum samples. The CV values ranged from 1.8 to 2.6%. The recovery data was obtained when piperine was added at two different concentrations to the rabbit serum sample. The average recovery at both concentrations was nearly 100%. The average recovery of piperine in tissue homogenates was about 100%. After bolus i.v. administration of 10 mg/kg of piperine, serum levels declined with time in a biexponential pattern as shown in Fig. 3. A two-compartmental open model was found suitable to describe the data

Table 1: Precision and accuracy on the determination of piperine in rabbit serum

Reproducibility[a] Piperine level (μg/ml)			Recovery[b] Piperine level (μg/ml)		
Mean	S.D	C.V (%)	Added	Found	Mean (%)
1.536	0.040	2.6	None	5.26	–
6.416	0.122	1.9	1.000	6.12	97.76
10.115	0.186	1.8	10.000	15.32	100.39

[a] Based on six determinations; [b] Based on five determinations

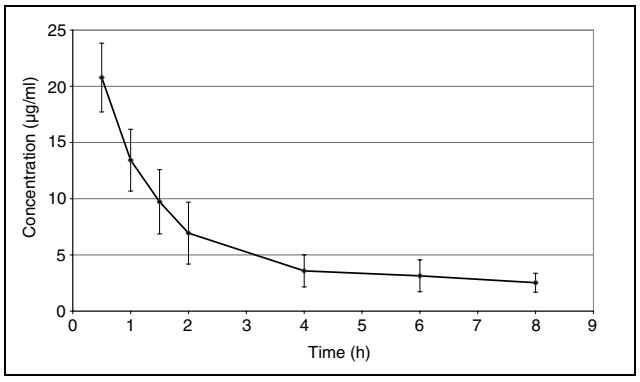

Fig. 3: Serum level of the drug declined with time in a bi-exponential pattern, after i.v. bolus administration of 10 mg/kg of piperine to rabbits (n = 6).

Table 2: Pharmacokinetic parameters for piperine after blous intravenous administration (10 mg/kg) to rabbits

Pharmacokinetic parameter	Average ± S.D
C_0 (μg/ml)	35.56 ± 2.310
A (μg/ml)	28.8 ± 1.900
B (μg/ml)	5.7 ± 3.530
α (h^{-1})	1.16 ± 0.136
β (h^{-1})	0.082 ± 0.046
$T_{1/2}$ (h)	10.33 ± 4.047
$AUC_{0\text{-}t}$ (μg · h/ml)	56.81 ± 15.350
$AUC_{0\text{-}\infty}$ (μg · h/ml)	94.13 ± 18.790
CL (ml/h/kg)	110.03 ± 22.950
V_d^{area} (ml/kg)	3632 ± 2146
V_d^{ss} (ml/kg)	2649 ± 1456
K_{12} (h^{-1})	0.615 ± 0.145
K_{21} (h^{-1})	0.247 ± 0.122
K_{13} (h^{-1})	0.378 ± 0.068

most adequately. The corresponding pharmacokinetic parameters, which were estimated by analysis of the data obtained from the individual rabbit, are given in Table 2. The elimination half-life in the terminal phase was 10.33 ± 4.04 h and the total body clearance was 110.03 ± 22.95 ml/h/kg.

Tissue levels of piperine along with corresponding serum levels are presented in the Table 3. Piperine levels in tissue were the highest 1 h after adminstration. The serum levels of piperine in rats ranged from 2.03 to 9.82 μg/ml. The piperine levels in highly perfused tissues viz. liver, spleen, kidney and brain were the highest at 1 h and declined with time.

3. Discussion

Determination methods for piperine in *Piper nigrum* using colorimetry [25, 26] and HPLC [27, 28] have been reported. Investigations of metabolites of piperine in bile and urine by HPLC [29] and detection of piperine in milk by HPLC [30] have also been reported. However, no paper concerning a method for piperine determination in serum and tissue homogenates has been published so far. We have developed a simple and precise HPLC method for the determination of piperine in plasma and tissue homogenates of rats. The method involves a simple solvent extraction and evaporation step. Rifampicin was employed as an internal standard since the solvent extraction step is involved. The rifampicin peak (5.6 min) and piperine peak (6.9 min) are well separated. The data listed in Table 1 indicates that the present method had satisfactory precision and accuracy.

Using the present determination method we have performed a pharmacokinetic study on piperine after i.v. administration. A simple plasma concentration – time graph indicated that piperine pharmacokinetics follow a two-compartment open model (Fig. 3). The distribution phase lasted about 3–4 h after which elimination phase started. Various pharmacokinetic parameters are calculated based on the two-compartment open model.

The proposed HPLC method was also used to study the tissue distribution of piperine in rats following i.v. bolus. The tissue levels of piperine also support the observation, that piperine follows two compartment pharmacokinetics. Piperine levels in brain were considerable and may explain the Central Nervous System (CNS) actions of piperine. It distributes to liver, spleen and kidney in significant amounts. Two compartmental behavior of piperine reveals

Table 3: Distribution of piperine in rat body tissue following i.v. bolus (50 mg/kg)

Time (hrs)	Amount of piperine in the tissue (μg/ml)* ± S.D				Piperine in the serum (μg/ml)* ± S.D
	Brain	Liver	Kidney	Spleen	
0.5	2.69 ± 0.61	4.58 ± 1.92	6.71 ± 1.76	5.98 ± 1.92	9.82 ± 2.69
1.0	4.19 ± 0.91	6.76 ± 2.15	6.96 ± 1.64	7.87 ± 1.51	6.89 ± 2.13
3.0	2.89 ± 0.72	5.12 ± 0.94	7.62 ± 1.19	6.69 ± 1.29	4.63 ± 1.98
6.0	1.82 ± 0.32	3.33 ± 0.19	6.45 ± 1.23	4.25 ± 1.23	3.10 ± 1.96
10.0	1.96 ± 0.79	3.18 ± 1.20	2.98 ± 0.93	3.12 ± 1.61	2.03 ± 1.52

* average of six determinations

the fact that it distributes not only to highly perfused tissues but also to the other group of tissues. Studies concerning protein binding of piperine are in progress.

4. Experimental

4.1. Materials

Piperine was purchased from Sigma Chemical Company Inc. USA. All other reagents were commercial products of analytical grade.

4.2. Animals

Male albino rats (Supplied by NIN, Hyderabad) weighing 250–300 g were used. Male rabbits (NIN, Hyderabad) weighing between 2.2–3.0 kg were used. Animals were fasted over night before the experiments. Drug was injected via tail vein and marginal ear vein to rats and rabbits respectively.

4.3. Drug administration

Piperine is insoluble in distilled water. Piperine was solubulized in a solvent system consisting of water, propylene glycol and ethanol (50 : 40 : 10). The rabbits were given a bolus i.v dose of 10 mg/kg of piperine through marginal ear vein. Piperine was injected into rats through tail vein (50 mg/kg) for tissue distribution studies.

4.4. HPLC assay

4.4.1. Extraction

To 500 μl of serum sample or tissue homogenate, 10 μg of rifampicin (100 μl of a methanolic solution of rifampicin) was added, 2 ml of ethyl acetate/dichloromethane (8 : 2) were added, and vortexed for 2 min. The mixture was centrifuged at 1.5×10^4 rpm for 5 min at room temperature and the supernatant (1 ml) was evaporated to dryness under vacuum. The residue was reconstituted in 200 μl of $CH_3OH : H_2O$ (75 : 25) and an aliquot (20 μl) was injected onto the chromatograph.

4.4.2. Chromatography

A Shimadzu model LC-6A pump (Shimadzu, Kyoto, Japan), equipped with a Shimadzu model SPD-6AV variable wavelength spectrophotometric detector was used. Samples were chromatographed with an analytical column of Bond Clone 10 C_{18} (4.6 mm i.d × 250 mm) at 40 °C. The mobile phase was methanol – water (75 : 25) with a flow rate of 1.0 ml/min. The eluates (rifampicin and piperine) were monitored by the spectrophotometric detector at 343 nm with a sensitivity of 0.005 a.u.f.s.

4.4.3. Calibration graph

To blank rabbit serum or rat tissue homogenate were added known amounts of piperine in the final concentration range of 0.2–20 μg/ml and rifampicin (10 μg) was used as an internal standard. These samples were treated as described above. The calibration curves were constructed by using the peak area of piperine.

4.4.4. Reproducibility

Blood samples were obtained from the rabbits at appropriate times after the administration of piperine. Aliquots of the serum samples were repeatedly analyzed.

4.4.5. Accuracy

To the serum samples obtained from the rabbits after administration of piperine, known amounts of piperine were added and then this compound was determined in the serum. The recovery was calculated by comparing the experimental value with the corresponding theoretical value. Similar studies were carried out with rat tissue homogenates following i.v. bolus administration of piperine.

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