Influence of Piperine on the Pharmacokinetics of Curcumin in Animals and Human Volunteers

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Abstract: **The medicinal properties of curcumin obtained from *Curcuma longa* L. cannot be utilised because of poor bioavailability due to its rapid metabolism in the liver and intestinal wall. In this study, the effect of combining piperine, a known inhibitor of hepatic and intestinal glucuronidation, was evaluated on the bioavailability of curcumin in rats and healthy human volunteers. When curcumin was given alone, in the dose 2 g/kg to rats, moderate serum concentrations were achieved over a period of 4 h. Concomitant administration of piperine 20 mg/kg increased the serum concentration of curcumin for a short period of 1–2 h post drug. Time to maximum was significantly increased ($P < 0.02$) while elimination half life and clearance significantly decreased ($P < 0.02$), and the bioavailability was increased by 154 %. On the other hand in humans after a dose of 2 g curcumin alone, serum levels were either undetectable or very low. Concomitant administration of piperine 20 mg produced much higher concentrations from 0.25 to 1 h post drug ($P < 0.01$ at 0.25 and 0.5 h; $P < 0.001$ at 1 h), the increase in bioavailability was 2000 %. The study shows that in the dosages used, piperine enhances the serum concentration, extent of absorption and bioavailability of curcumin in both rats and humans with no adverse effects.**

Key words: **Curcumin, piperine, pharmacokinetics, *Curcuma longa*, Zingiberaceae.**

Introduction

Curcumin is obtained from *Curcuma longa* L. (Zingiberaceae), a perennial herb widely cultivated in tropical regions of Asia. Its rhizome is extensively used for imparting colour and flavour to food. Current traditional Indian medicine claims the use of its powder, turmeric, against a wide variety of diseases (1). Extensive scientific research (2) on curcumin has demonstrated a wide spectrum of therapeutic effects which range from anti-inflammatory, wound healing, antispasmodic, anticoagulant, antitumor activities (3) and recently, with potential utility in autoimmune deficiency syndrome (4).

Pharmacokinetic properties of curcumin indicate that following oral administration, it is poorly absorbed (3) and only traces of the compound appear in the blood, while most of it is excreted in the faeces (5). The transformation of curcumin into an unidentified compound during absorption (6) and its glucuronidation in the liver (5, 7) are probably responsible for its low concentration in blood.

Black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.) have been in use as spices from ancient times throughout the world. A major component of the *Piper* species is the alkaloid piperine (1-piperoylpiperidine), which has been reported to enhance the bioavailability of drugs by inhibition of glucuronidation in the liver (8) and small intestine (9).

In view of the potential therapeutic utility of curcumin it appeared pertinent to examine the effect of piperine, a known hepatic and intestinal metabolic inhibitor, on the pharmacokinetic disposition of curcumin in animals and man, to provide a scientific rationale for assigning it a rightful place in the pharmacologists armamentarium.

Materials and Methods

Animal studies

Albino Wistar rats (n = 96) of both sexes (150–200 g) were chosen for the study. They were housed in well ventilated cages, fed on commercial rat pellets supplied by Hind Lever, Mumbai, with tap water ad libitum. They were divided into two sex and weight matched groups (n = 6/group/time cut), one group for administration of curcumin and the other for concomitant curcumin and piperine. Curcumin and piperine were supplied in pure powder form by Sami Chemicals and Extracts, Bangalore, India. Both the compounds were administered orally to fasted rats as an aqueous suspension. In the group that received both drugs, curcumin was administered first followed immediately by piperine. Control rats received water only. Curcumin was given in a dose of 2 g/kg and piperine, 20 mg/kg.

Under ether anaesthesia, pre and post drug jugular vein blood samples were collected from both groups of rats into centrifuge tubes at the following time intervals – 0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 5, and 6 h. The blood was allowed to clot at room temperature for about 1 h and then centrifuged at 3000 rpm for 10 min. The serum was separated out carefully using Pasteur pipettes into storage tubes and frozen at – 20 °C prior to analysis.

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Human volunteer studies

Ten healthy male volunteers, 20 to 26 years, weighing 50–75 kg (mean 60 ± 1.93) participated in a randomized cross over trial, to determine the comparative bioavailability and pharmacokinetic profile of curcumin when given alone and with piperine. Complete physical examination and an electrocardiogram were done. Laboratory tests comprising complete blood counts and haemoglobin percentage, blood biochemistry consisting of blood urea nitrogen (BUN), serum creatinine, total and conjugated bilirubin, alkaline phosphatase, aspartate transaminase (ASAT), alanine transaminase (ALAT), urine albumin, and sugar were performed to confirm that the subjects included in the study were normal. The study was formally approved by the Institutional Ethical Committee and informed consent was obtained from all subjects.

Subjects abstained from food since 10 pm of the previous evening and reported to the laboratory at 7.00 am. Venepuncture was done using a 20 g scalp vein set with heparin lock and left *in situ*. Blood samples (5 ml) were collected (without anticoagulant) at 0, 0.25, 0.50, 0.75, 1, 2, 3, 4, 5, and 6 h post drug. Blood was allowed to clot at room temperature for 1 h. Serum separation and storage until analysis was as explained earlier. Following basal blood sample collection 2 g of pure curcumin powder (4 capsules of 500 mg each) or 2 g of pure curcumin powder combined with 20 mg of pure piperine powder (4 capsules of 500 mg curcumin + 5 mg piperine each; identical capsules prepared by Sami Chemicals and Extracts, Bangalore, India) was given with 150 ml of water. Blood sampling after curcumin *per se* and curcumin with piperine was done on two occasions, separated by a two week wash out period on the same volunteers. The following precautions were taken during the trial: subjects refrained from smoking, consuming alcohol or beverages, and from taking drugs of any kind 24 hours prior to and during the trial. Standard meals were given to all the participants on the day of the test.

Analytical methods

Estimation of curcumin was done by reverse phase high pressure liquid chromatography (HPLC) using a modification of the method described by Tannesen et al. (10). The modification was done by Sami Chemicals and Extracts, Bangalore, India, and is detailed below: the mobile phase used was ethanol : methanol (60 : 40) instead of only ethanol and the flow rate was changed from 1.2 ml/min to 1 ml/min. HPLC grade methanol and low actinic glassware protected from light were used for the entire procedure.

Extraction and preparation of standard solution

Curcumin (25 mg) was dissolved and diluted to 25 ml with methanol in a volumetric flask; 0.1 ml (100 µl) of this was transferred to a volumetric flask and diluted with methanol upto 10 ml making a 10 ppm solution; 0.1 ml of this 10 ppm solution was transferred to another 10 ml volumetric flask and the volume made up with methanol making a 0.1 ppm solution.

Extraction of curcumin from serum and preparation of sample

Serum samples stored at −20 °C were equilibated to room temperature before analysis. A portion of 1 ml was transferred into a 10 ml volumetric flask and about 5 ml of methanol added. The mixture was shaken thoroughly and heated at 80 °C on a water bath for half an hour. After cooling to room temperature, methanol was added to make up the volume to 10 ml and mixed well. The turbid solution was transferred into a 15 ml centrifuge tube and centrifuged at 4000 RPM for 10 minutes. The supernatant was collected by means of a 25 ml syringe and 10 cm needle (Luer lock) and the clear solution filtered through a 0.45 µm, 13 mm millipore membrane filter, into a narrow end test tube. 20 µl of the solution were injected into the chromatograph for carrying out the HPLC analysis.

Samples were read by UV absorbance of 254 nM. The recovery rate experiments were carried out by adding a known amount of standard curcumin to the serum and the added curcumin extracted as per procedure and quantified. The recovery rate of curcumin from serum ranged from 87–89.9 %. The minimum level of detection of curcumin was 0.001 µg/ml.

Calculation

$$\text{Content of curcumin in } \mu g/ml \text{ in the test sample} = \frac{\text{Standard Reading} \times \text{standard concentration}}{\text{Standard Reading} \times \text{sample concentration}}$$

Treatment of pharmacokinetic data

For calculation of pharmacokinetic parameters (PK), curve fitting was carried out by a model independent method with non-linear least-square regression analysis using a computer designed programme "PHARMKIT". This programme uses an algorithm called "SIMPLEX" for calculating non-linear least squares. The various PK parameters calculated were: absorption half life ($t^1/_{2(a)}$), elimination half live ($t^1/_{2el}$), volume of distribution (Vd); and clearence (Cl). Areas under the concentration time curve ($AUC_{0\text{-}tn}$) was calculated using the trapezoidal method. Maximum concentration (C_{max}) and time to max (T_{max}) are the observed values. Relative bioavailability (F) was calculated using the formula:

$$F = \frac{\text{AUC Curcumin + Piperine}}{\text{AUC Curcumin}} \times 100$$

Statistical analysis

Serum concentration time curves and the PK parameters from animal data were analysed using the Student's "t" test, while the paired "t" test was used for comparing serum concentration curves in humans. PK parameters of curcumin when given alone in humans could not be calculated as curcumin could not be detected in most of the samples.

Results

Animal studies

Curcumin alone at 2 g/kg, or when combined with piperine, 20 mg/kg, was well tolerated by the rats as they showed no untoward effects for 48 h. Yellow coloured faecal pellets appeared at 30 h postdrug and continued upto 48 h. Perusal of Figure **1** indicates that when curcumin was given alone, peak serum concentrations of 1.00 ± 0.26 µg/ml were attained rapidly within 0.75 h and plateaued till 1 h. Thereafter, the levels declined gradually reaching zero at 5 h. The plasma

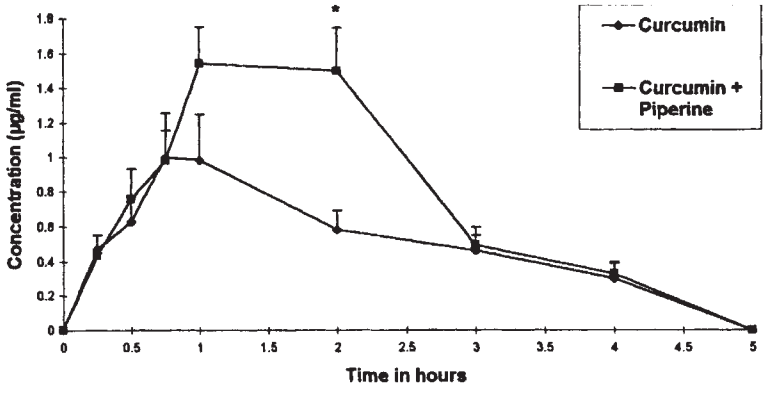

Fig. 1 Serum concentrations µg/ml (mean ± SEM) of curcumin 20 g/kg oral alone and with piperine 20 mg/kg in rats (n = 6/group/time cut). Significance as compared to curcumin alone; *P < 0.02.

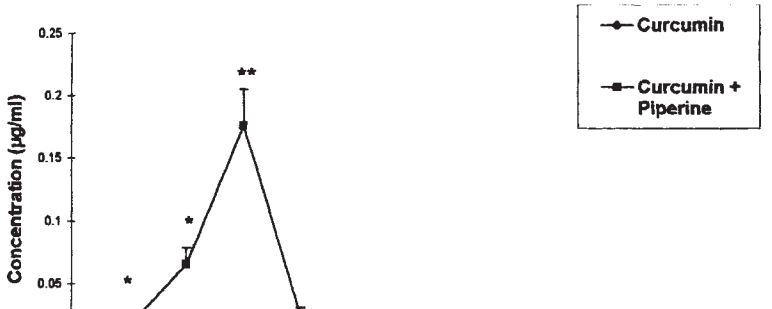

Fig. 2 Serum concentration µg/ml (mean ± SEM) of curcumin 2 g oral alone and with piperine 20 mg in humans (n = 8). Significance as compared to curcumin alone; * P < 0.01; ** P < 0.001.

concentration time curve of curcumin in combination with piperine followed a similar pattern from 0 to 0.75 h and 3 to 5 h. However, piperine produced higher serum concentrations of curcumin at 1 and 2 h (1.55 ± 0.21 and 1.50 ± 0.25 µg/ml) respectively, being significantly higher (P < 0.02) at 2 h. Thus piperine significantly enhanced the serum concentration of curcumin, albeit for a limited duration (although serum samples were collected upto 6 h, values are depicted till 5 h only, since the 6 h value was also "0" in all animals).

Table **1** shows the values (mean ± SEM) of the pharmacokinetic parameters of curcumin *per se* and when combined with piperine. C_{max} was increased from 1.35 ± 0.23 to 1.80 ± 0.16 µg/ml, but was not statisticallyy significant, while T_{max} was significantly increased from 0.83 ± 0.05 to 1.29 ± 0.23 h (P < 0.02). The $t^1/_{2(el)}$ significantly decreased from 1.70 ± 0.58 to 1.05 ± 0.18 h (P < 0.02). Though $t^1/_{2(a)}$ increased from 0.31 ± 0.07 to 0.47 ± 0.03 h and AUC increased from 2.36 ± 0.28 to 3.64 ± 0.31 µg/h/ml, these increases were not statistically significant. Cl significantly decreased from 713.00 ± 12.00 to 495.00 ± 37.00 L/h (P < 0.02), but the decrease in the Vd from 1366.00 ± 248.70 to 782.60 ± 193.90 L/kg was not significant. The relative bioavailability of curcumin when combined with piperine is 154 %.

Human volunteer studies

Curcumin alone or when combined with piperine was well tolerated by all the subjects and there were no adverse or untoward reactions; 2 subjects dropped out of the study for non-medical reasons. Therefore all calculations presented here are based on the data obtained from 8 subjects. In Figure **2** is shown the serum concentration of curcumin *per se* and when given with piperine. Although serum samples were collected upto 6 h, we have depicted values till 3 h, since the 4, 5, and 6 h values were also "0" in all subjects.

Serum levels of curcumin when given alone were either very low or undetectable at most time points in most subjects, explaining the almost flat serum concentration curve (Fig. **2**). However, when piperine was added the serum concentrations of curcumin were significantly increased at the time points

Table 1 Pharmacokinetic parameters (mean ± SEM) of oral curcumin 2 g/kg alone and in combination with piperine 20 mg/kg in rats (n = 6/drug/time cut).

Parameters	Curcumin alone 2 g/kg	Curcumin + Piperine 2 g/kg + 20 mg/kg
C_{max} (µg/ml)	1.35 ± 0.23	1.80 ± 0.16
T_{max} (h)	0.83 ± 0.05	1.29 ± 0.23*
$t^1/_{2(a)}$ (h)	0.31 ± 0.07	0.47 ± 0.03
$t^1/_{2(el)}$ (h)	1.70 ± 0.58	1.05 ± 0.18*
AUC(0–tn) (µg/h/ml)	2.36 ± 0.28	3.64 ± 0.31
Vd (L/kg)	1366.00 ± 248.70	782.90 ± 193.90
Cl (L/h)	713.00 ± 12.00	495.90 ± 37.08*

* P < 0.02: Statistical significance by Students "t"-test.
C_{max}: Maximum serum concentration.
T_{max}: Time to reach maximal serum concentration.
$t^1/_{2(a)}$: Absorption half-life.
$t^1/_{2(el)}$: Elimination half-life.
$AUC_{(0-tn)}$: Area under the concentration time curve.
Vd: Volume of distribution.
Cl: Total clearance.

upto 0.75 h; $P < 0.01$ at 0.25 h and 0.5 h; $P < 0.001$ and at 0.75 h. Subsequently there was a rapid decline upto 1 h and thereafter a gradual decline to zero by 3 h.

In Table **2** are depicted the PK parameters (Mean ± SEM) of curcumin when given alone and with piperine. C_{max} (observed values) when curcumin was given alone was only 0.006 ± 0.005 μg/ml at 1 h whereas when piperine was added the C_{max} (observed value) was increased to 0.18 ± 0.16 μg/ml and was attained earlier, i.e. at 0.75 h. Vd and Cl could not be calculated with curcumin alone as serum levels were not detected at most time points in most subjects. The mean $AUC_{(0-tn)}$, however, was calculated using the trapezoidal method and was found to be 0.004 μg/h/ml, the relative bioavailability of curcumin when given with piperine was therefore 2000 %.

Table 2 Pharmacokinetic parameters (mean ± SEM) of oral curcumin 2 g/kg alone in combination with piperine 20 mg/kg in normal healthy volunteers (n = 8).

Parameters	Curcumin alone 2 g/kg	Curcumin + Piperine 2 g/kg + 20 mg/kg
C_{max} (μg/ml)	0.006 ± 0.005	0.18 ± 0.03
T_{max} (h)	1	0.69 ± 0.07
$t^1/_{2(a)}$ (h)	*	0.11 ± 0.02
$t^1/_{2(el)}$ (h)	*	0.41 ± 0.17
$AUC_{(0-tn)}$ (μg/h/ml)	0.004	0.08 ± 0.01
Vd (L/kg)	*	202.60 ± 78.94
Cl (L/h)	*	7.33 ± 1.25
F (Relative bioavailability)		2000 %

C_{max}: Maximum serum concentration.
T_{max}: Time to reach maximal serum concentration.
$t^1/_{2(a)}$: Absorption half-life.
$t^1/_{2(el)}$: Elimination half-life.
$AUC_{(0-tn)}$: Area under the concentration time curve.
Vd: Volume of distribution.
Cl: Total clearance.
* Not calculated – see text.

Discussion

The results obtained in the study demonstrate that piperine enhances the oral bioavailability of curcumin in both rats and humans at doses that were devoid of adverse side effects. However, certain differences between rat and human with respect to curcumin were evident. Curcumin *per se* attained overall moderate serum concentrations over a 4 h period in rats with peak levels occurring between 0.75 h to 1 h. On the other hand, in humans when curcumin was given alone only negligible serum concentrations of curcumin were detectable the serum concentration-time curve being almost flat. This difference may be due to the high oral dose employed in the rat (2 g/kg), whereas the human dose was about 60 times less, approximately 33 mg/kg. Curcumin serum concentrations reached zero at 5 h in rats and 3 h in humans. Further in rats with the addition of piperine, curcumin achieved higher concentrations than in humans albeit for a short period, took a longer time to peak and declined slowly. Whereas in humans T_{max} was attained earlier and then declined rapidly. This rapidity in decline is more apparent probably because of the higher levels of curcumin achieved with piperine as compared to curcumin alone. There was an increase in the AUC though not significant and an increase in bioavailability of curcumin by about one and a half times as compared to curcumin given alone in both rats and humans. In rats when piperine was added to curcumin both Vd and Cl decreased which may have also contributed to the higher concentration, such a comparison was not possible in humans for reasons explained earlier. Our findings concerning absorption of curcumin in rats are in agreement with data obtained by Wahlstrom and Blennow (11), who showed that when Sprague Dawley rats were given curcumin 1 g/kg *p.o.*, measurement of blood plasma levels and biliary excretion indicated some absorption from the gut with no apparent toxic effects upto 5 g/kg *p.o.* Likewise, Khanna et al. (12) found that after curcumin, 100 mg/kg *p.o.*, 74 % was absorbed from the gastrointestinal tract within the first 5 h, while complete elimination occurred within 48 h. Our results are, however, in conflict with studies by Ravindranath and Chandrasekhara (6), who could not detect curcumin in portal or heart blood samples upto 24 h in rats given curcumin 400 mg/kg *p.o.* They, however, did report 60 % absorption of curcumin as determined by the amount excreted in the faeces.

There is evidence that piperine is a potent inhibitor of drug metabolism, and glucuronidation altering the disposition and bioavailability of a large number of drugs (8). Further piperine at 20 mg in humans has also been shown to produce earlier T_{max}, higher C_{max} and AUC of drugs like propranolol and theophylline (13). This property of piperine suggests that it may be involved in inhibiting the metabolism of curcumin and enhancing its bioavailability.

In conclusion, the study shows that piperine enhances the serum concentration and bioavailability of curcumin in rats and man probably due to increased absorption and reduced metabolism.

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