Relative Oral Bioavailability Study of Liposomal Curcumin

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Abstract

Background: Curcumin is a naturally derived yellow polyphenolic compound from the rhizome Curcuma longa. Curcumin modulates various signalling pathways, such as Cyclooxygenase-2 (COX-2), Matrix metallopeptidases (MMPs), glutathione, protein kinase C, ATPase, nuclear Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kb), Activator protein 1 (AP-1), P-glycoprotein 1 (P-gp), Multidrug resistance-associated proteins (MRP-1, MRP-2), Receptor tyrosine-protein kinase (ErbB2), Alpha-1-acid glycoprotein AGP), Cyclin D1 etc. Curcumin, as a free drug, has a short plasma half-life because it is rapidly metabolised in the liver. Its oral bioavailability is extremely poor, and hence Curcumin is classified as a class IV compound in the BCS system.

Materials and Methods: Authors of the current study have formulated Liposomal Curcumin and conducted a Relative Oral Bioavailability Study of Liposomal Curcumin in comparison with plain Curcumin in rats. The treatment group was given Curcumin 25% l lipo at the dose of 500 mg/kg body weight and the control group was given plain Curcumin 500 mg/kg body weight of the rats.

Observations: Oral administration of Liposomal Curcumin showed rapid absorption (t_{max} =15 min) with Peak plasma concentration (C_{max}), Area under Curve (AUC) and half-life ($t_{1/2}$) of 42.3 ng/ml, 244 ng.h/ml and 5.5 h, respectively. In the control group, the Curcumin levels were below the level of quantitation (5.43 ng/ml).

Conclusion: This study shows that Liposomal Curcumin has higher bioavailability as compared to the marketed formulations of Curcumin. Further extensive clinical studies are needed to prove efficacy of Liposomal Curcumin in various human applications.

Keywords: Liposomal Curcumin, bioavailability, pharmacokinetics

Conflict of Interest: Dr. Yogesh Dound is Proprietor, Shreepad Shree Vallabh SSV Phytopharmaceuticals.

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Introduction

Curcumin is a naturally derived yellow polyphenolic compound from the rhizome *Curcuma longa*.[1] Curcumin, as a free drug, has a short plasma half-life because it is rapidly metabolised in the liver.[2] The wide range of activities of Curcumin are confirmed to its ability to modulate various signalling pathways, such as Cyclooxygenase-2 (COX-2), Matrix metallopeptidases (MMPs), glutathione, protein kinase C, ATPase, nuclear Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kb), Activator protein 1 (AP-1), P-glycoprotein 1 (P-gp), Multidrug resistance-associated proteins (MRP-1, MRP-2), Receptor tyrosine-protein kinase (ErbB2), Alpha-1-acid glycoprotein AGP), Cyclin D1, etc.[3,4] Various pre-clinical and clinical studies have confirmed that Curcumin is significantly non-toxic even at very high doses.[5] Unfortunately, Curcumin faces different problems for its application, such as low-aqueous solubility, rapid systemic clearance, inadequate tissue absorption and degradation at alkaline pH values. Also, Curcumin is rapidly photodegraded by light, hence limiting its clinical use.[6,7] Consequently, its oral bioavailability is extremely poor, and Curcumin is classified as a class IV compound in the BCS system.[8]

To overcome these limitations, various strategies such as liposomes, solid dispersion, complex, emulsion, micelles, nanogels and microspheres have been employed to overcome poor absorption and other pharmacokinetic limitations of curcumin. Further, Curcumin is poorly water-soluble, <0.125 mg/L, and susceptible to enzymatic degradation in the blood stream, all of which limit the drug's bioavailability and therapeutic potential. To improve the bioavailability of curcumin, many different approaches, have been developed for sustained and efficient curcumin delivery.[9]

Materials

Curcumin 25% I lipo (Liposomal Curcumin) was obtained from Shreepad Shree Vallabh SSV Phytopharmaceuticals, Mumbai. Male Wistar rats (290–340 g) were housed in a temperature (23 ± 1^0C) and light-controlled room (12 hours light/dark cycle). They were allowed ad libitum access to food and water for 7 days. Rats were randomly divided into two groups of eight animals each. The control group was given plain Curcumin 500 mg/kg body weight of the rats and the treatment group was given Curcumin 25% I lipo at the dose of 500 mg/kg body weight. The study was performed as per ethical practices laid down in the CPCSEA guidelines[10] for animal care and use. The study was approved by the Institutional Animals Ethics Committee (IAEC) of the test facility.

Method

All the formulations were orally administered by oral gavage by means of flexible plastic tubes with 5 cm in length. At pre-determined interval (0, 5, 15, 30, 60, 120, 180, 240 and 480 min) after administration, blood was harvested from the abdominal Vena Cava of rats under light anesthesia (di ethyl ether) and placed into heparinized tubes to prevent clotting. Plasma was immediately prepared by centrifugation at 1000 x g for 15 min at 4^0C and stored at -80^0C until analysis. Plasma (200 mL) was mixed with 200 mL of 0.1 M sodium phosphate buffer (pH6.8) containing 0.1% EDTA, 200 mL of distilled water, and 600 mL of methanol. The mixture was vortexed for 3 min and, after the addition of 4mL of hexane, was shaken vigorously and centrifuged at 1000 x g for 10 min at 4^0C. The hexane layer was discarded, and 1.2 mL of distilled water and 3 mL of ethyl acetate were added to the mixture (aqueous-methanol layer). This was shaken vigorously and centrifuged at 1000 x g for 15 min at 4^0C, and the ethyl acetate layer was finally collected. This ethyl acetate extraction was repeated four times. The combined extraction phases were evaporated to dryness in vacuo and the residue was dissolved in 100 mL of methanol and was analysed for Curcumin content by HPLC method.

Results

Following oral administration of Curcumin 25% I lipo, Liposomal Curcumin showed rapid absorption (T_{max} =15 min) with Peak plasma concentration (C_{max}), Area under Curve (AUC) and half-life ($t_{1/2}$) of 42.3 ng/ml, 244 ng.h/ml and 5.5 h, respectively. In the control group, the Curcumin levels were below the level of quantitation (5.43 ng/ml). The Bioanalytical method was linear between 5.43 ng/ml and 3528.57 ng/ml and met all acceptance criteria. Table 1 shows the average values of the pharmacokinetic parameters in the test group and the control group. Curcumin plasma concentration versus time profiles in both the groups can be seen in Figure 1. Table 2 shows a comparison of the various bioavailability studies on different formulations[11-15] of Curcumin following oral administration in rats.

Polylactic-co-glycolic acid (PLGA) and PLGA-polyethylene glycol (PEG) (PLGA-PEG) blend nanoparticles are considered as potential carriers for the oral delivery of curcumin. The nanoparticles pro-

longed extensively the curcumin release. In an in vivo pharmacokinetic study, all parameters including mean half-life and the C_{max} were improved by curcumin nanoparticles. Compared to the curcumin aqueous suspension, the PLGA and PLGA–PEG nanoparticles increased the curcumin bioavailability by 15.6- and 55.4-fold, respectively [11].

K;kl;l in rats [12]. Curcumin incorporated into TMC-coated liposomes exhibited bioavailability (C_{max} = 46.13 ng/ml, T_{max} = 2.0 ± 0.62 hours), compared with curcumin encapsulated by uncoated liposomes (C_{max} = 32.12 ng/ml, T_{max} = 2.0 ± 0.30 hours) and curcumin suspension (C_{max} = 35.46 ng/ml, T_{max} = 1.0 ± 0.22 hours).

Curcumin powder extracted from Indian turmeric by using alcohol was evaluated *in vivo* for the plasma pharmacokinetics at the dose of 50 mg/kg and 300 mg/kg. For both the doses the T_{max} was 2 hours and the C_{max} was 13.0 ± 5.8 and 37.4 ± 36.1 ng/ml respectively.[13] In another study, the Curcumin powder at the dose of 250 mg/kg was evaluated for oral bioavailability studies *in vivo*.[14] The C_{max} was 32.29 ± 14.93 ng/ml and the T_{max} was around 1 hour. In a similar study with crystalline Curcumin powder at the dose of 100 mg/kg the C_{max} was 35 ± 8.0 ng/ml and the T_{max} was almost 80 minutes.[15]

Curcumin 25% I lipo when compared with other similar formulations or different formulations has shown improved pharmacokinetic profiles in terms of rapid absorption (T_{max}) with Peak plasma concentration (C_{max}), Area under Curve (AUC) and half-life ($t_{1/2}$) leading to higher bioavailability in comparison to various Curcumin formulations studied.

Table 1. Mean plasma concentration/time curves and mean pharmacokinetic parameters obtained after oral administration to rats in both the groups.

Pharmacokinetic Parameters	Curcumin 25% I lipo	Plain Curcumin
	Estimate	Estimate
K_e (1/h)	0.126	NE
$t_{1/2}$ (h)	5.520	NE
T_{max} (min)	15	NE
C_{max} (ng/ml)	42.280	NE
T_{last} (h)	8.000	NE
C_{last} (ng/ml)	11.388	NE
AUC_{last} (h*ng/ml)	154	NE
AUC_{INF} (h*ng/ml)	244	NE
$AUC_{\_\%Extrap}$	37	NE

NE: Not Estimated as Curcumin levels were BLOQ [5.43 ng/ml]
BLOQ: Below the limit of Quantitation

K_e - Elimination rate constant; $t_{1/2}$ - Elimination half life; T_{max} - Time of maximum drug concentration; C_{max} - Maximum plasma concentration; T_{last} - Time of C_{last}; C_{last} - last observed quantifiable concentration; AUC_{last} - Area under the plasma concentration-time curve from time zero to time of last measurable concentration; AUC_{INF} - Area under the plasma concentration-time curve from time zero to infinity; $AUC_{\_\%Extrap}$ - Area under the plasma concentration-time curve extrapolated from time t to infinity as a percentage of total AUC.

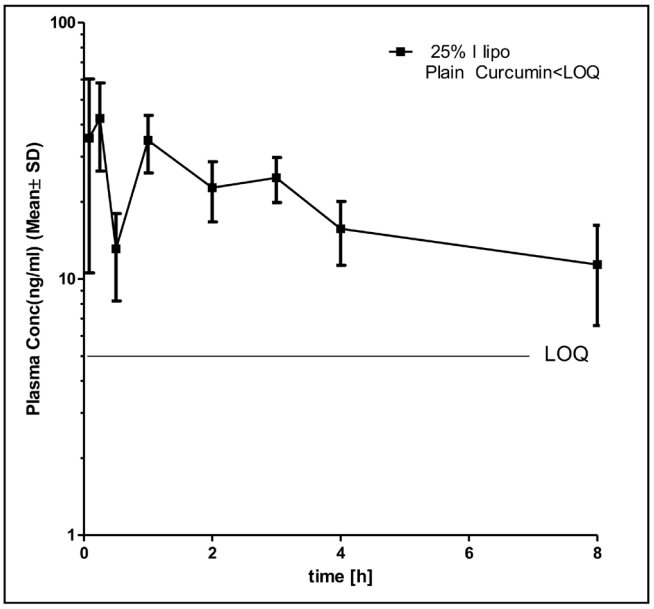

Figure 1. Curcumin plasma concentration versus time profiles in both the groups

Discussion

Liposomes are useful vehicles to deliver curcumin because of their ability to improve bioavailability and solubility of curcumin. They can be used as a potential targeted delivery system by folate-ligand surface modification, gradual release of curcumin in the body, and subsequent improved efficacy of treatment in cancer patients.[16,17] Liposomes have been used in the delivery of anticancer drugs and are able to alter the biodistribution and clearance of drug molecules.[18,19] Liposomal nanoconstructs are extensively studied to improve the bioavailability of Curcumin, as these drug delivery systems are approved by the Food and Drug Administration (FDA). [20,21]

Studies have shown that effective liposomal encapsulation of Curcumin can increase its solubility to 500 ug/mL as compared to 0.125 mg/L. The benefits of using a phospholipid bilayer nanoconstruct, include du-

Table 2. Peak plasma/serum concentration (C_{max}) and time to reach peak plasma/serum (T_{max}) concentration obtained from various studies with different formulations of Curcumin.

Curcumin formulation	Curcumin (g/kg)	C_{max} (ng/ml)	T_{max} (hour)	Ref.
Curcumin unformulated	0.05	4.066 ± 0.564	0.50	[11]
PLGA Curcumin nanoparticles	0.05	11.783 ± 0.454	2.00	[11]
PLGA–PEG Curcumin nanoparticles	0.05	29.778 ± 4.632	3.00	[11]
Curcumin suspension	0.25	35.46 ± 12.81	1.00	[12]
TMC-coated Curcumin liposomes	0.04	46.13 ± 5.31	2.00	[12]
Uncoated Curcumin liposomes	0.04	32.12 ± 9.42	2.00	[12]
Curcumin Powder	0.05	13.0 ± 5.8	2.00	[13]
	0.3	37.4 ± 36.1	2.00	[13]
Raw Curcumin	0.25	32.29 ± 14.93	0.58	[14]
Curcumin crystal suspension	0.10	35.00 ± 8.0	1.33	[15]

al loading of hydrophilic and hydrophobic drugs, improved bioavailability to target site cell and tissue, stability of encapsulated drugs, pharmacological inactivity and minimal toxicity of phospholipids. Several *in vitro* studies have shown the benefits of encapsulating curcumin in different cancer types such as pancreatic adenocarcinoma, osteosarcoma, liver cancer etc. [22-25] Liposomal delivery of Curcumin has also been tested in clinical trials for cancer therapy. Storka *et al.* found that short-term administration of intravenous doses up to 120 mg/m^2 are safe in healthy individuals. [26] Several products like Theracurmin® (Made with gum ghatti) is used to improve gut absorption and enhance the effects of curcumin in the body which range from cardiovascular health to free radical protection.[22,23] Other product Longvida® use solid lipid curcumin particle (SLCP) technology to enhance the bioavailability of curcumin by protecting curcumin from degradation in the stomach and promoting free drug absorption in the gut.

In the current study, the pharmacokinetic profile obtained for Liposomal Curcumin was compared with the pharmacokinetic profiles C_{max} and T_{max} of various formulations of Curcumin published in literature [11-15] and was found to be superior in comparison.

The results clearly show that Curcumin 25% I lipo (Liposomal Curcumin) is highly bioavailable and this property will help improve its efficacy for various human applications.

Conclusion

Curcumin 25% I lipo (Liposomal Curcumin) is highly bioavailable as seen by the pharmacokinetic profiles i.e. peak plasma/serum concentration (C_{max}) and time to reach peak plasma/serum (T_{max}) concentration. These pharmacokinetic values need to be extrapolated and correlated in humans. For the same, further extensive clinical studies are needed to be conducted. This will also prove the efficacy of Curcumin 25% I lipo (Liposomal Curcumin) in various indications in comparison to currently available formulations of Curcumin.

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