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Pharmacokinetics and tissue distribution of piperine lipid nanospheres

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Received October 18, 2007, accepted December 3, 2007

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Pharmazie 63: 352–355 (2008) *doi: 10.1691/ph.2008.7362*

The purpose of this study was to estimate the pharmacokinetic parameters and tissue distribution of positively charged stearylamine (LN-P-SA) and pegylated lipid nanospheres (LN-P-PEG) of piperine in BALB/c mice. Lipid nanospheres of piperine (LN-P), LN-P-PEG and LN-P-SA were prepared by homogenization followed by ultrasonication. The pharmacokinetics and tissue distribution of different lipid nanosphere formulations (piperine, LN-P, LN-P-PEG and LN-P-SA) were studied in male BALB/c mice. The pharmacokinetic parameters of LN-P-PEG and LN-P-SA were: AUC_{0-24}: 372.1 ± 71.6 and 162.2 ± 36.4 μg h^{-1} ml^{-1}, clearance 13 ± 2.5 and 32 ± 7.5 ml h^{-1}, $C_{max.}$ 24.7 ± 1.5 and 22.3 ± 1.0 μg ml^{-1}, Vd: 0.45 ± 0.02 and 0.66 ± 0.06 l kg^{-1}). Pharmacokinetics of piperine in lipid nanospheres showed a biexponential decline with significantly high AUC, a lower rate of clearance and a smaller volume of distribution than piperine.

1. Introduction

Leishmaniasis is a complex of disease syndromes, with a spectrum that has been divided into visceral, cutaneous and mucocutaneous forms, caused by protozoan parasites of the genus *Leishmania*. The disease is endemic in many tropical and subtropical regions of the world, with 500 000 people at risk (Boelaert et al. 2000). Visceral leishmaniasis (VL) has also emerged as an important opportunistic infection in immuno compromised patients, in particular those with HIV, in some regions of the World, most notably the mediterranean countries (Alvar et al. 1997; Gradoni et al. 1999). Cutaneous leishmaniasis (CL) continues to be a recurrent problem for populations in the Middle East and many parts of Latin America, with major outbreaks observed during the Iran-Iraq war and most recently in Afghanistan (Rowland et al. 1999).

Leishmaniasis occurs from tropical to mediterranean regions where the parasite is transmitted by female sandflies of the genus *Phlebotomus* in the old world and *Lutzomysa* in the new world (Senior et al. 1991).

The powder of dried seeds of black pepper (*Piper nigrum* Lin, Piperaceae) has been used in Indian medicine (Krishnamurthy et al. 1975) and the vapors of pepper species are used in treating epilepsy, ordinary cold, headache (Atal et al. 1975) and pepper is also used as abortificent in few areas of India (Chandhoke et al. 1978; Kholkute et al. 1979). Antifungal (Madhyastha et al. 1984) and antiamoebic activities (Ghoshal et al. 1996) of piperine have also been reported.

Piperine is a potent inhibitor of *Leishmania donovani* promasigotes (Kapali 1993). Piperine exhibited antileishmanial properties when tested in an experimental leishmaniasis model. It has been observed that, piperine inhibits type-I DNA topoisomerase, a vital enzyme of *Leishmania donovani*. A liposomal formulation of piperine has shown a significant improvement in antileishmanial activity compared to piperine (Raay et al. 1999). The purpose of this study was to estimate the pharmacokinetic parameters and tissue distribution of piperine lipid nanoparticles (LN-P), positively charged stearylamine (LN-P-SA) and pegylated lipid nanospheres (LN-P-PEG) of piperine in BALB/c mice.

2. Investigations and results

The plasma concentration vs time profiles of piperine obtained after the i.v. injection of piperine, LN-P, LN-P-PEG and LN-P-SA formulations are shown in Fig. 1. The pharmacokinetic parameters of LN-P, LN-P-PEG and LN-P-SA piperine were given in the Table.

Tissue distribution after administration of piperine, LN-P, LN-P-PEG and LN-P-SA were assessed in liver, spleen, kidney and brain.

Piperine concentration in liver after i.v. injection of piperine, LN-P, LN-P-PEG and LN-P-SA is given in Fig. 2.

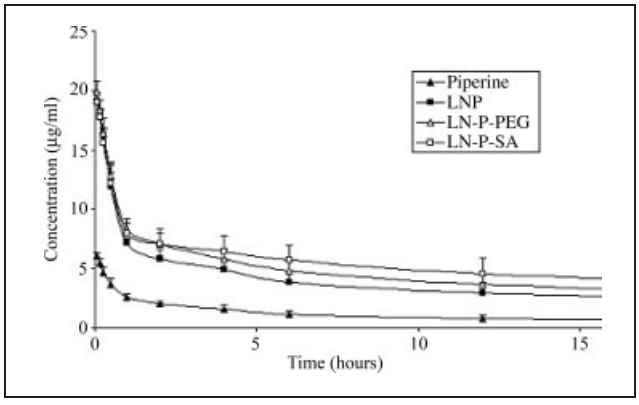

Fig. 1: Plasma concentration profiles of piperine following i.v. in mice

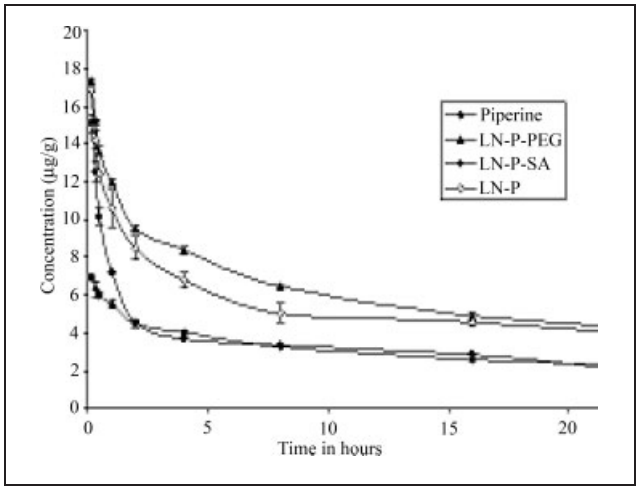

Fig. 2: Concentration profiles of piperine in mice liver

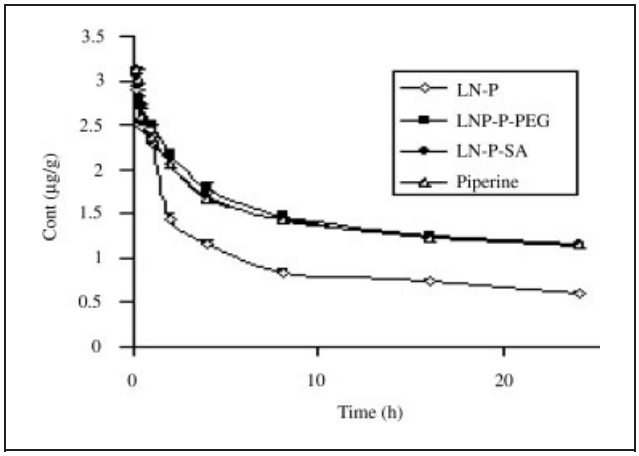

Fig. 4: Concentration profiles of piperine in mice kidney

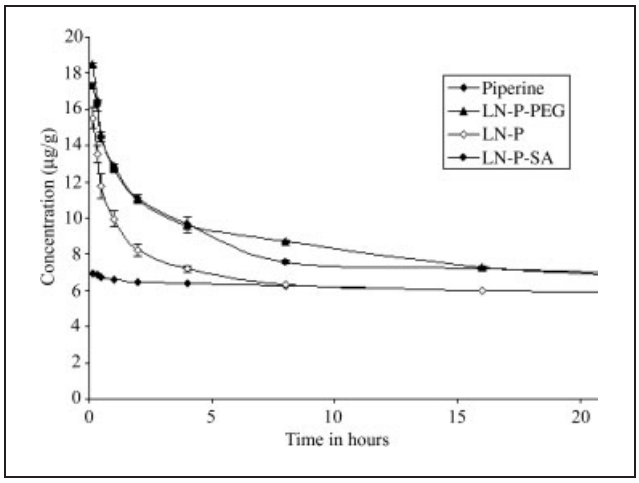

Fig. 3: Concentration profiles of piperine in mice spleen

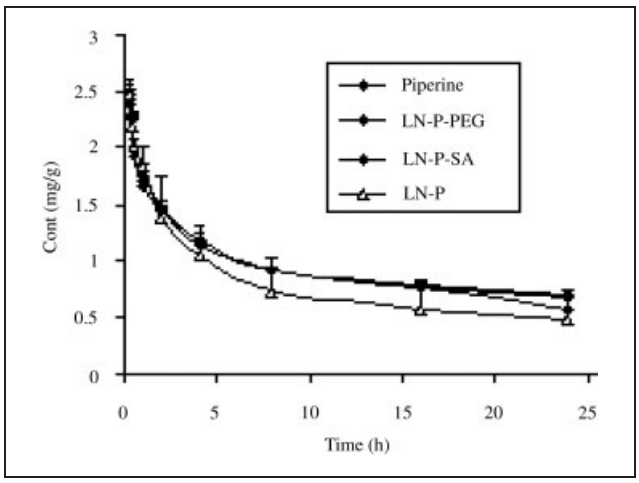

Fig. 5: Concentration profiles of piperine in mice brain

After 15 min of injection, the piperine concentration in the liver was in the order of LN-P-SA > LN-P > LN-P-PEG > piperine. There were large differences in liver uptake between LN-P, LN-P-SA, LN-P-PEG and piperine.

The piperine concentration in spleen after i.v. injection of piperine, LN-P, LN-P-PEG and LN-P-SA is shown in Fig. 3. After 15 min of injection, the piperine concentration in the spleen is in the order of LN-P-SA > LN-P > LN-P-PEG > piperine.

The piperine concentration in kidney after i.v. injection of piperine, LN-P, LN-P-PEG and LN-P-SA is given in Fig. 4. After 15 min of injection the piperine concentration in the kidney is in the order of piperine > LN-P-PEG > LN-P > LN-P-SA.

The piperine concentration in brain after i.v. Injection of piperine, LN-P, LN-P-PEG and LN-P-SA is given in Fig. 5. After 15 min of injection the piperine concentration in the brain is in the order of LN-P > piperine > LN-P-PEG > LN-P-SA.

3. Discussion

Piperine lipid nanospheres (LN-P), piperine positively charged stearylamine nanospheres (LN-P-SA) and pegylated piperine lipid nanospheres (LN-P-PEG) were prepared according to the method reported by Veerareddy et al. (2004). Piperine is administered at a dose level of 5 mg/kg body weight. The effect of multiple doses of varying amounts of piperine on hamsters was reported by Raay et al. (1999). Piperine solution has shown highest clearance and high volume of distribution when compared with that of lipid nanospheres. Encapsulation of piperine in LN improved the mean residence time (MRT) of piperine and area under the concentration (AUC) values were

Table: Pharmacokinetic parameters after i.v. administration of piperine lipid nanospheres in mice

Group	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	$AUC_{0-\alpha}$ (µg h ml^{-1})	CL (ml h^{-1}kg^{-1})	V_{ss} (l kg^{-1})	MRT (h)	C_{max} (µg ml^{-1})
Piperine	0.29 ± 0.10	8.50 ± 3.81	29.71 ± 10.85	188 ± 82.9	1.89 ± 0.21	11.46 ± 5.10	6.96 ± 0.09
LN-P	0.32 ± 0.02	21.56 ± 3.67	262.52 ± 71.93	21 ± 5.9	0.67 ± 0.11	30.16 ± 5.30	21.61 ± 0.77
LN-P-PEG	0.32 ± 0.07	23.77 ± 4.62	372.12 ± 71.57	13 ± 2.5	0.45 ± 0.02	33.72 ± 6.66	24.65 ± 1.47
LN-P-SA	0.36 ± 0.03	15.60 ± 1.86	162.15 ± 36.44	32 ± 7.5	0.66 ± 0.06	21.36 ± 2.89	22.32 ± 0.99

*Values in parentheses represent standard deviation.

also improved. Piperine encapsulated in pegylated LN has shown higher AUC and lower clearance. Coating of liposome surface with PEG increased the hydrophilicity of the liposome surface such that the nonspecific interaction of LN with plasma proteins is reduced. Additionally, PEG sterically prevents the coating of opsonins to the LN, resulting in a reduced specific interaction with the reticulo endothelial system (RES) cells (Kimura et al. 1986). Anchoring of lipid nanospheres with PEG improved the pharmacokinetic profile of piperine. LN-P-PEG and LN-P-SA showed higher AUC in comparison with that obtained following administration of piperine solution and LN-P. Both LN-P-SA and LN-P-PEG are bigger in size when compared with their LN-P formulations. It is reported that RES removes larger particles more quickly than smaller particles (Day et al. 2000). Further, positively charged particles are more easily taken up by the RES than negatively charged and neutral particles (Nakanishi et al. 1997). Since the lipid emulsions injected directly into the systemic circulation are foreign substances to the body, it could not be ruled out that LN are recognized by alternative pathways of complement activation and captured by macrophages in RES organs. It has been reported that, when lipid emulsions are administered i.v. they are rapidly taken up by the RES in liver and spleen (Daneshmend and Warnock 1983). Attempts have been made to avoid the trapping of lipid nanospheres by the RES by using surface-modified lipid microspheres (LM) (Schreoder et al. 1998), small LM (Kreuter et al. 1995) and negatively charged LM (Hultin et al. 1995). In order to avoid the rapid uptake by RES organs, LN were anchored with PEG-DSPE. It was found that the liver and spleen uptake is lowered with pegylation and improved the circulation time. The purpose of this formulation was to treat visceral leishmaniasis infections that need constant piperine levels in blood for a longer time.

All LN formulations tested have shown no significant difference in kidney concentrations and these results supported by the renal toxicity data of these formulations.

Many colloidal carrier systems have been studied to achieve blood brain barrier (BBB) penetration of drugs (Gopper and Müller 2003). Higher brain concentrations of piperine were seen following LN-P administration compared to piperine solution. Piperine in LN-P-PEG and LN-P-SA showed lower BBB concentrations than LN-P. It was observed that apolipoprotein E is responsible for transport of nanoparticles across the blood brain barrier (BBB). On intravenous injection of polysorbate-80 coated nanoparticles, apolipoprotein E adsorbed on to the nanoparticle surface. These particles then seem to mimic low density lipoprotein (LDL) particles and could interact with the LDL receptors leading to their uptake by the endothelial cells (Jain 1994). LN follow the same pathway, after adsorption of apolipoprotein E, the drug was released in endothelial cells and drug diffusion into the brain.

The physicochemical characteristics of the formulations such as surface characters like charge and coating with PEG-DSPE may play an important role for the pharmacokinetics of the formulation. The tissue distribution of these formulations varies widely, in liver and spleen, the piperine concentration was found to be higher following positively charged and pegylated lipid nanospheres administration. In summary, our results suggest that piperine LN formulations show improved pharmacokinetic parameters than piperine solution. Among the LN formulations, LN-P-PEG has shown the highest plasma levels. Piperine concentration was found to be higher in kidney and serum creatinine levels were also found to be higher in case of piperine solution. LN formulations were found to be safe with lower kidney concentrations.

4. Experimental

4.1. Materials

Piperine (Sigma Chemicals, USA), egg lecithin (Sigma Chemicals, USA), soybean oil (Cargil Foods, India), cholesterol (Qualigens Fine Chemicals, India), glycerol (Himedia, India), Sucrose (S.D. Fine, India), sodium oleate (Merck Ltd, India), propyl paraben, (Sigma Chemicals, USA), methanol HPLC grade, (Rankem Chemicals Limited, India), distearylphosphatidyl ethanolamine-polyethylene glycol (DSPE-PEG) (Sigma, USA), stearylamine (Sigma, USA), double distilled water.

4.2. Preparation of piperine lipid nanospheres

Positively charged and pegylated piperine lipid nanosphere formulation methods were reported by Veerareddy et al. (2004).

4.3. Animals

BALB/c mice weighing between 20 and 25 g were used for pharmacokinetic and tissue distribution studies. The animals were procured from National Institute of Nutrition (NIN), Hyderabad. All animal experiments were evaluated and approved by the animal and ethics review committee of the Faculty of Pharmaceutical Sciences, Kakatiya University, India. The animals were acclimatized for at least one week before the study, fed with standard NIN diet and received tap water *ad libitum*.

4.4. Pharmacokinetics and tissue distribution

BALB/c mice were divided into four groups. Piperine, LN-P, LN-P-SA and LN-P-PEG were administered at a dose of 5 mg/kg via the tail vein with a syringe equipped with a 28 $g^{1/2}$ needle, at predetermined time points (5, 10, 15 and 30 min and 1, 2, 4, 6, 12 and 24 h). Three mice were euthanized by cervical dislocation and dissected. Tissues of interest (liver, spleen, kidney and brain) and blood were collected.

4.5. Chromatographic conditions

The HPLC system (Shimadzu, Japan) consisted of LC-10AT solvent delivery module, SPD-M10A, VP UV-Visible spectrophotometric detector with 20 µl injection port. A Phenomenex C_{18} column (250 mm, 4 mm I.D, 5 µm particle size) was used for the analysis. A mobile phase consisting of methanol:water (75 : 25) was pumped through the column at a rate of 1 ml/min and the eluent was monitored at 343 nm. The sensitivity was set at 0.01 AUFS.

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