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In-Vivo Study of Phospholipid Assisted Nano-Suspensions of Efavirenz

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Abstract

Nanosystems are versatile drug delivery systems, with an ability to overcome physiologi c barriers and to guide the drug to specific cells or intracellular compartments due to th eir small size, typically in the 10 to 1000 anmarange. Nanosystems offer several advantages, such as the protection of drugs against degradation, targeting of drugs to specific sites, and tailorin the release kinetics to provide prolonged release of the dru gs.[8] Polymeric nanoparticles, solid lipidnanoparticles, liposomes, nanosuspensions and nanoemulsions have been reported to enhancethe effective delivery of the drugs.Pharmac okinetic studies involve the study of absorption, distribution, metabolism, and excretion of the drugs from the body. The current study involved oral dispersion and the nanosuspensions to male Wistar rats.Efavirenz is widely distributed and protein bound, primarily to albumin.[98] Organ distribution study was performed in rats to determine the EFV level in different organs over a specified period of time

Keywords: Efavirenz, Nanosuspension, Antiretroviral, Organ distribution, Pharmacokinetics

Introduction

The advent of anti-retroviral therapy has been one of the greatest research achievements in modern medicine. The therapy has helped improve longevity of patients, reduce viral load, sexual transmission of the virus and AIDS can be prevented.[4] By 1995-96, combination of anti- retroviral drugs was introduced and the regimen was called Highly Active Anti-retroviral therapy (HAART).[22] HAART generally includes a combination of three or more drugs which belong to different classes. The aim of the therapy is to reduce or prevent HIV replication by using drugs which target different stages of the lifecycle. [5]

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

NNRTIs are specific to the HIV1 and hen ce are being widely explored as a potentia l target. They act by induction and forma tion of a hydrophobic pocket near the act ive binding site which causes conformatio nal change of the enzyme and thus inhibi ts reverse transcriptase. Etravirine, delavir dine, efavirenz, and nevirapine are the F DA approved NNRTIs. [3]

Efavirenz, a BCS Class II drug, is practic ally insoluble in water with solubility <1

0µg/ml and low intrinsic dissolution rate of 0.037 mg/cm^2/min.[2] Due to its poor so lubility and erratic oral absorption,

The bioavailability of EFV is reported to be 40%.[13] Hence,lipid assisted drug deli very systems like nanosuspensions with p hospholipid as stabilizer was hypothesized to improve solubility and aid lymphatic uptake[15] of the poorly soluble Efavirenz.

Preparation and Characterization of E FV Nano- Suspensions

Phospholipid was dissolved in acetone wh ile the other stabilizers were dissolved i water.
Theorganic phase was added to the aqueo us phase under constant vortexing until a uniform dispersion was formed. The dispe rsion was stirred on a magnetic stirring ti ll complete evaporation of acetone.
Phospholipon® 90 G and Tween® 80 for med a nanosuspension which did not sho w any instability
immediately on preparation as well as for a week after preparation.[1] Hence, EFV nanosuspension using a mixture of phosp holipid and Tween® 80 (PL TNS) were prepared using the procedure mentioned above, where in EFV was disso lved with acetone along with the phospho lipid.

In Vivo Pharmacokinetic Study Materials:

Ethylene diamine tetraacetic acid (EDTA) was purchased from Loba chemie Pvt. Ltd.The animals were procured from Na tional Institute of Biosciences, Pune.

Methods:

The pharmacokinetic study was performed on healthy, male Wistar rats (180gto250 g). The experimental protocol was approv ed by the Institutional Animal Ethics Co mmittee ofBombay College of Pharmacy, Mumbai (protocol no. CPCSEA/BCP/2017 .01/06). After procurement, the animals w ere housed in the well..ventilated animal house with access to food and water ab l ibitum. The animals were divided into five treatment groups as
shown in Table.1**.**

Table.1. Grouping of animals for pharmacokinetic study

No.	Treatment	Dose and Route	Number of animals	Treatment duration
1	EFV dispersion	Oral (62 mg/kg)	8	Single dose
2	PL-T NS	Oral (62 mg/kg)	8	Single dose
3	PL-G NS	Oral (62 mg/kg)	8	Single dose
4	PL-T NS+ RTV NS	Oral (62 mg/kg) + Oral (21mg/kg)	8	Single dose
5	PL-G NS+ RTV NS	Oral (62 mg/kg) + Oral (21mg/kg)	8	Single dose
	Total		**40**	

The formulations were administered orally using the oral gavage syringe. Blood was collected by puncturing the retro orbital p lexus at predetermined time intervals of 1 ,2,4,6, 8, 12 and 24h into tubes containi ng EDTA. The blood was centrifuged a t 7000 rpm for 5min to separate
plasma.

The plasma was collected and stored at 20°C till further analysis. The plasma samples were analyzed for EFV concentra tio using HPLC bioanalytical method.[6]

In Vivo Organ Distribution Study:

The organ distribution study was performed on healthy male Wistar rats which were used for the pharmacokinetic study after a washout period of one week. The experimental protocol was approved by the Institutional Animal Ethics Committee of Bombay College of Pharmacy, Mumbai(protocol no. CPCSEA/BCP/2017/01/06). The animals were grouped as shown in Table2

Table.2. Grouping of animals for organ distribution study

No.	Treatment	Dose and Route	Number of animals
1	EFV dispersion	Oral (62 mg/kg)	12
3	PL-T NS	Oral (62 mg/kg)	12
4	PL-T NS+ RTV NS	Oral (62 mg/kg) + Oral (21mg/kg)	12

Each group was subdivided into 4 subgroups corresponding to the time points 2,4, 6,24 hr with three animals each corresponding to time points. At predetermined time point, blood was collected by puncturing the retro orbital plexus and the animal was anaesthetized. The thoracic cavity was opened and *in situ* whole body perfusion was performed by inserting the perfusion needle into the left ventricle and cutting the inferior vena cava. Tyrode solution was used as the perfusion fluid. Perfusion was carried out for 30 min, wherein the color of the organs turned pale and lungs were white indicating clearance of blood from the organ. Liver, spleen, kidneys, lymphnodes and heart were isolated. The organs were cut with sharp scissors and minced using high speed homogenizer. The homogenization process was adjusted to each tissue. The organs were weighed and homogenized in phosphate buffer pH 7.4. 0.1ml of the internal standard, Tenofovir disoproxil fumarate (TDF) was added to 0.5ml of homogenate with 0.4ml Acetonitrile which was used for extraction of EFV from the tissue homogenate. The samples were then analyzed by HPLC.

Result & Discussion

***In vivo* pharmacokinetic study:**

Pharmacokinetic studies involve the study of absorption, distribution, metabolism, and excretion of the drugs from the body. PK study plays an important role in the optimizing the drug delivery system along with the safety and efficacy assessments. Primarily, a PK study involves studying the concentration of the drug in the blood at various time points after administration until elimination. It also helps in the decision of a dose regiment with maintenance of desired blood concentration of drug with minimal side effects.[18] A few studies on the pharmacokinetics of EFV in various drug delivery systems have been reported in the literature which have shown merits of nanosystems in improving bioavailability of EFV. Patelet al demonstrated that lyophilized nano-suspensions showed a 1.90fold and 5.73fold increase in plasma peak concentration of EFV inrabbits (Cmax, ng/ml) than marketed formulation and standard EFV respectively.[12] Solid lipid nanoparticles loaded with EFV were shown to be superior to EFV suspension with 5.32 fold increase in *C*max and 10.98-fold increase in AUC_{0-24} after single oral administration to albino rats.[19] Oral delivery of EFV in self.nano emulsifying drug delivery systems (SNEDDs) to male Wistar rats showed a Cmax of 62.5µg/ml and an AUC (0 to t) of 717.2 µg/ml which was three fold higher than EFV dispersed in water.[15]

The current study involved oral administration of EFV dispersion and the nanosuspensions to male Wistar rats. Single oral dose of EFV and blood withdrawal over a period of

12 hours was used to determine various pharmacokinetic parameters using non compartment model after extravascular dose. Table.3. shows the various pharmacokinetic parameters of the formulations. Cmax is the maximum concentration attained over the specified time after a single.dose.

Tmax is the time taken to achieve the maximum concentration. (C_{max}). Cmax of EFV in the formulations is higher than the EFV dispersion which can be attributed to the nanosize of the formulations. Area under the Curve (AUC) is a measure of the variation of the drug concentration in the plasma as a function of time. AUC of PLT NS and PLG NS were found to be 15.997 µg/ml*hr and 16.398 µg/ml*hr which was 1.61 and 1.65fold higher than EFV dispersion. AUC is a measure of quantifying the bioavailability of the drug which is the rate and extent of drug absorption. The increase in bioavailability of EFV in the nanosuspensions can be attributed to the formulation factors viz presence of phospholipids and nanosize which aid in solubilization of EFV.

Ritonavir, a protease inhibitor is used as a pharmacokinetic enhancer to improve plasma concentrations of other antiretroviral drugs when given in combination.[19] Ritonavir acts mainly by inhibiting CYP3A4 which is the major enzyme responsible for metabolism of protease inhibitors and hence reducing first pass metabolism and improving halflife of the drugs. [22] EFV is mainly metabolized by CYP2B6 and by CYP3A4. However, it has been reported that CYP3A4 inhibition of NNRTIs like EFV mediated by ritonavir is offset by the inductive effects of CYP2B6.[17]

Hence, no dosage adjustment is required while coadministering RTV and EFV which means that there is no observed increase in plasma concentration of EFV in presence of RTV. This was evident by observing the Cmax and AUC readings of the two groups of rats which were administered ritonavir nanosuspension (RTV NS) along with PLT NS and PLG NS.

Table.3. Pharmacokinetic parameters

Formulation	tmax (hr)	Cmax (µg/ml)	AUC(0-t) (µg/ml*hr)
EFV dispersion	2	2.048±0.236	9.887±0.305
PL-T NS	4	3.453±0.473	15.997±1.118
PL-G NS	2	3.470±0.182	16.398±1.537
PL-T NS+ RTV NS	1	3.369±0.199	11.118±0.444
PL-G NS+ RTV NS	1.6	2.339±0.374	10.083±0.504

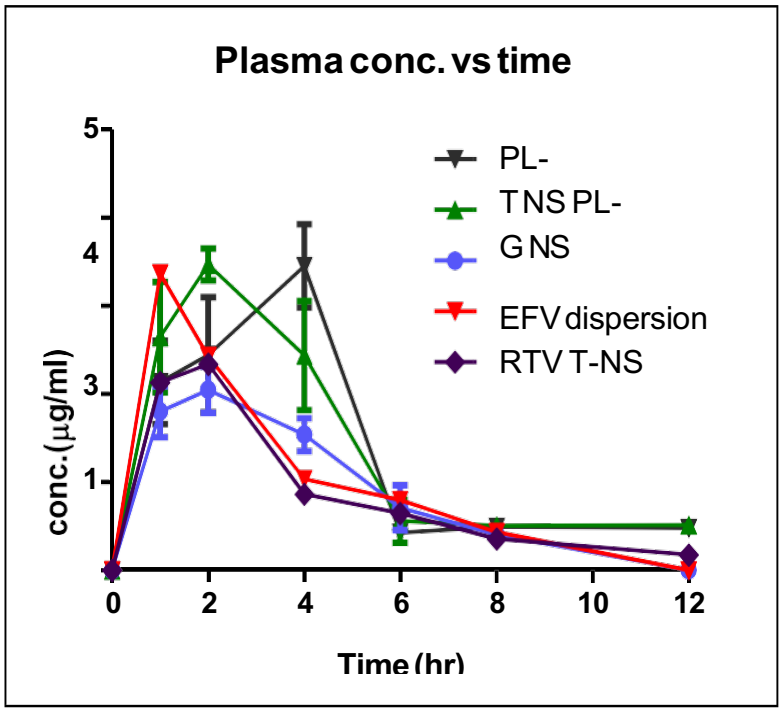

Figure 1. *In vivo* pharmacokinetic profile of nanosuspensions
Fig.1. shows the pharmacokinetic profile of the nanosuspensions.

***In vivo* organ distribution study**

Efavirenz is widely distributed and protei n bound, primarily to albumin.[18] Organ di stribution study was performed in rats to determine the EFV level in different orga ns over a specified period of time. Fig.2. shows the distribution of EFV from the f ormulations as a function of time. It can be observed that PLT NS showed higher amount of EFV in organs like lymph nod es and liver at the end of 24 hour ascom pared to EFV dispersion and combination of RTV NS and PLT NS. Amount of E FV in kidneys is maximum in case of th e combination nanosuspension which can be explained by its lower MRT as compa red to the others, which indicates higher renal clearance. It can also be observed t hat higher levels of EFV are found invari ous organs at 24 hour in case of EFV dispersion which can be attributed t o its micron size which took longer for s olubilization as compared to the nanosusp ensions. The highest concentration of EF V is found in lymph node at 4 hours which is the tmax of EFV in PL-T NS.

Fig.3. shows the distribution of EFV as a tissue based plot. Higher accumulation of EFV in lymph nodes was desired as it ha s been reported that HIV reservoirs are f ound invarious lymphoid tissues.[23]. Phosp holipids have been reported to improve ly mphatic absorption of lipophilic drugs. [21].

Presence of phospholipid in the nanosusp ensions can be attributed to higher amoun ts of EFV in the lymph node.

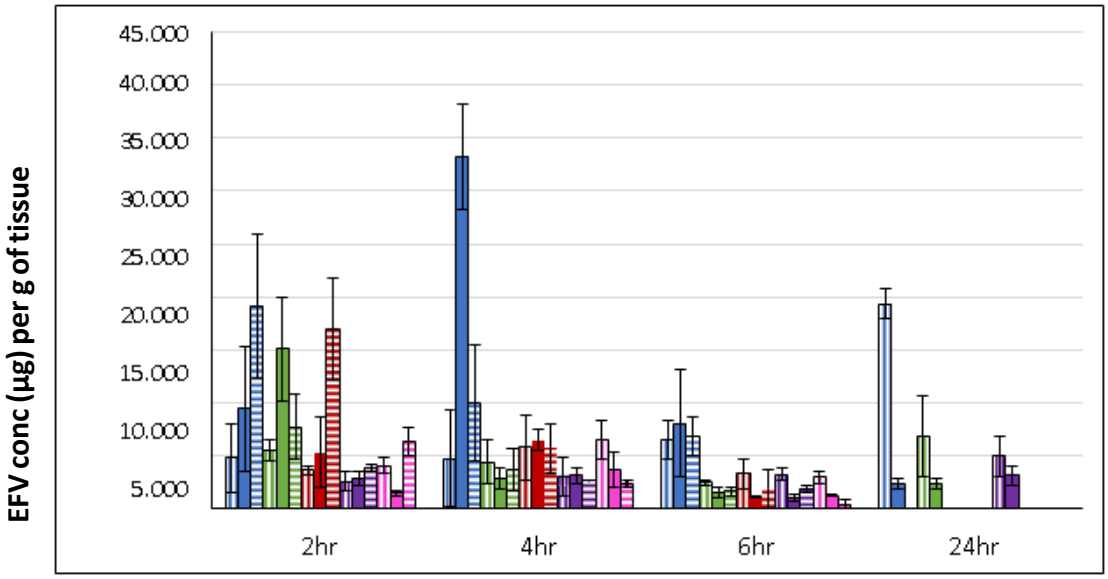

Fig.2. Summary of tissue distribution study, a kinetic plot. Tissues represented b color: blue Lymph nodes, green- liver, red – kidney, purple spleen, pink is heart.Vertical stripes denote EFV dispersion, solid column denote PLT NS and ho rizontal columns denote combination of RTV NS+ PL-T NS

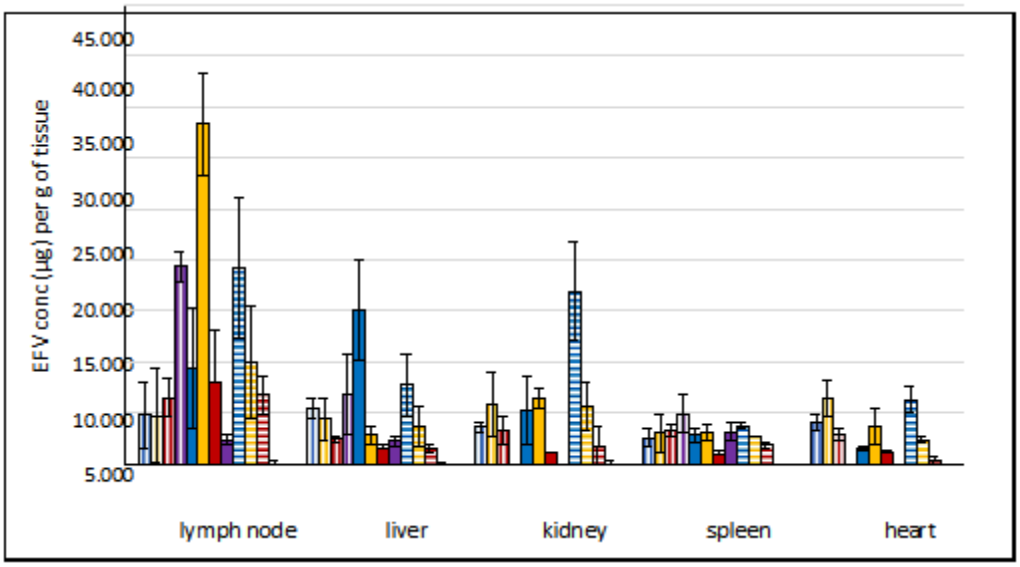

Fig.4.27. Summary of tissue distribution study, a tissuebased plot. Time points rep resented by color: blue – 2 hour, yellow – 4 hour, red- 6 hour, purple- 24 hour Vertical stripes denote EFV dispersion, solid column denote PLT NS and horizont al columns denote combination of RTV NS+ PL-T NS

Conclusion

Pharmacokinetic and organ distribution s tudies were carried out in male Wistar r ats with oral administration of the nanos uspension. To study the effect of a phar maco-kinetic enhancer Ritonavir (RTV) on the kinetics of EFV, RTV nanosuspensionsw ere administered in combination with E FV nanosuspension. PLT NS and PLG NS showed 1.66fold and 1.63fold increa se in AUC as compared to the EFV dis persion. Combination of RTV and EFV

nanosuspension did not enhance absorption of EFV but improved AUC as compared to the EFV dispersion. Higher amount of EFV was accumulated in thelymph nodes when PLT NS was administered to the rats as compared to the EFV dispersion, which was desirable due to the presence of HIV reservoirs in the lymphoid tissues.

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