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PRECLINICAL PHARMACOKINETIC EVALUATION OF EFAVIRENZ – βCD – POLOXAMER 407 – PVP K30 INCLUSION COMPLEXES

Sanjit Singh Lamba[1], K.P.R. Chowdary [1, 2*] and P. Suresh[3]

1. Eisai Knowledge Center, Eisai Pharmatechnology and Manufacturing Pvt., Ltd., Ramky Pharma City (SEZ), Parawada, Visakhapatnam
2. Former Principal, A. U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.
3. GITAM Institute of Pharmacy, GITAM University, Visakhapatnam

***Corresponding Author Email:** prof.kprchowdary@rediffmail.com

ABSTRACT

Efavirenz, a widely prescribed anti retroviral drug belongs to class II under BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility. Its oral absorption is dissolution rate limited and it requires enhancement in the solubility and dissolution rate for increasing its oral bioavailability. We reported earlier that combination of cyclodextrins (βCD and HPβCD) with Poloxamer 407 and PVP K30 have markedly enhanced the solubility and dissolution rate of efavirenz than is possible with them individually and the efavirenz –βCD –Poloxamer 407 - PVP K30 inclusion complexes could be compressed into tablets by both wet granulation and direct compression methods retaining their fast dissolution rate characteristics. The objective of the present study is to make a pharmacokinetic evaluation of efavirenz – βCD – Poloxamer 407 - PVP K30 inclusion complexes to assess their *in vivo* performance in comparison to efavirenz pure drug.

Pharmacokinetic evaluation was done on (i) Efavirenz; (ii) Efavirenz - βCD (1:2), (iii) Efavirenz – βCD (1:2) – Poloxamer 407(2%) and (iv) Efavirenz - βCD (1:2) - PVP K30 (2%) solid inclusion complexes in healthy rabbits weighing 1.5 – 2.5 kg (n=6) of either sex in a cross over RBD at a dose equivalent to 10 mg/kg of drug. Efavirenz was found to be absorbed slowly when given orally with an absorption rate constant (K_a) of 0.661 h^{-1}. All the pharmacokinetic parameters namely C_{max}, T_{max}, K_a and $(AUC)_0^{\infty}$ indicated rapid and higher absorption and bioavailability of efavirenz

when administered as CD complexes. . A 2.93, 5.83 and 6.49 fold increase in the absorption rate (K_a) and a 1.73, 1.88 and 1.92 fold increase in $(AUC)_0^\infty$ was observed respectively with efavirenz - βCD (1:2), efavirenz – βCD (1:2) – Poloxamer 407 (2%) and efavirenz - βCD (1:2) - PVP K30 (2%) inclusion complexes when compared to efavirenz pure drug. Combination of βCD with Poloxamer 407 or PVP K30 gave higher rates of absorption and bioavailability of efavirenz than is possible with βCD alone. The elimination characteristics of efavirenz have not changed when it was administered as βCD- Poloxamer 407/PVP K 30 inclusion complexes.

Key Words: Pharmacokinetics, Efavirenz, Cyclodextrin Complexation, Poloxamer 407, PVP K 30

INTRODUCTION

Efavirenz, a widely prescribed HIV- 1 specific non – nucleoside reverse transcriptase inhibitor drug belongs to class II under BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility. It is practically in soluble in water and aqueous fluids. As such its oral absorption is dissolution rate limited and it requires enhancement in the solubility and dissolution rate for increasing its oral bioavailability. Several conventional methods such as micronization, chemical modification, use of surfactants and solubilizers, solid dispersion and a few new emerging technologies such as cyclodextrin and its complexation, mucoadhesive contains microspheres, nanoparticles, nanosuspensions, micro emulsion and self-emulsifying systems are available to enhance the solubility, dissolution rate and bioavailability of poorly soluble BCS Class II drugs [1]. Among the various approaches complexation with cyclodextrins has gained good acceptance in recent years in industry for enhancing the solubility and dissolution rate of poorly soluble drugs. Cyclodextrins (CDs) are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity which can accommodate a variety of lipophilic drugs. As a consequence of inclusion process many physico-chemical properties such as solubility, dissolution rate, and bioavailability can be favourably affected [2, 3]. Cyclodextrins have been receiving increasing application in pharmaceutical formulation in recent years due to their approval by various regulatory agencies [4, 5]. Poloxamer 407 is a polyethylene oxide- polypropylene oxide- polyethylene oxide triblock co-polymer of non-ionic nature and is used as a solubilising agent [6-8].

We reported[9,10] earlier that combination of cyclodextrins (βCD and HPβCD) with Poloxamer 407 and PVP K30 have markedly enhanced the solubility and dissolution rate of efavirenz than is possible with them individually and the efavirenz –βCD – Poloxamer 407 - PVP K30 inclusion complexes could be compressed into tablets by both wet granulation and direct compression methods retaining their fast dissolution rate characteristics. The objective of the present study is to make a pharmacokinetic evaluation of efavirenz –βCD – Poloxamer 407 - PVP K30 inclusion complexes to assess their *in vivo* performance in comparison to efavirenz pure drug.

EXPERIMENTAL

Materials:

Efavirenz was a gift sample from M/s. Eisai Pharmatechnology and

Manufacturing Pvt. Ltd., Visakhapatham. β Cyclodextrin was gift sample from M/s. Cerestar Inc., USA. Methanol (Qualigens), poly vinyl pyrrolidone (PVP K30)

and Poloxamer 407 were procured from commercial sources. All other materials used were of pharmacopoeial grade.

Preparation of efavirenz- βCD-Poloxamer 407/ PVP K30 complexes:

Solid inclusion complexes of Efavirenz - βCD (1:2), Efavirenz – βCD (1:2) – Poloxamer 407(2%) and Efavirenz - βCD (1:2) - PVP K30 (2%) were prepared by kneading method. Efavirenz, βCD, Poloxamer 407 and PVP K30 were triturated in a mortar with a small volume of solvent consisting of a blend of water: methanol (1:1). The thick slurry formed was kneaded for 45 min and then dried at 55^{o}C until dry. The dried mass was powdered and sieved to mesh No. 120.

Pharmacokinetic Study:

Pharmacokinetic evaluation was done on (i) Efavirenz; (ii) Efavirenz - βCD (1:2), (iii) Efavirenz – βCD (1:2) – Poloxamer 407(2%) and (iv) Efavirenz - βCD (1:2) - PVP K30 (2%) solid inclusion complexes in healthy rabbits weighing 1.5 – 2.5 kg (n=6) of either sex in a cross over RBD at a dose equivalent to 10 mg/kg of drug. *In vivo* study protocols were approved by the Institutional Animal Ethics Committee (Regd. No 516/01/a/CPCSEA). A wash out period of one month was given between testing of two products.

After collecting the zero hour blood sample (blank), the product in the study was administered orally in a capsule shell with 10 ml of water. No food or liquid other than water was permitted until 4 hours following the administration of the product. Blood samples (2 ml) were collected from marginal ear vein at 0.5, 1, 2, 3, 4, 6, 8 and 12 hours after administration. The blood samples were collected in heparinized tubes and were centrifuged at 10000 rpm for 10 min and the plasma separated was collected into dry tubes. All the samples were stored under refrigerated conditions prior to assay on the same day. Plasma concentrations of efavirenz were determined by a known HPLC method[11] after revalidation as follows.

Estimation of Efavirenz in Plasma:

Instrumentation:

The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

Chromatographic Conditions:

Mobile Phase:

The mobile phase consists of mixture of 50 volumes of acetonitrile and 50 volumes of 0.86% w/v solution of ammonium dihydrogen phosphate, pH adjusted to 3.0 with orthophosphoric acid operated on isocratic mode. The flow rate is 1.5 ml/min. Chromatographic separation of Efavirenz (Drug) & Ziprasidone (ISTD) was performed on Genesis C_{18} (GRACE) column (50 X 4.6 mm id, 3µm).

Internal Standard: Ziprasidone

Detection: The column effluent was monitored at 252 nm.

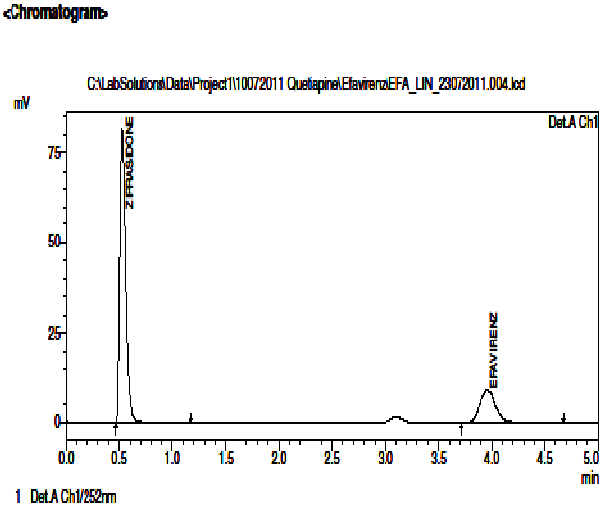
Retention Time of Efavirenz: 3.95 min

Retention Time of Internal Standard: 0.53 min

Estimation of Efavirenz in Plasma:

For the estimation of efavirenz in plasma samples, a calibration curve was constructed initially by analyzing plasma samples containing different amounts of efavirenz as follows:

To a series of tubes containing 0.5 mL of drug free plasma in each, 0.1 mL of internal standard solution containing 4µg of Ziprasidone and 0.1 mL drug solution containing 1.0, 2.5, 5.0, 7.5 and 10.0 µg of efavirenz were added and mixed. To each tube 1 mL of acetonitrile was added, mixed thoroughly and centrifuged at 5000 rpm for 20 min. The organic layer (0.5 mL) was taken into a dry tube and the acetonitrile was evaporated. To the dried residue 0.5 mL of mobile phase was added and mixed for reconstitution. Subsequently 20 µL were injected into the column for HPLC analysis. A model chromatogram was shown in Fig.1.



PeakTable

Detector A Ch1 252nm

Name	Ret. Time	Area	Theoretical Plate#	Tailing Factor
ZIPRASIDONE	0.52	305912	357	1.62
EFAVIRENZ	3.96	95792	3172	1.23

Fig.1: HPLC Chromatogram of Efavirenz (2.5 µg/ 0.5ml of plasma)

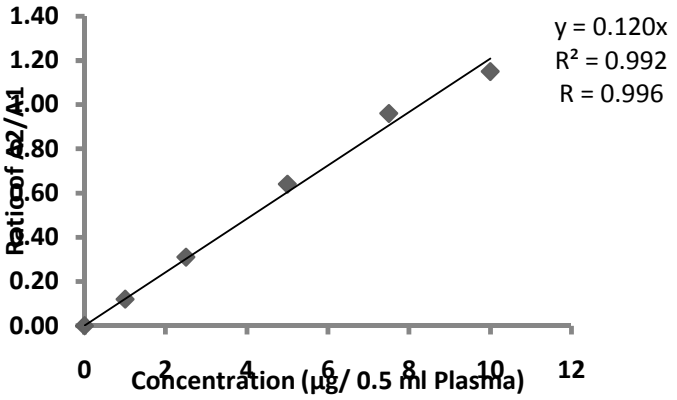


Fig. 2: Calibration curve for the Estimation of Efavirenz in Plasma Samples

The HPLC method validated was found suitable for the estimation of efavirenz in plasma samples. The mobile phase consists of mixture of 50 volumes of acetonitrile and 50 volumes of 0.86% w/v solution of ammonium dihydrogen phosphate, pH adjusted to 3.0 with orthophosphoric acid operated on isocratic mode. The retention time for efavirenz was 3.95 min and for internal standard (Ziprasidone) it was 0.53 min. Linearity of the method was in the concentration range 1- 10 µg/0.5 ml of Plasma. The accuracy and precision coefficient of variation for drug and internal standard was less than 0.680 % showing high accuracy and precision of the method. In the pharmacokinetic study 0.5 ml of plasma collected was used for the estimation of efavirenz as described above.

From the time versus plasma concentration data, various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak occurred (T_{max}), Area under the curve (AUC), elimination rate constant (K_{el}), biological half - life ($t_{1/2}$), percent absorbed to various times and absorption rate constant (K_a), were calculated in each case as per known standard methods[12,13].

RESULTS AND DISCUSSION

Pharmacokinetic evaluation was done on efavirenz - βCD (1:2), efavirenz – βCD (1:2) – Poloxamer 407 and efavirenz - βCD (1:2) - PVP K30 (2%) solid inclusion complexes in comparison to efavirenz pure drug with a view to evaluate the *in vivo* performance of drug- CD – Poloxamer 407/ PVP K30 complexes.

Plasma concentrations of efavirenz following the oral administration of efavirenz and its CD complexes are shown in Fig.3. Pharmacokinetic parameters estimated are summarized in Table 1.

The biological half- life ($t_{1/2}$) estimated from the elimination phase of the plasma level curves was found to be 5.02, 4.56, 4.43 and 4.49 h respectively following the oral administration of efavirenz and its CD complexes, efavirenz - βCD (1:2) , efavirenz – βCD (1:2)- Poloxamer 407 - and efavirenz - βCD (1:2) - PVP K30 (2%) complexes. The close agreement of the $t_{1/2}$ values obtained with the four products indicated that the elimination characteristics of efavirenz have not changed when it was administered as βCD- Poloxamer 407/PVP K 30 complexes.

Efavirenz was found to be absorbed slowly when given orally and a peak plasma concentration (C_{max}) of 10.22µg/ml was observed at 4.0 h after administration. The absorption rate constant (K_a) was found to be 0.661 h^{-1}. All the pharmacokinetic parameters (Table 2) namely C_{max}, T_{max}, K_a and $(AUC)_0^{\infty}$ indicated rapid and higher absorption and bioavailability of efavirenz when administered as CD complexes. Higher C_{max} values and lower T_{max} values were observed with the CD complexes when compared to those of efavirenz as such. The absorption rate constant (K_a) was found to be 1.937 h^{-1}, 3.855 h^{-1} and 4.290 h^{-1} respectively with efavirenz - βCD (1:2), efavirenz – βCD (1:2) – Poloxamer 407(2%) and efavirenz - βCD (1:2) - PVP K30 (2%) complexes. In the case of efavirenz, the K_a was only 0.661 h^{-1}. A 2.93, 5.83 and 6.49 fold increase in the absorption rate (K_a) was observed respectively with efavirenz - βCD (1:2), efavirenz – βCD (1:2) –

Poloxamer 407 (2%) and efavirenz - βCD (1:2) - PVP K30 (2%) inclusion (AUC) $_0^\infty$ (extent of absorption) was also much higher in the case of CD complexes when compared to efavirenz pure drug. (AUC) $_0^\infty$ was increased from 105.17 µg. h /ml for efavirenz pure drug to 182.28, 198.00 and 202.10 µg. h /ml respectively for efavirenz - βCD (1:2), efavirenz – βCD (1:2) – Poloxamer 407(2%) and efavirenz - βCD (1:2) - PVP K30 (2%) inclusion complexes when compared to efavirenz pure drug.

efavirenz - βCD (1:2) - PVP K30 (2%) inclusion complexes. A 1.73, 1.88 and 1.92 fold increase in (AUC) $^\infty_0$ was observed respectively with efavirenz - βCD (1:2), efavirenz – βCD (1:2) – Poloxamer 407(2%) and efavirenz - βCD (1:2) - PVP K30 (2%) inclusion complexes when compared to efavirenz pure drug.

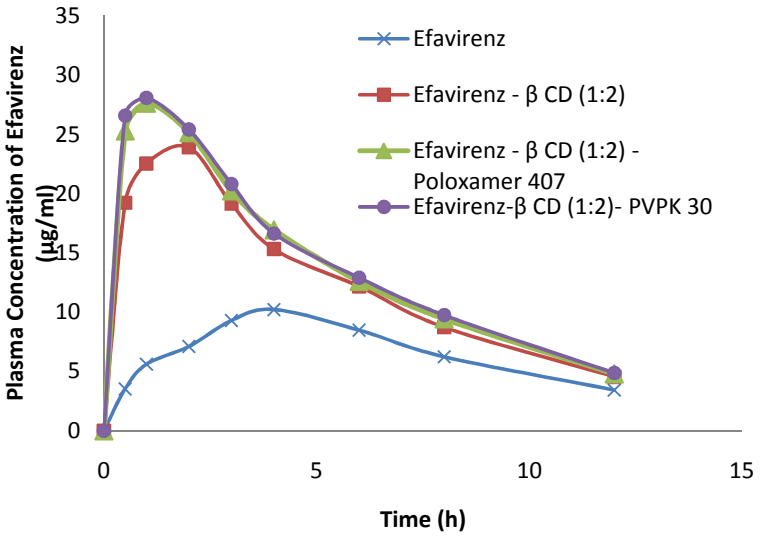


Fig 3: Plasma Concentration of Efavirenz Following Oral Administration of Efavirenz and Its Cyclodextrin Complexes in Rabbits

Table 1

Summary of Pharmacokinetic Parameters Estimated Following the Oral Administration of Efavirenz and its CD Complexes

Product	C_{max} (µg/ml)	T_{max} (h)	K_{el} (h^{-1})	$t_{1/2}$ (h)	$(AUC)_0^{12h}$ (µg.h /ml)	$(AUC)_0^{\infty}$ (µg.h/ml)	BA (%)	K_a (h^{-1})	Percent Absorbed		
									0.5 h	1.0 h	2.0 h
Efavirenz	10.22	4	0.138	5.02	80.28	105.17	100.00	0.661	24.64	40.69	56.70
Efavirenz - βCD (1:2)	23.92	2	0.152	4.56	152.28	182.28	173.32	1.937	68.74	85.60	100.00
Efavirenz – βCD (1:2)-Poloxamer 407	27.64	1	0.157	4.43	167.35	198.00	188.26	3.855	83.78	97.88	100.00
Efavirenz - βCD (1:2) - PVP K30 (2%)	28.10	1	0.154	4.49	170.48	202.10	192.16	4.290	87.23	98.63	100.00

Efavirenz - βCD (1:2) – Poloxamer 407 and efavirenz - βCD (1:2) – PVP K30 (2%) solid inclusion complexes exhibited markedly higher rates and extent of absorption of efavirenz when compared to efavirenz alone and efavirenz - βCD (1:2) inclusion complexes. Combination of βCD with Poloxamer 407 or PVP K30 gave higher rates of absorption and bioavailability of efavirenz than is possible with βCD alone.

CONCLUSION

Efavirenz was found to be absorbed slowly when given orally with an absorption rate constant (K_a) of 0.661 h^{-1}. All the pharmacokinetic parameters namely C_{max}, T_{max}, K_a and (AUC) $_0^\infty$ indicated rapid and higher absorption and bioavailability of efavirenz when administered as CD complexes. . A 2.93, 5.83 and 6.49 fold increase in the absorption rate (K_a) and a 1.73, 1.88 and 1.92 fold increase in (AUC) $_0^\infty$ was observed respectively with efavirenz - βCD (1:2), efavirenz – βCD (1:2) – Poloxamer 407 (2%) and efavirenz - βCD (1:2) - PVP K30 (2%) inclusion complexes when compared to efavirenz pure drug. Combination of βCD with Poloxamer 407 or PVP K30 gave higher rates of absorption and bioavailability of efavirenz than is possible with βCD alone. The elimination characteristics of efavirenz have not changed when it was administered as βCD- Poloxamer 407/PVP K 30 inclusion complexes.

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