

Research Article



PRECLINICAL PHARMACOKINETIC EVALUATION OF EFAVIRENZ – CYCLODEXTRIN – PVP INCLUSION COMPLEXES

K.P.R. Chowdary and Annamma Devi G.S*

University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

*Corresponding author's E-mail: annammadevi.sayam2011@gmail.com

Accepted on: 09-03-2012; Finalized on: 30-06-2012.

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 have earlier reported that a combination of CDs (β CD, HP β CD) with PVP K30 has markedly enhanced both the solubility and dissolution rate of efavirenz. In the present study pharmacokinetic evaluation of efavirenz - CD (β CD and HP β CD) - PVP K30 inclusion complexes in comparison to efavirenz pure drug was made with an objective of evaluating their *in vivo* performance. The efavirenz-CD (β CD/ HP β CD) - PVP K30 inclusion complexes were prepared by kneading method. Pharmacokinetic evaluation was done on efavirenz pure drug and efavirenz - β CD (1:2), efavirenz - β CD-PVP (1:2:0.3), efavirenz - HP β CD (1:2), efavirenz - HP β CD - PVP (1:2:0.3) solid inclusion complexes in healthy rabbits (n=6) of either sex in a cross over study at a dose equivalent to 10 mg/kg of drug. The close agreement of the $t_{1/2}$ values following the administration of efavirenz and its CD complexes indicated that the elimination characteristics of efavirenz have not changed when it was administered as CD complexes. Efavirenz was absorbed slowly when given orally and a peak plasma concentration (C_{max}) of 11.25 μ g /ml was observed at 4.0 h after administration. The absorption rate constant (K_a) was 0.467 h^{-1} . All the pharmacokinetic parameters estimated 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 3.97, 7.93, 4.99 and 6.5 fold increase in the K_a was observed respectively with efavirenz - β CD (1:2), efavirenz - β CD-PVP (1:2:0.3), efavirenz -HP β CD (1:2) and efavirenz -HP β CD-PVP (1:2:0.3) complexes when compared to efavirenz pure drug. A 1.84, 1.86, 1.63 and 1.91 fold increase in $(AUC)_0^\infty$ was observed respectively with these CD complexes. β CD and HP β CD have markedly enhanced both the rate (K_a) and extent (AUC) of absorption (i.e. bioavailability) of efavirenz. Addition of PVP has further enhanced both the rate of absorption and extent of absorption of efavirenz from efavirenz - β CD and efavirenz -HP β CD complexes.

Keywords: Pharmacokinetics, Efavirenz, Cyclodextrin Complexes, Absorption Rate, Bioavailability.

INTRODUCTION

About 95% of all new potential therapeutic drugs (APIs) exhibit low and variable oral bioavailability due to their poor aqueous solubility at physiological pHs and consequent low dissolution rate. These drugs are classified as class II drugs under BCS with low solubility and high permeability characters and pose challenging problems in their pharmaceutical product development process. Efavirenz belongs to Class II under BCS and exhibit low and variable bioavailability due to its poor aqueous solubility. As such it needs enhancement in the dissolution rate and bioavailability to derive its maximum therapeutic efficacy.

Several conventional methods such as micronization, chemical modification, use of surfactants and solubilizers, solid dispersion and a few new emerging technologies such as cyclodextrin complexation, mucoadhesive microspheres, nanoparticles, nanosuspensions, microemulsion and self-emulsifying systems are available¹ to enhance the bioavailability of BCS Class II drugs. Among the various techniques, cyclodextrin complexation is an efficient approach for enhancing the dissolution rate and bioavailability of BCS – Class II Drugs. Cyclodextrins (CDs) are cyclic torus-shaped molecules with a hydrophilic outer surface and 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, stability and bioavailability can be favorably affected.^{2,3} Cyclodextrins have been receiving increasing application in pharmaceutical products in recent years due to their approval by various regulatory agencies.^{4,5} It is reported in a few studies^{6,7} that addition of small amounts of water soluble polymers such as PVP, HPMC, PEG to cyclodextrin systems has improved both the complexing and solubilizing efficiencies of CDs. We have earlier reported⁸ that a combination of CDs (β CD, HP β CD) with PVP K30 has markedly enhanced both the solubility and dissolution rate of efavirenz, a BCS class II drug, than is possible with them individually. In the present study pharmacokinetic evaluation of efavirenz - CD (β CD and HP β CD) - PVP K30 inclusion complexes in comparison to efavirenz pure drug was made with an objective of evaluating their *in vivo* performance.

MATERIALS AND METHODS

Materials

Efavirenz was a gift sample from M/s Hetero Drugs Ltd., Hyderabad. β - cyclodextrin and hydroxypropyl β -cyclodextrin were gift samples from M/s Cerestar Inc., USA, Polyvinylpyrrolidone (PVP, K-30, Sigma Chemical Co.), dichloromethane (Qualigens), methanol (Qualigens)



were procured from commercial sources. All other materials used were of Pharmacopoeial grade.

Methods

Estimation of efavirenz

A UV Spectrophotometric method based on the measurement of absorbance at 245 nm in water containing 2% SLS was used for the estimation of efavirenz in *in vitro* studies. The method was validated for linearity, accuracy, precision and interference. The method obeyed Beer's law in the concentration range of 1-10 µg/ml. When a standard drug solution was repeatedly assayed (n=6), the relative error and coefficient of variance were found to be 0.68% and 1.02% respectively. No interference by the excipients used in the study was observed.

Preparation of Efavirenz-CD (βCD/ HPβCD) - PVP K30 Complexes

Solid inclusion complexes of efavirenz-CD (βCD / HPβCD) - PVP K30 were prepared by kneading method. Efavirenz, βCD/ HPβCD 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°C until dry. The dried mass was powdered and sieved to mesh No. 120.

Pharmacokinetic Evaluation

Pharmacokinetic evaluation of efavirenz-CD (βCD / HPβCD) - PVP K30 inclusion complexes prepared in comparison to efavirenz pure drug was done in healthy rabbits weighing 1.5 – 2.5 kg (n=6) of either sex in a cross over study at a dose equivalent to 10 mg/kg of drug. Pharmacokinetic evaluation was done on efavirenz pure drug and efavirenz - βCD (1:2), efavirenz - βCD -PVP (1:2:0.3), efavirenz - HPβCD (1:2), efavirenz - HPβCD - PVP (1:2:0.3) solid inclusion complexes. *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 known⁹ HPLC method after revalidation as follows.

Instrumentation

The HPLC system (Make: M/s Shimadzu Corporation, Japan.) consisted of UV-Visible detector (Shimadzu,

Model: SPD – 10AVP), C-18 column (Phenomenex, DESC: Gemini 5µ C18 110A, Size: 250 X 4.6 mm, S/No: 288063 – 23), 2 pumps (Model: LC – 10 ATPV) and a micro syringe of capacity 25µl (Model: Microliter® # 702, Mfd. by: M/s Hamilton).

Chromatographic Conditions

Mobile Phase: The mobile phase consists of a mixture of 0.1 M formic acid –acetonitrile-methanol (43:52:5). The mobile phase was filtered through a 0.45µm membrane filter before use and was run at a flow rate of 1 ml/min.

Internal Standard: Metadoxine (4µg/ml).

Detection: The column effluent was monitored at 266 nm.

Retention Time of Efavirenz: 7.027 min

Retention Time of Internal Standard: 2.975 min

For the estimation of efavirenz in plasma samples, a calibration curve (Fig. 1) 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 metadoxine and 0.1 mL drug solution containing 2, 4, 6, 8 and 10 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.

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^{10, 11}.

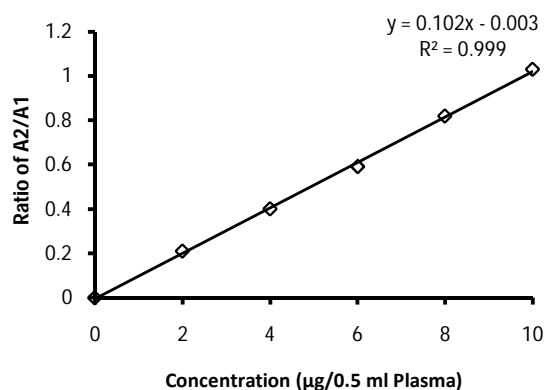


Figure 1: Calibration curve for the Estimation of Efavirenz in Plasma Samples



RESULTS AND DISCUSSION

The efavirenz-CD (β CD/ HP β CD) - PVP K30 inclusion complexes were prepared by kneading method with a view to enhance the solubility and dissolution rate of efavirenz, a BCS class II drug. All the solid inclusion complexes of efavirenz-CD (β CD/ HP β CD) - PVP K30 prepared were found to be fine and free flowing powders. Low coefficient of variation (c.v) values (< 1%) in the percent drug content indicated uniformity of drug content in each batch of solid inclusion complexes prepared. The dissolution rate characteristics of these efavirenz-CD (β CD/ HP β CD) - PVP K30 inclusion complexes were reported earlier⁸. In the present study pharmacokinetic evaluation was done on efavirenz-CD (β CD / HP β CD) - PVP K30 inclusion complexes in comparison to efavirenz pure drug with an objective of evaluating their *in vivo* performance. Plasma concentrations of efavirenz following the oral administration of efavirenz and its CD complexes are

shown in Fig.2. Pharmacokinetic parameters estimated are summarized in table 1.

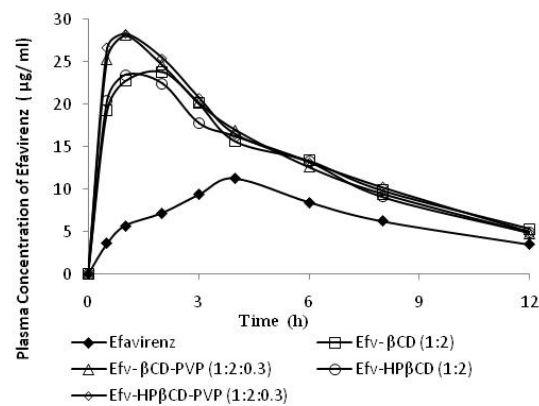


Figure 2: Plasma concentrations of efavirenz following the oral administration of efavirenz and its CD complexes

Table 1: Summary of Pharmacokinetic Parameters Estimated Following the Oral Administration of Efavirenz and 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 [∞] (µg.h/ml)	BA (%)	K _a (h ⁻¹)	MRT (h)	Percent Absorbed		
										0.5 h	1.0 h	2.0 h
Efavirenz	11.25	4.0	0.1464	4.73	82.1075	105.87	100.0	0.467	6.94	24.04	39.82	54.94
Efv-βCD(1:2)	23.80	2.0	0.1547	4.48	161.0325	195.29	184.46	1.857	5.53	67.15	84.37	100
Efv-βCD-PVP(1:2:0.3)	28.12	1.0	0.1614	4.29	167.825	197.688	186.72	3.705	5.67	82.04	97.53	100
Efv-HPβCD (1:2)	23.45	1.0	0.166	4.17	143.45	172.66	163.08	2.33	6.15	73.5	90.28	100
Efv-HPβCD-PVP (1:2:0.3)	28.32	1.0	0.166	4.17	172.545	202.36	191.14	3.043	5.54	88.1	95.22	100

The biological half- life (t_{1/2}) estimated from the elimination phase of the plasma level curves was found to be 4.73, 4.48, 4.29, 4.17 and 4.17 h respectively following the oral administration of efavirenz, and its CD complexes efavirenz - β CD (1:2), efavirenz - β CD -PVP (1:2:0.3), efavirenz - HP β CD (1:2), efavirenz - HP β CD - PVP (1:2:0.3) . The close agreement of the t_{1/2} values in the five cases indicated that the elimination characteristics of efavirenz have not changed when it was administered as CD complexes.

Efavirenz was found to be absorbed slowly when given orally and a peak plasma concentration (C_{max}) of 11.25µg /ml was observed at 4.0 h after administration. The absorption rate constant (K_a) was found to be 0.467 h⁻¹. All the pharmacokinetic parameters (Table 1) namely C_{max}, T_{max}, K_a and (AUC)₀[∞] 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 1.857h⁻¹, 3.705h⁻¹, 2.33 h⁻¹ and 3.043h⁻¹ respectively with efavirenz - β CD (1:2), efavirenz - β CD - PVP (1:2:0.3), efavirenz - HP β CD (1:2) and efavirenz -HP β CD - PVP (1:2:0.3)

complexes, whereas in the case of efavirenz K_a was only 0.467 h⁻¹. A 3.97, 7.93, 4.99 and 6.5 fold increase in the K_a was observed respectively with efavirenz - β CD (1:2), efavirenz - β CD-PVP (1:2:0.3), efavirenz -HP β CD (1:2) and efavirenz -HP β CD-PVP (1:2:0.3) complexes when compared to efavirenz pure drug. (AUC)₀[∞] (extent of absorption) was also much higher in the case of CD complexes when compared to efavirenz pure drug. (AUC)₀[∞] was increased from 105.87µg.h /ml for efavirenz to 195.29, 197.688, 172.66 and 202.36 µg.h /ml for efavirenz - β CD(1:2), efavirenz - β CD-PVP(1:2:0.3), efavirenz -HP β CD(1:2) and efavirenz -HP β CD-PVP(1:2:0.3) complexes respectively. A 1.84, 1.86, 1.63and 1.91 fold increase in (AUC)₀[∞] was observed respectively with efavirenz - β CD(1:2), efavirenz - β CD-PVP(1:2:0.3), efavirenz -HP β CD(1:2) and efavirenz -HP β CD-PVP(1:2:0.3) complexes when compared to efavirenz pure drug. Thus β CD and HP β CD have markedly enhanced both the rate (K_a) and extent (AUC) of absorption (i.e. bioavailability) of efavirenz. Addition of PVP has further enhanced both the rate of absorption and extent of absorption of efavirenz from efavirenz - β CD and efavirenz -HP β CD complexes.

CONCLUSION

1. The close agreement of the $t_{1/2}$ values following the administration of efavirenz and its CD complexes indicated that the elimination characteristics of efavirenz have not changed when it was administered as CD complexes.
2. Efavirenz was absorbed slowly when given orally and a peak plasma concentration (C_{max}) of 11.25 µg /ml was observed at 4.0 h after administration. The absorption rate constant (K_a) was 0.467 h⁻¹.
3. All the pharmacokinetic parameters estimated 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.
4. A 3.97, 7.93, 4.99 and 6.5 fold increase in the K_a was observed respectively with efavirenz -βCD (1:2), efavirenz - βCD-PVP (1:2:0.3), efavirenz -HPβCD (1:2) and efavirenz -HPβCD-PVP (1:2:0.3) complexes when compared to efavirenz pure drug. A 1.84, 1.86, 1.63 and 1.91 fold increase in $(AUC)_0^\infty$ was observed respectively with these CD complexes.
5. βCD and HPβCD have markedly enhanced both the rate (K_a) and extent (AUC) of absorption (i.e. bioavailability) of efavirenz. Addition of PVP has further enhanced both the rate of absorption and extent of absorption of efavirenz from efavirenz -βCD and efavirenz -HPβCD complexes.

REFERENCES

1. Chowdary, K.P.R., and Madhavi, B.L.R., Novel Drug Delivery Technologies for Insoluble Drugs, Indian Drugs, 42(9), 2005, 557.
2. Holvet, C., Heyden, Y.V. and Plaizer –Vercammen, J., Inclusion complexation of diazepam with different cyclodextrins in formulations for parenteral use, Pharmazine, 60(8), 2005, 598.
3. Veda, H., Ou, D., Endo, T., Narge, H., Tomono, K. and Nagari, T., Drug Dev. Ind. Pharm., 24(9), 1998, 863.
4. Thomson, D.O., Cyclodextrins-enabling Excipients: Their Present and Future Use in Pharmaceuticals. Crit. Rev. Ther. Drug Carriers Syst., 14, 1997, 1.
5. Hedges, A.R., Industrial Applications of Cyclodextrins. Chem. Rev., 98, 1998, 2035.
6. Koester, L.S., Mayorga, P. and Bassani, V.L., Carbamazepine/betaCD/HPMC solid dispersions. II. Physical characterization., Drug Dev. Ind. Pharm., 29(2), 2003, 139.
7. Mura, P., Faucci, M.T. and Bettinetti, G.P., The influence of polyvinylpyrrolidone on naproxen complexation with hydroxypropyl-beta-cyclodextrin., Eur. J. Pharm. Sci., 13, 2001, 2001, 187.
8. Chowdary, K.P.R. and Annamma Devi G.S., Effect of PVP on Cyclodextrin Complexation of Efavirenz for Enhancing Its Solubility and Dissolution Rate, Int. J. Res. Pharm. Chem., 2(2), 2012, 311-316.
9. Selo, Mogatle and Isadore, Kanfer., Rapid method for the quantitative determination of efavirenz in human plasma. J. Pharm. Biomed. Anal., 49 (5), 2009, 1308.
10. Wagner, J. G. and Nelson, Per cent absorbed time plots derived from blood level and/or urinary excretion data, E., J. Pharm. Sci., 52, 1963, 610.
11. Wagner, J. G. and Nelson, Kinetic Analysis of Blood Levels And Urinary Excretion in the Absorptive Phase After Single Doses of Drug, E., J. Pharm. Sci., 53, 1964, 1392.

