Journal of Applied Pharmaceutical Science Vol. 3 (4 Suppl 1), pp. S89-S92, May, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.34.S17
ISSN 2231-3354

Preclinical Pharmacokinetic Evaluation of Efavirenz Solid Dispersions in Two New Modified Starches

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ARTICLE INFO

Article history:
Received on: 08/09/2012
Revised on: 19/09/2012
Accepted on: 24/09/2012
Available online: 12/05/2013

Key words:
Pharmacokinetic,
Efavirenz,
Solid Dispersion,
Starch,
anti-retroviral drugs

ABSTRACT

Efavirenz, a widely prescribed anti retroviral drug belong to Class II under BCS and exhibit low and variable oral bioavailability due to its poor aqueous solubility and it requires enhancement in solubility and dissolution rate for increasing its oral bioavailability. In our earlier studies solid dispersion of efavirenz in two new modified starches namely (i) starch citrate and (ii) starch phosphate has markedly enhanced the dissolution rate and dissolution efficiency of efavirenz. The objective of the present study is to evaluate the *in vivo* performance and pharmacokinetics of the efavirenz solid dispersions in the two new modified starches. Pharmacokinetic evaluation of efavirenz- starch citrate (1:2) and efavirenz- starch phosphate (1:2) solid dispersions was done 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 comparison to efavirenz pure drug. 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 solid dispersion in the two new modified starches. A 9.90 and 9.14 fold increase in the absorption rate (K_a) was observed respectively with efavirenz- starch citrate (1:2) solid dispersion and efavirenz- starch phosphate (1:2) solid dispersion when compared to efavirenz pure drug. A 1.46 and 1.47 fold increase in $(AUC)_0^\infty$ was also observed respectively with these solid dispersions when compared to efavirenz pure drug. The solid dispersions of efavirenz in the two new modified starches (starch citrate and starch phosphate) exhibited markedly higher rates of absorption and bioavailability of efavirenz when compared to efavirenz alone in the *in vivo* evaluation.

INTRODUCTION

Efavirenz, a widely prescribed HIV-1 specific, non-nucleoside reverse transcriptase inhibitor (NNRTI) drug belong 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 solubility and dissolution rate for increasing its oral bioavailability. Several techniques (Chowdary *et.al.,* 2005) such as micronization, cyclodextrin complexation, use of surfactants and solubilizers, solid dispersion in water soluble and dispersible carriers, use of salts, prodrugs and polymorphs which exhibit high solubility , microemulsions and self emulsifying micro and nano disperse systems have been used to enhance the solubility, dissolution rate and bioavailability of poorly soluble drugs. Among the various approaches, solid dispersion in water dispersible excipients is a simple, industrially useful approach for enhancing the solubility, dissolution rate and bioavailability of poorly soluble drugs. We reported (Chowdary *et.al.,* 2011), (Chowdary *et.al.,* 2011) earlier that solid dispersion of efavirenz in two new modified starches namely (i) starch citrate and (ii) starch phosphate has markedly enhanced the dissolution rate and dissolution efficiency of efavirenz. These solid dispersions could also be compressed into tablets retaining their enhanced dissolution rate characteristics. The objective of the present study is to evaluate the *in vivo* performance and pharmacokinetics of the efavirenz solid dispersions in the two new modified starches.

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EXPERIMENTAL

Materials

Efavirenz was a gift sample from M/s Hetero Drugs Ltd., Hyderabad. Starch citrate and starch phosphate were prepared in the laboratory as described in our earlier papers (Chowdary *et.al.,* 2011), (Chowdary *et.al.,* 2011). All other materials used were of pharmacopoeial grade.

Methods

Preparation of Solid Dispersions of Efavirenz

Solid dispersions of efavirenz in two new modified starches (starch citrate and starch phosphate) were prepared in 1:2 ratio of drug: carriers by kneading method. Efavirenz (1 g) was dissolved in dichloromethane (10 ml) in a dry mortar to get a clear solution. Modified starch (1 g) was then added and mixed. The thick slurry was triturated for 15 min for complete evaporation of dichloromethane and then dried at 55^{o}C until dry. The dried mass was pulverized and sieved through mesh no. 100.

The solid dispersions of efavirenz in starch citrate and starch phosphate prepared were fine free flowing powders and found to contain 32.5 and 31.9% of efavirenz respectively.

In vivo Study

Pharmacokinetic evaluation of efavirenz- starch citrate (1:2) and efavirenz- starch phosphate (1:2) solid dispersions was done 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 comparison to efavirenz pure 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 (Mogatle *et.al.,* 2009) HPLC method after revalidation as follows.

Estimation of Efavirenz in Plasma

The HPLC method reported earlier (Mogatle *et.al.,* 2009) was revalidated employing an internal standard and was used in the present study for the estimation of efavirenz in plasma samples.

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 ATVP) 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 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. The inter day and intraday precision of the method was evaluated by analyzing plasma sample containing 8.0 µg/ 0.5 ml plasma of efavirenz repeatedly (n=6). 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 (Wagner *et.al.,* 1963), (Wagner *et.al.,* 1964).

RESULTS AND DISCUSSION

The HPLC method revalidated was found suitable for the estimation of efavirenz in plasma samples. The mobile phase consists of a mixture of 0.1 M formic acid –acetonitrile-methanol (43:52:5). The retention time for efavirenz was 7.027 min and for internal standard (metadoxine) it was 2.975 min. Linearity of the method was in the concentration range 2- 10 µg/0.5 ml of Plasma (Fig. 1). The intra and inter day coefficient of variation for drug and internal standard was less than 0.962 % showing high precision of the method. Plasma concentrations of efavirenz following the oral administration of efavirenz and its solid dispersions in modified starches are shown in Fig.2. 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 6.86, 7.87 and 7.87 h respectively following the oral administration of efavirenz, and its solid dispersions in modified starches, efavirenz-starch citrate (1:2) solid dispersion and efavirenz- starch phosphate (1:2) solid dispersion. The close agreement of the $t_{1/2}$ values obtained with the three products indicated that the elimination characteristics of efavirenz have not changed when it was administered as solid dispersions in modified starches. Efavirenz was found to be absorbed slowly when given orally and a peak plasma concentration (C_{max}) of 3.20±0.2 µg/ml was observed at 4.0 h after administration. The absorption rate constant (K_a) was found to be 0.3937 h^{-1}.

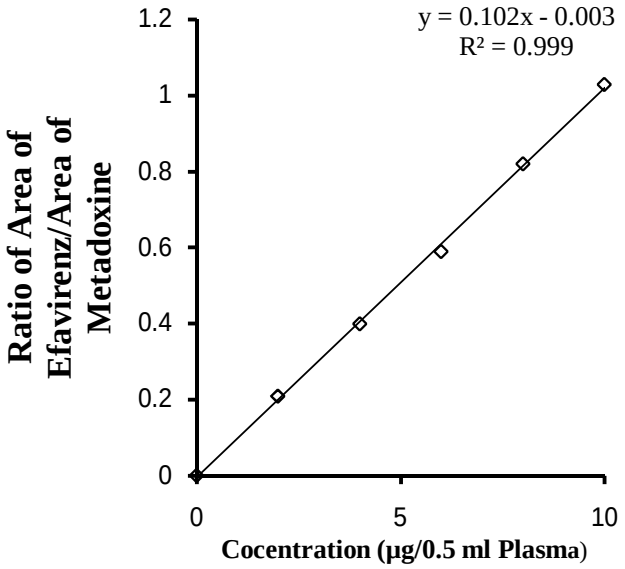

Fig. 1: Calibration curve for the Estimation of Efavirenz in Plasma Samples.

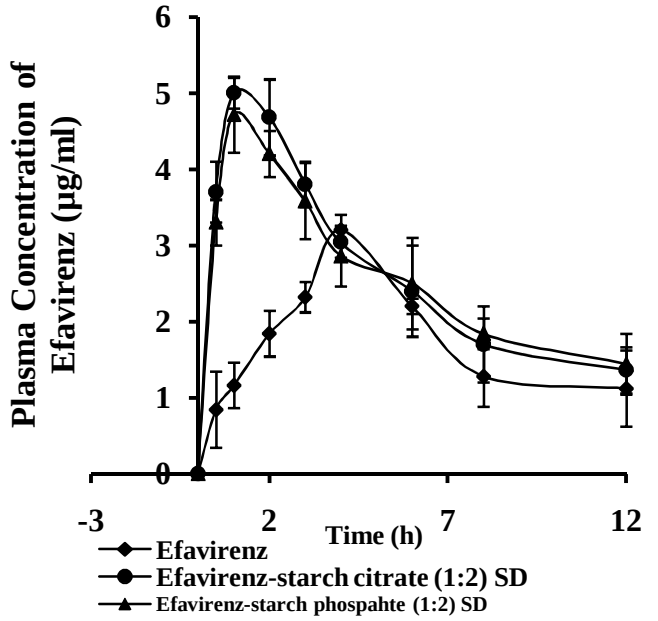

Fig. 2 : Plasma Concentrations of Efavirenz Following the Oral Administration of Efavirenz and its Solid Dispersions in Modified Starches in Rabbits.

Table. 1: Summary of Pharmacokinetic Parameters Estimated Following the Oral Administration of Efavirenz Products.

Parameter	Efavirenz	Efavirenz – Starch citrate (1:2) SD	Efavirenz – Starch phosphate (1:2) SD
C_{max} (µg/ml)	3.20	5.00	4.72
T_{max} (h)	4.0	1.0	1.0
K_{el} (h^{-1})	0.1010	0.0881	0.0880
$t_{½}$ (h)	6.86	7.87	7.87
$(AUC)_0^{12h}$ (µg. h /ml)	20.73	31.26	30.66
$(AUC)_0^{\alpha}$ (µg. h /ml)	31.82	46.72	47.02
BA (%)	100	146.8	147.7
K_a (h^{-1})	0.3937	3.899	3.602
Percent Absorbed			
0.5 h	22.00	70.27	67.86
1.0 h	31.46	97.97	99.00
2.0 h	52.73	100	100

All the pharmacokinetic parameters (Table. 1) namely C_{max}, T_{max}, K_a and $(AUC)_0^{\infty}$ indicated rapid and higher absorption and bioavailability of efavirenz when administered as solid dispersion in modified starches. Higher C_{max} values and lower T_{max} values were observed with the solid dispersions in modified starches when compared to those of efavirenz as such. The absorption rate constant (K_a) was found to be 3.899 h^{-1} and 3.602 h^{-1} respectively with efavirenz- starch citrate (1:2) solid dispersion and efavirenz- starch phosphate (1:2) solid dispersion, whereas in the case of efavirenz K_a was only 0.3937 h^{-1}. A 9.90 and 9.14 fold increase in the absorption rate (K_a) was observed respectively with efavirenz- starch citrate (1:2) solid dispersion and efavirenz- starch phosphate (1:2) solid dispersion when compared to efavirenz pure drug. $(AUC)_0^{\infty}$ (extent of absorption) was also much higher in the case of solid dispersions in modified starches when compared to efavirenz pure drug. $(AUC)_0^{\infty}$ was increased from 31.82 µg. h /ml for efavirenz pure drug to 46.72 and 47.02 µg. h /ml respectively for efavirenz- starch citrate (1:2) solid dispersion and efavirenz-starch phosphate (1:2) solid dispersion. A 1.46 and 1.47 fold increase in $(AUC)_0^{\infty}$ was observed respectively with efavirenz-starch citrate (1:2) solid dispersion and efavirenz- starch phosphate (1:2) solid dispersion when compared to efavirenz pure drug. Thus, solid dispersions of efavirenz in the two new modified starches (starch citrate and starch phosphate) exhibited markedly higher rates of absorption and bioavailability of efavirenz when compared to efavirenz alone.

CONCLUSION

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 solid dispersion in the two new modified starches. A 9.90 and 9.14 fold increase in the absorption rate (K_a) was observed respectively with efavirenz-starch citrate (1:2) solid dispersion and efavirenz- starch phosphate (1:2) solid dispersion when compared to efavirenz pure drug. A 1.46 and 1.47 fold increase in $(AUC)_0^{\infty}$ was also observed respectively with these solid dispersions when compared to efavirenz pure drug. The solid dispersions of efavirenz in the two new modified starches (starch citrate and starch phosphate)

exhibited markedly higher rates of absorption and bioavailability of efavirenz when compared to efavirenz alone in the *in vivo* evaluation.

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How to cite this article:

Seenivasan. R, Rekha. M, Indu. H, and Geetha.S Antibacterial Activity and Phytochemical Analysis of Selected Seaweeds from Mandapam Coast, India. J App Pharm Sci. 2013; 3 (4 Suppl 1): S89-S92.