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Preparation and Evaluation of Mucoadhesive Microspheres of Efavirenz for Enhancing Its Dissolution Rate and Bioavailability

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ABSTRACT

Efavirenz belongs to class II under BCS and exhibit low and variable bioavailability due to its poor aqueous solubility and needs enhancement in dissolution rate and bioavailability to derive its maximum therapeutic efficacy. A novel promising technology for enhancing the bioavailability is a combination of mucoadhesion principle and microsphere technology to result in mucoadhesion microspheres. The objective of the present study is to prepare and evaluate mucoadhesive microspheres for enhancing the dissolution rate and bioavailability of efavirenz.Mucoadhesive microspheres prepared were in fine discrete powder form. The size of the microspheres was in the range 19.0 – 25.0µ. Drug content was uniform (C.V.< 2.0%) in each batch of microspheres prepared. The dissolution of efavirenz from the mucoadhesive microspheres prepared was rapid and several times higher than the dissolution of the pure drug. A 17.02 and 10.42 fold increase in the dissolution rate (K_1) of efavirenz was observed with HPMC and Carbopol microspheres respectively when compared to efavirenz pure drug. The dissolution efficiency wad increased from 17.25% for efavirenz pure drug to 75.80 and 65.5% with HPMC and Carbopol microspheres respectively. Rapid absorption and bioavailability of efavirenz was observed when administered as mucoadhesive microspheres. A 3.58 and 7.80 fold increase in the K_a and 1.84 and 1.97 fold increase in $(AUC)_0^{\infty}$ was observed respectively with HPMC and Carbopol microspheres when compared to efavirenz pure drug. The mucoadhesive microspheres of efavirenz prepared employing HPMC and Carbopol exhibited marked enhancement in the dissolution rate, dissolution efficacy and bioavailability (both rate and extent of absorption) of efavirenz. Hence mucoadhesive microspheres are recommended to enhance the dissolution rate, dissolution efficiency and bioavailability of efavirenz, a BCS class II drug.

Key Words: Mucoadhesive microspheres, Efavirenz, Dissolution rate, Bioavailability.

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INTRODUCTION

Efavirenz, a widely prescribed HIV-1 specific, non-nucleoside reverse transcriptase inhibitor (NNRTI) drug belongs to Class II under BCS and exhibit low and variable oral bioavailability due to its poor solubility. As such it needs enhancement in dissolution rate and bioavailability to derive its maximum therapeutic efficacy. A novel promising technology for enhancing the bioavailability is a combination of mucoadhesion principle and microsphere technology to result in mucoadhesive microspheres. Mucoadhesion refers to attachment of polymers to the mucin layer of a mucosal tissue by means of interfacial forces. Several polymers such as HPMC, Carbopol, sodium CMC and polymethacrylic acid exhibit [1] mucoadhesive property. Mucoadhesive microspheres (1-1000 µm in size) consist of either entirely of a mucoadhesive polymer or having an outer coating of it enclosing the drug particles [2]. They are readily localized in the region applied and facilitate an intimate contact with the underlying absorption surface to improve and enhance the bioavailability of drugs. The mucoadhesive microspheres have additional advantage of providing efficient absorption and enhanced bioavailability of the drug due to a high surface to volume ratio. The objective of the present study is to prepare and evaluate mucoadhesive microspheres for enhancing the dissolution rate and bioavailability of efavirenz.

MATERIALS AND METHODS

Materials

Efavirenz was a gift sample from M/s Hetero Drugs Ltd., Hyderabad. Hydroxy propyl methyl cellulose (HPMC, 500 CPS) and Carbopol 934P were gift samples from M/s Natco Pharma Ltd., Hyderabad. Dichloromethane (Qualigens) and petroleum ether, 600 – 800 (Qualigens) were procured from commercial sources. All other materials used were of Pharmacopoeial grade.

Methods

Preparation of microspheres

Mucoadhesive microspheres of efavirenz were prepared by inversion microencapsulation method [3]. Hydroxy propyl methyl cellulose or Carbopol (4 parts) and efavirenz (1 part) were dissolved in dichloromethane (10 ml). The polymer-drug solution was added to a non-solvent, petroleum ether (100 ml) slowly while mild stirring. Stirring was continued for 2 h to form the microspheres due to removal of solvent into non-solvent. Microspheres so formed were separated by filtration and air dried. In each case three batches were prepared under identical conditions to assess the reproducibility of the method.

Evaluation of Microspheres

Size Analysis: Size analysis of the microsphere was done by microscopy. The microspheres were dispersed in light liquid paraffin and a smear of the dispersion was observed under compound microscope. The size of 100 microspheres was measured in each case against a calibrated eyepiece micrometer.

Content of active ingredient: From each batch of microspheres, four samples of 50 mg each were taken into 100 ml volumetric flask. Methanol was added and mixed to dissolve the drug and the solution was made up to 100 ml with methanol. The solution was then suitably diluted with water containing 2% SLS and assayed for efavirenz content at 245 nm.

Dissolution rate study: Dissolution rate of efavirenz and its mucoadhesive microspheres was studied in water containing 2% SLS (900 ml) employing USP 8 station Dissolution Rate Test Apparatus (M/s Labindia Disso 8000) with a paddle stirrer at 50 rpm. Sodium Lauryl Sulphate (SLS) was added to the dissolution fluid to maintain the sink condition as prescribed in I.P. 2010. A temperature of 37±1°C was maintained throughout the study. Efavirenz or its microspheres equivalent to 50 mg of efavirenz were used in each test. Samples of dissolution fluid (5 ml) were withdrawn through a filter (0.45 µm) at different intervals of time, suitably diluted and assayed for efavirenz at 245 nm. The dissolution fluid withdrawn at each sampling time was replaced with fresh dissolution fluid and suitable correction is made in calculating the amount dissolved. All dissolution rate experiments were conducted in triplicate (n=3).

Pharmacokinetic evaluation: Pharmacokinetic evaluation of mucoadhesive microspheres prepared in comparison to the 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. *In vivo* study protocols were approved by the Institutional Animal Ethics Committee (No. 516/01/a/CPCSEA). A wash out period of 1 month was given between testing of two products.

After collecting the 0 h blood sample (blank), the product in the study was administered orally in a capsule shell with the 10 ml of water. No food or liquid other than water was permitted until 4 h following the administration of the product. Blood samples (3 ml) were collected from marginal ear vie at 0.5,1,2,3,4,6,8 and 12 h 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 [4].

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 [5,6].

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RESULTS AND DISCUSSION

Mucoadhesive microspheres of efavirenz were prepared by phase inversion microencapsulation method employing HPMC (500 cps) and Carbopol 934P. To evaluate the reproducibility of the method of preparation of microspheres, three batches of microspheres were prepared in each case under essentially identical conditions and the resulting microspheres were evaluated. The microspheres prepared were in fine discrete powder form. Size analysis of the microspheres was done by microscopy. The average size (diameter) of the microsphere was found to be 21.5±3.6, 20.2±5.2 and 24.10±4.8 µ in the case of HPMC microspheres in batches I, II and III respectively. In case of Carbopol microsphere, the average size was 25.5 ± 6.5, 24.5±2.6 and 26.20±5.2 µ respectively in batches I, II and III.

Drug content of the microspheres was estimated by UV spectrophotometric method. The efavirenz content was found to be 21.4±0.40, 20.5±0.60 and 19.2±0.8 % in the case of HPMC microspheres in batches I, II and III respectively. The drug content was 20.5 ± 0.30, 20.8 ± 0.6 and 19.58 ± 0.60% in the case of Carbopol micropspheres in batches I, II and III respectively. Low c.v (<2.0%) in the percent drug content indicate uniformity of the drug content in each batch of microspheres. The results indicated that the microencapsulation method used was reproducible with regard to size and drug content of the microsphere with both the polymers.

The dissolution rate of efavirenz and its microspheres was studied in water containing 2% SLS. The dissolution parameters of efavirenz are given in Table -1.

Table-1: Dissolution Parameters of Efavirenz and its Mucoadhesive Microspheres

Product	T_{50} (min)	T_{90} (min)	PD_{10} (%)	DE_{20} (%)	$K_1(min^{-1})$	No.of folds of increase in K_1
Efavirenz	>60	>60	18.80±1.25	17.25	0.0079	----------
HPMC microspheres	2.0	20.0	85.7 ±0.90	75.80	0.1345	17.02
Carbopol microspheres	3.0	28.6	72.60±1.20	65.50	0.0823	10.42

The dissolution of efavirenz from the mucoadhesive microspheres prepared was rapid and several times higher than the dissolution of efavirenz as such. The dissolution data were fitted into zero order, First order and Hixson-Crowell's cube root dissolution models to assess the kinetics and mechanism of dissolution. The dissolution of efavirenz as such and from mucoadhesive microspheres followed first order kinetics and obeyed Hixson-Crowell's cube root dissolution model. The dissolution efficiency after 20 min (DE_{20}) values was calculated in each case as reported by Khan [7].

All the dissolution parameters ($T_{50,}$ PD_{10}, DE_{20} and K_1) indicated rapid and higher dissolution of efavirenz from mucoadhesive microspheres than that of efavirenz pure drug. Dissolution efficiency was increased from 17.25% for pure drug to 75.80 and 65.50% with HPMC and Carbopol microspheres. A 17.02 and 10.42 fold increase in the dissolution rate (K_1)

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was observed with HPMC and Carbopol microspheres respectively when compared to efavirenz pure drug. The dissolution of efavirenz as pure drug was very slow and low due to its hydrophobic and non- dispersible nature. Mucoadhesive microspheres of efavirenz gave rapid and higher dissolution of the contained drug due to the presence of hydrophilic coat (HPMC and Carbopol) on the hydrophobic drug particles. Due to small size (19-25µ) and hydrophilic nature of the coat the mucoadhesive microspheres dispersed rapidly giving good wettability and rapid dissolution of the contained drug.

Pharmacokinetic evaluation: Pharmacokinetic evaluation was done on mucoadhesive microspheres of efavirenz in comparison to the pure drug. Pharmacokinetic parameters estimated are summarized in Table-2.

Table-2: Summary of Pharmacokinetic Parameters Estimated following the Oral Administration of Efavirenz and its Mucoadhesive Microspheres.

Product	C_{max} (µg/mL)	T_{max} (h)	K_{el} (h^{-1})	$t_{1/2}$ (h)	$(AUC)_0^{\infty}$ (µg.h/mL)	BA (%)	K_a (h^{-1})	MRT (h)	% Absorbed		
									0.5h	1h	2h
Efavirenz	10.45	4.0	0.1466	4.72	106.88	100	0.469	6.95	24.1	39.8	54.9
HPMC microspheres	25.64	1.0	0.1584	4.37	196.77	184.10	1.68	5.66	70.3	90.0	96.5
Carbopol microspheres	26.80	1.0	0.1260	5.49	210.82	197.24	3.66	6.36	70.0	97.7	100.0

The biological half-life ($t_{1/2}$) was found to be 4.72, 4.37 and 5.49 h respectively following oral administration of efavirenz and its HPMC and Carbopol microspheres. The close agreement of the ($t_{1/2}$) values in the three cases indicated that the elimination characteristics of efavirenz have not changed when it was administered as mucoadhesive microspheres.

Efavirenz was found to be absorbed slowly when given orally and a peak concentration (C_{max}) of 10.45µg/ml was observed at 4 h after administration. The absorption rate constant (K_a) was found to be 0.469 h^{-1}. All the pharmacokinetic parameters (Table-2) namely $C_{max.}$ T_{max} ,K_a and $(AUC)^{\infty}_0$ indicated rapid and higher absorption and bioavailability of efavirenz when administered as mucoadhesive microspheres. Higher C_{max} values and lower T_{max} values were observed with the microspheres when compared to those of efavirenz as such. The absorption rate constant K_a was found to be 1.68 and 3.66 h^{-1} respectively with HPMC and Carbopol microspheres, whereas in the case of efavirenz K_a was only 0.469 h^{-1}. A 3.58 and 7.80 fold increase in the K_a was observed respectively with HPMC and Carbopol microspheres when compared to efavirenz pure drug. $(AUC)^{\infty}_0$ (extent of absorption) was also much higher in the case of mucoadhesive microspheres when compared to efavirenz pure drug. $(AUC)^{\infty}_0$ was increased from 106.88 µg h/ml for efavirenz to 196.77 and 210.82 µg.h/ml for HPMC and Carbopol microspheres respectively. A 1.84 and 1.97 fold increase in $(AUC)^{\infty}_0$ was observed respectively with HPMC and Carbopol microspheres when compared to efavirenz pure drug.

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CONCLUSIONS

1. Mucoadhesive microspheres prepared were in fine discrete powder form. The size of the microspheres was in the range 19.0 – 25.0µ. Drug content was uniform (C.V.< 2.0%) in each batch of microspheres prepared.
2. The dissolution of efavirenz from the mucoadhesive microspheres prepared was rapid and several times higher than the dissolution of the pure drug.
3. A 17.02 and 10.42 fold increase in the dissolution rate (K_1) of efavirenz was observed with HPMC and Carbopol microspheres respectively when compared to efavirenz pure drug.
4. The dissolution efficiency wad increased from 17.25% for efavirenz pure drug to 75.80 and 65.5% with HPMC and Carbopol microspheres respectively.
5. Rapid absorption and bioavailability of efavirenz was observed when administered as mucoadhesive microspheres.
6. A 3.58 and 7.80 fold increase in the K_a and 1.84 and 1.97 fold increase in $(AUC)_0^{\infty}$ was observed respectively with HPMC and Carbopol microspheres when compared to efavirenz pure drug.
7. The mucoadhesive microspheres of efavirenz prepared employing HPMC and Carbopol exhibited marked enhancement in the dissolution rate, dissolution efficacy and bioavailability (both rate and extent of absorption) of efavirenz.
8. Mucoadhesive microspheres are recommended to enhance the dissolution rate, dissolution efficiency and bioavailability of efavirenz, a BCS class II drug.

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