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Preparation and Evaluation (*In vitro* and *In vivo*) of Mucoadhesive Microspheres of Etoricoxib

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Etoricoxib 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 mucoadhesive microspheres. The objective of the present study is to prepare and evaluate mucoadhesive microspheres for enhancing the dissolution rate and bioavailability of etoricoxib. Mucoadhesive microspheres prepared were in fine discrete powder form. The size of the microspheres was in the range 19-25 µ. Drug content was uniform (C.V < 2 %) in each batch of microspheres prepared. The dissolution of etoricoxib from the mucoadhesive microspheres prepared was rapid and several times higher than the dissolution of the pure drug. A 17.11 and 9.26 fold increase in the dissolution rate (K_1) of etoricoxib was observed with hydroxy propyl methyl cellulose (HPMC) and carbopol microspheres respectively when compared to etoricoxib pure drug. The dissolution efficiency was increased from 16.45 % for etoricoxib pure drug to 76.05 and 62.71 % with hydroxy propyl methyl cellulose and carbopol microspheres respectively. Rapid absorption and higher bioavailability of etoricoxib was observed when administered as mucoadhesive microspheres. A 3.52 and 8.05 fold increase in the K_a and 1.86 and 2.06 fold increase in $(AUC)^{\infty}_0$ was observed respectively with hydroxy propyl methyl cellulose and carbopol microspheres when compared to etoricoxib pure drug. The mucoadhesive microspheres of etoricoxib prepared employing hydroxy propyl methyl cellulose and carbopol exhibited marked enhancement in the dissolution rate, dissolution efficacy and bioavailability (both rate and extent of absorption) of etoricoxib, a BCS-class II drug.

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

INTRODUCTION

Several modern organic drugs belong to class II category under BCS and exhibit low and variable dissolution rates. These drugs need enhancement in dissolution rate and bioavailability to derive their maximum therapeutic efficacy. Etoricoxib is a relatively new widely prescribed NSAID drug. Its mode of action is largely based on the inhibition of prostaglandin synthesis. Etoricoxib belongs to class II under BCS and exhibit low and variable bioavailability due to its poor aqueous 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 referes 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 exhibited[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 etoricoxib.

EXPERIMENTAL

Etoricoxib was a gift sample from M/s Natco Pharma Ltd., Hyderabad. Hydroxy propyl methyl cellulose (HPMC, 500 cps) and carbopol 934 p 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.

Preparation of microspheres: Mucoadhesive microspheres of etoricoxib were prepared by phase inversion microencapsulation method[3]. Hydroxy propyl methyl cellulose or carbopol (4 parts) and etoricoxib (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 microspheres 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 phosphate buffer of pH 7.4 and assayed for etoricoxib content at 272 nm.

Dissolution rate study: Dissolution rate of etoricoxib and its mucoadhesive microspheres was studied in 900 mL of phosphate buffer of pH 7.4 using Disso 2000 Dissolution rate test apparatus (Labindia) with a paddle stirrer at 50 rpm. A temperature of 37 ± 1 ºC was maintained throughout the study.

Etoricoxib or its microspheres equivalent to 50 mg of etoricoxib were used in each test. Samples of dissolution fluid (5 mL) were withdrawn through a filter (0.45 µ) at different intervals of time, suitably diluted and assayed for etoricoxib at 272 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 the 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 vitro* study protocols were approved by the Institutional Animal Ethics Committee (Regd. 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 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 vein 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 etoricoxib were determined by a known HPLC method[4].

From the time *versus* plasma concentration plots (Fig.1), 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 ($t_{1/2}$), per cent absorbed to various times and absorption rate constant (K_a), were calculated in each case as per known standard methods[5,6].

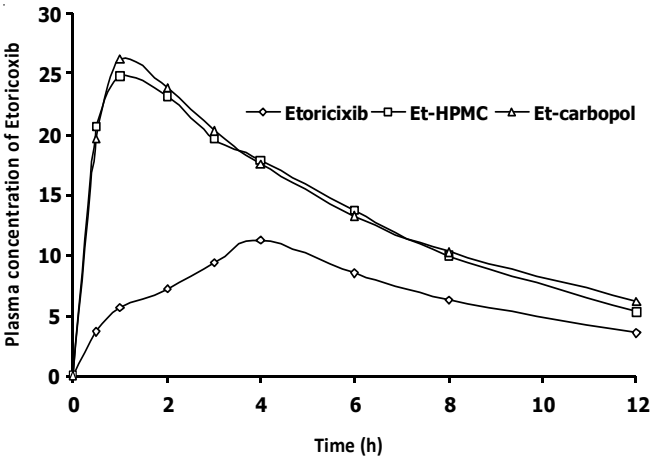

Fig. 1. Plasma concentrations of etoricoxib following the oral administration of etoricoxib and its mucoadhesive microspheres

RESULTS AND DISCUSSION

Mucoadhesive microspheres of etoricoxib were prepared by phase inversion microencapsulation method employing hydroxy propyl methyl cellulose (500 cps) and carbopol 934 P. 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 microspheres was found to be 20.5 ± 4.6, 19.2 ± 6.2 and 23.10 ± 5.8 µ in the case of hydroxy propyl methyl cellulose microspheres in batches I, II and III respectively. In the case of carbopol microspheres, the average size was 24.6 ± 5.5, 23.4 ± 3.6 and 25.10 ± 6.2 µ respectively in batches I, II and III.

Drug content of the microspheres was estimated by UV spectrophotometric method. The etoricoxib content was found to be 20.4 ± 0.40, 19.5 ± 0.60 and 19.6 ± 0.8 % in the case of hydroxy propyl methyl cellulose microspheres in batches I, II and III respectively. The drug content was 19.7 ± 0.30, 20.2 ± 0.6 and 20.48 ± 0.60 % in the case of carbopol microspheres in batches I, II and III respectively. Low c.v. (< 2.0 %) in the percent drug content indicated uniformity of the drug content in each batch of microspheres. The results indicated that the microencapsulation method used was reproducible with respect to size and drug content of the microspheres with both the polymers.

The dissolution rate of etoricoxib and its microspheres was studied in phosphate buffer of pH 7.4. The dissolution parameters are given in Table-1. The dissolution of etoricoxib from the mucoadhesive microspheres prepared was rapid and several times higher than the dissolution of etoricoxib as such. The dissolution data were fitted into zero order, First order and Hixson-Crowell's cube root models to assess the kinetics and mechanism of dissolution. The dissolution of etoricoxib 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 were 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 etoricoxib from

TABLE-1
DISSOLUTION PARAMETERS OF ETORICOXIB 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
Etoricoxib	>60	>60	18.60 ± 2.05	16.45	0.0078	---
HPMC microspheres	1.0	15.0	86.2 ± 0.80	76.05	0.1335	17.11
Carbopol microspheres	2.0	30.5	70.30 ± 1.10	62.7	0.0723	9.26

TABLE-2
SUMMARY OF PHARMACOKINETIC PARAMETERS ESTIMATED FOLLOWING THE ORAL ADMINISTRATION OF ETORICOXIB AND ITS MUCOADHESIVE MICROSPHERES

Product	C_{max} (µg/mL)	T_{max} (h)	K_{el} (h^{-1})	$t_{1/2}$ (h)	$(AUC)^{\infty}_0$ (µg.h/mL)	BA (%)	K_a (h^{-1})	MRT (h)	% Absorbed		
									0.5 h	1.0 h	2.0 h
Etoricoxib	11.25	4.0	0.1464	4.73	105.87	100	0.468	6.94	24.04	39.82	54.94
HPMC microspheres	24.84	1.0	0.1593	4.35	197.87	186.89	1.65	5.86	70.27	90.03	96.52
Carbopol microspheres	26.20	1.0	0.1259	5.50	218.72	206.65	3.76	6.32	70.03	97.68	100.0

mucoadhesive microspheres than that of etoricoxib pure drug. Dissolution efficiency was increased from 16.45 % for pure drug to 76.05 and 62.71 % with hydroxy propyl methyl cellulose and carbopol microspheres respectively. A 17.11 and 9.26 fold increase in the dissolution rate (K_1) was observed with hydroxy propyl methyl cellulose and carbopol microspheres respectively when compared to etoricoxib pure drug. The dissolution of etoricoxib as pure drug was very slow and low due to its hydrophobic and non-dispersible nature. Mucoadhesive microspheres of etoricoxib gave rapid and higher dissolution of the contained drug due to the presence of hydrophilic coat (hydroxy propyl methyl cellulose and carbopol) on the hydrophobic drug particles. Due to small size (19 - 25 µ) 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 etoricoxib in comparison to the pure drug. Pharmacokinetic parameters estimated are summarized in Table-2.

The biological half- life ($t_{1/2}$) estimated from the elimination phase of the plasma level curves was found to be 4.73, 4.35 and 5.5 h respectively following the oral administration of etoricoxib and its hydroxy propyl methyl cellulose and carbopol microspheres. The close agreement of the $t_{1/2}$ values in the three cases indicated that the elimination characteristics of etoricoxib have not changed when it was administered as mucoadhesive microspheres.

Etoricoxib was found to be absorbed slowly when given orally and a peak plasma concentration (C_{max}) of 11.25 µg/mL was observed at 4 h after administration. The absorption rate constant (K_a) was found to be 0.4677 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 etoricoxib when administered as mucoadhesive microspheres. Higher C_{max} values and lower T_{max} values were observed with the microspheres when compared to those of etoricoxib as such. The absorption rate constant (K_a) was found to be 1.65 and 3.76 h^{-1} respectively with hydroxy propyl methyl cellulose and carbopol microspheres, whereas in the case of etoricoxib K_a was only 0.4677 h^{-1}. A 3.52 and 8.05 fold increase in the K_a was observed respectively with hydroxy propyl methyl cellulose and carbopol microspheres when compared to etoricoxib pure drug. $(AUC)^{\infty}_0$ (extent of absorption) was also much higher in the case of mucoadhesive microspheres when compared to etoricoxib pure drug. $(AUC)^{\infty}_0$ was increased from 105.87 µg.h/mL for etoricoxib to 197.87 and 218.72 µg.h/mL for hydroxy propyl methyl cellulose and carbopol microspheres, respectively. A 1.86 and 2.06 fold increase in $(AUC)^{\infty}_0$ was observed respectively with hydroxy propyl methyl cellulose and carbopol microspheres when compared to etoricoxib pure drug.

Conclusion

Mucoadhesive microspheres prepared were in fine discrete powder form. The size of the microspheres was in the range 19.0 - 25.0 µ. Drug content was uniform (C.V. < 2.0 %) in each batch of microspheres prepared. The dissolution of etoricoxib from the mucoadhesive microspheres prepared was rapid and several times higher than the dissolution of the pure drug. A 17.11 and 9.26 fold increase in the dissolution rate (K_1) of etoricoxib was observed with hydroxy propyl methyl cellulose and carbopol microspheres respectively when compared to etoricoxib pure drug. The dissolution efficiency was increased from 16.45 % for etoricoxib pure drug to 76.05 and 62.71 % with hydroxy propyl methyl cellulose and carbopol microspheres respectively. Rapid absorption and higher bioavailability of etoricoxib was observed when administered as mucoadhesive microspheres. A 3.52 and 8.05 fold increase in the K_a and 1.86 and 2.06 fold increase in $(AUC)^{\infty}_0$ was observed respectively with hydroxy propyl methyl cellulose and carbopol microspheres when compared to etoricoxib pure drug. The mucoadhesive microspheres of etoricoxib prepared employing hydroxy propyl methyl cellulose and carbopol exhibited marked enhancement in the dissolution rate, dissolution efficacy and bioavailability (both rate and extent of absorption) of etoricoxib, a BCS - Class II drug.

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