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Ex vivo mucoadhesion and *in vivo* bioavailability assessment and correlation of ketoprofen tablet dosage forms containing bioadhesives

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The purposes of this study were to assess the mucoadhesion and bioavailability and their correlation for ketoprofen tablet dosage forms (F1-F6) containing polycarbophil (PC), sodium carboxymethylcellulose (Na CMC) as bioadhesives, Avicel pH 101 as direct compressible tablet vehicle or mixtures of these, and non compressible vehicles such as lactose and starch. For mucoadhesion assessment, we used sheep gastric mucosa and for bioavailability we used six human volunteers in an open randomized seven-way crossover study. Young's modulus (YM) and relative bioavailability (RB) parameters were used for evaluation of mucoadhesion and bioavailability, respectively. The results indicated that F2 containing Na CMC (72.5%) showed the highest value of YM (7.6 ± 0.76 pascals) and 119.4 ± 3.2% for RB. Decreasing the amount of Na CMC to 10% in F3 and F6 decreased the values of YM and RB to 1.4 ± 0.08 and 84 ± 2.05 in F3, 4.6 ± 0.43 and 114.7 ± 2.46 in F6, respectively. The highest RB (152.3 ± 2.56) was observed in F5 containing starch and Avicel pH 101. This formulation showed 6 ± 0.87 for YM. F4 containing PC (10%) showed 5.1 ± 0.43 and 74.15 ± 1.98 for YM and RB respectively. The lowest value of YM was observed in F1 containing Avicel pH 101 (0.27 ± 0.01) which also showed low RB (93.3 ± 2.3). In conclusion, formulations containing bioadhesives and/or starch in high concentration showed high values of YM and RB which indicate good correlation between mucoadhesion and bioavailability. Bioadhesives may show a high potential to improve bioavailability and therapeutic efficacy of ketoprofen in tablet dosage forms.

1. Introduction

It has been postulated that incorporating bioadhesives into drug dosage forms would improve the bioavailability through increasing the time of drug contact with absorption membrane and enhancing the paracellular transport through loosing or opening the tight junction of membrane cells. On adherence to cell surfaces, the high concentration of ionic groups inside the bioadhesive particles cause large influx of water by osmosis, swelling the particles until cross links are strained. In this sense, they can increase the solubility and bioavailability if used as tablet excipients (Bernkop-Schnurch et al. 2004). Bioadhesives have been incorporated in pharmaceutical dosage forms administered via almost all accessible routes of drug absorption, including oral (Akiyama et al. 1998), buccal (Perioli et al. 2004), ocular (Middleton et al. 1990), nasal (Iqbal et al. 2003), rectal (Dash et al. 1999) and vaginal (Justin-Temu et al. 2004). The main purpose of their incorporation was to retain the dosage form at the absorbing epithelial membrane, thereby prolonging drug release and thus decreasing dosage frequency compared to conventional dosage forms (Smart 2005). Another purpose was to adhere to mucosa of an appropriate organ for a sufficiently long time to improve the extent of release and absorption of the drug (Kockisch et al. 2003). Additionally, bioadhesives can be used as tablet binder and disintegrant because of their binding and swelling properties (Zaghloul 1995). The bioadhesive properties of a wide range of materials have been evaluated over the last two decades and some polymers such as polycarbophil, carbopol and Na CMC displayed good adhesion properties when tested *in vitro* (Smart 1991). However, *in vivo*, such performance may not be replicated, which explains why relatively few bioadhesive delivery systems have become commercially available (Parfitt 1996).

In a previous study (Zaghloul 1995) we prepared fifteen different ketoprofen tablet formulations containing different concentrations of bioadhesive polymers, directly compressible vehicles and regular tablet fillers. Out of them, six formulations were selected on the bases that they showed good physical characteristics such as uniformity of weight, thickness, hardness, friability, disintegration time, content uniformity and *in vitro* drug release in water and 0.1 N HCl.

The reasons for using ketoprofen as a drug model were because it has low water solubility, low bioavailability and

many side effects on the gastrointestinal tract (GIT) which limit its chronic use. Our purpose was to improve its bioavailability through keeping it in contact with the absorption membrane for longer time, meanwhile decreasing its side effects. Bioadhesives are thought to act as barriers between the drug and tissue cells, absorb water and swell admitting gradual release of the drug through the channel pores, thus decreasing, to some extent, the irritating side effects.

Therefore the overall objectives of this study were: to measure the mucoadhesion of the selected formulations using sheep gastric mucosa, to study their bioavailability using human volunteers, and to correlate between the results of mucoadhesion and bioavailability.

2. Investigations, results and discussion

2.1. Tablet characterization

The six formulations used in this study showed superior tablet characters regarding weight variation, tablet thickness, hardness, friability, disintegration time, drug content and dissolution compared to other formulations. Table 1 shows the results from which it is obvious that these parameters were within the limits stated by pharmacopoeias.

2.2. Mucoadhesion

Isolated sheep intestinal and vaginal mucosae have been used before to evaluate the mucoadhesion strength of different pharmaceutical dosage forms (Chary and Rao 2000; Vermani et al. 2002). In this study, we used the gastric mucosa as it was easy to obtain and fulfill the requirements for mucoadhesion measurement. In addition, since the drug is acidic in nature, the stomach is the preferred place for its absorption. For all formulations the crosshead speed of mucoadhesion tester was kept constant at 5 mm/min and the time of contact at 2 min. The data obtained from the instrument were compiled in Table 2. The instrument records the values of displacement at peak, the % strain at peak, the displacement at break, the % strain at break and Young's modulus. These values were normalized to the surface area of the film. Young's modulus (YM) is the parameter that correlates between stress and strain, that why it was taken as a parameter to assess the tablet mucoadhesion. The results in Table 2 showed that the highest YM value was observed in F2 (7.67 ± 0.76) containing 72.5% Na CMC while the lowest value was observed in F1 (0.271 ± 0.01) containing 72.5% Avicel pH 101. Decreasing the concentration of Na CMC to 10% decreased the values of YM to 1.4 ± 0.08 when combined with lactose (F3) and 4.69 ± 0.43 when combined with starch (F6). This may be attributed to the strong bioadhesive nature of Na CMC. Upon contact with gastric mucosa, it created strong bonds which needed high tensile strength to break. These results are in agreement with the study showing that Na CMC containing casting films were superior than those containing carbopol, PC, and methocel in terms of swelling, mucoadhesion and organoleptic characteristics (Perioli et al. 2004). Combination of Na CMC with starch in F6 significantly increased the value of YM compared to that obtained from its combination with lactose in F3 ($p < 0.05$). This could be explained if we know that starch itself may show mucoadhesion properties. This finding was in accordance with results reported by Illum et al. (2001) who used starch as a bioadhesive and combined it with absorption enhancers to synergistically enhance the nasal absorption of polypeptides. The mucodhesion of starch was obvious on using a combination of starch and PC in F4 and starch and Avicel pH 101 in F5 where both of them showed quit high values of YM. The low concentration of PC in F4 (10%) might be the reason for the low value of YM compared to Na CMC. The effect of this low concentration of PC on the tablet characters was obvious. F4 tablets showed low hardness (3.9 ± 1.7) and low disintegration time (0.157 ± 0.02) which indicate that the disintegrating effect of starch supersede the binding effect of PC. Avicel pH 101 in F1 and lactose in F3 showed low values of YM because no adhesive properties have been proven to them.

2.3. Bioavailability

The results of urine analysis showed that negligible amounts of dug appeared in urine samples collected after 24 h, that why the cumulative amount of drug excreted after 24 h was considered as a proper indicator of the ex-

Table 1: Physicochemical characteristics of different ketoprofen tablet formulations

Formula No.	Weight (mg)	Thickness (mm)	Hardness (kg)	Friability (% w/w)	Disinteg. Time (min)	Drug Content (%)
F1	200.33* ± 0.32	3.04 ± 0.02	4.8 ± 0.5	0.40 ± 0.09	9.29 ± 2.76	99.78 ± 1.45
F2	196.26 ± 5.35	2.99 ± 0.09	8.4 ± 0.79	0.21 ± 0.00	4.83 ± 0.59	99.17 ± 1.44
F3	200.52 ± 0.20	2.99 ± 0.02	5.4 ± 1.60	0.49 ± 0.00	0.42 ± 0.05	97.50 ± 2.49
F4	205.20 ± 7.05	3.01 ± 0.07	3.9 ± 1.70	0.20 ± 0.01	0.15 ± 0.02	98.33 ± 1.44
F5	202.80 ± 8.07	2.88 ± 0.05	3.8 ± 1.00	0.40 ± 0.10	0.83 ± 0.11	100.0 ± 0.00
F6	201.40 ± 4.39	3.21 ± 0.07	5.6 ± 1.70	0.50 ± 0.8	0.70 ± 0.13	96.70 ± 1.44

* ± SD

Table 2: Mucoadhesive measurement parameters of different ketoprofen tablet formulations

Form. No.	Displ@peak	%strain@peak	Disp@break	%strain@break	Young's modulus
F1	2.179* ± 0.17	4.359 ± 0.24	1.719 ± 0.12	3.439 ± 0.26	0.271 ± 0.01
F2	3.892 ± 0.23	7.784 ± 0.54	8.041 ± 0.55	16.082 ± 1.68	7.676 ± 0.76
F3	1.585 ± 0.10	3.17 ± 0.35	2.672 ± 0.25	5.345 ± 0.46	1.445 ± 0.08
F4	0.906 ± 0.05	1.811 ± 0.09	2.84 ± 0.22	5.68 ± 0.56	5.158 ± 0.43
F5	2.739 ± 0.19	5.478 ± 0.53	6.017 ± 0.46	12.034 ± 1.08	6.09 ± 0.87
F6	1.317 ± 0.08	2.634 ± 0.24	1.327 ± 0.08	2.653 ± 0.34	4.696 ± 0.43

* ± SD

Table 3: Pharmacokinetic parameters of different ketoprofen tablet formulations

Form. No.	Pharmacokinetic parameter						
	Peak exc. time, T_{max} (h)	Peak exc. rate, C_{max} (mg/h)	Mean cumulat. amount exc. Du^{∞} (mg)	Percent dose exc. (%)	Eliminat. rate const. K_{el}. $(h)^{-1}$	Eliminat. half life $T_{1/2}el$ (h)	Relative bioavailab. (%)
F1	1.5* ± 0.13	6.00 ± 1.10	16.83 ± 2.50	33.66 ± 3.21	0.44 ± 0.11	1.58 ± 0.12	93.34 ± 2.33
F2	0.5 ± 0.09	9.08 ± 1.26	21.53 ± 1.95	43.06 ± 3.85	0.40 ± 0.09	1.73 ± 0.19	119.41 ± 3.21
F3	1.5 ± 0.15	4.69 ± 1.09	15.18 ± 1.13	30.36 ± 3.42	0.41 ± 0.12	1.69 ± 0.13	84.19 ± 2.05
F4	1.5 ± 0.12	4.71 ± 1.08	13.37 ± 1.99	26.74 ± 2.56	0.30 ± 0.07	2.34 ± 0.23	74.15 ± 1.98
F5	0.5 ± 0.08	7.28 ± 1.10	27.47 ± 1.99	54.94 ± 5.42	0.30 ± 0.05	2.43 ± 0.25	152.36 ± 2.56
F6	1.5 ± 0.14	4.67 ± 1.06	20.68 ± 1.58	41.36 ± 3.89	0.27 ± 0.02	2.54 ± 0.24	114.7 ± 2.46
Market	1.5 ± 0.10	5.36 ± 1.09	18.03 ± 2.01	36.06 ± 2.99	0.22 ± 0.03	3.12 ± 0.33	–

* ± SD

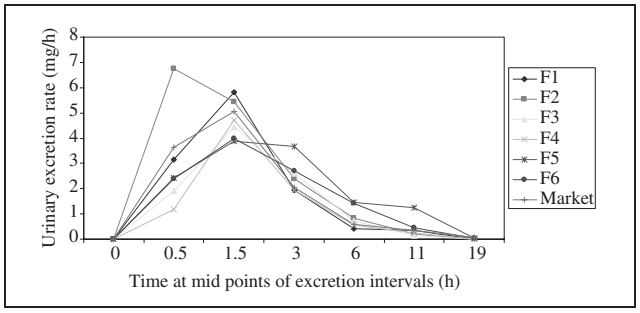

Fig. 1: Mean urinary excretion rates of ketoprofen tablet formulations

tent of ketoprofen absorption. As urinary excretion of the drug is, in most cases, directly proportional to the drug plasma concentration, the urinary excretion rate reflects the relative absorption rate of the drug. Similarly, the peak height of urinary excretion rate curve, C_{max}, and the time necessary to reach that peak, T_{max}, could be used as suitable parameters to describe the rate and extent of drug absorption (Shargel and Andrew 1999).

The pharmacokinetic parameters of ketoprofen formulations were shown in Table 3 and the mean urinary excretion rates in Fig. 1. From the Table, it is clear that F5 containing starch and Avicel pH 101 showed the highest amount of drug excreted. This may be attributed to the swelling properties of starch which enhanced the tablet disintegration, dissolution and hence absorption. On the other hand, F4 containing starch and PC showed the lowest amount of drug excreted. PC in F4, for being cross linking former, may entrap the drug inside the crosslinks and generate an effective barrier separating the drug contact with the absorption membrane. Also, it is obvious that F2 showed the highest value of C_{max} which may be attributed to the presence of a high concentration of Na CMC which in addition to its mucoadhesive properties, enhanced the drug absorption. The highest bioavailability (relative to the market preparation) was observed in F5 which contain Avicel and starch and the lowest one was in F4 containing PC and starch. These results were in agreement with those of Kato et al. (2002) who found an impairment of ciprofloxacin absorption by calcium polycarbophil. The mathematical modeling of the data indicated that drug release from F4 was best characterized by the Higuchi model (data not shown), suggesting a similarity to release from a matrix. When a tablet containing PC is immersed in water, it swells and forms a gel diffusion layer that may hinder the outward transport of the drug within the matrix. The results of RB could be arranged in descending order as follows: F5 > F2 > F6 > market > F1 > F3 > F4.

Correlating the results of mucoadhesion with that of bioavailability, it seems that including Na CMC or starch in the formulations significantly increased the YM compared to Avicel or lactose ($p < 0.05$). Similarly, with the exception of F4, including these excipients in F2, F5 or F6 significantly increased the RB compared to F1 and F3 containing Avicel or lactose ($p < 0.05$). Based on that, it seems that the higher the mucoadhesion formulation, the higher was its relative bioavailability. These results were in agreement with those of other authors who stated that the bioadhesive polymers improve the bioavailability in different ways. Firstly, the formulation is retained close to the absorption site for an extended period of time due to its bioadhesive nature. Secondly, the polymer absorbs water and swells forming a gel system. The gelled system provides a local high drug concentration in close contact with the absorptive site. Finally, the absorption of water by the dosage form from the mucous layer as it hydrates and gels could affect the passage of the drug through the paracellular tight junctions (Bjork et al. 1995).

In conclusion, the mucoadhesion between tablets containing bioadhesive polymers and the stomach biological substrate could be measured *ex vivo*. Na CMC, PC and/or starch containing formulations showed high YM values compared to ones containing Avicel pH 101 and lactose. Incorporating these polymers in high concentration also improved the bioavailability relative to the market preparation. Finally, incorporating bioadhesives in tablet dosage form in high concentration could potentially be an approach to improve the bioavailability of ketoprofen.

3. Experimental

3.1. Materials

Ketoprofen (Tourus SA, Switzerland), Polycarbophil (LEE lab. Pittsburgh, PA, USA), Avicel pH 101 and Na CMC (FMC, PA, USA), lactose and ethyl alcohol (Prolabo, France), hydrochloric acid and diethylether (BDH chemicals Ltd., Pool, England), acetonitrile (Romil chemicals Limited, England) and piroxicam (Secifarma, Milan, Italy). All other chemicals were of analytical grades and were used as received.

3.2. Preparation of ketoprofen tablets

Fifty milligram Ketoprofen were added to other tablet excipients (as shown in Table 4) and mixed with a high speed mixer (Erweka, SW1/S, Frankfurt, Germany) and then passed through # 40 sieves. The compression machine (Erweka, single punch EP1, Frankfurt, Germany) was set to produce flat-faced tablets, 8 mm in diameter and each has an average weight of 200 mg. The compression pressure applied was 1000 kg and dwell time was 4 s.

3.3. Characterization of ketoprofen tablets

The prepared tablets were characterized for uniformity of weight, thickness, hardness, friability, disintegration time, content uniformity and *in vitro* drug release in 0.1 N HCl. In brief, the methods of characterization were as follows.

Table 4: Composition of different ketoprofen tablet formulations

Form. No.	Types and amounts of tablet ingredients (mg)						
	Ketop.	Polycarb. (PC)	Avicel pH 101	Na CMC	Lactose	Starch	Aerosil 200
F1	50		145				5
F2	50			145			5
F3	50			20	125		5
F4	50	20				125	5
F5	50		20			125	5
F6	50			20		125	5

3.3.1. Uniformity of weight and thickness

The experiments were conducted according to the British Pharmacopoeia 2000 (PB 2000) guidelines. Briefly, a representative sample of twenty tablets were weighed individually to the nearest 0.1 mg (Sartorius 4503 microbalance, Germany). The thickness of each weighed tablet was measured (Micrometer, M & W, LTD, Sheffield, England). The average weight and thickness ± SD were determined.

3.3.2. Hardness and friability measurements

Ten tablets random sample from each batch were tested for hardness (Pharma Test Tablet Hardness Tester, Type PTB 301, Germany). For friability, twenty tablets random sample were brushed from adhering dust, accurately weighed and placed in the drum of the friabilator (Roch Friabilator, PTF1, China) and allowed to rotate for 5 min (100 rotation). The tablets were removed, carefully brushed and weighed. The percent of weight loss was taken as a measure of friability. The average hardness and friability ± SD were determined.

3.3.3. Disintegration time

Six tablets were weighed individually and tested for disintegration time (Pharma Test Disintegration Tester, PT 23, Germany). The average disintegration time ± SD was determined.

3.3.4. Content uniformity

Ten tablets were randomly taken, weighed and individually assayed for drug content as per BP 2000 guidelines. Criteria are met if the content uniformity lies within 90 to 110% of the label claim. The average drug content ± SD was determined. The results of weight, thickness, hardness, friability, disintegration time and drug content were tabulated in Table 1.

3.3.5. Dissolution

USP paddle method was employed for dissolution experiment using 900 ml of 0.1 N HCl (pH 1.2) as dissolution medium. The dissolution time was 150 min at 37 ± 0.5 °C and 100 rpm. Samples were withdrawn every 15 min and assayed spectrophotometrically at 260 nm (Pye, Unicam SP 6-550, Cambridge, England). Dissolution profiles are shown in Fig. 2.

3.4. Stomach tissue collection and preparation

Sheep stomach tissue was obtained from a local slaughterhouse, superficially cleaned with plain water just to remove the food debris without damaging the tissue coating layer. The samples were stored in a clean bottle surrounded with ice until transferring to 4 °C storing condition.

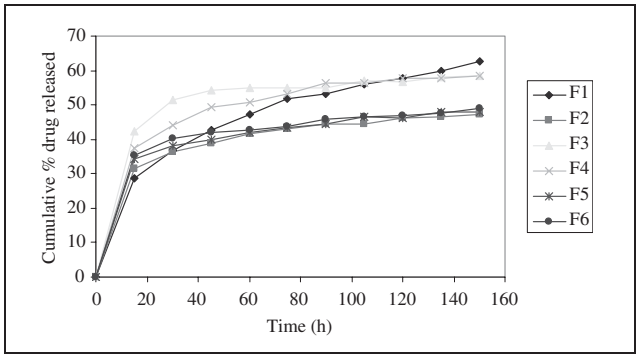

Fig. 2: *In vitro* dissolution profiles of ketoprofen tablet formulations in 0.1 N HCl

3.5. Measurement of mucoadhesion

The procedure used in this study was a modification of the method described by Shojaei et al. (2000). The adhesive forces of the prepared tablets in contact with sheep gastric mucosa were determined using an Instron materials testing system, Model 4442 (Instron Corp, Canton, MA, USA) equipped with a computer integrated data acquisition system. Before use, the samples were taken out to room temperature and clamped on equipment cross head. The tablet was affixed on a clean glass surface. To the bottom of a moving crosshead, the gastric tissue substrate was clamped and brought in contact with the tablet. Adhesion of the films to the substrate was brought on after the application of a constant force of 0.05 N. After a fixed time of contact, the crosshead was raised at a constant speed and the force required for complete detachment (break point) between the sample and the tissue was recorded. Before and during the measurements process, simulated gastric fluid (pH 1.2) was spread across the surface of the tissue periodically to maintain it hydrated throughout the experiment. The data were analyzed using Series IX software (Instron Corp.) and several parameters were recorded from which Young's modulus was chosen to assess the mucoadhesion. Experiments were run in triplicates and for each set of triplicate measurements, a new tablet and a fresh piece of tissue were used. Results of experiment are shown in Table 2.

3.6. Bioavailability study

3.6.1. Design

Six tablet formulations and a market preparation (Ketophan 25 mg tablet, Amyria Co., Alex., Egypt) were used in this study. The study was based on tracing of the drug in urine for 24 h after oral administration. Six healthy male volunteers, their ages ranged from 25 to 35 years and body weights from 60 to 85 kg, participated in an open randomized seven-way crossover study. The procedures followed in this study were in accordance with the ethical standards of the regional responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983 and informed consent was obtained from the participants. The volunteers were considered healthy on the basis of detailed medical history. Verbal assurance was taken from all of them that they had not administered any drugs during and for one weak preceding the study. The subjects fasted overnight before each treatment and for four hours after dosing. On the morning of the study, each subject was allowed to drink 200 ml of water at least one hour before dosing and after each urine collection. Complete urine collections were made at intervals of 1, 2, 4, 8, 14 and 24 h after drug administration. Blank urine samples were collected just before drug administration. The collected urine samples were refrigerated immediately until analyzed. A one week washout period was maintained between treatments. The areas under the curve (AUC) were calculated by the linear trapezoidal rule from zero to 24 h. The maximum plasma concentration, C_{max}, the time of its occurrence, T_{max}, and other pharmacokinetic parameters were calculated from the concentration-time data.

3.6.2. Drug extraction and assay set up

The method of drug extraction and assay adopted was a modification of Upton et al. (1980) method. In brief, the total ketoprofen, free and conjugated, in urine was obtained by including an alkaline hydrolysis step. This was carried out by adding 1 ml of urine to 1 ml of internal standard solution (piroxicam 100 µg/ml stock solution) and the mixture was vortex mixed for 10 min. To this mixture, 1 ml of 1 M sodium hydroxide solution was added. After allowing for 15 min hydrolysis at room temperature, 2 ml of 1 M phosphate buffer, pH 2, together with one ml of 1M HCL were added and vortex mixed for 15 min. The mixture was then extracted with 5 ml diethylether, vortex mixed for 1 min and centrifuged for 3 min at 3000 rpm. The upper organic phase was transferred to a 10 ml test tube and evaporated to dryness at 45 °C. The residue was reconstituted in HPLC mobile phase and 25 µl of this solution was injected into the HPLC column.

3.6.3. Chromatography

The HPLC system used was a Beckman System Gold® (Beckman Coulter Limited, Buckinghamshire HP11, 1JU, England, UK), supplied with a binary gradient 125 pump and a UV/VIS 166 detector at 262 nm. The column was Niclosil, C18, 25 cm × 4.6 mm, 5 µm and the mobile phase consisted of 0.05 M phosphate buffer, pH 3.5 and acetonitrile (55 : 45% V/V) at 1 ml/min flow rate.

3.7. Statistical data analysis

Statistical data analysis was performed using the student t test and ANOVA with $P < 0.05$ as the minimal level of significance.

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