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Enhancement of ketoprofen bioavailability by formation of microsponge tablets

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The release of ketoprofen incorporated into modified release ketoprofen microsponge 200 mg tablets and Profenid® Retard 200 mg was studied *in vitro* and *in vivo*. The formulation containing ketoprofen microsponges yielded good modified release tablets. An *in vivo* study was designed to evaluate the pharmacokinetic parameters and to compare them with the commercially available ketoprofen retard tablets containing the same amount of the active drug. Commercial ketoprofen retard tablets showed a more rapid absorption rate than modified release tablets and peak levels were reached within almost 3.6 h after administration. However, the new modified release tablets showed a slower absorption rate and peak levels were reached 8 h after administration.

1. Introduction

Taking drugs for a long period of time and taking several medicines simultaneously can lead to an increase in non-compliance. This problem tends to be serious for drugs with short biological half-lives because they must be taken more frequently. One method to solve such problems is to find a dosage form capable of releasing the drug gradually. Swellable polymers (Harland et al. 1988; Yamada et al. 2001), hydrophobic polymers etc. are frequently used as matrix materials or modified release microsponge tablets (Çomoğlu et al. 2002, 2003).

Ketoprofen is widely used for treatment of inflammation, pain or rheumatism (Upton et al. 1981; Jamali and Brocks 1990). In conventional immediate release formulations, ketoprofen is rapidly and efficiently absorbed with peak plasma levels occuring within 0.5–2 h, after which the therapeutic plasma concentration abruptly falls to very low levels. At a single dose of 150 mg, ketoprofen plasma concentration reaches values up to 15–25 μg/ml, which is much higher than the therapeutic concentration range (Jamali and Brocks 1990). The relativly high gastrointestinal concentration and plasma peaks associated with conventional formulations result in increased incidence of side effects and in the need for multiple daily administrations (Graham et al. 1984). When administered with food in the conventional form, the total bioavailability of ketoprofen remains unaltered, while the absorption rate is slowed by 1–2 h (Banwarth et al. 1988), which, however, is not enough to ensure appropriate therapeutic plasma levels for the entire day. The short half life and the low single dose administration make ketoprofen a very good candidate for the formulation of modified release dosage forms and considerable effort has been performed in this direction (Borsa et al. 1983; El Khodairy et al. 1992; Roda et al. 2002; Vergote et al. 2002). Several new formulations resist dissolution in the low pH of the gastric fluid and then gradually release the drug at a controlled rate in the higher pH environment of the small intestine. Various approaches have been adopted, including the use of pH dependent barrier films or capsules dispensed with microgranules, matrix pellets of nanocrystalline ketoprofen and tablets.

These show a tendency to decrease gastrointestinal side effects and have further therapeutic advantages due to less frequent dosing (Toft et al. 1985). Some dosage forms with slow release are commercially available (Roda et al. 2002). As ketoprofen is poorly soluble in acidic conditions due to its pH dependant solubility profile and as poor solubility is generally related to a low bioavailability, this presents a major challenge during drug formulation. A modified release dosage form may also improve the anti-inflammatory efficacy of ketoprofen and gastrointestinal tolerance without loss of bioavailability (Borsa et al. 1983).

In our previous studies, Eudragit RS 100 was chosen as a matrix polymer for the preparation of microsponges showing modified release of ketoprofen. The unique sponge-like texture of the microsponges encouraged us to investigate the direct compression behavior for the preparation of a matrix tablet of the drug and polymer (Çomoğlu et al. 2002, 2003). In the present study, ketoprofen was chosen as a model drug for investigation of the formulations showing modified release characteristics. We compared the *in vitro* dissolution rate of the experimental modified release formulation with that of a commercially available formulation. A single dose of the modified release formulation was then orally administered to 5 healthy volunteers and ketoprofen plasma levels were compared with those obtained in the same subjects after administration of commercially available retard tablets. Pharmacokinetic parameters, including time to peak (t_{max}), maximum plasma

concentration (C_{max}) and area under the curve (AUC) were calculated and the results were compared.

2. Investigations, results and discussion

2.1. In vitro study

Fig. 1 shows the dissolution profiles obtained from the developed modified release microsponge formulation compared with the commercially available Profenid® Retard tablets. Modified release microsponge formulation dissolved rapidly and efficiently (more than 48% during the first hour) in pH 7.4 buffer solution. Profenid® exhibited a much slower release profile, characterized by a constant dissolution rate during the entire period as shown by the slope of the curve; almost 35% of the active ingredient was dissolved after 7 h.

In order to investigate the mechanism of release, the percentage release versus time profile was evaluated for goodness of fit method. The details of this statistical technique are given by Bamba et al. (1979). For modified release ketoprofen microsponge 200 mg tablets and Profenid® Retard 200 mg tablets Higuchi's (Higuchi 1963) square-root equation ($100 - W = Kd\sqrt{t}$) shows a significantly better fit than first order ($\ln W = -Kf\ t + i$) and cube root ($\frac{1}{3}100 - \frac{1}{3}\ W = Kc\ t$) equations as determined by the F test. The release rate constants were determined from the slopes of the linear square root plots. The release rate values for the modified release ketoprofen microsponge 200 mg tablets and Profenid® Retard 200 mg tablets were 30.5 mg/h$^{-1/2}$ and 15.3 mg/h$^{-1/2}$ respectively.

2.2. In vivo study

Mean plasma ketoprofen concentrations resulted from the administration of Profenid Retard® 200 mg and modified release microsponge ketoprofen 200 mg tablets are presented in Fig. 2. Mean pharmacokinetic parameters determined from analysis of the single-dose data are presented in Tables 1, 2 and 3. The AUC values for Profenid and modified release microsponged tablets are 22.7 ± 5.5 μg.h/ml and 137.9 ± 35.9 μg · h/ml respectively. The statistical analysis indicated a significant difference among the C_{max}, t_{max} and AUC values of Profenid® and modified release microsponges tablets ($p \leq 0.05$). This analysis shows that the microsponge tablets lead to a significantly increased bioavailability compared to commercial sustained release tablets. This is probably due the use of Eudragit RS 100 and ketoprofen is most probably released in deeper segments of the intestines. This is supported by the fact that at pH 7.4, the drug release is enhanced.

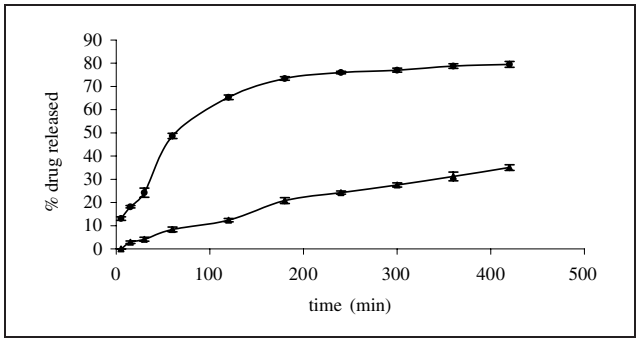

Fig. 1: *In vitro* drug release profiles from Profenid® Retard 200 mg (▲) and modified release ketoprofen microsponge 200 mg tablets (●)

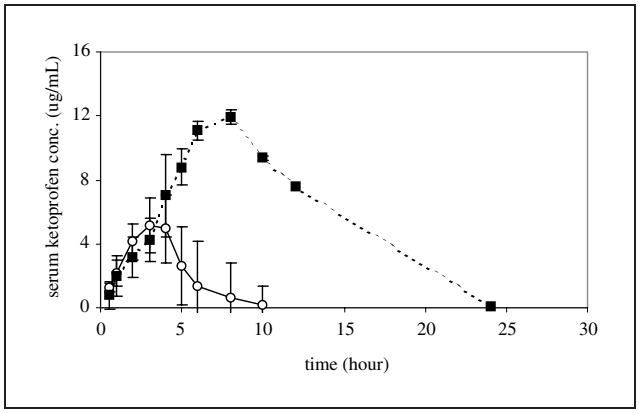

Fig. 2: Mean serum ketoprofen concentrations after the administration of a single dose of 200 mg ketoprofen
—○— commercial ketoprofen tablet; ···■··· modified release tablet

In vitro dissolution data were in agreement with *in vivo* plasma ketoprofen kinetic profiles obtained for both formulations. Oral administration of modified release microsponge tablets 200 mg produced a plasma steady-state ketoprofen level, which was achieved within 2 h after administration and maintained for about 12–14 h. This agrees with the constant drug release observed in the *in vitro* dissolution tests with the modified release microsponge tablet formulation.

Profenid ketoprofen retard tablets showed a more rapid absorption rate than modified release tablets and peak levels were reached within almost 3.6 h after administration. The modified release tablets showed a slower absorption rate and peak levels were reached 8 hours after administration. The apparent elimination phase was lengthened by the longer absorption period and in all subjects serum levels were detectable 24 h after dosing.

The results of this study demonstrate that modified release tablets of ketoprofen microsponges yielded ketoprofen levels higher than 5.4 μg/ml throughout a 12 h period. Since the mean steady state concentrations ranged from 0.4–5.6 μg/ml after the administration of 50 mg ketoprofen four times a day (Borsa et al. 1983), these results suggest that the new formulation may be administered only once daily leading to better patient's compliance.

The reported steady state concentrations were very similar to concentrations with double administration levels reached with 50 or 100 mg of ketoprofen. These concentrations are proved to give active synovial levels in patients with rheumatoid arthritis (Toft et al. 1985; Mc Crea et al. 1986).

Another advantage of this new formulation is the minimization of the interindividual variation caused by differences in transit time as the modified release tablets containing microsponges. Such microsponges, each with its own rate-limiting matrix structure, are widely scattered as they pass down the gastrointestinal tract. Also, high local concentrations are avoided.

This means that a correct dosage interval for modified release tablets (24 h) can be maintained for the well-known safety of ketoprofen and ketoprofen administered as commercial retard form tablets.

It can be concluded that the formulation developed in this study is indeed a modified-release preparation of ketoprofen with "smoother" concentration-time curves resulting in less fluctuation of blood levels. The new formulation pharmacokinetics should prompt chronic clinical studies that could lead to once-daily dosing, thereby improving patients' compliance to the treatment.

Table 1: Plasma ketoprofen concentrations (μg/ml) after single oral administration of one 200 mg Profenid® Retard tablet

Subject	Time (h)										
	0.5	1	2	3	4	5	6	8	10	12	24
1	1.53	3.81	5.90	7.37	8.53	4.04	2.25	nd	nd	nd	nd
2	2.45	2.89	3.62	4.80	2.26	1.02	0.75	0.36	nd	nd	nd
3	0.66	1.87	5.08	5.28	2.69	2.42	1.59	1.22	nd	nd	nd
4	0.51	2.03	3.66	4.81	6.24	3.27	1.23	0.30	0.05	nd	nd
5	nd	0.42	2.45	3.62	4.96	2.26	1.01	0.74	0.26	nd	nd
Mean ± SD	1.29 ± 0.90	2.20 ± 1.26	4.14 ± 1.28	5.17 ± 1.37	4.93 ± 2.59	2.60 ± 1.14	1.36 ± 0.58	0.65 ± 0.42	0.16 ± 0.15	–	–

nd: not detectable

Table 2: Plasma ketoprofen concentrations (μg/ml) after single oral administration of one 200 mg modified release microsponge tablet

Subject	Time (h)										
	0.5	1	2	3	4	5	6	8	10	12	24
1	0.34	0.63	1.40	2.86	3.52	5.27	7.42	9.84	10.77	8.97	0.10
2	0.78	2.13	4.23	6.73	9.01	11.43	14.61	10.29	7.66	5.48	0.23
3	0.92	2.54	4.01	6.17	6.88	7.51	9.27	11.16	8.65	7.23	0.12
4	0.67	1.95	2.72	5.79	8.59	10.24	12.31	14.60	9.51	8.35	0.14
5	1.12	2.71	3.32	5.49	7.14	9.43	11.83	13.75	10.20	7.73	0.05
Mean ± SD	0.77 ± 0.29	1.99 ± 0.82	3.14 ± 1.14	4.27 ± 1.73	7.03 ± 2.16	8.78 ± 2.42	11.09 ± 2.79	11.93 ± 2.13	9.36 ± 1.24	7.55 ± 1.33	0.13 ± 0.07

Table 3: Pharmacokinetic parameters (mean ± S.D.) obtained from 5 volunteers after single oral dose of Profenid® Retard (200 mg) and modified release ketoprofen microsponge tablets (200 mg)

Pharmacokinetic parameters	Profenid Retard 200 mg	Modified Release Ketoprofen Microsponge Tablets 200 mg
C_{max} (μg/mL)	5.96 ± 1.54	12.98 ± 1.88
t_{max} (h)	3.60 ± 0.55	8.00 ± 1.41
AUC_{0-24} (μg · h/ml)	22.74 ± 5.50	137.94 ± 35.94

3. Experimental

3.1. Materials

Ketoprofen (Dolder AG, Basel), acrylic copolymer Eudragit® RS 100 (Röhm Pharma GmbH, Darmstadt), spray-dried lactose Flow-Lac® 100 (Meggle, AG, Wasserburg, Germany), glyceryl palmitostearate Precirol® ATO-5 (Gattefosse, France), polyvinyl alcohol (PVA) 72000, etodolac (Farmatec, Spain), triethyl citrate (Morflex, USA), commercial ketoprofen retard tablets (Profenid Retard®, Eczacıbaşı, Turkey).

3.2. Methods

3.2.1. Preparation of microsponges

Microsponges containing ketoprofen and Eudragit® RS 100 were prepared by quasi-emulsion solvent diffusion method using an external phase containing 200 ml distilled water and 40 mg polyvinyl alcohol. The internal phase consisted of ketoprofen, ethyl alcohol, polymer and triethyl citrate which was added at a quantity of 20% of the polymer. Composition of the microsponges is given in Table 4.

At first, the internal phase was prepared at 60 °C and added to the external phase at room temperature. After emulsification, the mixture was continuously stirred for 2 h. Then the mixture was filtered to separate the microsponges. The product was washed and dried in a vacuum oven at 40 °C for 24 h (Çomoğlu et al. 2003).

Table 4: Composition of microsponges used in modified release tablet formulation

Inner phase		Outer phase	
Ketoprofen	1.056 g/ml	Distilled water	200 ml
Eudragit RS 100	0.096 g/ml	Polyvinyl alcohol 72.000	40 mg
Ethanol	10 ml		
Triethyl citrate	0.0192 g/ml		

3.2.2. Preparation of tablets containing microsponges

The modified release tablets were prepared containing 69% of microsponges corresponding to 200 mg of ketoprofen, 30.5% of Flow-Lac® 100 and 0.5% of Precirol® ATO-5. The tablets (total mass of 300 mg) were prepared by direct compression in an automatic hydraulic press (Ayaşlı Üçler, Turkey) using flat faced 10-mm diameter punches with a (196.2 MPa) 2000 kgf/cm^2 pressure (Çomoğlu et al. 2002).

3.2.3. Drug release studies

Release studies were carried out according to the USP 24 paddle method. The dissolution medium was phosphate buffer (pH 7.4, 900 ml) at 37 ± 0.5 °C and a stirring speed of 50 rpm was used. Six tablets were tested. The amount of ketoprofen present in each sample was determined spectrophotometrically, at 262 nm (Shimadzu 1202 UV visible).

3.2.4. Bioavailability study

3.2.4.1. Studied subjects

Five healthy volunteers aged 22–37 years, received a briefing on the purpose and nature of the study before providing written, informed consent. Before administration the first ketoprofen dose, medical examination and biochemical and hematological tests were performed to assess that subjects were healthy, while urine was screened for drugs or abuse. During the study all volunteers consumed a standard diet. No other medication was used for 2 weeks prior to dosing. The local Medical Ethics Committee approved the study protocol and the written informed consent form.

3.2.4.2. Study design

For each subject, the study comprised 1 day period, one for each formulation, separated by a 2 week washout period. In particular, one dose was administered at 8 h of either modified release microsponge tablet or Profenid® Retard 200 mg. Blood samples were collected immediately before administration and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after administration of the modified release microsponge and Profenid retard tablets. Plasma was separated by centrifugation and stored at −40 °C.

3.2.5. HPLC

The plasma samples were analyzed using a modified HPLC method (Corveleyn et al. 1996; Roda et al. 2002). A solution of 50 μg/ml etodolac (internal standard) was prepared using the mobile phase (water : acetonitrile : phosphate

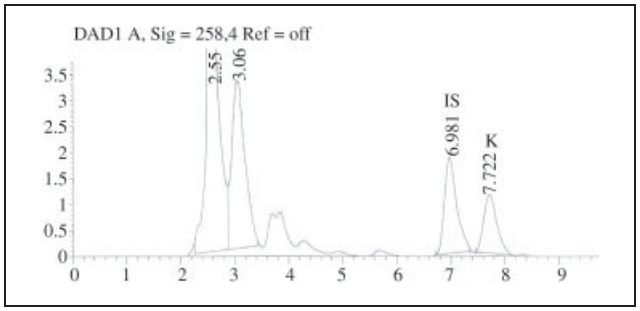

Fig. 3: Calibration chromatogram of ketoprofen
IS: Internal Standard (etodolac); K: ketoprofen

buffer (55 : 43 : 2; v/v/v) adjusted to pH 3.5) as a solvent. A solution of ketoprofen at a concentration of 100 µg/ml was also prepared using the mobile phase. From this stock solution dilutions were made using the same solvent: 1, 2, 3, 4, 5, 6 and 10 µg/ml. Calibration solutions were prepared by adding 50 µl of etodolac solution and 50 µl of a ketoprofen solution to 450 µl of blank plasma. All calibration curves were linear over the entire concentration range ($y = 0.793x + 0.0493$; $r^2 = 0.9980$). The plasma ketoprofen concentration in the unknown sample was calculated using a set of calibration curves ($n = 3$) (Fig. 3), obtained after linear regression of the peak area ratio (ketoprofen/etodolac) versus the ketoprofen concentration. Internal standard solution (50 µl) was added to 500 µl plasma sample. This solution was acidified by adding 1.0 ml of 1.0 M pH 2 phosphate buffer. After homogenizing and subsequent addition of 7 ml diethyl ether, the mixture was vortexed for 10 min and centrifuged for 10 min at 5000 × g. Next the organic layer was isolated and evaporated under a nitrogen stream. The residue was dissolved in 500 µl of mobile phase and 20 µl aliquot was injected into the chromatographic system. The HPLC system consisted of an isocratic pump (HP 1100, Model G 1311A), a reversed phase column Waters® spherisorb ODSI (250 × 4.6 mm; 5 µm) and a UV detector (HP 1100, Model G1314A) set at 262 nm. The flow rate of the mobile phase was 1 ml/min. The relative standard deviation of within day and between day reproducibility calculated at different concentrations ($n = 3$), were below 1.28 and 2.96% respectively.

3.2.6. Statistical methods

C_{max} and t_{max} were measured and AUC was calculated by the trapezoidal rule from 0 to 24 h for all subjects. The bioavailability of each formulation was determined and compared by calculating the mean AUCs.

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