Bioavailability Assessment of Ketoprofen Incorporated in Gelled Self-emulsifying Formulation: A Technical Note

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INTRODUCTION

Self-emulsifying formulations (SEF) are mixtures of oils and surfactants, ideally isotropic, and sometimes containing cosolvents, which emulsify spontaneously to produce fine oil-in-water emulsions when introduced into aqueous phase under gentle agitation.[1] Many workers have claimed various rational applications of SEF for enhancing bioavailability and site-specific targeting of highly lipophilic drugs (eg, WIN 54954,[1] N-4472,[2] idebenone,[3] coenzyme Q10,[4] halofantrine,[5] cyclosporin A[6]).

Ketoprofen (KPF) is a nonsteroidal anti-inflammatory drug (NSAID) with well-established analgesic and antipyretic properties. It is widely used in the treatment of rheumatoid arthritis, osteoarthritis, and a variety of other acute and chronic musculoskeletal disorders.[7,8] KPF is a poorly water-soluble drug (log P 0.98) and is absorbed rapidly by the oral route. Peak plasma levels occur within 0.5 to 2 hours, after which the therapeutic plasma concentration abruptly falls to very low levels. KPF is eliminated from the body by first-order kinetics ($k = 0.35/h$) and elimination half-life ($t_{1/2}$) ranges between 1.5 and 2 hours. At a single dose of 150 mg, KPF plasma concentration reaches up to 15 to 25 μg/mL, which is much higher than the therapeutic concentration.[9] When administered with food in conventional form, the total bioavailability of KPF remains unchanged, but the absorption rate is slowed by 1 to 2 hours.[10] Different formulation approaches that have been sought to increase bioavailability of KPF include matrix pellets of nanocrystals,[11] sustained-release microparticles,[12] and floating delivery systems.[13] Incorporation of drug in inert lipidic vehicles such as oils and surfactants is one of the most popular methods to enhance bioavailability.[14-16]

Recently, we have reported the effect of formulation variables on the preparation and evaluation of gelled self-emulsifying KPF formulation using 3^2 factorial design.[17] Gelled self-emulsifying KPF formulation consisted of diesters of caprylic/capric acids (Captex 200), C8/C10 mono-/diglycerides (Capmul MCM), polyoxyethylene 20 sorbitan monooleate (Tween 80), and colloidal silicon oxide (A 200). A 200 consists of small silica spheres with 12-nm diameter and a specific surface area of 200 m^2/g and acts as a gelling agent for oil-based systems. Gelling agent was incorporated with the intention that gelled SEF may require lesser excipients to convert in solid dosage forms such as tablets and capsules and may retard the drug release as well. We observed that the addition of colloidal silicon dioxide caused an increase in the viscosity of the liquid crystal phase, which in turn increased the average droplet size of the emulsion formed and slowed the drug release. Increasing the amount of cosurfactant was found to increase the drug release.

The purpose of the present work was to investigate the in vivo performance of a gelled self-emulsifying KPF formulation filled into hard gelatin capsules (Test), which was compared with pure KPF (active pharmaceutical ingredient [API]) filled into hard gelatin capsules (Reference) in healthy adult human volunteers under fasted conditions.

MATERIALS AND METHODS

Materials

KPF (batch no. K-02–003) was obtained as a gift sample from BEC Chemicals Pvt Ltd (Roha, India). Diesters of caprylic/capric acids (Captex 200) and C_8/C_{10} mono-/diglycerides (Capmul MCM) were generous gifts by Abitec Corp (Columbus, OH). Polyoxyethelene 20 sorbitan monooleate (Tween 80) was purchased from Merck Ltd (Mumbai, India). Colloidal silicon dioxide (Aerosil 200, Degussa Corp, Parsippany, NJ) and naproxen (used as an internal standard) were gift samples from Get Rid Pharmaceuticals (Pune, India). Hard gelatin capsules, especially designed for liquid formulations (Licaps), were provided by Capsugel (Colmar, France). All other reagents were of analytical grade and were used as received.

Methods

Preparation of Gelled Self-emulsifying KPF Formulations

A mixture of Captex and Tween 80 (4:3 parts, by volume) was prepared by simple mixing, and KPF (1 g/7.0 mL) was

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dissolved in it to get a clear solution. Capmul MCM (3 mL) and Aerosil 200 (100 mg) were added to this mixture and mixed well. This system (10 mL) was then poured into a plastic injector and volumetrically filled into hard gelatin capsules (Licaps) so as to contain 1-mL system (equivalent to 100 mg KPF) per capsule (Test). For Reference product, pure KPF (100 mg/capsule) was filled into each hard gelatin capsule manually. Both the Test and Reference products were evaluated for weight variation and drug content. The products were stored in tightly closed containers at ambient temperature until further evaluation.

Determination of Drug Content

KPF from preweighed quantities of Test and Reference was extracted in methanol using sonication technique. The methanolic extracts of Test, Reference, and blank SEF were analyzed spectrophotometrically at 258 nm, using a Jasco V 530 spectrophotometer (Tokyo, Japan). KPF content was calculated in comparison with a working standard API.

In Vivo Study Design

The study protocol was approved by the Ethical Committee of Bangalore Medical College, Bangalore, India. Eight healthy, male volunteers between 22 and 28 years old (mean, 24.75; SD, 2.55 years) and weighing from 59 to 65 kg (mean, 61.5; SD, 2.00 kg) participated in the study after providing written consent. All the volunteers were ambulatory adults with no negative past medical history and had not taken any medication at least 7 days before starting the study. They were not in the habit of smoking or drinking alcoholic beverages. The study was conducted according to a single-dose, 2-way crossover design with 4 subjects in each of the 2 treatment groups and a washout period of 1 week between 2 phases of study. Overnight fasted subjects were randomly divided into 2 groups, and dose was administered with 200 mL of water in the morning. Food and drinks were withheld for at least 2 hours after dosing. A low fat content breakfast and lunch were served at 2 hours after sampling and 4 hours after dosing, respectively. The volunteers were required to refrain from other food during the conduct of study. Water was allowed ad libitum. Venous blood samples (5 mL each) were drawn just before administration and at 0.5-, 1-, 1.5-, 2-, 3-, and 8-hour intervals after administration and collected in heparinized glass vials. Plasma was separated by centrifugation and stored at frozen condition prior to assay of KPF by high performance liquid chromatography (HPLC).

Analysis of Plasma KPF Concentration

Plasma level of KPF was analyzed using a reversed-phase (RP) HPLC method described by Satterwhite and Boudinot[18] with some modifications. Stock solutions of KPF and naproxen were prepared in 0.01 M phosphate buffer (pH 6.0) containing 1.0% vol/vol acetonitrile. Plasma standards (1 mL) were prepared by adding appropriate KPF solution to drug-free plasma to obtain concentrations in the range of 0.045 to 20 μg/mL. Calibration and clinical plasma samples were processed and submitted to HPLC analysis in an identical manner. In brief, 50 μL of naproxen (internal standard) solution (10 μg/mL) was added to 1 mL of plasma, which was then acidified with 0.2 mL of 1.0 M phosphate buffer at pH 2.0. The sample was then extracted with 5 mL diethyl ether and vortex-mixed for 5 minutes. The upper organic phase was separated and evaporated to dryness at 40°C under stream of nitrogen gas. The dry residue was dissolved in 0.3 mL of mobile phase for HPLC analysis.

The HPLC analysis system consisted of high-pressure pump (Shimadzu LC-10 ATVP, Tokyo, Japan), a sample injection valve with 20-μL sample loop, and a variable-wavelength ultraviolet detector (Shimadzu SPD-10 AVP). Samples (20 μL each) were injected, and KPF and naproxen were separated using an RP C-18 column (SGE, Mumbai, India) at room temperature. The mobile phase consisted of 0.01 M phosphate buffer (pH 7.0):acetonitrile (80:20 parts, by volume). KPF and naproxen were eluted isocratically at a flow rate of 1.5 mL/min and monitored at 254 nm. The retention time for KPF and naproxen was 10.58 minutes and 8.59 minutes, respectively. The method produces a linear calibration curve over the range of 0.045 to 20 μg/mL of KPF in plasma ($r = 0.9847$). The calibration curves were made by fitting KPF to naproxen peak area ratios with KPF concentration. Plasma to mobile phase extraction of KPF and naproxen was 99.89% ± 0.07% and 99.91% ± 0.05%, respectively. Detection and quantification limits of method were 0.015 μg/mL and 0.045 μg/mL, respectively. Intraday and interday coefficient of variation values were 1.59% and 1.97%, respectively.

Data Analysis

The pharmacokinetic parameters viz maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were directly obtained from plasma analysis data. Area under the plasma concentration-time profile curve ($AUC_{(0\text{-}8\ h)}$) was calculated using trapezoidal formula.[19] The values of C_{max} and AUC obtained with 2 preparations were analyzed using an analysis of variance (ANOVA) procedure. Mean values for pharmacokinetic parameters were determined and SD, SEM, and 90% confidence intervals (CI) were calculated. Different ratios of Test:Reference were compared (Table 1).

RESULTS AND DISCUSSION

Mean weight of each filled capsule was 498.9 ± 11.76 mg and 205.3 ± 0.15 mg for Test and Reference, respectively.

Table 1. Summarized Bioavailability Data of Test and Reference Products*

Parameter	Ref (n = 8)	Test (n = 8)	Ratio (Test/Ref)		90% Confidence Intervals	
			Result	Limits	Result	Limits
C_{max} (μg/mL)	7.1658	8.0755	1.1270	0.8-1.2	110.65-114.79	80-120
$AUC_{(0\text{-}8\ h)}$ (μg/mL/h)	33.6192	37.9718	1.1295	0.8-1.2	112.28-113.62	80-120
Ln C_{max}	1.9693	2.0889	1.0607	0.8-1.2	105.12-107.02	80-125
Ln AUC	3.5151	3.6368	1.0346	0.8-1.2	103.29-103.63	80-125

*Ref indicates Reference.

Drug content was 99.38% ± 1.251% wt/wt and 99.72% ± 1.024% wt/wt for Test and Reference, respectively.

Comparative data of KPF plasma concentration of Test and Reference at each sampling time in all the volunteers is shown in Table 2. The time to reach maximum plasma concentration (t_{max}) in all the volunteers was 2 hours for both the products tested. Plasma concentration-time profiles for both the products are shown in Figure 1. Test showed slightly higher values of plasma concentration as compared with Reference at each sampling time for all the volunteers.

The bioavailability parameters viz C_{max}, t_{max}, and $AUC_{(0\text{-}8\ h)}$ for both the products are shown in Table 3. Marginal increase in C_{max} value was observed for Test (8.0755 μg/mL) over Reference (7.1658 μg/mL). Also, $AUC_{(0\text{-}8\ h)}$ for Test (37.9718 μg/mL/h) was slightly higher than that of Reference (33.6192 μg/mL/h). Summarized bioavailability data of Test and Reference, Test/Reference ratios, and 90% CI are shown in Table 1. The limits for bioequivalence mentioned are in accordance with US Food and Drug Administration Center for Drug Evaluation and Research (USFDA CDER) guidance for bioavailability and bioequivalence studies.[20] For C_{max}, the Test/Reference ratio was 1.1272 with 90% CI between 1.11 and 1.15. It was observed that C_{max} ratios (Test/Reference), as such and log transformed, were well within the prescribed limits for bioequivalence, indicating no significant difference between both the products in terms of C_{max}. Since t_{max} was also the same (2 hours) for both the products (Table 3), it can be concluded that there was no significant difference in the rate of absorption of KPF from these products.

$AUC_{(0\text{-}8\ h)}$ ratios (Test/Reference), as such and log transformed, are shown in Table 1. $AUC_{(0\text{–}8\ h)}$ values for Test

Table 2. Ketoprofen Plasma Concentration of Test and Reference, at Each Sampling Point (n = 8)*

Volunteer	Plasma Concentration of Reference, μg/ml Sampling Time, hours							Plasma Concentration of Test, μg/mL Sampling Time, hours						
	0	0.5	1	1.5	2	3	8	0	0.5	1	1.5	2	3	8
V1	0	2.324	5.289	6.158	7.123	6.167	1.072	0	3.428	6.432	7.253	7.836	6.934	1.348
V2	0	2.393	5.364	6.167	7.146	6.119	1.064	0	3.449	6.287	7.268	7.924	6.881	0.926
V3	0	2.648	4.933	6.148	7.198	6.138	1.067	0	3.436	6.673	7.248	8.39	6.829	0.887
V4	0	2.439	5.136	6.153	7.171	6.173	1.073	0	3.438	6.479	7.251	8.053	6.881	1.048
V5	0	2.228	5.338	6.235	7.371	6.118	1.064	0	3.674	6.423	7.523	7.983	6.834	1.231
V6	0	2.432	5.612	6.415	7.239	6.367	1.107	0	3.447	6.345	7.514	8.17	7.127	1.172
V7	0	2.334	5.236	6.049	7.022	6.129	1.065	0	3.528	6.919	6.936	8.237	6.529	1.029
V8	0	2.687	5.028	6.083	7.056	6.118	1.063	0	3.128	6.218	6.988	8.011	7.012	0.998
Mean	–	2.436	5.242	6.176	7.1657	6.166	1.072	–	3.441	6.472	7.248	8.076	6.878	1.080
SD	–	0.1586	0.2124	0.1116	0.1092	0.0840	0.0147	–	0.1513	0.227	0.211	0.1803	0.173	0.158
SEM	–	0.0561	0.0751	0.0394	0.0386	0.0297	0.0052	–	0.0535	0.080	0.075	0.0637	0.061	0.056
Upper 90% CI	–	2.5419	5.3843	6.2507	7.2389	6.2224	1.0817	–	3.5423	6.624	7.389	8.1963	6.994	1.186
Lower 90% CI	–	2.3294	5.0997	6.1013	7.0926	6.1098	1.0620	–	3.3397	6.320	7.106	7.9547	6.763	0.974

*CI indicates confidence interval.
– indicates not applicable.

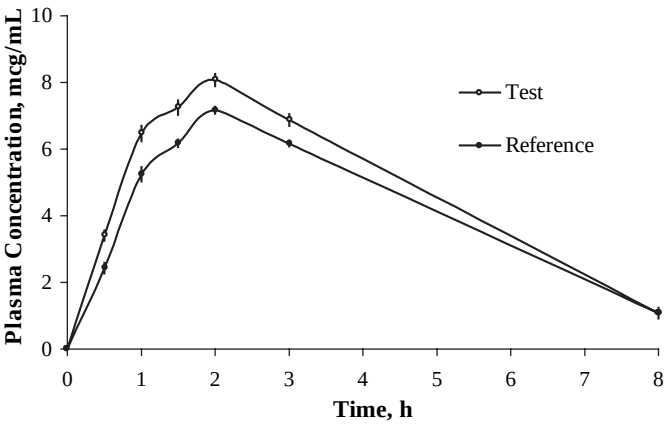

Figure 1. Comparative plasma concentration-time profiles of Test and Reference products.

were not significantly different than those for Reference. These observations suggested that the Test and Reference were not significantly different in terms of bioavailability.

When used with lipids, A 200 retards the drug release. The surface of A 200 nanospheres is covered with hydroxyl groups that interact with each other via hydrogen bonding. Our earlier observation indicated that with incorporation of A 200 in self-emulsifying systems, the average droplet size of the resultant (micro) emulsion increased and drug release from the droplets slowed. This effect of A 200 was attributed to its gelling in oils due to formation of hydrogen bonds between polar silanol (Si-OH) groups.[21]

Many attempts have been reported in the literature for delivery of lipophilic moieties using the SEF approach, claiming enhanced bioavailability.[16] Lipophilic drugs are generally transported via transcellular pathways and the majority of such drugs have been shown to be the substrates for efflux mechanisms at apically polarized cell membrane of enterocytes. These efflux systems comprise P-gp and CYP3A4 carriers, which carry the drug molecules from cytosol to apical cell membrane, thus contributing to low oral bioavailability of such drugs. Lipids as well as surfactants have been shown to be the inhibitors of different efflux carriers, thus contributing to enhanced cellular uptake of lipophilic drugs. Nerurkar et al[22] reported that apical (AP) to basolateral (BL) flux of a model peptide drug, which is not a substrate for efflux carriers, did not increase in the presence of Tween 80 when used at a concentration as high as 1% wt/vol across Caco-2 monolayers. Similar study for another model peptide drug, which is a substrate for efflux carriers, revealed that its AP to BL flux across Caco-2 monolayers increased significantly ($\backsim$3 times) in the presence of Tween 80 at the concentration 0.05% wt/vol.

KPF, a propionic acid derivative, is a relatively polar drug, owing to the presence of keto (C=O) and carboxylic acid (-COOH) functional groups. Adequate oral bioavailability and rapid absorption of KPF are indicative of its cellular uptake process that is not subject to efflux system. Cellular uptake of KPF may perhaps be mediated primarily through the paracellular pathway. Probably the effect of the used system components on this uptake route is not prominent, hence no significant increase in bioavailability was observed when KPF was administered in SEF by the oral route. However gelling of SEF with A 200, which has shown its effect on liquid crystal viscosity and the drug release in vitro, did not retard the rate and extent of KPF absorption from such gelled system.

Table 3. Bioavailability Parameters for Test and Reference Products*

	C_{max}, µg/ml		t_{max}, hours		$AUC_{(0–8\ h)}$, µg/mL/h	
Volunteer	**Reference**	**Test**	**Reference**	**Test**	**Reference**	**Test**
V1	7.123	7.836	2	2	34.5338	38.6055
V2	7.146	7.924	2	2	33.3385	37.4030
V3	7.198	8.39	2	2	33.3445	37.6755
V4	7.171	8.053	2	2	33.4438	37.8868
V5	7.371	7.983	2	2	33.4428	38.3768
V6	7.239	8.170	2	2	34.5273	39.0915
V7	7.022	8.237	2	2	33.1205	37.0288
V8	7.056	8.011	2	2	33.2025	37.7063
Mean	7.1658	8.0755	2	2	33.6192	37.9718
SD	0.1092	0.1803	–	–	0.5732	0.6759
SEM	0.0386	0.0637	–	–	0.2026	0.2390
Upper 90% CI	7.2389	8.1963	–	–	34.0032	38.4246
Lower 90% CI	7.0926	7.9547	–	–	33.2365	37.5190

*CI indicates confidence interval.
– indicates not applicable.

CONCLUSION

Based on the results of the present study, it is apparent that the gelled SEF containing KPF did not significantly alter its bioavailability as compared with that of an immediate release solid dosage form when administered to human volunteers by the oral route.

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