Chronobiological and chronopharmacological studies of ketoprofen and its solid dispersion form using adjuvant arthritis model in rats

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Received 30 January 2004; revised 8 October 2004

Chronobiology of rheumatoid arthritis (RA) was studied using a standard adjuvant arthritis animal model. Chronopharmacology of ketoprofen, and its solid dispersion forms was also studied. Temporal variations in the degree of articular inflammation (paw volume) and progression of articular destruction were studied by injecting Freund’s Complete Adjuvant (FCA) at 0800 and 2000 hrs. Temporal variations in anti-inflammatory effects and ulcerogenic effect were also studied by administration of plain ketoprofen (20 mg/kg) and its solid dispersion with hydroxypropyl β-cyclodextrin (equivalent to 20 mg/kg of ketoprofen) at the same time points (0800 and 2000 hrs) twice weekly for 22 days. Solid dispersion of ketoprofen was found to be more effective in inhibiting progression of RA. The incidence and severity of ulcers was found to be less with the solid dispersion. The protective effect of ketoprofen and its solid dispersion was significantly higher when these were administered at 0800 hrs. The incidence of ulceration was more in 2000 hrs group. Thus, it was observed that in the adjuvant induced arthritis model, inflammation and articular damage was significantly greater in the rest period of diurnally active rats than in the activity phase. KPF and its solid dispersion showed better protection from inflammation in the morning than in the evening.

Keywords: Chronopharmacology, Gastric ulceration, Ketoprofen, Rheumatoid arthritis

Mammalian body is characterized by a complex time structure of biological rhythms. These rhythms have different frequency range and are found at all levels of biological organization ranging from substrate concentration to enzymatic activity, sub-cellular particles to cell, tissue cultures to isolated organ and to the organism as a whole. Biological rhythm is an essential component of homeostasis.

Biological rhythm is a regular variation in biophysical or biochemical processes with time, occurring in a predictable manner. The most studied rhythm is the one which has a period of approximately 24 hr, known as circadian rhythm. The environmental factors that maintain the periodicity of a biological rhythm are the light-dark, sleep-activity and feeding-fasting periods.

Chronopharmacology is the study of rhythmic predictable time differences in the effects and/or pharmacokinetics of drugs. It investigates the effects of drugs upon temporal changes in biological functions as well as drug effects as a function of biological timing. Also, it is likely that the ebb in the disease manifestation may influence therapeutic effectiveness of drugs. Hence, it becomes important to study the rhythmic pattern of the disease in itself. Thus, due to these temporal variations in drug effects and pharmacokinetics and the temporal fluctuations in the disease, the concept of chronotherapy has emerged. This concept of chronotherapy can be applied to treat human diseases such as inflammation, ulcers, cancer, asthma, hypertension etc., so that therapeutic effects can be optimised and undesired or toxic effects to be minimised.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by joint pain and swelling with multiple extra-articular manifestations. The signs and symptoms of RA vary within days and between days. The classic morning stiffness observed in patients with RA is so characteristic that has become one of the diagnostic criteria of the disease. Clinically it is treated with various nonsteroidal anti-inflammatory drugs like tolmetin, indomethacin, piroxicam, ketoprofen, etc.

Ketoprofen’s rapid onset of action together with its accumulation in synovial fluid makes it well suited for treating acute inflammation and chronic arthritis.
All NSAIDs including ketoprofen are known to produce gastrointestinal irritation and ulceration due to poor solubility of their crystals in gastric fluid and inhibition of cytoprotective prostaglandins. Solid dispersion of ketoprofen with hydroxypropyl β-cyclodextrin has been reported to improve solubility and dissolution rate and also reduce the ulcerogenic effect of the drug.

It was therefore proposed to study both, the circadian variations in the experimental inflammatory process using adjuvant arthritis model and circadian variations in the pharmacological effects of ketoprofen and its solid dispersion form to optimize the therapy.

**Materials and Methods**

Ketoprofen (KPF) and hydroxypropyl β-cyclodextrin (HPB) were generously donated by Rhone Poulenc (India) and Amiazo (USA) respectively. Freund’s complete adjuvant (FCA) was obtained from BARC, Mumbai, India.

Preparation of solid dispersion of ketoprofen — Solid dispersion was prepared by using KPF and HPB (1:1 molar ratio) by co-evaporation method using ethanol as a solvent and evaluated as described by Nagarsenkar et al. In this co-evaporation method, 125 mg of KPF (MW=254.29) and 674.23 mg of HPB (MW=1371.6) were used. This mixture was evaporated to get a co-precipitate with 1:1 water alcohol mixture. KPF was dissolved in 5 ml alcohol in an evaporating dish. HPB was dissolved in 4 ml of double distilled water (DDW) and added drop-wise to the alcoholic solution of KPF being stirred by a magnetic stirrer. On complete addition of HPB solution, 1 ml of DDW was added to the test-tube of HPB solution, rinsed and the rinsing was transferred to the evaporating dish. The evaporating dish was covered with a glass plate and the contents stirred for 1 h at room temperature with a magnetic stirrer. At the end of 1 h the evaporating dish was uncovered and the contents evaporated at a temperature of 45°-50°C. When almost dry, the evaporating dish was transferred to a dessicator and stored overnight at room temperature. The solid was then scraped off and sieved through an 85-mesh sieve to get a powder. The yields in all cases were about 95%.

Chronobiology and Chronopharmacology of ketoprofen (KPF) and its solid dispersion (KPF-HPB) using adjuvant induced rheumatoid arthritis model — Three weeks prior to the experiment, the inbred albino rats of either sex weighing between 150-200 g were housed in room on a 12 hr light dark cycle (Lights on 0700 to 1900 hrs) and thereafter throughout the study. The animals had free access to food and water. All experimental study protocols were reviewed and approved by Institutional Animal Ethics Committee. The experiments were performed in two (0800 and 2000 hrs) temporal series within a period of 24 h. For each series the animals were subdivided into three groups of nine, seven and eight rats each, i.e. control group, KPF treated group and KPF-HPB treated group, respectively. The study was carried out using progressive arthritis model to study the effect of drug on the progression of the disorder.

Progressive arthritis model — Arthritis in all the six groups was produced by injecting 0.1 ml Freund’s complete adjuvant (FCA) in the plantar region in the left hind paw of rats on day 0. One set of animals received injection of FCA at 0800 hrs and another set of animals received injection of FCA at 2000 hrs during the 24 hr day-night cycle. It was proposed to study these two time points since they represent the start of rest and activity period respectively. As mentioned earlier, each of these sets was divided into 3 groups, i.e., control group (n=9), KPF treated group (n=7) and KPF+HPB treated group (n=8). A dose of solid dispersion equivalent to 20 mg/kg of KPF and KPF alone (20 mg/kg) suspended in water using tragacanth 1% as suspending agent, were administered orally twice weekly at 0800 hrs to one set of animals and at 2000 hr to the other set from day 1 to 22 respective to the time of FCA insult. Control group received vehicle only. The dose of KPF was chosen based on the human dose of 150 mg/70 kg adult and was converted to rat dose by surface area ratio method. The hind paw volume (right and left) was measured just before injection and then twice weekly from day one onwards at both 0800 and 2000 hrs for every group irrespective of time of FCA insult, using plethysmometer (Ugo Basile-Italy). As per recorded data, the FCA type of arthritis usually peaks within 14 to 16 days. Hence paw volume was measured twice a week until the 12th day and then was measured daily until the 18th day. Also, preliminary studies in our laboratory did not show any significant difference in volume on daily measurements. Hence, paw volumes were measured at 0800 hrs and at 2000 hrs in order to investigate any temporal changes in volume irrespective of adjuvant insult time. Twice a
week schedule consisted of dosing and measuring paw volume on the 4th, 7th, 11th, 14th, 18th and 21st day.

At the end of the study (22nd day), the animals were sacrificed, hind limbs were resected and immediately fixed in formalin (10%). The radiographs were obtained with a conventional radiographic unit (Siemens 300 mA Pleophus-D) at 3 KV and 4 mA for 30 sec. The radiographs were evaluated by method of Kawai et al. and Clark et al. Four features i.e. bone demineralization, bone erosion, periostitis and phalanges alignment were evaluated using grades of 0 to 4 (with 0 including no or nominal and 4 severe change) for each feature. The sum of these four findings, defined as articular score was obtained for each paw.

Stomachs of the same animals were removed, opened along the greater curvature, washed with physiological saline and examined for ulceration. The degree of stomach injury was evaluated as described by Nambu et al. Evaluation of the articular score and that of ulceration was carried out by two evaluators who had no knowledge of treatment.

Statistical analysis—Statistical analysis was performed using SAS 8.02 (Statistical Analysis Software Inc., USA). Results are expressed as mean ± standard deviation using MS excel. One-way analysis variance for pharmacokinetic parameters and PROC GLM (procedure general linear model) followed by Dunnett’s t and t test (parametric test) and Kruskal Wallis test (non-parametric test) were used for edema, % inhibition in progressive arthritis model, articular destruction and ulcer score to test significance level in ketoprofen and solid dispersion of ketoprofen treated groups as compared to control.

### Results

#### Progressive arthritis model

**Hind-paw volume**—The effects of various treatments on adjuvant injected left hind paw inflammation (0800 and 2000 hrs groups) is shown in Table 1. There was sharp increase in edema till 18th day and thereafter almost a plateau was observed in all the groups. There was significantly greater percentage of edema at the resting phase (0800 hrs) than at the activity phase (2000 hrs) in the rats. This temporal variation was observed in the untreated control group and was irrespective of the time of FCA insult and the time of drug administration. KPF and its dispersion form produced a significant reduction in left hind paw volume when compared with control group (0800 hrs). The percentage inhibition of edema was found significantly more in KPF+HPB treated animals as compared to animals which received plain KPF (P<0.05) at both time points (0800 and 2000 hrs).

**Radiographic changes**—Table 2 shows the effect of KPF and KPF+HPB on articular destruction in the hind paws of the rats at 0800 and 2000 hrs groups. The articular score in the untreated control group was significantly greater in rats injected with FCA at 0800 hrs than 2000 hr, which was applicable to the individual scores too. This temporal variation in articular damage was observed in the right uninjected paw and the drug treated groups. Twice weekly dose of KPF as well as KPF+HPB significantly inhibited the progression of articular damage in both hind paws, as shown by the articular score and the individual items with the exception of erosion. Solid dispersion form of ketoprofen was found to be significantly superior to plain ketoprofen in inhibiting the articular damage.

### Table 1—FCA induced maximum % edema and inhibition of edema by KPF and KPF+HPB in the left hind paw of rats in progressive arthritis model measured at two time points at 0800 and 2000 hrs for each group on same day

<table>
<thead>
<tr>
<th>Time of FCA administration</th>
<th>Control (n=9)</th>
<th>KPF (n=7)</th>
<th>KPF+HPB (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0800</td>
<td>79.22 ± 9.5</td>
<td>41.82 ± 5.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.77 ± 4.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2000</td>
<td>68.59 ± 8.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.27 ± 10.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.64 ± 8.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2000</td>
<td>71.48 ± 8.67&lt;sup&gt;e&lt;/sup&gt;</td>
<td>38.91 ± 10.25&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22.14 ± 9.42&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>0800</td>
<td>74.28 ± 6.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.18 ± 6.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.19 ± 7.55&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> significant to control group by using one way ANOVA (P<0.05)
<sup>b</sup> significant to KPF treated group by using one way ANOVA (P<0.05)
<sup>c</sup> significant to 0800 hrs group when drug was administered at 0800hr by using one way ANOVA. (P<0.05). (comparison within the group)
<sup>d</sup> significant to 0800 hrs group when drug was administered at 0800 hrs by using one way ANOVA. (P<0.05)
<sup>e</sup> significant to 2000 hrs group when drug was administered at 2000 hrs by using one way ANOVA. (P<0.05)
The representative radiographs of hind paw of rats from control and treated group are shown in Fig. 1, which basically show the edema and demineralization. In the right hind paw, though there was significant protection with both the treatments, there was no significant difference between 0800 and 2000 hrs results. This suggested that no time related changes were observed for the secondary lesions in arthritis.

Ulcer formation—Temporal variation in ulcer formation was found to be associated with chronic treatment with ketoprofen and its solid dispersion form. Table 3 shows the ulcer scores observed for the treated and untreated groups at both time points. In comparison to the untreated control group, which showed no ulcers, KPF and its dispersion treated groups showed significant gastric ulceration. The 2000 hrs treated animals showed greater incidence of ulceration than 0800 hrs treated animals. Solid dispersion form of ketoprofen showed a significant reduction in the ulcer score when administered at both 0800 and 2000 hrs.

Discussion

The present study demonstrated that ketoprofen and its solid dispersion form were able to inhibit the progression of articular destruction in rat adjuvant arthritis. In addition, both temporal variations in anti-inflammatory effect of ketoprofen and its solid dispersion form (reduction in hind paw volume) as well as an inhibitory effect on articular destruction in rat model of human rheumatoid arthritis, was observed.

It has been observed that irrespective of time of FCA insult, the progression of disease was more during the resting phase (0800 hrs) than in the activity phase (2000 hrs), that is similar to usual observation in human RA, where the stiffness and swelling of the joints is more in the early morning (during rest).

The medical implications of chronobiology are being increasingly acknowledged with the knowledge that biological rhythms affect pathophysiological process. It is, therefore, logical that the drug administration should be adjusted to mimic body rhythms.
rhythms. Labrecque and Reinberg\(^2\) have revealed a circadian, circannual variation in effectiveness, toxicity, and pharmacokinetics with NSAIDs and side effects produced by NSAIDs like gastric irritation and ulceration. In the present study, the temporal variation in anti-inflammatory and ulcerogenic effect has been observed with ketoprofen and its solid dispersion form. Ketoprofen like other NSAIDs is known to produce gastric damage due to poor solubility of its crystals in gastric fluid and inhibition of cytoprotective prostaglandins. A solid dispersion of ketoprofen with hydroxypropyl \(\beta\)-cyclodextrin has been reported to improve solubility and dissolution rate and also reduces the ulcerogenic effect of the drug\(^2\).

In the present study, the solid dispersion of ketoprofen not only improved the anti-inflammatory effect but also produced less gastric damage when compared to plain ketoprofen. The pharmacokinetic studies of ketoprofen and its solid dispersion form in rats, carried out in our laboratory (data shown in Table 4) showed increased plasma KPF levels when administered in the morning 0800 hrs than evening 2000 hrs although this observation was not statistically significant. Absorption of drug from its solid dispersion form was significantly faster than plain drug as indicated by smaller \(T_{\text{max}}\) values (Table 4). Thus, better solubility and increased bioavailability of drug from solid dispersion form must be the factor contributing to enhanced anti-inflammatory activity and reduction in ulcerogenic effect.

Savarino et al.\(^10\) have shown time dependent ulcerogenic effect of indomethacin on gastric mucosa. Ulceration has been found to be greater in dark period of cycle. A similar phenomenon appears to be occurring in the present study, where KPF caused increased ulceration during activity period of the animal.

![Fig. 1](image_url)

**Fig. 1**—Representative radiographs of rat hind paws after 22 days adjuvant injection. KPF and KPF+HPB groups showing marked inhibition of soft tissue swelling and articular destruction as compared with control in both the time points. Right (A) and Left (B) hind paws of Control, KPF and KPF+HPB treated groups at 0800 hrs showing tissue swelling and articular destruction. Right (A) and Left (B) hind paws of Control, KPF and KPF+HPB treated groups at 2000 hrs.
The circadian changes in the inflammatory process may be because of time dependent changes in the secretion and metabolism of inflammatory autacoids or sensitivity of tissue to inflammatory agents. Although, various cytokines might influence articular destruction in rheumatoid arthritis in man, IL-1 and TNFα seem to make a major contribution to tissue damage by inducing release of proteolytic enzymes from synovial cells and chondrocytes. Davidson et al. have also found that COX-inhibitor, KPF enhanced the increased phagocytic activity in presence of endotoxin and IL-1, indicating suppression of endogenous prostaglandins. These actions of KPF may help reduce pain and inflammation in arthritic patients.

In conclusion, the chronopharmacological studies with KPF and its solid dispersion have provided experimental evidence to support clinical impression that morning ingestion of solid dispersion to diurnally active rats was superior to evening ingestion.

The conventional assumption that a constant rate of drug delivery provides constant blood levels and effects over 24 hr period needs reconsideration. The anti-inflammatory activity of KPF and its solid dispersion was maximal in the morning with minimal side effects. This data could be used in clinical situations to optimize and to individualize drug treatment of arthritic diseases. Time of drug administration may be an important factor that could explain unexpected variations in the drug effects.

Acknowledgement

The authors are thankful to AICTE, New Delhi for awarding R&D grant for the present work. The authors also thank Dr. Shailendra Bhuse, X-ray Sonography & Echo-Cardiography Clinic, Mumbai for radiographic evaluation and Ms. Shalaka Kale, Bio-statistician, SIRO Clinpharm Pvt. Ltd., Mumbai for helping with the statistical analysis.

References


