

FORMULATION AND PHARMACOKINETIC EVALUATION OF COMPRESSED SLOW RELEASE SUPPOSITORIES OF DICLOFENAC SODIUM IN RABBITS

RAMAKRISHNA SISTLA AND PRAKASH V. DIWAN*

Pharmacology Division, Indian Institute of Chemical Technology, Hyderabad 500 007, INDIA

Compressed slow release suppositories of diclofenac sodium (25mg) (CAS 15307-79-6) were formulated employing stearic acid or bees wax at different concentrations (5, 10 and 15%) in polyethylene glycol (PEG) 4000 base. In vitro drug release studies were conducted to optimize the concentration of stearic acid and bees wax. Selected suppositories were further evaluated for pharmacokinetic performance in New Zealand rabbits by comparing the results with those of conventional (immediate release) suppositories. Results revealed significant reduction in C_{max} and prolonged t_{max} values for slow release suppositories. However the extent of absorption represented by $AUC_{0-\infty}$ did not change significantly ($p>0.05$) between slow release and conventional suppositories. Sustained plasma concentrations of diclofenac sodium were observed for a period of 12 hrs in case of slow release suppositories compared to conventional plain suppositories indicating potential usefulness of the dosage forms for sustaining the therapeutic activity.

Keywords: *Compressed slow release suppositories; Nonsteroidal anti-inflammatory drugs (NSAID); Pharmacokinetics; (CAS 15307-79-6); Diclofenac sodium.*

Introduction

Rectal administration of nonsteroidal anti-inflammatory drugs (NSAID) have been proposed with a view to minimise the gastrointestinal toxicity of these drug upon chronic oral administration. We have earlier reported about the formulation and pharmacokinetic evaluation of compressed suppositories of diclofenac sodium (CAS 15307-79-6) (1,2). The studies revealed bioequivalence of diclofenac sodium from rectal suppositories compared to oral administration in rabbits and healthy human volunteers at the doses of 25 mg and 50 mg respectively. As diclofenac sodium has a short biological half-life of 1-2 hrs and also it undergoes extensive first pass elimination (3,4), a sustained rectal suppository formulation would be more beneficial than an oral slow release dosage form. Hence, attempts were made to formulate slow release suppositories of diclofenac sodium for rectal administration, employ-

ing water insoluble excipients like stearic acid or bees wax (at different concentrations of 5, 10 and 15%) in a water soluble polyethylene glycol (PEG) 4000 base. The concentration of stearic acid or bees wax in these formulations was optimized by conducting in vitro drug release studies. Selected formulations were evaluated for pharmacokinetic performance in healthy male rabbits.

Materials and Methods

Polyethylene glycol (PEG) 4000, beeswax, stearic acid, magnesium stearate and talc were of pharmaceutical grade. Acetonitrile, ethyl acetate and methanol were of HPLC grade (Qualigens, Bombay). Other reagents were of high pure grade and were obtained commercially. Diclofenac sodium was a gift sample from M/s Tablets India Ltd, Madras, India.

Formulation of Slow Release Matrix Suppositories: Diclofenac sodium (25 mg per suppository) was dispersed with continuous stirring in molten mass of PEG 4000 containing 5, 10 and 15% of stearic acid or bees wax separately. The dispersion was allowed to solidify while stirring. The solidi-

* Correspondence: Dr. Prakash V. Diwan: e-mail: diwan@iict.ap.nic.in

fied mass was then pulverized and passed through 20 mesh screen. The resultant granules were lubricated with talc and magnesium stearate (1% each) and were compressed into 0.8 g suppository formulations (3 with beeswax and 3 with stearic acid) each in 50 number were prepared. Conventional (immediate release) suppositories containing 25 mg drug, without stearic acid or bees wax were also prepared for comparison. The hardness of all suppository formulations was studied employing a previously reported method (5). For comparison, plain suppositories containing 25 mg. diclofenac sodium were also included in the experiment. A volume of 600 ml 0.2 M phosphate buffer (pH 7.4) was used as the dissolution medium. Each suppository was placed directly in a metallic basket without any artificial membrane or filter paper and immersed in the dissolution medium maintained at $37 \pm 5^\circ\text{C}$ with a magnetic stirrer bar placed in the dissolution medium rotating at 150 rpm. Aliquots of samples (1 ml) were drawn at fixed time intervals up to 12 hrs, each time replacing the fluid with drug free dissolution medium. The samples were assayed for drug content at 275 nm using a double beam spectrophotometer (Shimadzu, Japan), after suitable dilution with drug free dissolution medium. The experiment was performed in quadruplicate for each suppository. The mean percent drug released at various time intervals from different suppositories was calculated and plotted against time. From These graphs, T 50%, and T90% (time for 50% and 90% drug release) values were calculated.

Pharmacokinetic evaluation of slow release matrix suppositories of diclofenac soduim in rabbits: Male New Zealand white rabbits weighting range 2.5-3.0 kg were procured from Biological E Ltd, Hyderabad, India. Animals were housed in air conditioned room maintained at $25 \pm 1^\circ\text{C}$ with a relative humidity of $55 \pm 5\%$. Water was provided *ad libitum* and animals were fed with standards rabbit pellets (Hindustan Lever Ltd, Bombay, India).

Drug products: Slow release suppositories containing either 10% stearic acid or 10% bees wax were used as test preparations. Plain (immediate release) suppositories were used as reference product. All suppositories contained 25 mg of diclofenac sodium.

Procedure: The study was conducted as three way randomized cross over manner in six rabbits with a washout period of one week between each admini-

stration. Rabbits were fasted for 24 hrs prior to conducting the experiment. Water was provided during fasting period but not after drug administration.

For rectal administration of different suppositories, rabbit was secured in supine position and a suppository was inserted 2 to 3 cm inside the anus which was closed with plastic clip, after insertion, to prevent possible leakage. Blood samples (1 ml) were collected from marginal ear vein of each rabbit into heparinized glass tubes at predetermined time intervals up to 12 hrs after the administration of the suppositories. Plasma was separated immediately by centrifugation of blood samples and stored at -20°C until further analysis.

The plasma were analysed for diclofenac sodium using a sensitive HPLC method (6) with minor modifications. Briefly, the method involves acidification of 0.2 ml of plasma containing 20 μg of naproxen (internal standard) with 0.5 ml of 5M HCl. After vortexing for 1 min, the samples were extracted with 5 ml of ethyl acetate by mechanical shaking for 10 mins. A after centrifugation of samples at 3000 rpm for 15 min, 4 ml of organic phase was withdrawn, into a separate clean glass tube and was evaporated to dryness under a thin stream of nitrogen. The dried residue was reconstituted with 200 μl of acetonitrile and a volume of 25 μl was injected into HPLC system (Shimadzu LC 6A) equipped with reverse phase C_{18} column (5x250 mm, 5 μ Machery Nagel, Duren Germany). Mobile phase consisted of acetonitrile: water (50:50) and the pH was adjusted to 3.2 using phosphoric acid. Detector wavelength was 278 nm, flow rate was 1 ml/min and analysis was performed at 0.08 aufs. Linearity was observed in the concentration range of 2 to 20 μg with this method. Interday and intraday variations were found to be less (CV less than 2.1% for interday and 2.7% for interday). The mean percent drug recovered for a concentration of 10 μg was 96.8 (interday) and 96.2 (intraday) indicating that the method was sufficiently precise and reproducible.

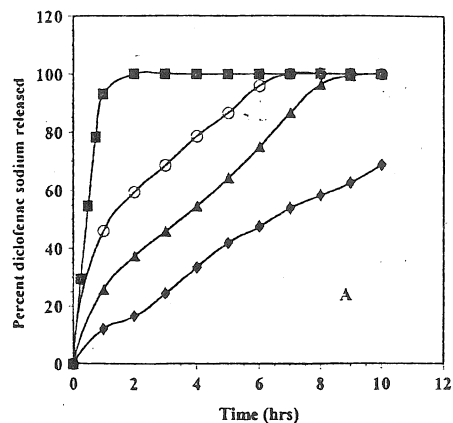
Pharmacokinetic and statistical analysis: Peak plasma concentration (C_{max}) and time of their occurrences (t_{max}) were read directly from the individual plasma concentration-time profiles. The other pharmacokinetic parameters such as terminal half-life ($t_{1/2}$), area under the curve (AUC_{0-x}) and mean residence time (MRT) were calculated for each animal by using equations based on noncompartmental analysis of pharmacokinetic data (7). Statistical significance of the differences between the pharmacokinetic parameters obtained after administration of different suppositories of diclo-

fenac sodium were evaluated using ANOVA followed by Dunnet's multiple comparison procedure (8).

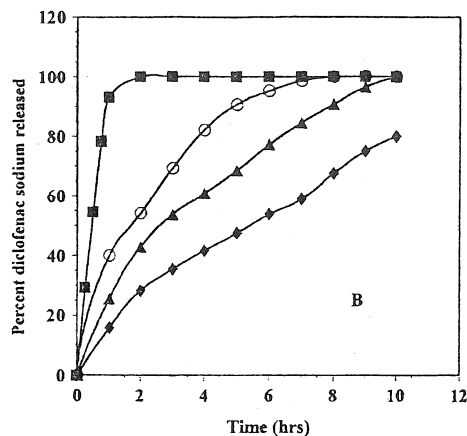
Results

The mean percent release Vs time profiles of diclofenac sodium from compressed slow release suppositories with different concentrations (5, 10 and 15%) of either stearic acid or bees wax are shown in Figs. 1A and 1B respectively. Suppositories containing stearic acid or bees wax displayed slow release characteristics and the slow release was dependent on the amount of either stearic acid or bees wax present.

The plasma concentration time profiles (mean \pm SE) of diclofenac sodium obtained after administration of plain suppositories and slow release suppositories containing either stearic acid or bees wax (10%) in rabbits are shown Fig. 2. The different pharmacokinetic parameters obtained are given in Table 1. Slow release suppositories displayed significant ($p > 0.05$) lowering of C_{max} values and their t_{max} values were significantly prolonged compared to those of plain suppositories. There was about 50% reduction in C_{max} values in case of slow release suppositories. Relatively high plasma concentrations of diclofenac sodium were observed at 12 hrs for slow release suppositories. However $AUC_{0-\infty}$ values did not differ significantly ($p > 0.05$) between plain suppositories and slow release matrix suppositories indicating that the extent of absorption of the drug remained unchanged in this single dose study. The differences in all pharmacokinetic parameters were insignificant ($p > 0.05$) between suppositories containing 10% stearic acid and 10% bees wax.



■ Plain suppositories ▲ 10% Bees wax
 ○ 5% Bees wax ◆ 15% Bees wax



■ Plain suppositories ▲ 10% Stearic acid
 ○ 5% Stearic acid ◆ 15% Stearic acid

Fig. 1. *In vitro* release profile of diclofenac sodium from compressed slow release matrix suppositories containing bees wax (A) or stearic acid (B). (Mean of 4 experiments)

Table 1. Pharmacokinetic parameters obtained after rectal administration of slow release suppositories of diclofenac sodium (25 mg) in rabbits

Parameter	Plain Suppositories	Suppositories containing	
		10% Bees wax	10% Stearic acid
C_{max} ($\mu\text{g/ml}$)	63.65 \pm 3.69	30.59 \pm 0.47	30.94 \pm 1.78
t_{max} (hr)	1.0 \pm 0.18	2.83 \pm 0.31	3.0 \pm 0.37
$t_{1/2}$ (hr)	1.84 \pm 0.08	3.44 \pm 0.12	3.31 \pm 0.18
MRT (hr)	3.11 \pm 0.12	5.86 \pm 0.24	6.03 \pm 0.14
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h/ml}$)	196.83 \pm 15.50	201.38 \pm 6.23	188.59 \pm 5.78
% Bioavailability	100.00	102.3	95.8

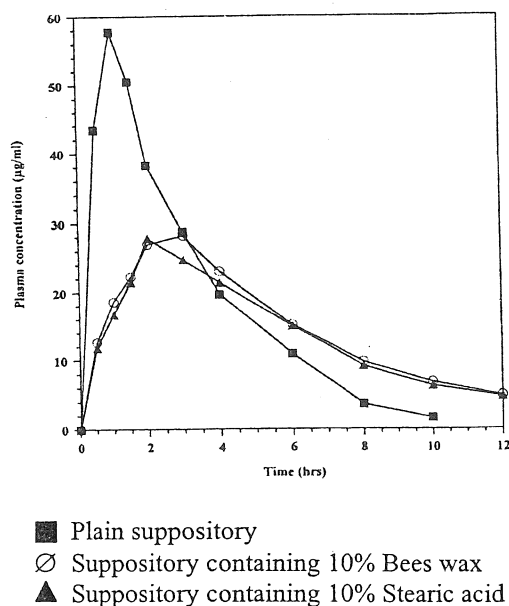


Fig. 2. Plasma concentration-time profile of diclofenac sodium (25 mg) after rectal administration of different slow release suppositories in rabbits

Discussion

Rectal administration of NSAIDs is gaining much attention in the recent years and is used to avoid the gastrointestinal irritation of these drugs upon oral administration. This route of administration is also useful in delivering drugs that are prone to extensive first pass elimination by oral route. Conventional fatty bases are not suitable to tropical climates like India. Hence, polyethylene glycol (PEG) 4000 was selected as the suppository based on its physiological inertness and temperature stability.

Diclofenac sodium, a potent acidic NSAID is known to cause severe gastrointestinal side effects upon oral administration. The drug has short biological half-life and needs to be administered in multiple doses daily (4). Sustained release formulations of diclofenac sodium are administered with the convenience of once daily regimen. It is known that diclofenac

sodium undergoes extensive first pass metabolism (3). Since rectal administration partially avoids first pass metabolism of drugs, sustained release dosage forms of diclofenac sodium for rectal administration are more suitable than for oral administration.

In the present investigation, compressed PEG 4000 suppositories of diclofenac sodium (25 mg) containing different proportions of stearic acid/bees wax were formulated with a view to develop slow release matrix type suppositories for slow sustained release of diclofenac sodium. It is clear from Figs. 1A and 1B that incorporation of either stearic acid or bees wax significantly prolonged the release of diclofenac sodium compared to plain suppositories. The decrease in drug release was dependent on the amount of either stearic acid or bees wax present. Suppositories containing 10% bees wax and 10% stearic acid displayed $T_{90\%}$ values of 7.4 and 6.8 hrs respectively, indicating 90% drug release in about 7.5 hrs whereas $T_{90\%}$ values for plain suppositories were around 1 hr. Suppositories containing more than 10% concentration of either beeswax or stearic acid displayed very slow release and $T_{90\%}$ values were more than 10 hrs. Thus, blending a water insoluble carrier with water soluble PEG 4000 resulted in suppositories with slow drug release characteristics.

As the prevailing plasma concentrations are indicative of pharmacological activity of drugs from dosage forms, diclofenac sodium (25 mg) suppositories containing 10% stearic acid and 10% bees wax were selected for further pharmacokinetic evaluation in rabbits based on *in vitro* drug release studies. The study was conducted as single dose randomized crossover design in six healthy male rabbits. Slow release suppositories displayed significant ($P < 0.05$) reduction in C_{max} val-

ues. The maximum concentration of drug obtained with conventional plain (immediate release) suppositories was $63.65 \pm 3.69 \mu\text{g.ml}^{-1}$ which was reduced to 30.59 ± 0.47 and $30.94 \pm 1.78 \mu\text{g.ml}^{-1}$ in case of slow release suppositories containing 10% beeswax and 10% stearic acid respectively. There was about 50% reduction in C_{max} values for slow release suppositories compared to conventional plain suppositories. The times to reach maximum concentrations of drug were prolonged in case of slow release suppositories, which were around 3.0 hrs as compared against plain suppositories which produced very short t_{max} values ($1.0 \pm 0.18\text{h}$). The apparent elimination phase was lengthened for both slow release formulations with drug elimination half-life of 3.3-3.4 hrs which differed significantly ($P < 0.05$) compared to conventional plain suppositories ($t_{1/2}$: 1.84 ± 0.08 hrs). The mean residence time of the drug was also prolonged in case of slow release suppositories indicating slow absorption and elimination of diclofenac sodium from slow release suppositories. The plasma of the drug at 12th hr for slow release suppositories were similar to those of plain suppositories at 8th hrs. However, insignificant ($p > 0.05$) differences among $\text{AUC}_{0-\infty}$ values for plain suppositories and slow release suppositories indicate that the extent of absorption of the drug was comparable between plain and slow release suppositories. The relative bioavailability of diclofenac sodium from slow release suppositories calculated with reference to $\text{AUC}_{0-\infty}$ values was 102.3% and 95.8% for bees wax and stearic acid containing suppositories respectively compared to conventional plain (immediate release) suppositories which were used as reference products.

Thus, addition of excipients like bees wax and stearic acid to water soluble PEG 4000 base may be useful for formulating slow release matrix suppositories of diclofenac sodium for maintaining prolonged plasma levels of drug avoiding multiple dosing. Though, pharmacokinetic studies in rabbits revealed slow and sustained release of diclofenac sodium from these suppositories, studies in healthy human volunteers need to be conducted to confirm these findings so these studies are in progress.

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