INTRODUCTION

Chronotherapeutics refers to a treatment method that is in accordance with a patient's daily, monthly, seasonal or yearly biological clock, in order to achieve maximum therapeutic benefits and minimal adverse effects. Chronotherapeutic drug delivery systems (ChrDDS) with a preset lag time are helpful for patients suffering from allergic rhinitis, rheumatoid arthritis (RA) and related disorders, asthma, cancer, cardiovascular diseases and peptic ulcer disease as the drug is released at a predetermined time and the maximum concentration ($C_{\text{max}}$) of the drug will be reached when the symptoms of the disease are at its peak [1-3].

Rheumatoid arthritis (RA) is a chronic, autoimmune inflammatory disease characterized by joint swelling, tenderness and progressive destruction of synovial joints, leading to severe disability and premature mortality [4]. Morning stiffness that lasts for hours in one or more of the smaller joints is a common early sign of RA. Chronopharmacotherapy for RA has been recommended to ensure that the highest blood levels of the drug coincide with the incidence of pain and stiffness. Compression coated tablets (CCT) are dosage forms consisting of an immediate core release tablet embedded in an outer layer made of a hydrophilic or hydrophobic polymer or a combination of both. The outer layer disintegrates or dissolves slowly to release the drug after a predetermined lag time [5, 6].

This technique is simple, economical and eliminates the need for a tedious and time-consuming granulation or coating processes. It can be used to physically separate two incompatible drugs within the same dosage form and also improves the stability of the drug by protecting it from moisture [7]. Current drugs that are available in the market are conventional film-coated, and enteric coated tablets of NSAIDs and these dosage forms are to be taken twice or thrice a day post meals. This does not reduce the pain and stiffness of RA patients, especially in the early morning hours. This necessitates the present study on the development of compression coated tablets and to study differences in the pharmacokinetic patterns of a compression coated tablet and an immediate release tablet (conventional), both containing the same dose of lornoxicam.

MATERIALS AND METHODS

Materials

Lornoxicam was a generous gift sample from Lifecare formulations, Puducherry, India. Ethyl acetate-AR grade, methanol-HPLC grade, and Milli-Q water-HPLC grade were procured from SD fine chemicals ltd, India.

The study protocols were approved by the institutional animal ethics committee (IAEC) of M. S Ramaiah College of Pharmacy, Bangalore, India (CPCSEA approval no: 220/PO/adbc/2000/CPCSEA and renewal approval no: XVIII/MSRFPH/F-10/8.2.17)

Methods

a) Dosage forms

Immediate release core tablets containing 8 mg lornoxicam and compression coated with an interpenetrating polymer network (IPN) of chitosan-poly (acrylamide) were chosen as dosage forms for the study.

b) Animals

The pharmacokinetic study [8] was performed using New Zealand white rabbits (2.5-3 kg) of either sex. The animals were housed in polycarbonate cages in a room maintained under constant environmental conditions (22±1 °C, 50±5% RH). Food and water were given ad libitum. Six rabbits were divided into two groups (A and B); containing three rabbits each. The numbers of animals were selected as per the recommendations of the IAEC. Group I rabbits was administered immediate release core tablets (IR) of lornoxicam, and group II rabbits were administered compression coated tablets (CCT) of lornoxicam [9-13].

RESULTS

In the case of IR tablets, the drug was detected within 15 min after oral administration and a $C_{\text{max}}$ of 1269.57±4.04 ng/ml were attained at 2±0.15 h. With CCT, the drug was detected only after 5 h and a $C_{\text{max}}$ of 1279.24±12.76 ng/ml were attained at 8±0.10 h. The CCT showed maximum drug release at the second hour of study.

CONCLUSION

The predominant lag time prior to drug release from CCT is an indication that it is consistent with the requirements of chronopharmacological drug delivery. The results suggest that the compression coated tablet is a promising approach for chronotherapeutic management of rheumatoid arthritis.
The study was conducted in a crossover design with a washout period of three weeks in between the two experiments. The dosage forms were administered using sterile internal stomach pumps.

c) Dose fixation

The human dose of lornoxicam is 8 mg and the dose given to each rabbit was adjusted to 0.4 mg/kg according to Paget and Barnes [14].

d) Blood sampling

The rabbits were fasted overnight for 12 h prior to the study. The tablet was administered using sterile feeding tubes. The animals were fed 4 h after drug dosing and had free access to water throughout the study period. 2 ml of blood samples were collected through the marginal ear vein into heparinized tubes at predetermined intervals of 0, 15, 30, 60, 120, 240, 360, 480 and 600 min postdose. The plasma was separated immediately by centrifugation at 10000 rpm for 5 min and maintained at -20 °C until further analysis.

e) Analytical method development for determination of lornoxicam in rabbit plasma

Drug levels in the plasma were analyzed by LC-MS/MS [8].

Preparation of lornoxicam standard stock solution

10 mg of accurately weighed lornoxicam working standard was transferred into a 10 ml volumetric flask, dissolved in methanol and the volume was made with methanol to produce a stock solution of strength 1000 µg/ml. This solution was stored in a refrigerator at 2–8 °C. Working internal standard solutions in the concentration range 5.0–2200.00 ng/ml were prepared by suitably diluting the stock solution with methanol fresh before use.

Spiking of blank plasma samples

180 µl of drug-free rabbit plasma was spiked with 20 µl of lornoxicam standard calibrants (5.0–2137.45 ng/ml), separately extracted with 800 µl ethyl acetate and analyzed by LC-MS. The response obtained for different levels of calibrants was analyzed by linear regression and regression equation obtained was used for estimating lornoxicam concentration in unknown rabbit plasma samples processed similarly.

Regression equation used for calculating lornoxicam in unknown samples:

\[ Y = 54.43 X + 888.54 \]

Where \( Y \) = peak area of lornoxicam, and \( X \) is the concentration of lornoxicam in ng/ml of plasma samples.

Preparation of plasma samples

All the frozen rabbit plasma samples were thawed at room temperature and vortexed to ensure uniform mixing of contents.

Lornoxicam was selectively isolated from 200 µl plasma by liquid-liquid extraction using ethyl acetate as the extraction solvent. 200 µl plasma was vortexed with 800 µl ethyl acetate for 5 min at 2500 rpm. The mixture was centrifuged at 12000 rpm for 5 min and the supernatant collected. The extract was then evaporated to dryness under a stream of nitrogen followed by reconstitution with 100 µl of mobile phase (acetoni trile: 2 mmol ammonium formate buffer in the ratio 70:30 v/v). 10 µl of the prepared sample was then injected into the LC-MS/MS system and analyzed for drug content.

Instrumentation and chromatographic conditions

Shimadzu PROMINENCE LC-20AT series HPLC was used. The equipment consisted of an AGILENT ZORBAX XB-C18 column (5 µm particle size; 50 mm *4.6 I. D). The mobile phase used was acetoni trile: 2 mmol ammonium formate buffer (70:30 v/v) and was run at a flow rate of 0.4 ml/min. The temperature of the column oven was maintained at 45 °C and that of the autosampler at 10°C. The injection volume was 10 µl and the total run time was 10 min.

LC-MS/MS analysis was performed on SCIEX series API 4000 triple, quadruple mass spectrometer equipped with electrospray ionization (ESI) interface with turbo ion spray. Positive ions were measured in mode (ionspray voltage: 5300 KV, declustering potential: 86.50 eV, entrance potential: 12.50 eV, temperature of source: 450 °C). The mass spectrometer was programmed to monitor the precursor ion [M+H]+ of lornoxicam at m/z 372 and fragment ion [M+H]+at m/z 121.60

f) Data analysis

Pharmacokinetic parameters like maximum concentration (Cmax), time taken to reach maximum concentration (Tmax), area under the curve (AUC_{0-24}), area under the first moment curve (AUMC_{0-24}), elimination half-life (t_{1/2}), and mean residence time (MRT) were determined by Kinetica 5.0 (Thermo Fisher Scientific, PK/PD software, USA) for each sample.

RESULTS AND DISCUSSION

The primary goal for developing any dosage form is to deliver the required concentration of an active drug substance to the site of action and to achieve optimum efficacy. The ability of compression coated tablets as a drug delivery system to release drugs in a predetermined time-release manner was investigated in New Zealand white rabbits. The core and compression coated tablets of lornoxicam, prepared and evaluated under laboratory conditions were considered for pharmacokinetic studies.

A rapid and selective analytical method was developed for the quantitative analysis of lornoxicam in rabbit plasma. A retention and separation of lornoxicam were obtained by using acetonitrile: 2 mmol ammonium formate buffer (70:30 v/v) as the mobile phase. The correlation coefficient for lornoxicam over the concentration range of 5.0 ng/ml–2137.45 ng/ml was found to be 0.9984. The average slope and intercept of regression equations were 54.43 and 888.5 respectively (table 1 and fig. 1).

![Fig. 1: The calibration curve of lornoxicam in rabbit plasma](image-url)

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Table 1: Data for standard calibration curve of lornoxicam in rabbit plasma

| Concentration (ng/ml) | Counts±SD (peak area)* |
|-----------------------|-------------------------|
| 5.00                  | 683.00±1.15             |
| 10.05                 | 1232.00±0.577           |
| 50.23                 | 3601.00±1.52            |
| 502.30                | 27666.00±0.577          |
| 1004.60               | 59396.00±1.00           |
| 2137.45               | 115583.00±0.577         |

*Each value is represented as a mean±standard deviation of three observations (n=3)

The LC-MS/MS method was found to satisfy the requirement of routine analyses as it had a short run time (3 min). The MS optimization was performed by direct injection of lornoxicam into the mass spectrometer. The mass parameters were optimized to obtain better ionization of lornoxicam molecules. The full scan spectrum [15] was dominated by protonated molecule m/z 372.00 for lornoxicam and the major fragment ions observed in the product spectrum was at m/z 121.60 (fig. 2-3).

Fig. 2: Mass scan of lornoxicam standard

Fig. 3: Product ion spectrum of lornoxicam standard
The retention time obtained for lornoxicam was 1.24 min. The representative chromatograms of blank plasma and plasma samples spiked with lornoxicam are shown in fig. 4.

The developed LC-MS/MS method was successfully applied for pharmacokinetic study in rabbits. In the case of IR, the drug was detected within 15 min after its oral administration in rabbits. The chromatograms of plasma samples withdrawn at different intervals of time for IR are shown in fig. 5.

In the case of CCT, the drug was detected only after 5 h. This indicates that there was a predominant lag time before the drug release. The chromatograms of plasma samples withdrawn at different intervals of time for CCT are shown in fig. 6 [16].
The drug absorption was rapid in the case of IR, i.e., a Cmax of 1269.57±4.04 ng/ml was attained at a Tmax of 2±0.15 h (120 min) h. In CCT, a Cmax of 1279.24±12.76 ng/ml was attained at a Tmax of 8±0.10 h (480 min), but no drug was detected till 5 h in all the three rabbits, indicating a similar correlation with in vitro release. The AUC0-24MRT is defined as the average time spent by the drug in the body and can be used to compare the in vivo performance of different dosage forms. The difference in MRT values (3.45 and 9.12 h for IR and CCT respectively) is an indication of the pulsatile release pattern of compression coated tablet. The other pharmacokinetic parameters [9] determined were AUMC for animals administered IR was found to be 4503.59±42.0 ng/ml h and 4598.36±74.1 ng/ml h for animals administered CCT.

Table 2: Summary of pharmacokinetic parameters of all the groups

| Pharmacokinetic parameters | Group I (Core tablets of lornoxicam)* | Group II (Compression coated tablets of lornoxicam)* |
|----------------------------|--------------------------------------|-----------------------------------------------------|
| Cmax (ng/ml)               | 1269.57±12.89                       | 1279.24±12.76                                       |
| Tmax (h)                   | 2±0.15                               | 8±0.10                                              |
| Lag time (h)               | 0±0                                  | 5±0.10                                              |
| AUC0-24 (ng/ml h)          | 4503.59±42.0                         | 4598.36±74.1                                        |
| AUMC0-24 (ng/ml * [h];)    | 1555.24±26.92                        | 41968.23±808.25                                     |
| t ½ (h)                    | 1.43±0.01                            | 1.53±0.025                                          |
| Ke (h-1)                   | 0.45±0.005                           | 0.45±0.005                                          |
| MRT (h)                    | 3.45±0.036                           | 9.12±0.03                                           |

* Each value is represented as a mean±standard deviation of three observations (n=3)

Fig. 7: Plasma drug concentration-time curves for pharmacokinetic study in rabbits, *All points are presented as mean±SD of observations (n=3)
CONCLUSION
A rapid and selective LC-MS/MS method was developed for the quantitative analysis of lornoxicam in rabbit plasma. An acceptable retention and separation of lornoxicam were obtained by using acetonitrile: 2 mmol ammonium formate buffer (70:30 v/v) as the mobile phase. The developed method was found to be highly sensitive and suitable for the detection of lornoxicam in plasma in concentrations as low as 5 ng/ml. The results of the pharmacokinetic studies indicated that drug release from compression coated tablet took place only after a lag time of 5 h. Thus, drug release, consistent with requirements for chronopharmaceutical drug delivery, was achieved from the compression coated tablet. Thus the formulation can be considered as one of the promising tools for chronotherapeutic management of rheumatoid arthritis with improved patient compliance.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally

CONFLICT OF INTERESTS
The authors declare that there are no conflicts of interest regarding the publication of this article.

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