

Investigations on Analgesic, Anti-inflammatory and Ulcerogenic Potential of Meloxicam Solid Dispersion Prepared with Skimmed Milk

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(Received February, 6, 2006; Accepted April 18, 2006)

Meloxicam, a non-steroidal anti-inflammatory drug is used in the treatment of rheumatoid arthritis and osteoarthritis. It is practically insoluble in water leading to poor dissolution, variations in bioavailability and gastric irritation on oral administration. In order to modulate its gastric side effect and to increase aqueous solubility, physical mixture and solid dispersion of the drug were prepared with skimmed milk. The analgesic, anti-inflammatory and ulcerogenic effects were assessed for physical mixture and solid dispersion in comparison to pure meloxicam. The results indicate that solid dispersion possess better analgesic and anti-inflammatory properties with less ulcerogenic potential as compared to pure meloxicam.

Key words—meloxicam; solid dispersion; analgesic; anti-inflammatory; ulcerogenic study; skimmed milk

INTRODUCTION

Meloxicam (MLX), a non-steroidal anti-inflammatory, analgesic and antipyretic agent is used in the treatment of rheumatoid arthritis, osteoarthritis and other joint diseases.¹⁾ Like other non-steroidal anti-inflammatory drugs (NSAIDs), MLX is also practically insoluble in water and therefore exhibits poor dissolution, variations in bioavailability and gastric irritation on oral administration.^{2–5)} Solid dispersion in water-soluble carriers is a potential mean of improving the dissolution rate and bioavailability.^{2,3,6)} The solid dispersions results in the reduction of particle size of such drugs to nearly molecular level, due to transformation of the drug from the crystalline to the (partial) amorphous state, and/or to increase the saturation solubility.⁷⁾ In this study, physical mixtures and solid dispersion of MLX were prepared with skimmed milk using rotary vacuum evaporation technique⁸⁾ in order to enhance aqueous solubility, while maintaining the original pharmacological activity of the drug. We assumed that this method could result in the increased and more predictable dissolution rate benefiting the therapeutic response. The solid dispersion may be considered as a potential dosage form modification for MLX and other poorly water soluble or insoluble drugs that are commonly administered orally, where an increase in the bioavailability, enhancement of therapeutic effects and lower-

ing of side effects are desirable. Therefore, the physical mixture and solid dispersion of MLX were subjected to analgesic, anti-inflammatory and ulcerogenic studies using rodents in comparison to the pure MLX.

MATERIALS AND METHODS

Drug The Meloxicam B.P. was obtained as gift sample from Sun Pharmaceuticals Ltd. (Mumbai, India). Skimmed milk (fat content 1.5% maximum) procured from Hisar-Jind Co-op Milk Producers Union Ltd., Jind (Haryana, India). Acetic acid was purchased from S.D Fine Chemicals (Mumbai, India). Carrageenan type 4 was procured from Sigma Co. USA and all other chemicals/solvents used were of analytical grade.

1) Preparation of Skimmed Milk Powder 100 ml of skimmed milk (SM) was dried in a rotary vacuum evaporator (Steroglass, Italy) at 100 rpm, 35°C under vacuum for 6 h. The obtained powder was dried in an oven (100 ml SM yielded about 12.10 g powder), passed through a sieve (75–150 μm) and stored in an airtight container till further use.

2) Preparation of Solid Dispersion Meloxicam (1 g) was mixed in 50 ml of SM on a water bath at 50°C and stirred for 30 min using a magnetic stirrer. The resulting suspension was dried in a rotary vacuum evaporator at 100 rpm, 35°C under vacuum for 6 h forming solid dispersion of the drug in a powder form.⁸⁾ The obtained powder was dried in an oven, passed through a sieve (75–150 μm) and stored in an

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airtight container till further use.

3) Preparation of Physical Mixtures 1 g of MLX was uniformly mixed with 6.05 g of powdered SM using a spatula in a mortar. The prepared mixtures were passed through a sieve (75–150 μm) and stored in an airtight container till further use.

Animals Swiss Albino mice (20–30 gm) and Wistar Albino rats (180–200 gm) of either sex were used for pharmacological studies. The animals were housed under standard laboratory conditions in polypropylene cages and were provided food and water *ad libitum*. The animals were acclimatized to the laboratory environment for at least one week before starting of experiments. The experimental protocol was approved by Institutional Animal Ethical Committee. Meloxicam (MLX), physical mixture of meloxicam (PM) and solid dispersion of meloxicam (SD) were suspended in 1% w/v sodium carboxyl methyl cellulose suspension for administration to animals. The drugs were administered at a dose of 4 mg/kg of MLX (28.2 mg/kg of PM and SD equivalent to 4 mg/kg of MLX).

Analgesic Study

1) Acetic Acid Induced Abdominal Writhing

Mice were divided randomly into four groups of five animals each. The test drugs (MLX, PM and SD) or vehicle control (1% sodium carboxyl methyl cellulose) was administered to different group of animals. The abdominal writhing syndrome was elicited by an interaperitoneal injection of 1% (v/v) acetic acid at a dose of 10 ml/kg body weight. The analgesic response was assessed by counting the number of abdominal writhings in 20 min⁹⁾ after 5 min of acetic acid injection. The drug substances were administered 30 min before acetic acid injection.

Anti-inflammatory Study

1) Carrageenan Induced Paw Oedema

The rats were fasted for 18 h and water was provided *ad libitum*. The different groups of animals were orally pretreated with the test drugs and the control. The paw oedema was induced by sub plantar injection of 0.1 ml of a 1% w/v freshly prepared suspension of carrageenan in normal saline into the right hind paw of each rat. The paw volume was measured before (0) h and 1, 3 and 5 h after the injection of carrageenan using a plethysmometer.¹⁰⁾ All the treatments were given orally 30 min prior to the injection of carrageenan. The oedema was expressed as an increase in paw volume due to carrageenan injection.

Ulcerogenic Study The ulcerogenic potential of PM and SD was studied in rats by the method reported by Nagarsenker et al.,¹¹⁾ and compared with MLX. The animals were randomly divided into four groups comprising four animals each. The first three groups were treated with MLX, PM or SD for seven consecutive days. Similarly, the fourth group (control) was administered orally with 1% w/v sodium carboxyl methyl cellulose suspension. On the seventh day, the rats were starved for 24 h and water was provided *ad libitum*. On day eight, the rats were sacrificed and the abdomen was opened. The stomach was removed, incised along the greater curvature and gently washed with water. Hemorrhagic lesions, produced in the glandular portion were observed under a dissection microscope ($\times 20$ magnifications) and evaluated by the following score.

- 0.0—Normal (no injury, bleeding and latent injury)
- 0.5—Latent injury or widespread bleeding
- 1.0—Slight injury (2 to 3 dotted lines)
- 2.0—Severe injury (continuous lined injury or 5–6 dotted injuries)
- 3.0—Very severe injury (several continuous lined injury)
- 4.0—Widespread lined injury or widened injury

Statistical Analysis The data are presented as mean \pm S.E.M. The difference between the groups was determined by analysis of variance (ANOVA) followed by Tukey-Karmer Multiple Comparisons Test. In ulcerogenic studies, the data were analysed using Kruskal-Wallis test. The results were considered significant at values of $p < 0.05$ in both tests used.

RESULTS AND DISCUSSIONS

The present study was aimed to enhance aqueous solubility of the drug by the use of PM and SD with skimmed milk to get formulations with better analgesic and anti-inflammatory activity and lower side effects than the pure drug.

MLX, PM and SD significantly reduced the number of abdominal writhing after 1% v/v acetic acid injection (10 ml/kg, *i.p.*) in comparison to the vehicle control (Table 1). Both PM and SD considerably improved the analgesic activity (37.24 and 42.85% respectively) in comparison to the MLX (32.14%). The increase in analgesic activity with SD was significant in comparison to the same dose of MLX. The results are in accordance with analgesic effects of

poorly water soluble NSAID in PM and SD.^{6,12)}

Figure 1 illustrates the anti-inflammatory effect of MLX, PM and SD. Both PM and SD showed significant increase in anti-inflammatory effect, in the carrageenan induced paw oedema, as compared to MLX at 3 and 5 h after carrageenan injection. The SD showed the maximum anti-inflammatory activity at 3 h, which is consistent with reported results.^{11,13–15)}

In the ulcerogenic studies, MLX, PM and showed significant ulcerogenic potential than the control in rats treated chronically for seven consecutive days (4 mg/kg MLX, ≡ 4 mg/kg of MLX in PM or SD, *p.o.*). The PM and SD showed less ulcerogenic potential (Fig. 2) with the ulcer score of 1.38 ± 0.38 and 0.75 ± 0.14 , respectively as compared to MLX (1.75 ± 0.14).

Further, SD possesses no significant difference in ulcerogenic potential as compared to control. The results (Table 2) indicate that SD protect the gastric mucosa from injury, which is in accordance with ulcerogenic effects of poorly water soluble NSAID in PM and SD.^{6,16)}

It has been reported that crystals of non-steroidal anti-inflammatory agents are poorly soluble in gastric acid and remain in contact with the stomach wall for a longer period, thus producing a highly dangerous local concentration. This leads to local irritation of the stomach wall followed by ulceration.^{11,17)} It is expected that in the complexed form, the drug dissolves fast and show an accelerated absorption. Moreover, it will not come in direct contact with the stomach wall in crystalline state, as it remains encapsulated by the

Table 1. Analgesic Effect of MLX, PM and SD

Treatment	Number of abdominal writhing	% Inhibition
Control	39.2 ± 1.2	—
MLX	$26.6 \pm 1.6^{***}$	32.14
PM	$24.6 \pm 0.98^{***}$	37.24
SD	$22.4 \pm 1.63^{***}$	42.85

Data were expressed as mean \pm S.E.M., $n=5$, *** $p < 0.001$ compared with control.

Table 2. Ulcerogenic Potential of MLX, PM and SD

Treatment	Ulcer index
Control	0.00 ± 0.00
MLX	$1.75 \pm 0.14^*$
PM	$1.38 \pm 0.38^*$
SD	0.75 ± 0.14

Data were expressed as mean \pm S.E.M., $n=4$, * $p < 0.05$ compared with control.

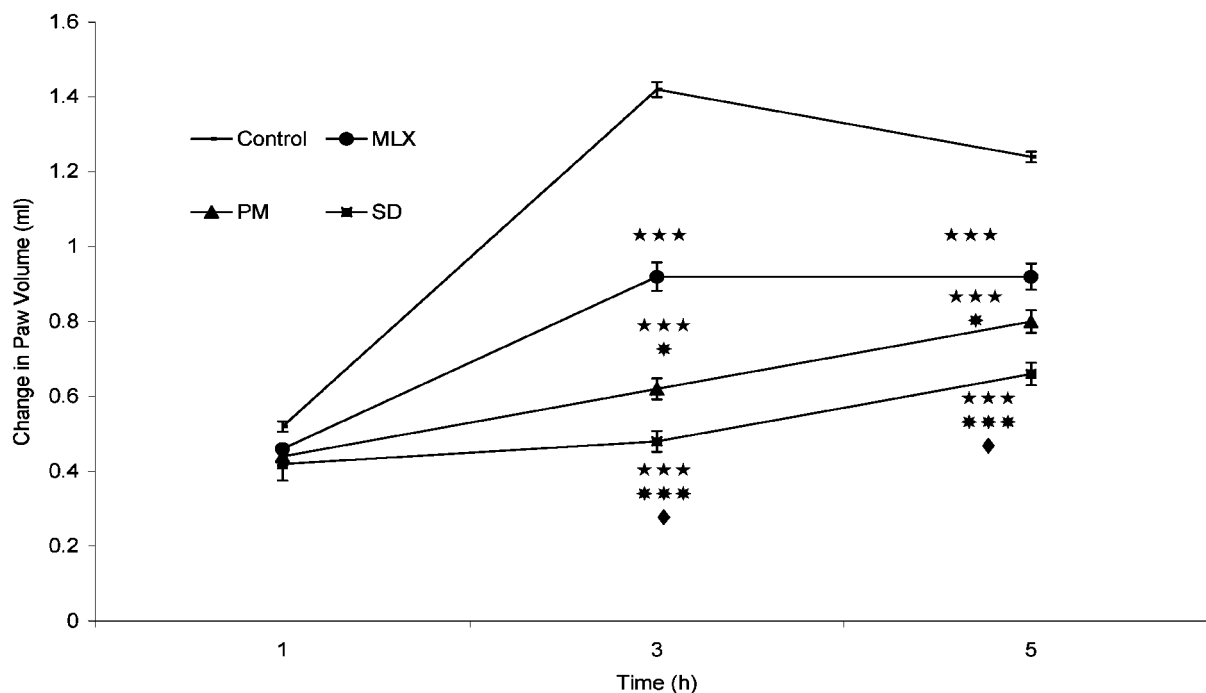


Fig. 1. The Effect of MLX, PM and SD on Rat's Hind Paw Oedema Induced by Carrageenan

Data were expressed as mean \pm S.E.M. ($n=5$). *** $p < 0.001$ compared with control, * $p < 0.05$, *** $p < 0.001$ compared with same dose of MLX, ♦ $p < 0.05$ compared with same dose of PM.

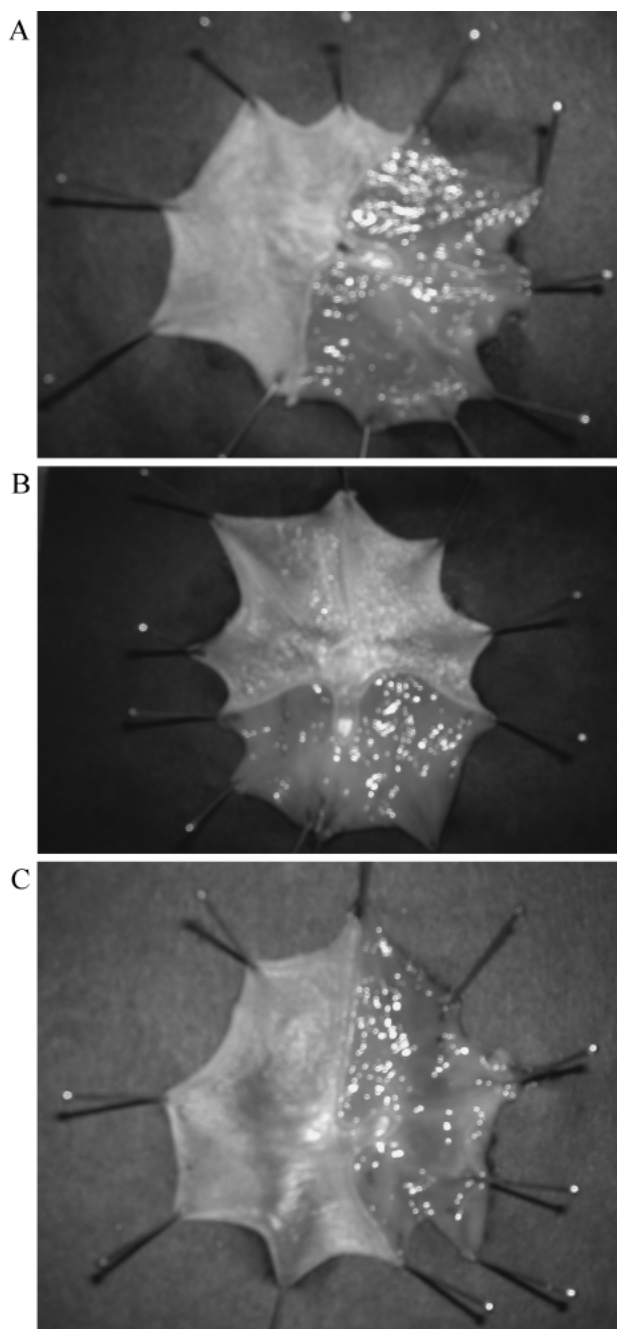


Fig. 2. Representative Stomachs of Rats Treated with (A): Powdered SM, (B): MLX at a Dose of 4 mg/kg Body Weight, and (C): SD Equivalent to 4 mg of MLX/kg

polymer, until its dissolution.

It was concluded that SD of MLX with skimmed milk possess better analgesic and anti-inflammatory properties with less ulcerogenic potential as compared to the pure drug.

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