tion Products, San Jose, USA), series 200 autosampler (Perkin Elmer, Wellesley, USA) and Coulochem II electrochemical detector (Environmental Sciences Assoc., Chelmsford, USA) with +850 mV (guard cell), +500 mV (E1) and +780 mV (E2). The Waters Spherisorb C-8 analytical column (Waters Corp., Milford, USA), 5 µ, 150 × 4.6 mm was used. The mobile phase was composed of acetonitrile : methanol : acetate buffer (50 : 10 : 40) with pH 7.5. The flow rate was 1.3 ml/min. The calibration curve of clarithromycin ranged from 90 to 3600 ng/ml. The limit of quantitation was 18 ng/ml. The % recovery, % accuracy and within-run precision (% C.V.) of clarithromycin in spiked plasma samples at the concentrations of 90, 360 and 1800 ng/ml were 99.6, 84.5, and 89.1%, 95.7, 101.3 and 100.0% and 7.2, 3.7 and 8.1% respectively. The % recovery and % C.V. of the internal standard in spiked plasma samples were 90.7% and 8.8% respectively.

3. Pharmacokinetic and statistical analysis

A non-compartmental pharmacokinetic method was employed to determine the pharmacokinetic parameters of clarithromycin. The time to peak plasma concentration (T_{max}) and the peak concentration (C_{max}) were obtained directly from the plasma clarithromycin concentrations. WinNonlin Standard (version 3.0) was used to determine area under the concentration-time curve ($AUC_{0-\infty}$) and half-life ($t_{1/2}$). $AUC_{0-\infty}$ was calculated by linear trapezoidal method.

An analysis of variance (ANOVA) was performed on the pharmacokinetic parameters C_{max} and $AUC_{0-\infty}$, using general linear models (GLM) procedures, in which sources of variation were sequence, subjects within sequence, period, and preparation. Then the 90% confidence intervals of the test/reference ratios for C_{max} and $AUC_{0-\infty}$ (log transformed) were determined. Bioequivalence between two formulations could be concluded when the 90% confidence intervals for these pharmacokinetic parameters of two products are found within the acceptable range of 80–125%.

References

1 Langtry, H. D.; Brogden, R. N.: Drugs **53**, 973 (1997)
2 McCarty, J. M.: Clin. Ther. **22**, 281 (2000)
3 Alvarez-Elcoro, S.; Enzler M. J.: Mayo Clin. Proc. **74**, 613 (1999)
4 Carbon, C.; Poole, M. D.: J. Chemother. **11**, 107 (1999)
5 Rodvold, K. A.: Clin. Pharmacokinet. **37**, 365 (1999)
6 Fraschini, F.; Scaglione, F.; Deinartini, G.: Clin. Pharmacokinet. **25**, 189 (1993)
7 Chu, S. Y.: Wilson, D. S.; Deaton, R. L.: J. Clin. Pharmacol. **33**, 719 (1993)
8 Chu, S. Y.; Sennello, L. T.; Sonders, R C.: J. Chromatogr. **571**, 199 (1991)

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Meloxicam complexation with β-cyclodextrin: influence on the anti-inflammatory and ulcerogenic activity

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to provide effective anti-inflammatory and analgesic therapy to patients with arthritis. However, they are associated with a high incidence of gastrointestinal (GI) side effects, which can reduce patient compliance and discourage physicians from prescribing them [1]. Therefore there is a need for a delivery system for NSAIDs with improved GI tolerability, while retaining efficacy.

Cyclodextrins are widely used in the pharmaceutical field owing to their high aqueous solubility and ability to stabilize drug molecules. They are known for their ability to encapsulate a wide variety of drugs into their hydrophobic cavity without the formation of any covalent bonds [2–6].

Meloxicam is a preferential COX-2 inhibitor with strong anti-inflammatory activity. It is practically insoluble in water and has a longer onset of action. Its prolonged use is associated with incidence of side effects like GI perforations, ulcerations and bleeding. Therefore the aim of the present study was to investigate the effect of inclusion of meloxicam with β-cyclodextrin (β-CD) upon anti-inflammatory activity and GI mucosal toxicity.

The β-CD complex of meloxicam was prepared by the freeze-drying (FD) method [7] and showed a faster onset of anti-inflammatory activity than the pure drug, freeze dried similarly (pH 5.0 ± 0.1), indicating a maximum inhibition of oedema. A peak inhibition of 80.0% was obtained at 2 h in the group of mice, which was treated with meloxicam-β-CD (FD) solution (pH 4.9 ± 0.1), but for

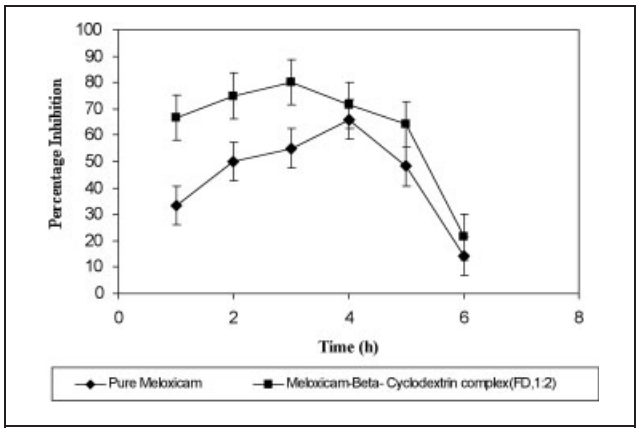

Fig.: Anti-inflammatory studies of meloxicam and meloxicam/β-CD complex by rat hind paw odema method (mean ± SD; n = 4)

Table: Degree of injury to the stomach of rats (mean ± SD; n = 4)

Sample	Degree of injury
Pure meloxicam	2.25 ± 0.96
Pure β-CD	0.00 ± 0.00
Meloxicam-β-CD complex (PM)	$1.75 \pm 0.43^*$
Meloxicam-β-CD complex (FD)	$0.9 \pm 0.25^*$

PM: Physical mixture FD: Freeze dried. * $p < 0.05$

mice having recieved pure meloxicam, the peak inhibition was only 66% after 4 h (Fig.). The difference was significant at 5% level of significance indicating that in complexed form the drug shows an improvement in the rate and extent of absorption.

In the ulcerogenic studies, at the dose level of 3 mg/kg of meloxicam, the meloxicam-β-CD complex prepared by freeze drying method gave a significant lower score; 0.9 ± 0.25 ulceration as compared to pure meloxicam which gave 2.25 ± 0.96 ulceration (Table). The difference was statistically significant at 5% level of significance ($p = 0.0032$). The complex prepared by physical mixing (PM) showed an intermediate effect (score: 1.75 ± 0.43).

It is reported [8] that crystals of non-steroidal anti-inflammatory agents being poorly soluble in gastric acid remain in contact with the stomach wall for a long period of time, resulting in a dangerously high local concentration. This leads to local irritation of the stomach wall and to ulceration. It is expected that in the complexed form, the drug will dissolve fast and show an accelerated absorption. Moreover, it will not come in direct contact with the stomach wall in crystalline state since until it is dissolved it remains encapsulated within the cyclodextrin matrix.

These results clearly demonstrate a significant decrease in the gastric ulcerogenic activity of meloxicam through complexation with cyclodextrins. Even though the physical mixture of meloxicam with cyclodextrins reduces ulcer formation, it is the freeze-dried complex, which minimizes gastric ulceration. These findings are important from a commercial point of view as the prepared complex removes a major drawback for meloxicam in therapy.

The inclusion complex of meloxicam with β-CD was also found to have better anti-inflammatory activity.

Experimental

1. Chemicals

Meloxicam was obtained as a gift sample from Sun Pharmaceuticals (India) Ltd. β-CD was purchased from S.D. Finechem. (India). Other reagents and chemicals were of analytical reagent grade.

2. Procedures

2.1. Anti-inflammatory studies

Anti-inflammatory studies were performed by the carrageenan-induced rat hind paw oedema method [8]. Wistar male rats, weighing between 130–200 g were fasted overnight prior to experiment but water was allowed *ad libitum*. The animals were divided into 3 groups of 4 animals each. Group 1 received 3 mg/kg of pure meloxicam suspended in sodium CMC. Group 2 received pure β-CD solution prepared in water and group 3 received meloxicam β-CD freeze-dried complex at a dose of 3 mg/kg equivalent to meloxicam. One hour after drug administration, 0.1 ml of 1.0% carrageenan in sodium CMC, was injected into the surface of hind paw and the paw volume was measured with the help of plethysmometer at 1, 2, 3, 4, 5 and 6 h after administration of carrageenan. ANOVA single factor test was applied as the test of significance.

2.2. Ulcerogenic studies

The potential of the prepared inclusion complexes in producing gastric ulceration was studied in the "Fasted rat-model" as described by Nambu et al. [8].

Wistar male rats weighing 140–210 g were fasted for 24 h prior to experimentation and water was allowed *ad libitum*. The animals were divided into 5 groups of 4 animals each. Group 1 received pure meloxicam suspended in sodium carboxy methylcellulose at dose of 3 mg/kg and served as control. Group 2 received pure β-CD solution. Group 3 received meloxicam β-CD inclusion complex prepared by grinding method. Group 4 received meloxicam β-CD inclusion complex prepared by the freeze drying method. 3 mg/ml/kg body weight, volume was administered orally with a mouth feeder to the animals for a total period of 7 days.

The rats were sacrificed under ether anesthesia after 7 days. The isolated stomach was opened up along the greater curvature and its contents was carefully washed under tap water. Hemorrhagic lesions, produced in the glandular portion, were observed under a dissection microscope in 20 magnification 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 (continuos lined injury or 5–6 dotted injuries)
3.0 – Very severe injury (several continuos lined injury)
4.0 – Widespread lined injury or widened injury.

ANOVA single factor test was applied as the test of significance.

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References

1 Langman, M. J. S.; Weil, J.; Wainwright, P.: Lancet **343**, 1075 (1994)
2 Dhanaraju, M. D.; Kumaran, K. S.; Baskaran, T.; Rama Moorthy, M. S.: Drug Dev. Ind. Pharm. **24**, 583 (1998)
3 Ahn, H. J.; Kim, K. M.; Choi, J. S.; Kim, C. K.: Drug Dev. Ind. Pharm. **23**, 397 (1997)
4 Dhanaraju, M. D.; Senthi Kumaran, K.; Baskaran, T.; Sree Rama Moorthy, M.: Drug. Dev. Ind. Pharm. **24**, 583 (1998)
5 Loftsson, T.; Brewster, M. E.: J. Pharm. Sci. **85**, 1017 (1996)
6 Loftsson, T.; Olafsdottir, B. J.; Fridriksdottir, H.; Jansdottir, S.: Eur. J. Pharm. Sci. **1**, 95 (1993)
7 Baboota, S.; Agarwal, S. P.: Indian J. Pharm. Sci. **64**, 408 (2002)
8 Nambu, N.; Kikuchi, K.; Kikuchi, T.; Takahashi, Y.; Ueda, H.; Nagai, T.: Chem. Pharm. Bull. **26**, 3609 (1978)