was determined and the precipitate was vacuum dried and then the procedure of method I was repeated. Method III – Twenty milligrams of microspheres were added to 1 ml dichloromethane with stirring and the supernatant containing the dissolved polymer was discarded after centrifugation. The pellet was vacuum dried and extracted with 1 ml PBS. This procedure was performed twice. Method IV – Two milliliters of 1 N NaOH was used to digest 20 mg microspheres by sonication for 15 min and incubation at 37 °C for 2 days. The solution was analyzed after neutralization with HCl. Method V and VI – Twenty milligrams of microspheres were dissolved in 1 ml dichloromethane (V) or acetonitrile (VI). After centrifugation, the precipitated protein was centrifuged and supernatant containing polymer was discarded. After being vacuum dried, the pellet was re-dispersed in 1 ml PBS and centrifuged again. The supernatant was retained and the residue was dissolved in 1 ml of 0.1 N NaOH, then vortexed thoroughly and centrifuged. The protein in the PBS and alkaline extractions was neutralized with HCl before estimation. Method VII – The non-entrapped protein in the aqueous phase was collected during the preparation and the protein in the supernatant was determined.

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Molecular inclusion of rofecoxib with cyclodextrin: pharmacological properties in laboratory animals

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Rofecoxib is practically insoluble in water and its prolonged use is associated with the incidence of side effects like gastro intestinal perforations, ulcerations and bleeding. Therefore, an attempt has been made to improve the aqueous solubility of the drug by making an inclusion complex using dimethyl-β-cyclodextrin (DIMEB). The complexes were prepared by kneading and by the spray drying method. The prepared complexes showed better anti-inflammatory activity and decreased ulcerogenic potential than the pure drug.

Water insoluble drugs are usually characterized by a low bioavailability, because their absorption is dissolution rate limited and consequently slow (Kamada et al. 2002; Fernandes et al. 2002; Peeters et al. 2002; Baboota et al. 2003). The potential use of cyclodextrins as a novel drug carrier material is to control the drug release at the desired level.

Rofecoxib, a selective COX-2 inhibitor with strong anti-inflammatory activity, is practically insoluble in water and has a longer onset of action. Its prolonged use is associated with side effects like gastro intestinal (GI) perforations, ulcerations and bleeding (McEvoy 2001; Schrefer 2000). Therefore the aim of the present study was to investigate the effect of inclusion of rofecoxib with dimethyl-β-cyclodextrin (DIMEB) upon anti-inflammatory activity and GI mucosal toxicity.

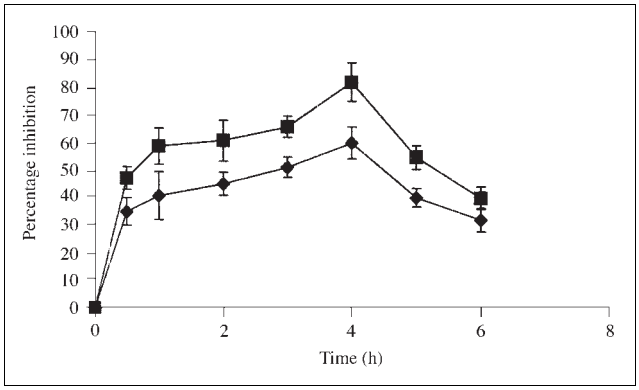

Fig. Anti-inflammatory studies of rofecoxib and rofecoxib DIMEB complex by rat hind paw odema method (mean ± SD; n = 4).
—◆— Pure rofecoxib, —■— Rofecoxib-DIMEB SD complex

The DIMEB complex of rofecoxib was prepared by spray-drying (SD) and showed a faster onset of anti-inflammatory activity than the pure drug spray dried similarly (pH 5.5 ± 0.2), indicating maximum inhibition of oedema. A peak inhibition of 82.0% was obtained at 4 h in the group of mice, which had received the rofecoxib-DIMEB (SD) solution (pH 4.0 ± 0.1), but for mice treated with pure rofecoxib, the peak inhibition was only 60% after 4 h (Fig). The difference was significant at 5% level of significance indicating that in the complexed form the drug shows an improvement in the rate and extent of absorption ($p = 0.011$).

In the ulcerogenic studies, at the dose level of 200 mg/kg/ml of rofecoxib, the rofecoxib-DIMEB complex prepared by spray drying gave a significantly lower score; 0.75 ± 0.25 as compared to pure rofecoxib which gave an ulceration score of 1.80 ± 0.63 (Table). The difference was statistically significant at 5% level of significance ($p = 0.026$).

It is reported (Nambu et al. 1978) that crystals of non-steroidal anti-inflammatory agents being poorly soluble in gastric acid remain in contact with the stomach wall for a long 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 it remains encapsulated within the cyclodextrin matrix until dissolution.

These results clearly demonstrate a significant decrease in the gastric ulcerogenic activity of rofecoxib after complexation with cyclodextrins. Even though the physical mixture of rofecoxib with cyclodextrins reduces ulcer formation, it is the spray-dried complex, which minimizes gastric ulceration.

The inclusion complex of rofecoxib with DIMEB was also found to have better anti-inflammatory activity.

Experimental

1. Chemicals

Rofecoxib and DIMEB were obtained as a gift sample from Ranbaxy Pharmaceuticals (India) Ltd and Cyclo Labs, Budapest (Hungary) respectively. Other reagents and chemicals were of analytical reagent grade.

2. Procedures

2.1. Preparation of inclusion complexes

Rofecoxib/DIMEB complexes were prepared in the molar ratio of 1 : 1. Kneaded mixture was prepared by triturating both powders for 30 min. together in a clean, dry pestle and mortar. Thereafter, the powder mixture was wetted with a mixture of dichloromethane and ethanol (1 : 1) and kneaded. Kneading was continued until the product started drying on the walls of mortar. The product was further dried in hot air oven at 60 °C for 20 min. or to a constant weight. The dried material was powdered and stored in a desiccator.

The Spray Dried (SD) product was prepared by spray drying a mixture of rofecoxib and DIMEB dissolved in dichloromethane. The solid obtained was sieved through 85 mesh B.S.

Table: Degree of injury to the stomach of rats

Sample	degree of injury
Pure rofecoxib	1.80 ± 0.63
Pure DIMEB	0.00 ± 0.00
Rofecoxib-DIMEB complex (KN)	$0.88 \pm 0.41^*$
Rofecoxib-DIMEB complex (SD)	$0.7 \pm 0.25^*$

KN: Kneaded mixture; SD: spray dried; * $p < 0.05$; mean $\pm$ SD; $n = 4$

2.2. Anti-inflammatory studies

Anti-inflammatory studies were performed by the carrageenan-induced rat hind paw oedema method (Nambu et al. 1978). Wistar male rats, weighing between 150–210 g were fasted overnight prior to the experiment but water was allowed *ad libitum*. The animals were divided into 3 groups of 4 animals each. Group 1 received 200 mg/kg/ml of pure rofecoxib suspended in sodium CMC. Group 2 received pure DIMEB solution prepared in water and Group 3 received rofecoxib DIMEB spray-dried complex at a dose of 200 mg/kg/ml equivalent to rofecoxib. One hour after drug administration, 0.1 ml of 1.0% carrageenan in sodium carboxymethylcellulose was injected into the plantar surface of the hind paw. The volume of the normal saline displaced by the paw, just before the carrageenan injection and every hour for 6 h was measured with a digital plethysmometer. Anova single factor test was applied as the test of significance.

2.3. Ulcerogenic studies

The potential of the prepared inclusion complexes to produce gastric ulceration was studied in Fasted rat-model' as described by Nambu et al. (1978).

Wistar male rats weighing 150 to 210 g, were fasted for 24 h prior to the experiments and water was allowed *ad libitum*. The animals were divided into 5 groups of 4 animals each. Group 1 received pure rofecoxib suspended in sodium carboxy methylcellulose at a dose of 200 mg/kg/ml and served as control. Group 2 received pure DIMEB solution. Group 3 received rofecoxib DIMEB inclusion complex prepared by the kneading method. Group 4 received rofecoxib DIMEB spray-dried complex. 200 mg/ml/kg body weight, were administered orally with a mouth feeder to the animals for a total period of 4 h.

The rats were sacrificed under ether anesthesia after 4 h. 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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