Synthesis and Pharmacological Evaluation of Cyclodextrin Conjugate Prodrug of Mefenamic acid

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In the present investigation mefenamic acid prodrug of ß-cyclodextrins was synthesized. The primary hydroxy group of ß-cyclodextrins was used to block the acid group. The synthesis involved a series of protection and deprotection reaction. The ester was evaluated for stability in simulated gastric and intestinal fluid. The hydrolysis of cyclodextrin conjugate in colon is confirmed by the hydrolysis kinetics studies in rat faecal material. The ester was also evaluated for ulcerogenicity. Results of these studies established the primary aim of masking the ulcerogenic potential of free drug, by using 12-fold dose of the normal dose of mefenamic acid and equivalent doses of the ester.

Cyclodextrins (CDs) belongs to family of cyclic-oligosaccharides; the most common being α-, β- and γ-CDs consisting of 6, 7 and 8 glucopyranosyl units, respectively, linked by a (1→4) glucosidic bonds. CDs are obtained by enzymatic degradation of starch, glucosyl transferase a type of amylase of bacterial origin obtained usually from Bacillus macerans, Bacillus megaterium, Klebsiella pneumoniae M5, and Bacillus stercrothermophilus. CDs are moderately soluble in water, methanol and readily soluble in strongly polar aprotic solvents. After oral administration, CDs are not hydrolyzed during their transit through the stomach, hydrolysis occurs only in colon by colonic microflora. The oral administration of CDs does not result in toxicity. Thus, CDs were thought to be one of the most suitable promoieties to reduce the ulcerogenic tendencies of mefenamic acid since they eliminate the exposure of free drug in stomach and small intestine but release the drug in colon.

Mefenamic acid is a nonsteroidal antiinflammatory and analgesic compound belonging to the family of N-aryl anthranilic acid. It is also described as 2-[(2,3-Dimethylphenyl) amino] benzoic acid. The main side effects include GIT disturbance, peptic ulceration and gastric bleeding. Mefenamic acid is widely used in rheumatic disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis.

The major factor, which limits the use of mefenamic acid, is its gastric effect due to local irritation of gastric mucosa by free COOH group of the drug. Hence efforts have been made to mask the free COOH group by the primary hydroxy group of CD and releasing the drug in colon and preventing the exposure of free drug to the stomach.

MATERIALS AND METHODS

All melting points were determined by open capillary method and are uncorrected. TLC ascertained the purity of the compounds on precoated silica gel-60 F254 plates. Solvent used was butanol:ethanol:water:acetic acid (3:2:3:0.1). Thus different spots for reference and test substance were detected using iodine vapours or by charring the plates using 5% methanolic sulfuric acid. The final compound was re-crystallized from water, after extracting the impurities with ethyl acetate. The IR spectrum of the synthesized compound was recorded on a FT-IR spectrophotometer, in potassium bromide (anhydrous IR Grade) pellets. The λ max of the synthesized compounds were determined on a FT-NMR spectrophotometer. The NMR spectra of the synthesized compounds were determined on a FT-NMR spectrophotometer. The λ max of the synthesized compounds was determined on UV/Vis double beam spectrophotometer by scanning the compounds between 400-200 nm in various solvents.

Mefenamic acid was obtained as a gift sample from P&B Laborotries, Mumbai. CDs were obtained as gift sample from S. A. Pharmachem, Mumbai. All the other chemicals used were of synthetic grade.

Mefenamic acid ester of ß CD (SA-001):
Synthesis of mefenamic acid ester of ß-CD involved 5 steps. First step involved tritylation of one of the primary...
Acetylated monotritylated β-CD (0.0036 mol, 6 g) was dissolved in N, N-dimethyl formamide (DMF, 200 ml) in round bottom flask. The cation exchange resin *T-63 MP resin, (d), Mefenamic acid free acid and, (e), 1,3-dicyclohexyl carbodiimide, (g) was added slowly into the reaction flask. The reaction mixture was stirred at room temperature and TLC ascertained reaction completion. The reaction mixture was stirred at 0° for 2 h and then at room temperature for 12 h. The reaction mixture was then filtered and dried under reduced pressure to give acetylated monotritylated β-CD (6.0 g). Selectively detritylated ß-CD (0.002 mol, 4 g) was dissolved in N, N-dimethyl formamide (DMF, 200 ml) in round flask. The cation exchange resin *T-63 MP (MP) (d) (3 g) was added slowly into the reaction flask. The reaction mixture was stirred at room temperature and TLC ascertained reaction completion. The reaction mixture was stirred at room temperature and TLC ascertained reaction completion. The reaction mixture was stirred at 0° for 2 h and then at room temperature for 12 h. The reaction mixture was then filtered to separate the precipitate of N, N-dicyclohexyl urea, the by-product. The filtrate was concentrated under reduced pressure to give acetylated mefenamic acid ester of β-CD (6.0 g)9.

Hydrolysis kinetics:
Hydrolysis of β-CD ester was studied in simulated gastric fluid and simulated intestinal fluid (0.05 M HCl buffer, pH 1.2 and 0.05 M phosphate buffer, pH 7.4). The ester showed negligible release in both the hydrolysis media. The hydrolysis of the ester was studied in rat faecal material (pH 7.4) to confirm the colonic hydrolysis of the esters12. The release of drug in rat faecal material was almost complete. The results of hydrolysis in rat faecal content are quoted in Table 1.

Release studies in 0.05 M HCl buffer, pH 1.2 and 0.05 M phosphate buffer, pH 7.4:
Same procedure was followed for the synthesized ester, β-CD ester was dissolved in HCl buffer and in phosphate buffer so that the final concentration of the ester is equivalent to 1 µg/ml of mefenamic acid. To each of the flasks of dissolution apparatus (Veego Scientific DA 6D model) 900 ml of buffer was added when the temperature reached 37±1°C the ester solution was added to the flask.
and stirred at 100 rpm. The ester showed negligible release in both the hydrolysis media.

**Release study in rat faecal matter, pH 7.4:**

The ester was dissolved in phosphate buffer (pH 7.4) so that final concentration of solution was 250 µg/ml. Fresh fecal material of rats was weighed (about 1 g) and placed in different sets of test tubes. To each test tube, 1 ml of the ester solution was added and diluted to 5 ml with phosphate buffer (50 µg/ml). The sets of test tubes were incubated at 37° for different intervals of time. For analysis the free drug was extracted in 5 ml CHCl₃ and directly estimated on double beam UV-spectrophotometer (Jasco, V-530 model, Japan). The concentration of free drug was determined using K and b values obtained from calibration curve.

**Antiinflammatory activity:**

The animal study protocols have met with the Institutional Animal Ethics Committee’s (IAEC) approval. Carrageenan-induced rat hind paw oedema method was used for determining antiinflammatory activity. Sprague Dawley rats of either sex (150-200 g) were selected. They were kept for a week and sacrificed 3 h after drug administration and the number of writhes was recorded for each animal. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The time period with the greatest percent of inhibition is considered the peak time. Compounds with less than 70% inhibition are considered to have minimal activity. The results are given in Table 2.

**Analgesic activity:**

The analgesic activity was evaluated by acetic acid induced writhing method. Albino mice of either sex with a weight range between 20 and 25 g were used. Acetic acid (0.6%) was used as irritant and 0.25 ml of this solution was injected intraperitoneally. An aliquot of groups of 6 animals were used for control and treated mice. Test animals were administered the drug or the standard at various pretreatment times prior to administration of acetic acid. The mice were placed individually into glass beakers and five minutes were allowed to elapse. The mice were then observed for a period of ten minutes and the number of writhes was recorded. The results are summarized in Table 2.

**RESULTS AND DISCUSSION**

The studies have revealed that the CD ester of mefenamic acid has retained its pharmacological activity in

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**TABLE 1: HYDROLYSIS KINETICS OF SA-001 IN RAT FAECAL MATTER**

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Drug released with various time intervals in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>SA-001</td>
<td>0</td>
</tr>
</tbody>
</table>

The hydrolysis of SA-001 in rat faecal contents was studied with time (n = 6).

**TABLE 2: ANTIINFLAMMATORY, ANALGESIC AND ULCEROGENIC ACTIVITY OF SA-001**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antiinflammatory activity (% inhibition of oedema)</th>
<th>Analgesic activity (%)</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6h</td>
<td>24h</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>NIL</td>
<td>NIL</td>
<td>NIL</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>21.49</td>
<td>08.08</td>
<td>70.2±3.06</td>
</tr>
<tr>
<td>SA-001</td>
<td>44.65</td>
<td>52.03</td>
<td>17.8±5.85</td>
</tr>
</tbody>
</table>

SA-001, Mefenamic acid (20.0 mg/kg or equivalent) and vehicle were administrated orally to Wistar rats and corresponding parameters were measured after specific time intervals after inducing either inflammation or hyperalgesia with carrageenan.
behavior of ester in gastrointestinal tract. This can be further confirmed by improved analysis, one approach to it is to isolate the specific strain of microorganism responsible for hydrolysis of CD and study the hydrolysis of conjugates in presence of them. The present study clearly indicates that conjugation of β-CD with mefenamic acid is a good method of masking the -COOH group and thus reducing the ulcerogenicity, a major drawback. The synthesized compound need to be further studied before being considered as potentially useful prodrugs.

ACKNOWLEDGEMENTS

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REFERENCES


Fig. 1: Photographs of ulcers produced by mefenamic acid and SA-001
Top panel shows the ulcerogenic effect of mefenamic acid treatment while the bottom panel depicts that after treatment with SA-001