A new approach: Enhancement of solubility of rofecoxib

M V Ramana, M Himaja, Kamal Dua, V K Sharma, Kavita Pabreja

Department of Pharmaceutical Chemistry, School of Science & Humanities, Vellore Institute of Technology, Vellore-632 014., Tamil Nadu, 1Department of Pharmaceutics and Pharmaceutical Chemistry, DJ College of Pharmacy, Niwari Road, Modinagar - 201 204, Uttar Pradesh, 2Research Scholar, ISF College of Pharmacy, Moga, Punjab, India.

The present study is aimed at improving the dissolution of poorly soluble drug, rofecoxib. Rofecoxib, being a drug, basic in nature shows a better solubility in acidic environment. Hence, an attempt was made to provide an acidic microenvironment around the drug molecules by incorporating various freely soluble acidic substances. Addition of such additives provides a dual effect of not only providing an acidic microenvironment but also imparts solubilizing effects due to the free water-soluble nature of the additives used. In the present work, β-cyclodextrin (β-CD) complexes of rofecoxib were prepared and solubilizing additives such as citric acid and ascorbic acid were incorporated in various proportions. Dissolution studies were performed in both HCl buffer (pH 1.2) and phosphate buffer (pH 7.4). The results have shown an enhanced dissolution rate of rofecoxib in both media from beta-cyclodextrin complex, and a further enhancement of dissolution was found in presence of ascorbic acid as well as citric acid. Differential scanning calorimetry (DSC) and infrared spectroscopy (IR) spectral studies performed on the solid complexes have shown that there is no interaction of the drug with β-CD and the additives. Hence, β-CD enhances the solubility of rofecoxib, and by creating an acidic microenvironment around the drug molecules, solubility of rofecoxib can be enhanced further, which in turn will enhance the absorption of rofecoxib and produce a better pharmacological activity.

Keywords: acidic microenvironment, ascorbic acid, β-cyclodextrin, citric acid, dissolution rate, rofecoxib, solubility

INTRODUCTION

It is well known that many drugs show bioavailability problems due to their low water solubility, slow dissolution rate, and instability in the gastrointestinal tract. Several methods were applied to overcome this problem. Among these methods, cyclodextrins (CDs) have been extensively studied to improve solubility,[1] dissolution,[2] and bioavailability[3] of various drugs. Due to its price, availability, and cavity dimensions (7.5 Å) β-cyclodextrin (β-CD) is widely used. It is the least toxic among all the other natural CDs.

The cavity size is suitable for common pharmaceutical drugs with molecular weights between 200 and 800 g/mol.[4,5] Though CDs have been investigated widely during the last two decades, their commercial application in pharmaceutical formulation was started only in recent years with drugs such as piroxicam and nimesulide.[6-9] Cyclooxygenase-2 (COX-2) inhibitors constitute a new group of non steroidal anti-inflammatory drugs (NSAIDs). Rofecoxib, is about 100-1000 times more selective on the COX-2 than on the COX-1 isoform with lower incidence of gastric bleeding and other gastrotoxic effect.[10-17] It is indicated for the treatment of symptoms and signs of osteoarthritis. It is sparingly soluble in acetone, slightly soluble in ethanol, and insoluble in water.[18]

The objective of the present study was to investigate the possibility of improving the solubility and dissolution rate of rofecoxib in presence of solubilizing agents such as ascorbic acid and citric acid, which are incorporated into β-CD complex.

MATERIALS AND METHODS

Rofecoxib (Dr. Reddys Lab., Hyderabad, India) β-CD (Cerestar USA, Inc., Hammond, IN, USA) of commercial purity grade were used. All other chemicals used were of analytical reagent grade. UV spectrophotometer (Jasco V530, Jasco Inc., Easton, MD, USA) and Dissolution test apparatus USP XXIV (Erweka-DT-1, Commerce Dr., Easton, MD-21601, Germany) were used for drug content estimation and dissolution studies respectively.

Address for correspondence:
M V Ramana, Pharmaceutical Chemistry, School of Science & Humanities, VIT University, Vellore - 632 014., India.
E-mail: kamalpharmacist@rediffmail.com, kamalpharmacist@gmail.com
Phase solubility studies
The phase solubility studies were carried out according to the method reported by Higuchi and Connors.\textsuperscript{19,20} Excess amount of rofecoxib was added to the aqueous solution of β-CD at various concentrations (0.002-0.02 M) and was shaken for 24 h at room temperature on rotary flask shaker. After equilibrium, the solutions were filtered and the portions of the solutions were analyzed for the drug content using JascoV-530 UV spectrophotometer at 261.0 nm. The experiment was performed in triplicate.

Preparation of β-cyclodextrin complexes
The solid complexes of rofecoxib and β-CD were prepared in 1:1 and 1:2 molar ratios and also with various solubilizing additives such as citric acid or ascorbic acid in 1:1:0.5 and 1:2:0.5 molar ratio using kneading method.\textsuperscript{[21]}

In kneading method, accurately weighed quantity of β-CD (3.61 g or 7.22 g for 1:1 and 1:2, respectively) was mixed with sufficient quantity water to obtain a smooth and homogeneous paste. Weighed quantity of rofecoxib (1 g) along with various solubilizing additives (citric acid, 0.61 g or ascorbic acid, 0.29 g) was added slowly by grinding. The mixture was ground for one hour. During this process, appropriate quantity of water was added to maintain suitable consistency. Finally, the paste was dried in oven at 40 °C for 48 hours.

The prepared solid mass was stored in dessicator and dried under vacuum to a constant weight. The dried products were removed, pulverized, and passed through sieve no. 100 and finally stored in closed airtight container.

Preparation of physical mixtures
The physical mixtures were prepared by mixing mesh. No 100-sieve fractions of rofecoxib and β-CD along with citric acid or ascorbic acid in the same proportions that were used in βCD complexes.

Characterization
Percentage yield
Rofecoxib: β-CD complexes in different molar ratios were prepared under similar set of conditions. The percentage yield and drug content were estimated to confirm that there is no degradation of the drug and expected amount of the drug is present in the product.

Drug content
Content of rofecoxib in β-CD complexes was estimated by UV spectrophotometric method using JascoV-530 UV spectrophotometer at 261.0 nm. β-Cyclodextrin complexes equivalent to 10 mg of pure drug was accurately weighed and dissolved in 100 ml of 1% v/v acetic acid, from that 1 ml was diluted to 10 ml and absorbance was measured. Drug content was determined from the regression equation generated from the standard plot for rofecoxib.

Hygroscopic studies
βCD-rofecoxib complexes were dried in a dessicator under anhydrous CaCl₂ for 2 days. Hundred milligrams of each of the dried samples were exposed to ambient atmospheric conditions (70 ± 5% RH, 30 ± 2°C) and accelerated humidity condition (99 ± 1% RH, 30 ± 2°C) for 2 days. The gain in their weight was determined and the percentage moisture absorbed was calculated.\textsuperscript{[22]}

Compatibility studies
Compatibility of the rofecoxib with β-CD and the solubilizing additives was confirmed by comparing the IR spectra and DSC thermograms taken for the drug, β-CD complexes with solubilizing agents.

In vitro dissolution study
Dissolution studies were performed separately in 900 ml HCl buffer (pH 1.2) and phosphate buffer (pH 7.4), maintained at 37 ± 0.5°C using an USP XXII type 2 dissolution rate test apparatus at a stirring speed of 50 rpm. Samples equivalent to 100 mg of rofecoxib were taken for dissolution studies. Aliquot (5 ml) was withdrawn at different time intervals up to 2 h, filtered, and replaced with the same volume of fresh dissolution medium. The samples were estimated for the amount of rofecoxib dissolved, by measuring the absorbance at 277.6 nm. The dissolution experiments were performed in triplicate.

In situ rat gut technique
The experiments were carried out as per the guidelines of Animal Ethics Committee. Comparison of extent of intestinal absorption of drug from a selected β-CD complex (which showed good in vitro performance), and pure drug was performed using in situ rat gut technique.\textsuperscript{[23]} Suspension of selected β-CD complex containing 100 mg of rofecoxib in 10 ml of 0.6% w/v sodium carboxy methyl cellulose (CMC) was introduced into the rat intestine. Samples of 0.1 ml were withdrawn at different time intervals. The collected samples were analyzed for rofecoxib content by the UV-spectrophotometric method at 261 nm. The studies were performed in duplicate and mean values were taken. The results obtained for the pure drug and β-CD complex were compared.

Analgesic and anti-inflammatory activity
Analgesic activity was studied by writhing method\textsuperscript{[24]} using mice and anti-inflammatory activity by using rat paw edema method.\textsuperscript{[24]} The animals used for both the activities were divided into three groups, each group containing six animals. One group served as control, which were administered with a suspension of 0.6% w/v sodium CMC, second group was administered with a suspension of pure rofecoxib in 0.6% w/v sodium CMC and the third group was administered with a suspension of selected β-CD complex in 0.6% w/v sodium CMC orally. The analgesia was induced by 0.6% v/v acetic acid intraperitoneally and inflammation was induced by injecting...
0.1% w/v carrageenan in the subplanter region of hind paw of rat. Percentage efficiency of analgesia of the drug/test formulations is calculated using the following formula:

\[ \text{Percent inhibition} = \frac{W_c - W_f}{W_c} \times 100, \]

where \( W_c \) is the average number of writhes in the control group and \( W_f \) the number of writhes in the formulation group.

The percentage inhibition in edema by the drug/test formulation was calculated using the formula:

\[ \text{Percentage edema inhibition} = \frac{V_c - V_t}{V_c} \times 100, \]

where \( V_c \) is the volume of paw edema in the control group and \( V_t \) the volume of paw edema in the formulation treated group.

**DISCUSSION**

Phase solubility studies of rofecoxib β-CD systems in water at 25°C revealed that the solubility of rofecoxib increased linearly with the increase in the concentration of β-CD, showing a typical A\(_1\)-type phase solubility curve. This curve may be ascribed to the formation of a stoichiometric 1:1 complex of rofecoxib and β-CD. The apparent 1:1 stability constant (\( K_c \)) was calculated from the straight line of the phase solubility diagram by using the following equation:

\[ K_c = \frac{\text{Slope}}{\text{Intercept (1-Slope)}}. \]

The constant value was found to be 24.57/M\(^1\).

Solid inclusion complexes of rofecoxib were prepared by kneading method in two molar ratios (1:1 M and 1:2 M) along with addition of solubilizing additives such as citric acid or ascorbic acid (1:1:0.5 M). The presence of solubilizing additives appears to modify the dissolution behavior of the drug by altering its surrounding environment.

Low values of standard deviation in drug content of β-CD complexes in respect of drug content indicated uniform drug distribution in all the complexes.

The particle size of the β-CD complexes ranged from 3.27 to 163.5 µ and the average diameter was found to be in the range of 58.3-63.2 µ. Smaller particle size of the complexes is also responsible for the enhanced solubility of the drug.

The hygroscopic studies revealed that the maximum moisture absorption was observed in complexes containing citric acid as a solubilizing additive, whereas complexes prepared with ascorbic acid are less hygroscopic. The results obtained has been shown in Table 1.

DSC thermograms of rofecoxib β-CD complexes containing solubilizing agents showed peaks at 209°C, 260°C, 190°C, and 153°C, which corresponds to the melting point of rofecoxib, β-CD, ascorbic acid, and citric acid, respectively.
Table 1: Physical characteristics of the β-cd-rofecoxib complexes prepared by kneading method

<table>
<thead>
<tr>
<th>Composition</th>
<th>Ratio</th>
<th>Drug content (theoretical value) in μg/ml</th>
<th>Average particle size (μm)</th>
<th>% Moisture absorption 70 ± 5% RH, 30 ± 2°C</th>
<th>% Moisture absorption 70 ± 5% RH, 30 ± 2°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF: β-CD</td>
<td>1:1 M</td>
<td>9.84 ± 0.29 (10)</td>
<td>59.32</td>
<td>1.1 ± 0.18</td>
<td>3.7 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>1:2 M</td>
<td>9.90 ± 0.49 (10)</td>
<td>60.12</td>
<td>1.0 ± 0.37</td>
<td>3.1 ± 0.27</td>
</tr>
<tr>
<td>RF: β-CD: AA</td>
<td>1:1:0.5 M</td>
<td>9.58 ± 0.37 (10)</td>
<td>58.34</td>
<td>2.3 ± 0.42</td>
<td>7.3 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>1:2:0.5 M</td>
<td>9.62 ± 0.34 (10)</td>
<td>63.21</td>
<td>2.7 ± 0.46</td>
<td>7.7 ± 0.35</td>
</tr>
<tr>
<td>RF: β-CD:CA</td>
<td>1:1:0.5 M</td>
<td>9.76 ± 0.28 (10)</td>
<td>68.92</td>
<td>6.2 ± 0.63</td>
<td>12.6 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>1:2:0.5 M</td>
<td>9.89 ± 0.59 (10)</td>
<td>59.93</td>
<td>7.3 ± 0.41</td>
<td>13.2 ± 0.43</td>
</tr>
</tbody>
</table>

Values given in the parenthesis indicate theoretical values; RF = rofecoxib; β-CD = β-cyclodextrin; AA = ascorbic acid; CA = citric acid; M = molar

Absence of additional peaks indicated that there was no interaction between the drug and carriers [Figure 1].

IR spectra for the drug and complexes have shown no additional peaks, which also gave evidence that there is no interaction between the drug and carriers [Figure 2].

In vitro dissolution studies revealed that the solubility of rofecoxib was found better in acidic media. It is also observed that the β-β-CD complexes exhibited faster dissolution rate than the pure drug and their corresponding physical mixtures, but physical mixtures exhibited faster dissolution rate than the pure drug. The Dissolution Profile obtained for β-cd-rofecoxib complexes has been shown in Table 2.

Increase in dissolution from physical mixtures and solid complexes is exhibited due to the surface tension lowering effect of the β-CD, resulting in wetting of hydrophobic drug surface. The increase in dissolution rate is due to the formation of water-soluble inclusion complexes with the β-CD. Moreover, the dissolution rate of the drug β-CD inclusion complexes containing citric acid in 1:2:0.5 M ratio is superior than all other formulations. This could be due to creation of acidic microenvironment around the drug molecules and also imparts solubilizing effects due to free water-soluble nature of the additives used, which enhanced the drug dissolution.

Studies on in situ rat gut technique revealed that the extent of intestinal absorption was more in β-CD complex containing citric acid as solubilizing agent in comparison to the pure drug. The first-order rate constant obtained for the pure drug was less (0.003/min), in comparison to that of β-CD complex containing citric acid 1:1:0.5 M (0.015/min).

The in vivo performance of selected β-CD complex showed better analgesic and anti-inflammatory activity than the pure drug. The enhanced results of the above-mentioned biological activities are due to its enhanced dissolution rate from β-CD complex system. In vivo performance for Analgesic and Anti-Inflammatory Activity of β-CD complex has been shown in Tables 3 and 4 respectively.
CONCLUSIONS

The present study concludes that complexation of rofecoxib with β-CD can enhance the dissolution rate of the rofecoxib. Dissolution rate of rofecoxib can be enhanced further by incorporating acidic substances like citric acid and ascorbic acid into the formulations, and thereby improve its bioavailability and have the potential to produce a faster onset of action.

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REFERENCES


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