DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF CYCLODEXTRIN COMPLEXES OF ETORICOXIB

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Abstract: Etoricoxib is an anti-inflammatory drug largely used in a variety of acute and chronic inflammatory diseases, but is associated with low aqueous solubility and poor dissolution leading to a delayed rate of absorption and onset of action. This study focuses on the development and pharmacological evaluation of a series of binary systems of etoricoxib with cyclodextrins. The binary systems of etoricoxib with β-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) were prepared by the kneading method. Drug-cyclodextrin interactions in solution were investigated by the phase solubility analysis. X-ray diffractometry studies were carried out for the characterization of all binary systems. In vivo studies were performed using the tail flick and Eddy’s hot plate apparatus. The results of the phase solubility studies indicated an increase in etoricoxib solubility upon complexation with β-cyclodextrin (stability constant, K, value of 198.6 and 209.9 L/mol for 1:1 and 1:2 β-CD complexes of the drug, respectively) and a further increase on complexation with HP-β-CD (stability constant, K, value of 265.3 and 355.8 L/mol for 1:1 and 1:2 HP-β-CD complexes of the drug, respectively). Results of the in vivo drug activity studies also pointed towards an enhanced antinociceptive effect of etoricoxib upon cyclodextrin complexation with 1:2 drug HP-β-CD complex showing maximum effect.

Keywords: β-cyclodextrin (β-CD), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), kneading method, phase solubility study, antinociceptive activity

Etoricoxib is a selective COX-2 inhibitor. Chemically, it is 5-chloro-6′-methyl-3-[4-(methylsulfonyl)phenyl]-2,3′-bipyrindine, and is used in the treatment of rheumatoid arthritis, osteoarthritis, gout, spondylitis and other inflammatory disorders (1). It reduces the prostaglandin generation from arachidonic acid, by inhibiting COX-2 enzyme. However, it shows delayed rate of absorption and onset of action, owing to its very low aqueous solubility and poor dissolution resulting in formulation problems and limited therapeutic application (2). An additional effect of the drug was found to be its antinociceptive effect, by which decreased stimuli to pain was observed in the subject. Cyclodextrins, also known as cycloamyloses, belong to the family of cyclic oligosaccharides, and are made up of 6 or more α-1,4-linked D-glucopyranose units. These contain 6 to 8 glucose monomers in a ring, forming a conical shape (3). β-Cyclodextrin, a seven sugar ring molecule has been studied extensively in spite of its low aqueous solubility. Cyclodextrins contain a lipophilic central cavity and a hydrophilic exterior (4). Drug complexation with cyclodextrins has resulted in increased drug solubility, stability and bioavailability, by modifying physical, biological, and chemical properties. 2-Hydroxypropyl-β-cyclodextrin is being preferred, owing to its greater water solubility and less toxicity than β-cyclodextrin (5–7).

The present study was designed to prepare cyclodextrin complexes of etoricoxib by kneading method and to evaluate the formulated complexes for phase solubility studies and in vivo animal studies, for studying the effect of cyclodextrin complexation of the drug on its pharmacological activity. XRD analysis of the prepared complexes was also carried out.

MATERIALS AND METHODS

Materials and reagents

Etoricoxib was received as a gift sample from Helios Pharmaceuticals, India. 2-Hydroxypropyl-β-cyclodextrin was kindly gifted by Cadila
β-Cyclodextrin was procured from CDH, India. All the chemicals and reagents were of analytical grade and were used as such.

**Preparation of drug cyclodextrin complexes**

Cyclodextrin inclusion complexes were prepared in 1:1 and 1:2 (drug : cyclodextrin) ratio by the kneading method (8). The drug, i.e., etoricoxib, and the cyclodextrins were weighed accurately and mixed thoroughly by trituration in a mortar. The physical mixture was wetted in a mortar using a minimum volume of water and kneaded thoroughly with a pestle to obtain a paste, which was then dried under vacuum at room temperature, sieved through 0.25 mm sieve and stored in desiccator until further evaluation. The above defined method was employed for preparing β-CD and HP-β-CD complexes of etoricoxib in 1:1 and 1:2 molar ratios.

**XRD analysis**

The drug and drug-cyclodextrin complexes were subjected to X-ray diffraction study for the confirmation of complex formation. X-ray powder diffraction patterns were recorded on an X-ray diffractometer (Model X’Pert, Philips, Netherlands) using Ni-filtered, Cu K radiation, voltage of 40 kV and 25 mA current. The scanning rate employed was one minute over the 0–100° diffraction angle (2θ) range.

**Phase solubility studies**

An excess of etoricoxib was added to aqueous solutions of increasing cyclodextrin concentrations (0–30 mM) in stoppered conical flasks. The samples were stirred at room temperature and an aliquot was filtered through a 0.45 μm membrane filter (Millipore). The samples were spectrophotometrically assayed for drug content at 284 nm (Systronics 2202, India). The apparent stability constant (Kc) of the drug-cyclodextrin complex was calculated according to equation 1 (9):

\[
K_c = \frac{\text{slope}}{S_0} \times (1 - \text{slope})
\]

where, Kc is the apparent stability constant, slope is obtained from the linear portion of the phase solubility diagram, and S0 is the aqueous solubility of etoricoxib. The phase solubility studies were performed on etoricoxib:β-CD complexes (1:1 and 1:2) and etoricoxib:HP-β-CD complexes (1:1 and 1:2). Each experiment was carried out in triplicate.

**In vivo study**

Analgesic effect of etoricoxib was selected as a parameter for assessment of the time dependent pharmacological activity of the treatments. Tail flick latency and reaction latency were used to quantitate the antinociceptive effect of etoricoxib. Nociceptive threshold in rats was measured using the tail flick apparatus and Eddy’s hot plate method (10). The tail flick latency was considered as the time between tail exposure to radiant heat and tail withdrawal. Electrically heated nichrome wire was used as a source of radiant heat in the analgesimeter (INCO, India). The intensity of radiant heat was regulated in order to obtain pretreatment latency between 2 and 3 s in the animals. The reaction latency was considered as the time between contact of the animal with the hot plate and the consequent reaction. Eddy’s hot plate (INCO, India) was used as a source of heat in reaction latency experiment. A cut off latency time was fixed at 10 s. Tail flick latency and reaction latency were expressed as a percentage of the maximum possible effect (MPE):

\[
\text{MPE (%) } = \frac{(\text{Post treatment latency} - \text{pre treatment latency})}{(\text{Cut off time} - \text{pre treatment latency})} \times 100
\]

Thus, tail flick latency and reaction latency were observed immediately before and 15, 30, 45, 60, 90 min after the respective treatment(s) according to the in vivo study protocol. The protocol of study was approved by animal ethics committee of the department and the experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India (Chitkara College of Pharmacy Animal Facility Registration Number: 1181/ab/08/CPCSEA).

**In vivo study protocol**

**Group I (Vehicle control group)**

Tail flick latency and reaction latency were observed immediately before and at various time points after vehicle (10 mL/kg, oral) administration.

**Group II (Etoricoxib control group)**

Tail flick latency and reaction latency were observed immediately before and at various time points after etoricoxib (6 mg/kg, i.p.) administration.

**Group III (Etoricoxib β-CD treatment group)**

Tail flick latency and reaction latency were observed immediately before and at various time points after administration of β-CD complexes of etoricoxib (the amount of drug equivalent to 6 mg/kg, i.p.).
Figure 1. Phase solubility diagram for etoricoxib at increasing β-CD concentrations

\[ y = 0.0057x + 0.035 \]
\[ R^2 = 0.9897 \]

Figure 2. Phase solubility diagram for etoricoxib at increasing HP-β-CD concentrations

\[ y = 0.0097x + 0.065 \]
\[ R^2 = 0.9917 \]

Figure 3. Effect of various treatment(s) on the tail flick latency in rats. Results are expressed as the mean % MPE (Maximum possible effect)
Figure 4. Effect of various treatment(s) on the reaction latency in rats. Results are expressed as the mean % MPE (Maximum possible effect).

Figure 5. XRD of a) drug, b) 1:2 drug β-CD complex, c) 1:2 drug HP-β-CD complex.
Group IV (Etoricoxib HP-β-CD treatment group)

Tail flick latency and reaction latency were observed immediately before and at various time points after administration of HP-β-CD complexes of etoricoxib (the amount of drug equivalent to 6 mg/kg, i.p.).

Statistical analysis

All experimental data were analyzed using Student’s t-test and one way ANOVA, wherever necessary, to determine statistical difference in the results. A probability value $p < 0.05$ was considered statistically significant.

RESULTS

Etoricoxib is a sparingly soluble drug. The solubility of etoricoxib in distilled water at pH 6.8 at room temperature was determined to be 27.0 μg/mL and was notably affected by the presence of β-CD and HP-β-CD. The solubility of etoricoxib in 3 mmol/L β-CD aqueous solution was 0.05 mmol/L while it was 0.12 mmol/L in 15 mmol/L β-CD solution. The obtained phase solubility diagram was linear (Fig. 1) and could be classified as $A_t$ type according to Higuchi and Connors (10). The slope of solubility diagram was less than one; it was therefore assumed that the solubility increase could be attributed to the formation of the complexes of the drug with the cyclodextrin molecule. Stability constant ($K_c$) value for etoricoxib β-CD 1:1 and 1:2 complexes was found to be 198.6 and 209.9 L/mol, respectively. These observations point towards the stability of the complexes in the defined ratios. Furthermore, the solubility of etoricoxib in 3 mmol/L HP-β-CD aqueous solutions was 0.05 mmol/L while it was 0.21 mmol/L in 15 mmol/L HP-β-CD solution (Fig. 2), indicating an elevated increase in the solubility of drug complexation with HP-β-CD compared to β-CD. Stability constant ($K_c$) value obtained for the etoricoxib HP-β-CD complexes was 265.3 and 355.8 L/mol for 1:1 and 1:2 complexes, respectively, demonstrating that the etoricoxib HP-β-CD complexes have higher stability than the β-CD complexes of the drug.

Figure 5 shows the XRD patterns of etoricoxib, drug β-CD complex and drug HP-β-CD complex. Characteristic peaks of the drug in the XRD pattern indicate that the drug is present as a crystalline material. Furthermore, a reduced number of signals, of markedly lower intensity, are noticeable in the cyclodextrin complexes of the drug, indicating the amorphous nature of the complexes compared with the free drug molecules. Moreover, in case of HP-β-CD complexes of etoricoxib, a marked reduction in peak intensity was observed as compared to β-CD complexes of etoricoxib, indicating more amorphous nature of the former.

The results of in vivo drug activity studies show that the vehicle administration did not exert any effect on tail flick latency and reaction latency in rats at any of the time points. However, etoricoxib per se did produce a marked antinociceptive effect in rats. Peak time of antinociceptive effect of the drug was observed to be 45 min after the administration. On administration of the etoricoxib β-CD complex, the analgesia occurred at a peak time of 60 min, whereas etoricoxib HP-β-CD complex showed the same peak time as etoricoxib β-CD complex but with increased % MPE illustrating enhanced antinociceptive effect (Fig. 3). Similar results were obtained with Eddy’s hot plate method, in which peak time for etoricoxib control group was found to be 45 min and that for etoricoxib β-CD and etoricoxib HP-β-CD complex treatment groups was found to be 55 min with etoricoxib HP-β-CD complex showing enhanced effect than etoricoxib β-CD complex (Fig. 4).

DISCUSSION

The results of the phase solubility studies carried out on the drug and its cyclodextrin complexes indicate that etoricoxib when administered alone, exhibits a very low aqueous solubility and poor dissolution, resulting in delayed rate of absorption and onset of action. On complexation with β-cyclodextrin in a molar ratio of 1:2, the stability of drug was markedly increased, which is evident from the value of the stability constant ($K_c$) obtained from the phase solubility diagrams, which was found to be 209.9 L/mol for the etoricoxib β-CD complex and 355.8 L/mol for the etoricoxib HP-β-CD complex. It is thus evident that of the complete series of the complexes that were prepared and evaluated, the 1:2 complex of the drug with HP-β-CD exhibited the most desirable physicochemical properties owing to its high stability constant value.

The results of the in vivo drug activity studies throw light on an enhanced antinociceptive effect of etoricoxib on its complexation with cyclodextrins. It was established that the etoricoxib HP-β-CD complex exhibited a more favored response than the etoricoxib β-CD complex, which further ascertained the 1:2 complex of the drug with HP-β-CD to be the most effective combination for the best possible effect. The enhanced antinociceptive effect observed on the cyclodextrin complexation of the
drug may be attributed to the properties of the cyclodextrins such as their high aqueous solubilities resulting in an improved availability of the hydrophobic drug molecules aiding them in reaching and/or remaining at their site of action.

Thus, the development of an inclusion complex for etoricoxib makes it possible to handle the limiting physicochemical and toxicological properties, such as solubility. In addition, these systems improve the therapeutic effect of etoricoxib, favoring its clinical use.

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