INCLUSION COMPLEX OF DEXIBUROFEN AND IT’S IN VITRO AND IN VIVO EVALUATION

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ABSTRACT

Dexibuprofen, a poorly water-soluble nonsteroidal anti-inflammatory drug has better anti-inflammatory effect than Ibuprofen. The aqueous solubility and dissolution rate of Dexibuprofen (DI) with β-cyclodextrin (β-CD) was investigated. The Dexibuprofen was complexed with β-CD in 1:1 and 1:2 ratio by freeze drying and kneading method. The aqueous solubility of Dexibuprofen was determined by phase solubility method. The inclusion complexes were prepared and characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry techniques (DSC). FTIR spectra and DSC thermograph of Dexibuprofen, β-CD, and Dexibuprofen-β-CD (DI-β-CD) complexes indicate there is no chemical interaction and confirmed the stability of the drug with its complex. The invivo analgesic activity by Tail-flick and Eddy’s hot plate method and anti-inflammatory activity by paw edema method were performed. Invitro studies showed that the solubility and dissolution rate of Dexibuprofen were significantly improved by complexation with β-cyclodextrin. In contrast, freeze-dried complexes showed higher dissolution rate than the other complexes and better analgesic and anti-inflammatory activity with respect to the drug alone.

Key words: Dexibuprofen, Inclusion complex, Phase solubility, Eddy’s hot plate method, Paw edema method.

INTRODUCTION

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are recognised as effective analgesics in acute and chronic pain. NSAIDs are associated with a number of adverse effects. These include effects on the kidney, and exacerbating asthma in some people, but the most important adverse effect of NSAIDs is that on the gastrointestinal tract. These can cause symptoms of an ulcer in some people, the ulcers may bleed, and indeed some people may die of a bleeding ulcer caused by NSAIDs. Most patients receive an ulcer-protective drug as prophylaxis during long-term treatment. Patients with osteoarthritis more often develop symptomatic liver disease than patients with rheumatoid arthritis. Liver function should be monitored regularly during long-term treatment. NSAIDs are associated with adverse renal [kidney] effects caused by the reduction in synthesis of renal prostaglandins (Brater DC et al., 2002) and also induce warm antibody haemolytic anaemia by inducing antibodies to Rh antigens. The poor aqueous solubility and wettability of NSAIDs, however, give rise to difficulties in the design of pharmaceutical formulations and lead to variable oral bioavailability.

Ibuprofen, a NSAID is very effective for the systemic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Ibuprofen was formulated into many topical preparations to reduce the adverse side effects and avoid the hepatic first-pass metabolism. But it is difficult to maintain effective concentrations by topical

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delivery of ibuprofen due to its poor skin permeation ability (Yano T et al., 1986).

Dexibuprofen (S (+) - isomer ibuprofen), which has better anti-inflammatory effects than ibuprofen and less gastric damage belongs to class II of Biopharmaceutical Classification System (BCS) having low water solubility which is rate limiting step in absorption(Adams S et al., Bonabello A et al., 2003) of drug in GI tract.

β-cyclodextrin (β-CD) was used since this posses a special ability to complex with drugs enabling them to increase solubility, reduce bitterness, enhance stability and decrease tissue irritation upon dosing(Mosher GL et al., 2002). One of the most common applications of cyclodextrins cited in the pharmaceutical literature is the enhancement of bioavailability. The use of cyclodextrins in oral dosage forms, and many which specifically address the effect of cyclodextrins on oral absorption and/or bioavailability (Lofthson T et al., 2004). The objective of this research was to improve solubility, dissolution and bioavailability of Dexibuprofen by forming inclusion complexes with β-cyclodextrin.

MATERIALS AND METHODS

Materials

Dexibuprofen was obtained as a gift sample from Alkem laboratories Pvt. Ltd, (Mumbai). The following chemicals β-cyclodextrin, Carboxy methyl cellulose and Carrageenan, were purchased from Himedia laboratories Pvt. Ltd (Mumbai). All other chemicals were of analytical grade.

Methods of preparation

Kneading method: (KM)

The inclusion complexes of Dexibuprofen with β-CD were prepared in 1:1 and 1:2 molar ratios (Osadebe PO et al., 2008) using acetone as solubilizing additive. Dexibuprofen was accurately weighed and dissolved in acetone to which required quantity of β-CD was added in a mortar so as to obtain a homogenous paste. The thick slurries were kneaded for one hour and dried at room temperature.

Freeze drying method: (FD)

Dexibuprofen was accurately weighed and added to 50ml of aqueous solution of β-CD in 1:1 and 1:2 molar ratios. The suspension was stirred for five days and lyophilized (Cao FT, Guo J et al., 2005).

Phase solubility studies

The phase solubility studies were carried out according to the method reported by Higuchi and Connors (Higuchi T et al., 1965). An excess of drug was added to 25 ml portions of distilled water each containing variable amount of β-CD, such as 0.30x 10^{-2}, 0.60x10^{-2}, and 0.90x10^{-2}, 1.2x10^{-2} and 1.5x10^{-2}mol/L. All the above solution was shaken for 72 hr using mechanical stirrer at 150rpm. The solubility of Dexibuprofen in β-CD solution was calculated. The apparent solubility constant (k_c) was calculated using the equation.

K_c = slope/intercept (1-slope)

Invitro dissolution studies

The invitro dissolution studies of different formulations of Dexibuprofen- β-CD complexes were performed using USP XXII rotating basket method (USP, 2005). The samples were placed in a hard gelatin capsules. 900ml of 0.1N Hcl was used as dissolution media at 37±0.5°C and maintaining stirring speed at 50rpm. The samples were withdrawn and replaced with same volume of fresh dissolution media at different time intervals and estimated at 220nm by U.V. Spectrophotometer.

Differential scanning calorimetry

The DSC thermograms were recorded on a DSC (model Dsc-60, Shimadzu). Samples of were heated in hermetically sealed aluminium pans over temperature range of 10¹⁰⁰⁰ – 300 °c at a constant rate of 10³⁰⁰⁰°C/min under nitrogen purge.

Fourier Transform Infra Red Spectroscopy

FTIR spectra were obtained on FTIR-8400S (Shimadzu). Samples were prepared in KBr disks. Data were collected over a spectral region from 4000 to400cm⁻¹.

Experimental animals

Wister male albino rat weighing 150-200g were used for the study. The animals were housed in the animal house maintained under standard hygienic conditions, at 20 ± 2°C, 12 hour day and night cycle and fed with standard pellet diet. The study was carried out as per CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals) norms after obtaining approval from the Institutional Animal Ethical Committee (SVCP/IAEC/04-0037).

The animals were divided into three groups of 6 animals each.

Group I: Control (2% w/v carboxy methyl cellulose suspension).

Group II: Standard (Dexibuprofen 50 mg/kg).

Group III: Test (freeze dried DI-β-CD 1:2 complex).

Eddy’s hot plate method

The animals were individually placed on the hot plate maintained at 55±1 °C, one hour after their respective treatments (Turner RA et al., 1965). The response time was noted as the time at which animals reacted to the pain.
stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 30 seconds.

**Tail flick method**  
After one hour of drug administration, the tip of tail was dipped up to 5 cm into hot water maintained at 50-55°C. The response time was noted as the sudden withdrawal of the tail from the hot water (OL Davies et al., 1946). Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded.

**Anti-inflammatory activity**  
Acute inflammation was induced in all groups by injecting 0.1 ml of 1% w/v carrageenan into the subplantar region of the right hind paw of rats (Winter CA et al., 1962). Mean paw volume was measured 1 h prior to carrageenan injection using plethysmometer and at 0, 1, 2, 3 and 4hr after the carrageenan injection.

**Statistical analysis**  
Data were statistically analyzed by analysis of variance (ANOVA) with the level of significance set at \( p < 0.05 \). Critical differences between means were evaluated by Dunnett’s multiple comparison test at \( p < 0.05 \).

**RESULTS AND DISCUSSION**

**Fourier Transform Infrared Spectroscopy**  
The FTIR spectra of Dexibuprofen showed prominent peaks at 3087, 2994, 1707, 1466, 1320, 950 cm\(^{-1}\) corresponding to O-H stretching, C-H stretching, C=O stretching, C-C stretching, C-O stretching, O-H bending respectively. FTIR spectra of Dexibuprofen, \( \beta \)-CD, and Dexibuprofen -\( \beta \)-CD complexes obtained indicates there is no chemical interaction between the drug and \( \beta \)-CD.

**Differential Scanning Calorimetry**  
The DSC thermo grams of the Dexibuprofen exhibits a characteristic endothermic fusion peak at 61.75°C, \( \beta \)-CD shows a broad endothermic effect at 118.34°C and Dexibuprofen -\( \beta \)-CD complex shows the persistence of the endothermic peak of Dexibuprofen for the kneaded and freeze-dried product. Furthermore, the characteristic endothermic effect of \( \beta \)-cyclodextrin is slightly shifted to higher temperatures for the freeze-dried products, indicating that Dexibuprofen has complexed with \( \beta \)-cyclodextrin.

**Phase solubility studies**  
The aqueous solubility of Dexibuprofen increased linearly as a function of \( \beta \)-CD concentration as shown in figure 3. The aqueous solubility of drug increases linearly as a function of \( \beta \)-cyclodextrin concentration. It is clearly observed that the solubility diagram of Dexibuprofen in presence of \( \beta \)-cyclodextrin can be classified as the classified as AL type according to Higuchi and Connors. The linear DI-\( \beta \)-CD correlation with slope of less than 1 suggested that the formation of soluble inclusion complexes with respect to \( \beta \)-cyclodextrin. The apparent stability (Kc) was calculated as 175M\(^{-1}\) indicating that the DI-\( \beta \)-CD complexes are adequately stable.

**Invitro dissolution studies**  
The dissolution profile of Dexibuprofen in 0.1N Hcl at 120min showed 43.09%. The inclusion complexes prepared by kneading method showed 78.15% and 88.01% for 1:1 and 1:2 molar ratios respectively whereas freeze dried showed 95.03% and 96.05% for 1:1 and 1:2 molar ratios respectively at 120 min. The freeze dried product of 1:2 showed marked increases in drug release compared with other methods.

**Analgesic activity by using tail-flick method and Eddy’s hot plate method**  
The analgesic activity of freeze dried 1:2 complex was determined by using Eddy’s hot plate method and tail-flick method respectively (figure5 & 6). The complex exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared to the pure drug and control.

**Anti-inflammatory activity by Carrageenan-induced Paw edema**  
Anti-inflammatory activity of freeze dried 1:2 complex was found significant at the level of \( P<0.05 \), when compared to control and less compared to pure drug (Dexibuprofen).

**Figure 1.** Pure drug (A), \( \beta \)-CD (B), Kneading 1:1(C) &1:2 (D), Freeze drying 1:1(E) & 1:2(F)
Figure 2. Pure drug (A), β-CD (B), Kneading 1:1(C) &1:2 (D), Freeze drying1:1(E) & 1:2(F)

Figure 3. Phase solubility studies

Figure 4. Invitro dissolution of inclusion complexes

Figure 5. Analgesic activity by Eddy’s hot plate method of freeze dried 1:2, n=6, mean± SEM at p<0.05.

Figure 6. Analgesic activity by Tail flick method of freeze dried 1:2, n=6, mean± SEM at p<0.05.

Figure 7. Injection of carrageenan into subplantar region
DISCUSSION

Inflammation has different phases the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. The development of carrageenan induced paw edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins (Larsen GL, et al., 1983, Brooks PM, et al., 1991, Vane J, et al., 1987). We observed that freeze dried 1:2 complex at 50mg/kg p.o shows significant inhibition of inflammation when compared to pure drug (Dexibuprofen).

The analgesic activity by tail-flick method and Eddy’s hot plate method of freeze dried 1:2 complex posses better analgesic activity by increase threshold potential of pain may be due to above mentioned mechanism.

REFERENCES


