Long Acting Parenteral Formulation of Heparin

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Received on: 02-05-2009; Accepted on:09-07-2009

ABSTRACT

The aim of the present work was to develop and evaluate of micro particles (MPs) as potential drug carriers for low molecular weight heparin. This study investigates the potentiality of micro particles as prolong release systems for subcutaneous administration of Enoxaparin (LMWH). Owing to the high solubility of the drug in water, the Double emulsification technique was chosen as one of the most appropriate method of preparation. Biodegradable polymer poly [D, L-lactic-co-glycolic acid] 85:15 (PLGA) was used for the preparation of MPs. The drug loading has been optimized by varying the concentration of the polymer in the organic phase. The systems were characterized by particle size analysis for their mean size, size distribution and surface morphology by scanning electron microscopy (SEM). The average diameter of the microparticles of the optimized formulation was 8.26 µm. Differential scanning calorimetric analysis (DSC) suggested that Enoxaparin was molecularly dispersed in the polymeric matrices. To evaluate the influence of the polymeric carrier on the drug release, an in vivo study using New Zealand variety of rabbits was performed. Wright’s method, Duke’s method was used as simple and non-invasive techniques for analysis of blood. Results from the animal studies demonstrated that the release process of Enoxaparin from MPs is affected by the molecular weight and concentration of the polymer employed.

Keywords: Low Molecular Weight Heparin, Enoxaparin, Subcutaneous injection, prolonged release formulation, Biodegradable, poly (D,L- lactide-co-glycolide), micro particles

INTRODUCTION

The growing interest in controlled drug release in human medicines can be attributed to the promise of increased patient compliance due to a reduced frequency of administration, improvement in the safety and efficacy of drug substance (particularly those with narrow therapeutic index) and a reduction in undesirable side effects. Parenteral preparations are intended for injection through the skin or other external boundary tissues, rather than through the alimentary canal, so that the active substances are administered directly into the blood vessel, organ, tissue, or lesion.

The oral route represents the most convenient administration pathway which is highly accepted by all patients. However, low molecular weight heparin is not absorbed from the gastrointestinal tract (presumably owing to their molecule size and their strong anionic charge provoking ionic repulsions from negatively charged mucus and epithelial tissue). Polymeric drug delivery systems have been widely developed and provide an attractive alternative for progressive long term delivery of therapeutic agents. These polymeric dosage forms offer many advantages like:

- Drugs can be delivered to tissues in a sustained and continuous fashion
- Drugs are well protected
- Site specific delivery may be achieved and repeated drug administration can not be required.

Because of their desirable biocompatible and biodegradable properties, poly (DL lactide-co-glycolide) has been widely studied for use as microparticulate vehicle for long-term sustained release preparation. Increasing attention has also been paid to the colloidal particles of these polymers as injectable drug carriers which would enable long systemic circulation.

The materials utilized for the preparation of microparticles should ideally fulfill the following prerequisites. Longer duration of action, control of content release, increase of therapeutic efficiency, protection of drug, reduction of toxicity, biocompatibility, sterilizability, relative stability, water soluble or dispersability, bioresorbability, targetability, polyvalent.

Low Molecular Weight Heparins (LMWH)

Heparin has been fractionated into LMW forms by different methods. LMW heparins have a different anticoagulant profile. They selectively inhibit factor Xa with little effect on factor II.

Advantages of LMW Heparins

- Better subcutaneous bioavailability compared to unfractionated heparin.
- Longer and more consistent monoexponential t½.

Enoxaparin

Enoxaparin is prepared by alkaline degradation of heparin benzyl ester obtained from the intestinal mucosa of pig. The molecular mass ranges between 3500 to 5500.

Mechanism of action

Enoxaparin binds to and accelerates the activity of antithrombin III. By activating antithrombin III, Enoxaparin preferentially potentiates the inhibition of coagulation factors Xa and IIa. The anticoagulant effect of Enoxaparin can be directly correlated to its ability to inhibit
factor Xa. Factor Xa catalyzes the conversion of prothrombin to thrombin. So Enoxaparin’s inhibition of this process results in decreased thrombin and ultimately the prevention of fibrin clot formation.

**Monitoring**
- Enoxaparin does not affect the INR, PT or aPTT
- Anti-factor Xa levels can be measured, and are often used to monitor Enoxaparin activity

**Absorption**
Mean absolute bioavailability of Enoxaparin, after 1.5 mg/kg given subcutaneously, based on anti-Factor Xa activity is approximately 100% in healthy volunteers.

**MATERIALS AND METHODS**
Enoxaparin gift sample was obtained from Gland Pharma, Hyderabad. PLGA was purchased from Sigma Aldrich, Bangalore. Biodegradable microparticles of Enoxaparin with polymer PLGA was prepared by Double emulsification method. The method was based on two step emulsification process. Firstly, 1ml of aqueous Enoxaparin solution (5000 IU) was emulsified in 10 ml of methylene chloride containing the polymer (ratio 1: 0.25, 0.5, 0.75, 1.0) by mixing 5 minutes followed by sonication for 1min, the resulting w/o emulsion was poured into 50 ml of poly vinyl alcohol aqueous solution (0.1% w/v) and homogenized at high stirrer for 5 min involving the formation of the second w/o/w emulsion. The stirring was continued until evaporation of methylene chloride completely, resulting in precipitation of micro particles of

### Tables 1: Blood clotting time profile

<table>
<thead>
<tr>
<th>Clotting time of controlled group (Wright’s method) min</th>
<th>Clotting time of controlled group (Duke’s method) min</th>
<th>Pure drug clotting time (Wright’s method) hour</th>
<th>Pure drug clotting time (Duke’s method) hour</th>
<th>Clotting time of formulation 3 (F3) (Wright’s method) hour</th>
<th>Clotting time of formulation 3 (F3) (Duke’s method) hour</th>
</tr>
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<tbody>
<tr>
<td>3.87</td>
<td>4.00</td>
<td>6.44</td>
<td>6.44</td>
<td>18.50</td>
<td>18.56</td>
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**Figure 1:** Comparison of in-vitro permeation profile of formulations F1, F2, F3 and F4

**Figure 2:** Scanning electron microscopy of formulations F1, F2, F3, and F4.

**Figure 3:** Differential scanning Calorimetry of PLGA, Enoxaparin and Formulation 3
Enoxaparin which was separated by freeze drying.

RESULTS
The prepared micro particles had given the percentage yield of 81, 50, 63.5, and 69% for formulations F1, F2, F3 and F4. The formulations had given 80, 82, 85 and 85% of drug content and 68, 73.1, 76.4 and 70.5% of encapsulation. Mean particle size for the formulations was measured as 8.07, 7.66, 8.26 and 15.86 µm and for blank particles it was 8.34 µm. Scanning electron microscopy studies were carried out for the formulations; pictures revealed that the Enoxaparin microparticles were smooth and spherical. Differential scanning calorimetry of the pure drug was taken, the melting point of the pure drug was seen at 67.8°C at melting phase and 243.57°C was seen at cooling phase, and the Differential scanning calorimetry isotherm of formulation does not contain any exothermic and endothermic peaks, suggesting that the drug is encapsulated in the polymer. And in case of the Infrared spectrum of the pure drug, aliphatic CH stretching was present at 2934 cm⁻¹, C = O of acid at 1749 cm⁻¹, C = O of amide (CONH) at 1687 cm⁻¹ and glycoside linkage (C-O-C) at 1222 cm⁻¹. And in the formulation the prominent peaks has been obtained at 2945 cm⁻¹ aliphatic CH, 1747 cm⁻¹ for C = O of acid, 1649 cm⁻¹ for C = O of amide and 1236 cm⁻¹ for glycoside linkage.

The formulations have shown 79.3, 78.4, 76.3, 82.5% of drug permeation in 24 hour in phosphate buffer pH 7.4. Animal studies were carried out on New Zealand variety of rabbits and the clotting time of pure drug was measured by Wright’s method and Duke’s method, and clotting time was observed as 6.40 hour for Wright’s method and 6.44 hour for Duke’s method. Clotting time of formulation F3 was measure by same methods and the time was 18.50 and 18.56 hours.

DISCUSSION
The standard graph of Enoxaparin was plotted using distilled water, the data obtained was found to be linear in concentration range of 10 to 80 µg/ml, same procedure was repeated six times and average values of the absorbance obtained were calculated, the data was subjected to regression analysis and regression co-efficient (R²) was calculated. Microparticles were prepared by using emulsification technique, and optimized to obtain MPs of very small particle size, as a result w/o/w emulsion was produced with very small particle size, and the prepared MPs were freeze dried and were found to be free flowing. Microparticles were formulated to obtain highest drug content and drug entrapment efficiency, and total Enoxaparin content was determined using colorimetric method of analysis of samples. The results showed good entrapment efficiency and drug content. Scanning electron microscopy studies were carried out for the above formulations. Pictures revealed that the Enoxaparin MPs were smooth and spherical. The infrared spectra (IR) of Enoxaparin and formulation 3 were Studied. Distinct peaks on the region 2934 cm⁻¹, 1687 cm⁻¹ and 1222 cm⁻¹ are identical to that of the pure drug which conforms the intactness of the drug in the microparticles. The thermal behavior of Enoxaparin and its microparticles were studied using Differential scanning calorimetry to observe the effect of polymer on thermal behavior of Enoxaparin. DSC thermogram indicates that there is no interaction of drug and polymer, and polymer is encapsulated in the polymer.

ACKNOWLEDGMENT
The corresponding author would like to Thank his guide Dr. Sarasija Suresh for her excellent technical assistant and timely availability.

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Source of support: Nil, Conflict of interest: None Declared