Development and Preclinical Evaluation of Diclofenac Sodium loaded Hydroxyethyl Starch Nanoformulation for Parenteral Use

ABSTRACT

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Inflammation research is gaining immense interest in mainstream medicine since researchers uncover evidence that inflammation ignites and feeds chronic illness. It is a protective response to begin the healing process by removing harmful stimuli, including damaged cells, irritants, or pathogens. Inflammation occurs in various disease conditions such as arthritis, diabetes, cardiovascular, neurological, cancer and other autoimmune diseases. Progression of the disease condition affects major organs of the body including brain, kidneys, liver, lungs, muscle, bones etc. and hence disease management at early stage is essential. Disease is currently managed mainly via non-steroidal anti-inflammatory drugs (NSAIDs) and it is estimated that 30 million people intake NSAIDs worldwide. Diclofenac Sodium is the most commonly prescribed NSAIDs with its name on seventy four national essential medical lists (EMLs). Although this drug has proven its efficacy in the treatment of pain and inflammation, it often presents safety and tolerability issues, including serious concerns related to gastro-intestinal (GI), cardiovascular (CV), and renal toxicity. Toxicity of DS results from the high clinical doses administered and the route of drug delivery adopted. Specifically, GI related toxicity is common when the drug is administered orally since diclofenac is a weak acid.

The side effects associated with the oral route can be mitigated by adopting parenteral administration. While intravenous drug delivery helps the drug molecules to reach systemic circulation easily and has excellent therapeutic outcome, it is accompanied by a rapid decline in plasma levels and drug clearance. This is primarily due to the short plasma half-life and protein binding of the drug, thereby demanding multiple injections. In this regard, biopolymeric nanodrug delivery systems can be a good alternative to resolve the limitations of intravenous drug delivery. Various natural and synthetic polymers are now being tested for their capability for development as parenteral drug delivery carriers. Of the natural polymers that have gained interest, starch and its derivatives are relatively less explored for drug delivery applications and the properties such as biocompatibility, biodegradability, non-toxicity, ease of availability and

economical concerns make starch a good starting material for developing nanocarriers. However, hydrophilicity is a serious practical limitation for starch based materials. Hence, modification of starch with hydrophobic units would be a viable option for increasing the stability and degradation of nanoparticles, *in vivo*. However, despite the intense efforts done by researchers, the procedures including hydrophobic modification and synthesis of starch nanoparticles still involve extreme labour and time, restricting its use as a translatable, pharmaceutically acceptable drug delivery vehicle.

This study focuses on the development of a pharmaceutically acceptable and translatable nanomatrix system for parenteral delivery of diclofenac sodium based on Hydroxyethyl Starch (HES), a FDA approved polymer that is relatively unexplored in drug delivery research. Towards this, HES nanoparticles were prepared through a simple, two-step crosslinking-precipitation route, yielding spherical particles with low polydispersity index and high colloidal stability. The influence of processing parameters on size and entrapment efficiency was studied. A detailed physico-chemical characterization including its drug entrapment efficiency, release and *in vitro* modelling were performed. Stable diclofenac sodium loaded hydroxyethyl starch nanoparticles of size ~170nm and entrapment efficiency ~72% were obtained with the release kinetics following Higuchi model. Additionally, to test its scale-up ability which is a pre-requisite for translational nanomedicine, the formulation was scaled up to 5 ampoules and no significant change in size or entrapment efficiency was observed.

An extensive hemo/biocompatibility study of HES nanocarriers was performed both *in vitro* and *in vivo* since the formulation is intended for parenteral application. Toxicity of nanodiclo, was tested on macrophages and lymphocytes up to a concentration of 1mg/ml. The toxicological impact of nanodiclo on blood and its components was also tested using a detailed panel of hemocompatibility assays over a wide range of concentrations. The toxicological assays demonstrated excellent compatibility of the developed nanoformulation. Further, acute toxicity studies as per OECD guidelines performed on Sprague Dawley (SD) rats revealed that the toxicity induced by bare drug can be alleviated upon its entrapment within HES nanomatrix. Histopathology done on kidney and liver tissues treated with bare as well as nanodrug substantiated the findings of acute toxicity studies.

Furthermore, a detailed *in vivo* analysis of diclofenac sodium loaded HES nanoformulation was performed to understand the influence of surface modification, presence of unreacted excipients and dosage on pharmacokinetics in SD rats. Organ

distribution studies were carried out to validate the findings of PK. This was followed by a pharmacodynamic study in rat paw edema model induced by carrageenan. Overall, excipient removal and surface modification using PEG improved the performance of the nanoformulation in terms of its kinetics and dynamics with better performance exhibited at a lower dosage, suggesting the possibility of treatment using a lower dosage by exploiting the platform of nanotechnology.