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Targeting of diacerein loaded lipid nanoparticles to intra-articular cartilage using chondroitin sulfate as homing carrier for treatment of osteoarthritis in rats

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Abstract

Targeted delivery of antiosteoarthritic drug diacerein to articular tissue could be a major achievement and soluble polysaccharide chondroitin sulfate (ChS) may be a suitable agent for this. Therefore, diacerein loaded solid lipid nanoparticles modified with ChS (ChS-DC-SLN) were prepared for synergistic effect of these agents to combat multidimensional pathology of osteoarthritis (OA). Prepared formulation were of size range 396 ± 2.7 nm, showed extended release up to 16 h and increased bioavailability of diacerein by 2.8 times. ChS-DC-SLN were evaluated for their effect on histopathology of femoro-tibial joint of rat knee and amount of ChS and rhein (an active metabolite of diacerein) at targeted site. Concentration of rhein was significantly higher in case of ChS-DC-SLN (7.8 ± 1.23 μg/ml) than that of drug dispersion (2.9 ± 0.45 μg/ml). It can be stated that ChS served as homing to articular cartilage for targeting of drug. Thus, ChS-DC-SLN have great potential to enhance the overall efficacy of treatment for OA.

Key words: Diacerein; Chondroitin sulfate; Articular cartilage; Nanoparticles; Histopathology

Background

Osteoarthritis (OA) is a degenerative heterogeneous disease which is characterized by multi tissue failure and knee joints are commonly affecting joints by this. OA is histologically manifested as changes of cells as well as matrix leading to softening, fibrillation, loss of articular cartilage, sclerosis and eburnation of subchondral bone, osteophytes and subchondral cyst.[1–5] Diacerein (DC) is novel chondroprotective agent intended for the treatment of OA. Diacerein is a prodrug of rhein; undergoes acetyl esterase-mediated hydrolysis to give rhein. It selectively inhibits the synthesis of interleukin-1 (IL-1) and subsequent production of nitric oxide (NO); agents responsible for the degeneration of the cartilage.[6–8]

Chondroitin sulfate (ChS), a biologically active polysaccharide, is the major component of articular cartilage. ChS is widely distributed to extracellular matrix of body tissues which is reported to modify the chondrocyte death process and to maintain the metabolic process of cartilage matrix.[9–11] The concentration of ChS decreases at the site of osteoarthritic lesions which is normally characterized by softening and erosion of cartilage.[12] ChS has also been reported for inhibitory action towards certain enzymes involved in cartilage breakdown and to have general anti-inflammatory action. Apart from these value added properties for the treatment of OA, ChS has capability to be used as potential carrier for drug delivery. It can be synthesized and exist with specific structure as a hollow spheres. The use of this property can have an impact in the treatment of OA.[13] Moreover, keeping in view of the side effects, low bioavailability and other attributes of DC; site specific or targeted delivery of DC to articular tissue could be a major

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achievement and for this purpose ChS may be a suitable agent. Both DC and ChS may be used in the same formulation to achieve synergistic effect to combat the multidimensional pathology of OA. In recent year's use of colloidal lipid dispersions as nanoparticulate drug carrier has gained more attention. Further, development of lipid nanoparticles using solid lipid instead of liquid oils has come out with better results. Thus, solid lipid nanoparticles were introduced as alternative carrier system for drug delivery which offers potential for sustained or controlled drug release by immobilization of the drug within a solid matrix. The physical and chemical stability of these lipid nanoparticles also get increased due to the presence of solid particle core. It combines the advantages such as biocompatibility of ingredients and ease of production with those of polymeric nanoparticles such as solid matrix.[14] Therefore, in the present study, diacerein loaded solid lipid nanoparticles prepared with stearic acid and modified with ChS (ChS-DC-SLN) were developed.

As histological and biochemical changes are of main concern for OA, prepared ChS-DC-SLN were evaluated for their effect on histopathology and the amount of ChS and rhein were estimated at the targeted site. Further, the pharmacokinetic study, *in-vitro* evaluation and other general characterization of solid lipid nanoparticles were also performed.

Methods

Materials

Diacerein was received as a gift sample from Dr. Reddy's Laboratories Ltd, India. Rhein, chondroitin sulfate and monosodium iodoacetate were procured from Sigma-Aldrich. Stearic acid was purchased from SRL, India. Pluronic F 68, soya lecithin and dialysis membrane were procured from Hi-Media, India. Nanosep® was purchased from Pall Life Sciences, India. All other chemicals used were of analytical reagent grade and solvents used were of HPLC grade. Millipore Direct Q® 3UV water was used in studies.

Preparation of solid lipid nanoparticles

Diacerein loaded solid lipid nanoparticles modified with chondroitin sulfate (ChS-DC-SLN) were prepared by the method described by Jain et al. with modification.[15] Briefly, 20 mg of soya lecithin was dissolved in 2-3 ml of chloroform and was mixed with 25 mg of diacerein suspended in 3-4 ml of methanol. This mixture was added to 125 mg of melted stearic acid to make a primary emulsion at 800 rpm. This hot emulsion was immediately added to 50 ml of hot aqueous phase (temp was kept 10 °C above the melting point of lipid) containing 750 mg of pluronic F68 and 20 mg of citric acid under high speed homogenization at 20000 rpm using IKA Ultra-Turrax® (T 25 digital) for 10 min. After this, 50 ml of aqueous solution containing ChS was added to resulting suspension under similar above mentioned condition for 10 min. Resulting suspension was ultrasonicated for 2 cycles of 5 min each at 60% amplitude using probe ultra sonicator (Hielscher® UP200H). Finally, 55-60 ml of water along with organic solvent from this 100 ml of suspension was evaporated under vacuum using rotary evaporator (IKA® RV 10 digital). Compositions of different batches are presented in Table 1.

Table 1
Composition of diacerein loaded solid lipid nanoparticles modified with chondroitin sulfate (ChS-DC-SLN).

Ingredients (%w/v)	Batch1	Batch2	Batch3	Batch4	Batch5	Batch6
Diacerein (DC)	0.025	0.025	0.025	0.025	0.025	0.025
Stearic acid (SA)	0.125	0.125	0.125	0.125	0.125	0.125
Pluronic F68	0.75	0.75	0.75	0.75	0.75	0.75
Soya lecithin	-	0.02	0.02	0.02	0.02	0.02
Tri ethyl citrate (TEC)	-	-	0.02	-	-	-
Citric acid	-	-	-	0.02	0.02	0.02
Chondroitin sulfate (ChS)	0.25	0.25	0.25	0.25	0.125	0.5
Solvent	5-7 ml					
Purified water	q.s. (100 ml)					

Particle size, polydispersity index (PDI) and zeta potential determination

Mean particle size, polydispersity index and zeta potential were determined by Delsa™Nano C (Beckman Coulter, USA). Distilled water was used as dispersant and the system was maintained at 25 °C.

Total drug content (TDC), entrapment efficiency (EE) and drug loading (DL)

Diacerein amount was estimated for free dissolved drug present in ChS-DC-SLN in order to calculate the EE using Nanosep® centrifuge tubes.[16] TDC and DL were calculated as per method described by Rawat et al. (2011). Calculation were performed using following equations[17]:

$$\text{Total drug content } (\%) = \{(\text{Concentration} \times \text{Dilution factor} \times \text{Volume of formulation}) \div \text{Drug amount used}\} \times 100$$

$$\text{Entrapment efficiency } (\%) = \{(\text{Total drug content} - \text{Free dissolved drug}) \div \text{Drug amount used}\} \times 100$$

$$\%\,\textit{Drug loading} = \{(\text{Total drug content} - \text{Free dissolved drug}) \div ((\text{Drug amount used} - \text{Free dissolved drug}) + \text{Weight of lipid})\} \times 100$$

In-vitro release study

In-vitro release studies were performed in pH5.8 phosphate buffer under sink condition using the dialysis bag diffusion technique using dialysis membrane having molecular weight 12,000-14,000 daltons.[18,19] Dialysis membrane was soaked in double-distilled water for overnight before use. ChS-DC-SLN aqueous nanosuspension or control (diacerein dispersed in 0.3% aqueous sodium carboxymethylcellulose (NaCMC)) equivalent