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Preparation, characterization and anti-inflammatory effects of curcumin loaded carboxymethyl cellulose acetate butyrate nanoparticles on adjuvant induced arthritis in rats

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ABSTRACT

Rheumatoid Arthritis is a destructive illness and results in damage of joints and cartilages, followed by loss of function. Curcumin proved to be more effective in alleviating the symptoms of rheumatoid arthritis like tenderness and swelling of joints compared to regular drugs. However, its utility as a therapeutic agent is limited by its poor absorption, rapid metabolism and rapid systemic elimination. In our study, curcumin was molecularly dispersed in amorphous polymer and nanoparticles were produced by a rapid precipitation technique. Dynamic light scattering showed the particle size was 166.5 ± 4.2 nm. Differential Scanning Calorimetry (DSC) results showed that the drug in the nanoparticles was in the amorphous state. Drug loading efficiency in the nanoparticles was 73.41 ± 2.4%. The drug from the nanoparticle showed higher release compared to the pure as-received drug. Behavioral studies were conducted such as thermal hyperalgesia, mechanical hyperalgesia, mechanical allodynia, hematological assessment, radiographic analysis and various disease development parameters such as mobility score, joint stiffness, paw volume and locomotor activity was assessed after induction of arthritis using complete Freund's adjuvant in rats. Studies revealed that nanoparticles act as better anti-inflammatory agents and could give faster relief from pain.

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory disease that often leads to localized damage to articular cartilage, bone, tendon and ligament [12]. It primarily influences synovial joints resulting in pain, swelling, stiffness and deformity. Most patients suffer progressive disability. About 0.5–1% of the worldwide population is suffering from RA [20]. This mostly occurs between 40 and 70 years of age, typically increases with age and is 2–3 times more likely in women than men [12]. It causes cardiovascular diseases and infections, leading to an increased mortality and a 5–10 year reduction in mean life expectancy [36]. A review of contemporary treatment methods shows that medical science has not been able to significantly improve the long-term outcome of this disease.

Current therapy only aims at accomplishing complete and long lasting remission. Frequent relapses, partial remission and unresponsive treatments are very common problems which the patients face with the available therapeutics. In addition, anti-rheumatic and immune modulating drugs carry severe or even life threatening effects. Also, current RA therapeutics often requires application of high dosages frequently due to rapid clearance and lack of specificity of most of the drugs for the target tissues [31]. Efforts are required to design therapeutic agents that can be used for enduring effects and with little or no side effects. The most efficient way is to

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deliver the drug to the desired site of action in the body and decrease or avoid side effect at non-target sites. A number of targeted micro- and nanoparticle delivery methods, liposomes and micelles have shown the potential for targeted delivery [13,14,27]. Polymer based nanoparticles have are by-far the most promising approach for improving efficacy, solubility and bioavailability, resulting in controlled drug delivery and better targeting [29,33,39].

Curcumin (CUR), a plant-derived chemical and crystalline in nature is the active pharmaceutical ingredient (API) of interest in this study. It is used as a natural yellow pigment in the food and textile industries has shown beneficial anti-oxidant, anti-cancer, and anti-inflammatory effects [17]. It has proved to be more effective in alleviating the symptoms of rheumatoid arthritis (RA) like tenderness and swelling of joints compared to regular RA drugs. The suggested daily dose of CUR is 12 g, with no significant toxicity [2]. However, the major disadvantage of CUR is its poor aqueous solubility (11 ng/mL at pH 5.0). It shows chemical instability, degrades faster in neutral or alkaline pH condition [19,28] and metabolic susceptibility which leads to poor bioavailability in both animals and humans [1]. Hence, there is a significant need to develop an effective and efficient drug delivery system for CUR that would increase its solubility and chemical stability. Some of the methods currently under investigation include incorporation of CUR in liposomes, phospholipids, cyclodextrins and solid dispersions [5,11,22–24,30,32,37], nanoparticles [11,24], micelles [7,38] and self-micro emulsifying drug delivery system (SMEDDS) [4]. Another study showed an improvement in CUR bioavailability upto 20 fold by using piperine to inhibit glucuronidation [15].

Recently, amorphous solid dispersions (ASD) have been used to enhance CUR solubility. Several authors have reported solubility enhancement of CUR by ASD such as Cremophor and PEG 20000 was used to get a concentration of 18 mg/mL of CUR [18]. Enhancement of CUR solubility and bioavailability upto 9–16 folds was achieved by preparation of CUR nanocrystals, HPMCAS solid dispersions, and nanoemulsions [35]. Polyvinylpyrrolidone (PVP) was also used to form spray dried ASDs which showed higher solubility and dissolution rate compared to pure CUR [26]. In 2011, Li et al. [21] showed enhancement of CUR solubility by spray dried ASDs with several polysaccharides such as hydroxypropylmethyl cellulose acetate butyrate (HPMCAS), carboxymethyl cellulose acetate butyrate (CMCAB) and cellulose acetate adipate propionate (CAADP).

In this study a cellulose based polysaccharide carboxymethyl cellulose acetate butyrate (CMCAB) is used synthesize the polymeric nanoparticles. The drug is dispersed, ideally molecularly in the amorphous polymer matrix. The highly crystalline drug needs to surmount high kinetic energy barrier to dissolve in the bodily fluid. Therefore only a very less portion of the drug which is soluble is bioavailable. In order to suppress crystalline activation energy barrier, the drug need to be in the amorphous form so that it need to surmount only a small amount of energy barrier to become soluble in the fluid. The creation of a miscible drug-polymer blend leads to a high energy, amorphous state of the drug, where the polymer inhibits drug crystallization. Therefore, the kinetic energy barrier is reduced and solution concentration of the drug is increased. The smaller size and enhanced surface area of the nanoparticles help in enhanced mass transfer of the drug in the dissolution medium when administered orally. This synergistic approach of crystallinity suppression by polymer and nanoparticle size has not been studied systematically, specifically in ameliorating RA induced pain and inflammation.

The complete freund's adjuvant (CFA) induced arthritic rat model has been used extensively to study various pain and disease development parameters [3,6,25] since it resembles very closely various characteristics of human rheumatoid arthritis. Cheng et al. [8] reported the inefficiency of CUR in attenuating joint edema by oral administration of CUR in CFA induced arthritic rats. However, they suggested that repeated intrathecal administration inhibits glial activation and production of inflammatory mediators in spinal cord thereby reducing CFA induced pain. In another instance, Jeengar et al. [16] reported significant reduction in proinflamatory mediators by topical application of by CUR-emu oil combination. There are no literature which have reported the efficacy of CUR complexed with CMCAB via oral route in arthritic model.

Here, we investigate the effect of CUR-CMCAB nanoparticles on behavior of adjuvant-induced arthritic rats. The nanoparticles were prepared by a rapid precipitation method using Multi Inlet Vortex Mixer (MIVM). The effect of processing parameters on particle size and drug loading of nanoparticles has already been described in our previous studies [9,34]. The nanoparticles were characterized to study their size and drug loading. The *in vitro* release profile of CUR showed initially burst release followed by a slow release. Finally, the behavior of CFA adjuvant rats was studied to understand the potential of administered drug nanoparticles compared to pure drug in treating RA.

2. Materials and methods

2.1. Materials

Curcumin was purchased from Sigma Aldrich, India. Carboxymethyl Cellulose Acetate Butyrate (CMCAB™ 641–0.2 Cellulose Ester) was from Eastman Chemical Company, USA. CMCAB was used in its free acid form. Tetrahydrofuran (analytical reagent grade, MOLYCHEM, India) was used for polymer/drug nanoparticles formation without further purification. Sodium chloride, potassium chloride, di-sodium hydrogen phosphate dehydrate and potassium dihydrogen phosphate (Hi Media, Mumbai, India) were used for release studies. All other chemicals used were of analytical grade. Millipore water (18.2 MΩ cm at 25 °C ultrapure) was used for all the experiments. Ethanol (analytical reagent grade, MOLYCHEM, India) was used for preparing calibration curve for CUR.

2.2. Animals

Adult male Wistar rats (150–250 g) were raised in Central Animal House facility of Panjab University. Three to four animals were used in each group. The rats were housed in polyacrylic cages (38 × 23 × 10 cm) and maintained under standard laboratory conditions with natural dark and light (12:12 h) cycle and had free access to food (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. Animals were acclimatized to laboratory conditions before all the behavioral tests. All experiments were carried out between 09:00 and 17:00 h. The experimental protocols were approved by the Institutional Animal Ethics Committee of Paunjab University and performed in accordance with the guidelines of the Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

2.3. Preparation of curcumin CMCAB nanoparticles by flash nanoprecipitation technique using MIVM

The CUR-CMCAB drug complexes were prepared using a four-jet MIVM [9,34]. CUR and CMCAB were dissolved in THF solvent, mixed using a vortex mixer and injected into the MIVM along with three other water (anti-solvent) streams. The four inlet