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Hyaluronic acid based micelle for articular delivery of triamcinolone, preparation, *in vitro* and *in vivo* evaluation

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ABSTRACT

A novel triamcinolone loaded polymeric micelle was synthesized based on hyaluronic acid and phospholipid for articular delivery. The newly developed micelle was characterized for physicochemical properties including size, zeta potential, differential scanning calorimetry (DSC) analysis and also morphology by means of transmission electron microscopy. The biocompatibility of micelle was explored by histopathological experiment in rat model. Also biological fate of micelle was investigated in rat by means of real time *in vivo* imaging system. Triamcinolone loaded micelle was in the size range of 186 nm with negative zeta potential charge. Micelles were spherical in shape with core shell like structure. Triamcinolone was released from micelle during 76 h with almost low burst effect. DSC analysis showed the conversion of crystalline triamcinolone from its crystalline state. Histopathological analysis showed no evidence of tissue damage or phagocytic accumulation in knee joint of rat. The real time *in vivo* imaging analysis suggested at least three days retention time of micellar system in knee joint post injection.

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1. Introduction

Joint related disorders including arthritis rheumatism (RA) and osteoarthritis (OA) are among big challenges in recent years. This complication afflicted over 40 million people only in US and it is predicted to increase to 60 million till 2020 (Brooks, 2002). Surgery and pharmacotherapy are the most two indicated remedies for these kind of disorders the first of which is more invasive and indicated for end-stage patients only. The pharmacotherapy of articular disorders is based on the administration of non- steroidal anti-inflammatory (NSAIDs) drugs and also corticosteroids (Bradley et al., 1991; Minas and Nehrer, 1997). As most of these drugs are administered orally, various kinds of side effects like gastrointestinal complication, cataracts and ulcer may occur during therapy (Duru et al., 2007; Da Silva et al., 2006). One approach to bypass these side effects is the intra-articular (IA) injection of drugs to joints, which shows a relatively high concentration of the drug in the active site and eliminates gastrointestinal upset; but the major problems with this method is high drug clearance from joints which needs frequent injections and can potentially lead to infection and joint disability and post injection flare (Gerwin et al., 2006). Moreover, due to the crystalline nature of some drugs and especially corticosteroids, inflammatory events may happen upon IA injection; a condition known as crystalline-induced arthritis (Ellman and Becker, 2006).

Local articular delivery is a concept developed for articular complications treatment that has significant clinical outcomes including reduced joint inflammation, reduced side effects and increased patient compliance (Dingle et al., 1978). Till now, various kinds of delivery systems have been adapted for local delivery to joints. These systems include microspheres (Brown et al., 1998), nanospheres, liposomes (Lopez-Garcia et al., 1993) and hydrogels (Inoue et al., 2006) and have several advantages. Prolonged drug retention time in active site, reduced drug clearance from joints and also increased patient compliance through the reduction of administration frequency are among the advantages of these delivery systems (Raynauld et al., 2003; Horisawa et al., 2002a). Although these systems may improve drug efficacy, each of them has some limitations. Microspheres with the average size of 50–100 μm can be trapped by the articular immune system and triggered a granulation reaction. Moreover, microsphere drug delivery represented other implications like syringe clogging during administration and painful injection (Kempe and Mäder,

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2012). Nishide et al. (1999) reported an increase in the immune system responses following IA injection of microspheres from 5 to 40 μm. Beside microparticle related immune responses the shape of particles has been reported to be an important issue in increasing immunological responses. Ratcliffe et al. (1984) reported synovial membrane and subsynovial lining inflammation after IA injection of irregularly shaped PLA and poly (butyl cyanoacrilate) microparticles. Liposome drug delivery is an ideal platform for articular drug delivery but less stability and drug leakage are two important defects of this system (Gregoriadis and Davis, 1979). Hydrogel is another delivery system which is used in IA delivery. Due to high viscosity of this kind of delivery system a specific syringe with defined needle size is needed. Moreover, administration of this system needs slow and careful injection.

Polymeric micelle is a nano-sized particle formed spontaneously in an aqueous media. This system is composed of at least two different block polymers with hydrophobic and hydrophilic characteristics. When such a co-polymer disperses in water, the hydrophilic part faces with water while the hydrophobic segment moves far from it in order to reach maximum stability. The hydrophobic and hydrophilic segments of these particles form the core and shell part of micelle, respectively. Due to hydrophobic characteristics of micelle core, it could be considered as a cargo part which can dissolve hydrophobic drugs. The hydrophilic part of micelle interacts with the surrounding water and makes micelle more stable (Nakanishi et al., 2001). Polymeric micelle has been used as drug delivery system due to its stability and solubilizing characteristics (Jiang et al., 2012). Moreover, micelles have the ability to target different tissues due to various targeting moieties which can be decorated on the surface of this system.

Hyaluronic acid (HA) is a naturally occurring polymer in human tissues like cell matrix, connective tissues and also synovial fluid in different joints (Palumbo et al., 2006). HA is composed of repeating disaccharide units including D-glucuronic acid and *N*-acetyl glucosamine linked by β (1→4) and β (1→3) glucosidic bands (Brown and Jones, 2005). HA is a biocompatible and biodegradable polymer which has numerous applications in medicine and drug delivery. HA has been used as a scaffold and also different types of drug delivery systems including nanoparticles (Choi et al., 2010), polymeric micelles (Lee et al., 2009) and hydrogels (Luo et al., 2000). HA also has an important biological role in cell proliferation, differentiation and also angiogenesis by binding to the cell specific receptors like CD44 and receptor for HA-mediated motility (RHAMM) which are increasingly over-expressed on the surface of tumor cells. HA also has various functional groups like hydroxyl and carboxylic acid, which makes it an attractive polymer for further modification.

Phospholipids (PEs) are a group of lipids which play an important role in cell structure and also exist in different tissues like the nervous system and lungs. Due to biocompatibility and biodegradability, they have been used excessively in different drug delivery systems such as liposomes, solid lipid nanoparticles (SLN) (Westesen and Siekmann, 1997) and also cochleates (Papahadjopoulos et al., 1975). PEs which are composed of one or two fatty acids esterifies with glycerol, are synthesized by most cells. Regarding the biocompatibility and also the desirable hydrophobic characteristics of these substances, they could be considered as a hydrophobic micelle cores. Wang et al. (2010) reported attractive micellar characteristics of dextran–PE conjugates for drug delivery. Also Gill et al. (2011) reported sustained release kinetic of the drug from PEG–PE micelle due to impressive hydrophobic core part of this micelle.

Triamcinolone suppresses inflammation by increasing production of anti-inflammatory mediators like serum leuko-proteas inhibitors which leads to limited production of cyclooxygenases 1 and 2 (Mangal et al., 2014). Triamcinolone has limited solubility in water around 0.018 mg/ml (Miro et al., 2012). In this study a novel polymeric micelle based on HA and 1,2-distearoylphosphatidylethanolamine (DSPE) was synthesized for sustained delivery of triamcinolone (M_w = 476, log *P* = 3.2), a highly used corticosteroid in OA and RO. Physicochemical properties of the drug loaded micelle and also the *in vitro* release profile of triamcinolone was investigated. Moreover, understanding the biological fate of micelles, IA injection of the labeled micelle into the knee joint of rat model was performed and *in vivo* real time imaging analysis was explored accordingly.

2. Materials and methods

2.1. Materials

Hyaluronic acid (molecular weight of 10 kDa) was purchased from Freda Biochem Co., Ltd. (China). 1,2-Distearoylphosphatidylethanolamine (DSPE) were purchased from lipoid, (Germany). Triamcinolone was obtained from Jaber pharmaceutical company. *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and pyrene were bought from Sigma–Aldrich Co. (USA). Regenerated cellulose dialysis bag (molecular weight cut off 3500 Da) was purchased from Spectrum Laboratories Inc. (Canada). Cy 7.5 amine was bought from Lumiprobe Corporation (USA). All other reagents and materials are from analytical grade and used as received.

2.2. Methods

2.2.1. Synthesis of HA–DSPE co-polymer

HA–DSPE co-polymer was synthesized as described previously (Saadat et al., 2014). 100 mg of HA was dissolved in dionized water with 2 molar excess of NHS and EDC and mixed for 2 h at room temperature in order to activate carboxylic acid groups. DSPE (5 M excess of HA) was dissolved in tert-butanol and deionized water (10%; v/v) in the presence of 0.1 mol triethylamine and mixed at 55 °C. HA solution was added dropwise to DSPE solution and stirred at 60 °C for 6 h followed by stirring at room temperature for additional 24 h. The resultant mixture was dialyzed against ethanol/water solution (70:30, 50:50, 30:70; v/v) for 24 h to remove impurities followed by lyophilization.

2.2.2. Preparation of triamcinolone loaded micelle

For preparation of loaded micelle, dialysis method was selected. Briefly, 2 mg of triamcinolone was dissolved in methanol and added dropwise to 10 ml of HA–DSPE dispersion (1 mg/ml). The mixture was stirred for 12 h at room temperature. To remove the unloaded drug from the micellar system the whole mixture was poured in dialysis tube (Molecular cut off 3500 Da) and dialyzed against water/methanol (50:50; v/v) for 24 h. For further purification, the resultant dispersion was centrifuged (5000 rpm) for 15 min in order to remove any unloaded drugs and other impurities. The obtained polymeric micelle dispersion was lyophilized and kept in refrigerator for further experiments.

2.2.3. Characterization of triamcinolone loaded micelle

The hydrodynamic size diameter and also zeta potential of triamcinolone loaded micelle were obtained by dynamic light scattering method (Zetasizer ZS, Malvern, UK). 1 ml of triamcinolone loaded micelle was diluted in deionized water and the micelle size determined at the wave length of 633 nm at 25 °C with detection angle of 90°. For the evaluation of the loaded amount of drug in polymeric micelle, 500 μl of micelle dispersion was mixed with an equal amount of methanol and vortex vigorously. The whole mixture was centrifuged and 20 μl of supernatant was taken and analyzed by HPLC. The mobile phase was methanol with the

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