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Intraarticular application of superparamagnetic nanoparticles and their uptake by synovial membrane—an experimental study in sheep

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Abstract

A superparamagnetic iron oxide nanoparticle, coated with polyvinyl alcohol, (PVA-SPION) and its fluorescently functionalized analogue (amino-PVA-Cy3.5-SPION) were compared in vivo as proof of principle for future use in magnetic drug targeting in inflammatory joint diseases. They were injected either intraarticularly or periarticularly and their uptake by cells of the synovial membrane was evaluated. Uptake was completed in 48 h and was enforced by an extracorporally applied magnet.

Keywords: Magnetic drug targeting; Sheep; Knee joint; Inflammation; SPION; Intraarticular medication; Osteoarthritis; Fluorescent nanoparticles; Cy3.5; Biocompatibility; Synovial cells; Phagocytosis; PVA

1. Introduction

Chronic aseptic inflammatory diseases of the joint such as rheumatoid arthritis or osteoarthritis require long-time therapy of patients with analgesic, anti-inflammatory, immune-modulating or chondroprotective drugs to release pain and modulate the degree of disease symptomatically [1]. Prolonged medication periods are commonly associated with negative side effects of the drugs, such as nephrotoxicity, hepatitis or gastrointestinal ulceration leading to forced discontinuation of

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the medication [2]. In the last years several attempts were made to find new successful therapy strategies reducing the unwanted side effects while at the same time leading to optimal well being of the patients [3–5]. If possible, replacement of systemic drug administration through intraarticular injections is the most effective route to treat joint diseases [6]. However, the efficiency of the injected drug may be limited through a relatively short drug persistence or failure to maintain adequate drug concentrations in the joint cavity [7–9]. Different approaches are made to optimize drug persistence and efficiency in the joint either with biodegradable drug carriers, conjugated drug formulations or targeted drug systems [10–13]. Of those, superparamagnetic iron oxide nanoparticles (SPIONs) seem highly interesting for clinical applications because of their superparamagnetic properties and their possible use in magnetic drug targeting including gene therapy [14,15]. Up to this time of writing, the use of SPIONs for joint therapy has not been reported.

The goal of this study was to determine whether intraarticularly (i.art.) or periarticularly (p.art.) injected SPIONs with PVA coatings are biocompatible and successfully taken up by synovial cells in vivo. In addition, the effect of an extracorporally applied magnet on nanoparticle persistence within the synovial membrane was tested. Last but not least, a fluorescent molecule, Cy3.5, was covalently attached to the surface of the nanoparticles for (i) tracking the nanoparticles within the tissues and at the same time (ii) acting as a model cargo to evaluate nanoparticle stability, performance and biocompatibility with respect to drug targeting applications after i.art. injection using sheep as an experimental model.

2. Materials and methods

2.1. Preparation of nanoparticles

Polyvinyl alcohol (PVA; (Mowiol® 3-83, average molecular weight Mw: 14 000 g/mol, hydrolysis degree: 83% supplied by courtesy of CLARIANT)) stabilized iron oxide nanoparticles were produced as described elsewhere [16]. For this work, 10 wt% of the PVA was exchanged by vinyl alcohol/vinyl amine copolymer (M12, average molecular weight of 80 000–140 000 g/mol, supplied by courtesy of Erkol, (E)). To limit the number of amine group per nanoparticle, a vinyl alcohol/vinyl alcohol copolymer mass ratio of 45 was chosen. The overall ratio by weight of iron to polymer content was for all samples fixed at 13. The concentration of the amino-PVA functionalized nanoparticles (amino-PVA-SPIONs) in aqua's solution was 4.6 mg Fe/ml. The results of a more detailed characterization of the nanoparticles are given elsewhere [17].

Amino-PVA-SPIONs were further derivatized by covalent coupling of a fluorescent dye to the nanoparticles via the polymer. For this work Cy 3.5: derivatized CyDye™ (NHS ester; Eurogentec) was chosen that carries one reactive group on each dye molecule for accurate labelling of amine groups. The absorption maximum of this dye is at 581 nm, the maximal emission at 596 nm.The final dispersion contains 0.3 mg iron/ml dispersion and approx. 3 dye molecules/nanoparticle.

2.2. Experimental animals and surgical technique

A total of 14 Swiss Alpine sheep between 2 and 4 years of age was used for application of PVA-SPIONs and amino-PVA-Cy3.5-SPIONs into the stifle and carpometaphalangeal joints. Eight additional sheep without SPIONs as well as without magnets and slaughtered for other reasons than infectious or systemic disease served as controls. Two main experiments were conducted with the nanoparticles, such that in experiment 1, PVA-SPIONs were injected either intraarticularly (i.art.; into the joint cavity) or periarticularly (p.art.;into joint capsular tissue) and in experiment 2, amino-PVA-Cy3.5-SPIONs were injected only i.art.

The sheep were checked for their overall health, routinely wormed as well as vaccinated. After an adaptation period of 14 days prior to surgery, they were kept in groups in a stall, routinely fed with hay and with free access to water. All experiments were conducted under general anesthesia. The animals were fasted 24 h before sedation with medetomidine (5 μg/kg, i.m., Domitor®, Orion-Farmos, Turku, Finland) and induction with