European Journal of Pharmaceutics and Biopharmaceutics 96 (2015) 388–395

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Efficacy study of two novel hyaluronic acid-based formulations for viscosupplementation therapy in an early osteoarthrosic rabbit model

Sema Kaderli [a], Eric Viguier [b], Dorothée Watrelot-Virieux [b], Thierry Roger [b], Robert Gurny [a], Leonardo Scapozza [a], Michael Möller [a], Caroline Boulocher [b,*,1], Olivier Jordan [a,*,1]

[a] School of Pharmaceutical Sciences, University of Lausanne and Geneva, Quai Ernest-Ansermet 30, 1211 Geneva, Switzerland
[b] UPSP ICE 2011.03.101, University of Lyon, VetAgro Sup, Veterinary Campus of Lyon, 69280 Marcy l'Etoile, Lyon, France

ARTICLE INFO

Article history:
Received 8 July 2015
Revised 8 August 2015
Accepted in revised form 7 September 2015
Available online 11 September 2015

Chemical compound studied in this article:
Hyaluronic acid (PubChem CID: 24728612)
4-Aminoresorcinol (432827)
Chitosan (PubChem CID: 21896651)

Keywords:
Osteoarthrosis
Viscosupplementation
Hyaluronic acid
Chitosan
Antioxidant
Rabbit
Anterior cruciate ligament transection

ABSTRACT

Viscosupplementation (VS) is a therapy for osteoarthrosis (OA) consisting of repetitive intra-articular injections of hyaluronic acid (HA). It is known to be clinically effective in relieving pain and increasing joint mobility by restoring joint homeostasis. In this study, the effects of two novel HA-based VS hydrogel formulations were assessed and challenged against a pure HA commercial formulation for the first time and this in a rabbit model of early OA induced by anterior cruciate ligament transection (ACLT). The first formulation tested was a hybrid hydrogel composed of HA and reacetylated chitosan, a biopolymer considered to be chondroprotective, assembled thanks to an ionic shielding. The second formulation consisted of a novel HA polymer grafted with antioxidant molecules (HA-4AR) aiming at decreasing OA oxidative stress and increasing HA retention time in the articulation.

ACLT was performed on rabbits in order to cause structural changes comparable to traumatic osteoarthrosis. The protective effects of the different formulations were observed on the early phase of the pathology in a full randomized and blinded manner. The cartilage, synovial membrane, and subchondral bone were evaluated by complementary investigation techniques such as gross morphological scoring, scanning electron microscopy, histological scoring, and micro-computed tomography were used.

In this study, ACLT was proven to successfully reproduce early OA articular characteristics found in humans. HA and HA-4AR hydrogels were found to be moderately protective for cartilage as highlighted by μCT. The HA-4AR was the only formulation able to decrease synovial membrane hypertrophy occurring in OA. Finally, the hybrid HA-reacetylated chitosan hydrogel surprisingly led to increased subchondral bone remodeling and cartilage defect formation. This study shows significant effects of two innovative HA modification strategies in an OA rabbit model, which warrant further studies toward more effective viscosupplementation formulations.

1. Introduction

According the WHO, OA is defined as "an heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage in addition to related changes in the underlying bone and joint margins" [1]. Viscosupplementation (VS), that is the supplementation of the joint with elastoviscous hyaluronic acid (HA) hydrogel or its derivative, has been shown therapeutically effective in the early phase of OA since HA acts as a mediator between cells, enzymes, and inflammatory factors [2–7]. However, HA short residence time in the joint together with its disputed clinical efficacy has spurred the search for more efficient, long-lasting VS formulations.

Abbreviations: ACLT, anterior cruciate ligament transection; CS, chitosan; EPIC-μCT, equilibrium partitioning of iodine contrast micro-computed tomography; FOV, field of view; HA, hyaluronic acid; HA-4AR, hyaluronic acid linked to 4-aminoresorcinol; MMP-3, matrix metalloproteinase-3; MIP, maximal intensity projection; OA, osteoarthrosis; PBS, phosphate buffer saline; SEM, scanning electron microscopy; VS, viscosupplementation.

* Corresponding authors. Tel.: +33 478 87 25 31; fax: +33 4 78 87 82 62 (C. Boulocher). Tel.: +41 22 37 965 86; fax: +41 22 37 965 67 (O. Jordan).

E-mail addresses: sema.kaderli@unige.ch (S. Kaderli), eric.viguier@vetagro-sup.fr (E. Viguier), dorothee.watrelot@vetagro-sup.fr (D. Watrelot-Virieux), thierry.roger@vetagro-sup.fr (T. Roger), robert.gurny@unige.ch (R. Gurny), leonardo.scapozza@unige.ch (L. Scapozza), michael.moeller@unige.ch (M. Möller), caroline.boulocher@vetagro-sup.fr (C. Boulocher), olivier.jordan@unige.ch (O. Jordan).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejpb.2015.09.005
0939-6411/

In this work, the therapeutic efficacies of two new HA-based VS formulations have been evaluated for the first time for their potential of preventing OA development, as compared to a commercial reference in order to assess their clinical potential. A rabbit model of early traumatic OA induced by the transection of the anterior cruciate ligament (ACLT) was used for this purpose. The first tested formulation is a so-called hybrid hydrogel, containing HA and reacetylated chitosan from fungal origin. The hybrid approach advantageously combines compounds having a proven track record of safety to facilitate clinical translation. Chitosan is an attractive polymer candidate for OA therapy, potentially adding therapeutic strength to HA, due to its chondroprotective characteristics and also due to the therapeutic effect of its monomer; N-acetylglucosamine [8–12]. Previously, the biocompatibility of this formulation has been assessed *in vivo* [13], and in this study its therapeutic efficacy was assessed and challenged against a pure HA commercial formulation. The second challenged formulation is a newly synthesized chemically modified HA grafted with antioxidant moieties, which has proven to resist *in vitro* oxidative stress such as found in OA articulations and proven to be biocompatible [14–16]. The addition of an antioxidant could be beneficial for HA protection leading to a longer acting formulation but also for its intrinsic antioxidant activity known to be beneficial for OA pathology [17].

OA has been long thought to be a cartilage-driven pathology. Yet today, it is clearly established that multiple structures of the joint are affected by the disease [18]. In addition to this, there is a need of a more complete characterization of the ACLT rabbit model which is a routinely-used preclinical animal model for VS therapeutic evaluation. These are the reasons why, the cartilage, synovial membrane, and subchondral bone were assessed using different complementary techniques such as micro-computed tomography (μCT), gross morphological and histological scoring (cellularity, proteoglycan), immunohistochemistry (matrix metalloproteinase 3; MMP-3), and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Formulation preparation

A hybrid hydrogel HA-based formulation composed of 1.3% (m/V) HA and 0.5% reacetylated fungal chitosan, in a phosphate buffer with added sodium chloride as a stabilizing agent (Na_2HPO_4 0.13%, NaH_2PO_4 0.03% and 1.2% NaCl) was prepared, sterilized, and analyzed according to our previously developed methods [13]. The final pH of the formulation was 6.8, its viscosity of 722 ± 18 mPa s and its osmolarity of 415 mOsmol. HA of GMP grade was used from Streptococcus origin (HTL, La Boitardière, France, molecular weight of 1300–2200 kDa). Chitosan GMP grade KIOmedine-CsU® from Agaricus bisporus origin (Kitozyme, Herstal, Belgium) was reacetylated to a degree of deacetylation of 48% and molecular weight of 161 kDa.

HA-4 aminoresorcinol (HA-4AR) was synthesized as described in our previous work and sterilized at 2.7% polymer concentration [15]. The polymer concentrations were optimized in order to have formulations within the same viscosity range. The formulation included NaCl (0.83%) as an isotonic agent and phosphate salts as buffering agents (Na_2HPO_4 0.32%, NaH_2PO_4 0.03%). The mean pH was 7.0 and the viscosity was 858 ± 267 mPa s.

Both formulations were tested for sterility and endotoxin levels and were found to be suitable for intra-articular injection. Sterility was demonstrated by direct inoculation according to Ph. Eur. and pyrogen content found to be below the acceptable limit of 5.5 UI/m by the Ph. Eur. chromogenic kinetic test (the limit calculated for rabbits is 100 UI/ml of formulation) [13,15].

As a control, HA of commercial source was used (i.e. HA; Ostenil®, TRB Chemedica, Switzerland; 1% HA with phosphate buffer and sodium chloride as isotonic agent, measured pH of 7.0 and viscosity of 1130 ± 21 Pa s).

2.2. Surgical procedure

The animal experimentation was performed under authorization of the ethical committee of VetAgro Sup (Lyon, France, authorization number 1373) and in accordance with European legislations. Twenty-four male adult New Zealand rabbits (5 months of age, 3.7 kg on average) were provided by Centre Lago (Vonnas, France) and kept 2 weeks in acclimation in individual boxes. The pre-surgical treatment, surgical procedure as well as the post-surgical care and the sacrifice are detailed in Supplementary material.

2.3. Formulation administration

The operators of the injections and of the evaluations were blinded to the formulations. The rabbits were randomized after the surgery in 4 groups as follows: hybrid hydrogel ($n = 6$), HA-4AR ($n = 6$), HA commercial formulation ($n = 6$), and control-operated (i.e. saline injected, $n = 6$). At weeks (W) 1, 2, 3, 4, and 5 post-ACLT, the rabbits were anesthetized for a short time (Ketamine 1000® 40 mg/kg and Domitor® 80 μg/kg), and after careful disinfection (Vetedine® soap and solution) an intra-articular injection was performed in the operated knees with 0.2 ml of formulation. The contralateral knees were kept intact (control-unoperated).

2.4. Micro-computed tomography imaging of the knees

A μ-CT eXplore Locus system (General Electric, Fairfield, USA) was used at a 90 μm^3 isotropic resolution with the following source parameters: 80 kV and 450 μA. The acquisitions were performed with a voxel size of 90 μm^3 and with a field of view (FOV) of 80 mm in diameter and 35 mm in depth. A maximal intensity projection (MIP) from the coronal plane image of both knees simultaneously was then obtained using the MicroView software ABA 2.2 (General Electric, Fairfield, USA). After acquisition and reconstruction, 16-bit images were calibrated with a phantom containing hydroxyapatite, water, and air and expressed in Hounsfield Units (HU: air 1000 HU, fat 150 HU, water 0 HU, and calcified tissues >100 HU).

Each knee was graded blindly by a trained veterinarian surgeon based on front views for osteophyte presence from 0 (no osteophyte) to 3 (severe) [19]. The control-unoperated group was composed of all contralateral knees ($n = 24$).

2.5. Micro-computed tomography imaging of the medial tibial cartilage and subchondral bone

After careful dissection of the knees and saw section of the proximal part of the tibia, equilibrium partitioning of iodine contrast micro-computed tomography (EPIC-μCT) was performed. The tibial plates were immersed in an iodinated contrast agent (Hexabrix®, Guerbet, Roissy, France) diluted at 40% in phosphate buffer saline (PBS) at room temperature for 10 min. The specimens were then rinsed with saline and entirely immersed in colza oil for the acquisition, which was performed with a 45 μm^3 isotropic resolution at 80 kV and 450 μA, and a voxel size of 45 μm^3 with a FOV of 80 mm in diameter and 35 mm in depth. The method of picture treatment as well as the method of calculation is detailed in Supplementary material. For the cartilage as well as for the subchondral bone, the control-unoperated group was composed of 2 contralateral knees per group ($n = 8$).