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Effects of intraarticular treatment with stanozolol on synovial membrane and cartilage in an ovine model of osteoarthritis

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ABSTRACT

Aim of the study was to verify the clinical and morphological effects of intra-articular stanozolol or placebo treatment, lasting 3 and 9 months, in sheep in which a femoro-tibial osteo-arthritis (OA) were surgically induced (medial bilateral meniscectomy).

Twenty healthy sheep divided into four groups and two control animals group, after surgical medial bilateral meniscectomy, were weekly injected in femoral-tibial joint (FTJ) with stanozolol or placebo. Lameness evaluation was performed and synovial fluid was collected from all sheep at each treatment time. Necropsies were performed after 3 or 9 month as described in experimental design. Gross pathologies were described and specimen tissues collected from femoro-tibial articular joints were processed for routine histological examination.

The gross anatomy of the FTJ was well-preserved in stanozolol-treated sheep; this also applied to the histological features of articular cartilage. Joint aseptic inflammation and fibrosis were observed in placebo-treated sheep, associated with a different degree of severity of condylar and tibial plate cartilage degeneration.

Stanozolol intra-articular treatment reduces osteophytes formation and subchondral bone reaction and promotes articular cartilage regeneration.

1. Introduction

The pharmacological treatment of osteo-arthritis (OA) is an ongoing challenge in equine clinical practice. Many treatments have been proposed, including those tested in experimental and clinical trials; however, response to treatment is not always satisfactory (Goodrich and Nixon, 2006; Frisbie et al., 2009; Pearson et al., 2009).

A variety of compounds used for the treatment of OA, including steroidal and non-steroidal drugs, inhibitor of nitric oxide production (NO) (Saleri et al., 2004). NO may be responsible for cartilage destruction in OA (Blanco et al., 1995), by damaging chondrocyte function, inhibiting collagen and proteoglycan synthesis, activating metalloproteinase, decreasing the expression of IL-1 receptor antagonist, inhibiting chondrocyte proliferation, and inducing apoptotic death (Studer et al., 1999). Stanozolol is a synthetic derivative of testosterone; its properties include anabolic/androgenic activity (Zannetti, 2004), probably associated with its affinity for androgenic and, at lower doses, glucocorticoid receptors (Fernandez et al., 1994). Because of its strong anabolic effects, studies on the effects of stanozolol treatment on OA are not performed in human medicine (Belch et al., 1986; Ellis et al., 1994): the same promising results (Zannetti, 2004; Dondi et al., 2008; Adamama-Moraitou et al., 2009) has led to renewed interest in the use of the product in veterinary medicine for companion animals.

Systemic stanozolol is used in horses as an anabolic steroid and doping agent (Yamada et al., 2008; You et al., 2009). However, stanazolol reduces apoptosis in equine chondrocytes *in vitro*, by reducing the production of NO and stimulating IGF-1 production (Saleri et al., 2004).

Lateral (Amstrong et al., 1994) or medial (Oakley et al., 2004) sheep meniscectomy is considered the best widely used animal model to investigate possible therapeutic approaches for degenerative joint disease in human and companion animals. Meniscectomy induces lesions both in the articular cartilage and in subchondral bone with the characteristic features of OA: articular space narrowing, osteophyte formation, focal cartilage lesions and decreased histochemical staining for proteoglycan (Moskowitz and Goldberg, 1987; Moskowitz et al., 1979). The technique of bilateral medial meniscectomy described by Oakley et al. (2004) is considered the golden standard for experimental AO investigation. For these reasons this model has been considered ideal for the present study.

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The aim of this study was to verify the clinical and morphological effects of intra-articular treatment with stanozolol on surgically induced OA of the stifle joints of sheep after 3 and 9 months of treatment.

2. Materials and methods

The experiment was performed according to the legislative and ethical requirements on animal care. The study protocol followed the "Principles of good laboratory practice" of OECD (Organization for Economic CO-operation and Development) and was approved by the University of Bologna Ethics Committee (119-120/2004 C). The experiment took place in the spring 2005.

2.1. Treatment preparation

The stanozolol[1] suspension and placebo were produced according to the following protocol: the test product was produced by adding stanozolol to a sterile apyrogenic water suspension containing sodium chloride and phosphate salts as a buffering system; the placebo did not contain any stanozolol molecules. The syringes were filled of drug, or placebo, and capped in a sterile environment. Analytical controls of the product were carried out before use.

2.2. Animals and experimental design

Twenty, 1 year-old, female Bergamasca sheep, weighing 42.85 ± 3.7 kg, were used in the study.

Fifteen days before surgery, twenty sheep were housed in small inside stalls with controlled temperature (18 °C) and relative humidity (70%); they were given a regular feeding regimen and divided randomly into six groups: 4 groups of 4 animal each and two groups of 2 animal each. Sixteen animals underwent bilateral medial meniscectomy as described by Oakley et al. (2004). Sheep were subjected to blood tests (hematological and biochemical profile) to verify their health status and orthopedic examination was performed to exclude signs of lameness in the hind limbs. A radiographic examination of the right and left stifle was also performed to rule out arthritic alterations of the stifle before the start of the study.

2.2.1. Group STAN3

Four sheeps (weighing 42.0 ± 1.4 kg) underwent meniscectomy and were treated, 7 days post-surgery (p.s.), with weekly bilateral FTJ (femoral-tibial joint) intra-articular administration of 1 mg of stanozolol in 0.4 ml of aqueous suspension for three months. Sheep were euthanized 3 months after the first treatment applied 6 days after meniscectomy.

2.2.2. Group MEN3

Four sheeps (weighing 42.0 ± 4.2 kg) underwent meniscectomy and were treated 7 days p.s., with weekly bilateral FTJ intra-articular administration of placebo in 0.4 ml solution for three months. The animals were euthanized 3 months after the first treatment applied 6 days after meniscectomy.

2.2.3. Group STAN9

Four sheeps (weighing 43.5 ± 0.7 kg) underwent meniscectomy and were treated 7 days after the surgery, with weekly bilateral FTJ intra-articular administration of a dose of 1 mg of stanozolol in 0.4 ml of aqueous suspension for three months and then every two weeks for the following six months. Sheep were euthanized 9 months after the first treatment applied 6 days after meniscectomy.

2.2.4. Group MEN9

Four sheeps (weighing 48.5 ± 2.1 kg) underwent meniscectomy and were treated 7 days after the surgery, with weekly bilateral FTJ intra-articular administration of placebo in 0.4 ml solution for three months; then every two weeks over the next six months, they were treated with placebo in 0.4 ml solution; the sheep were euthanized 9 months after the first treatment applied 6 days after meniscectomy.

2.2.5. Group CON1

Two sheeps (weighing 42.5 ± 2.1 kg) without meniscectomy, were treated bilateral FTJ intra-articular administration of 1 mg of stanozolol in 0.4 ml of aqueous suspension; one sheep CON(1–3) received the same treatment plan and timing of the STAN3 and was euthanized at the same time as this group (after 3 months of treatment); the other one CON(1–9) followed the same treatment plan and timing of the STAN9 and was euthanized at the same time as this group (9 months of treatment).

2.2.6. Group CON2

Two sheeps (weighting 41 ± 1.4 kg) without meniscectomy were treated bilateral FTJ intra-articular administration of placebo in 0.4 ml solution; one sheep CON(2–3) received the same treatment plan and timing of the MEN3 and was euthanized at the same time as this group (3 months of treatment); one CON(2–9) followed the same treatment plan and timing of the MEN9 and was euthanized at the same time as this group (9 months of treatment).

To induce OA, sixteen sheep were subjected to open bilateral medial meniscectomy under general anesthesia (premedication with xylazine[2] 0.1 mg/kg im and butorphanol[3] 0.05 mg/kg im; induction with ketamine[4] 2.2 mg/kg; maintenance with isoflurane[5] and 100% O_2) and dorsal recumbency.

Before the incision, a sample of synovial fluid was taken from all FTJ joints to determine normal baseline values. Post-surgery (p.s.) sheep, stabled individually, were treated with ampicillin[6] (20 mg/kg, IM, q 12 h) and gentamicin[7] (4 mg/kg, IM, q 24 h) and sheep flunixine meglumine[8] (1 mg/kg, IM, q 12 h) five days p.s. Any bandage was placed following surgery.

After a period of 30 days of confinement in the stall, the 20 sheep were forced to walk in a paddock 50 × 50 m. Handlers forced them to walk twice a day, at least for half-an-hour until the euthanasia for all animals.

Treatment with stanozolol or placebo began at day six p.s. For 3 months, the 20 sheep received weekly intra-articular injections. As described previously, after 3 months the sheep in groups MEN3, STAN3 and CON1 (1–3) and CON2 (2–3) were euthanized. The remaining sheep grouped in MEN9, STAN9 and CON1 (1–9) and CON2 (2–9) were treated every two weeks for a further 6 months and subsequently euthanized.

The dosage and interval times between injections were based on the results of an *in vitro* study on the effects of stanozolol on primary chondrocyte cultures (Saleri et al., 2004) and from a pharmacological study performed after intra-articular injection on sheep to assess tolerability and pharmaco-kinetics (unpublished data).

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[2] Nargesic, ACME, Cavriago (RE), Italy.

[3] Ketavet, Intervet, Aptilia (LT), Italy.

[4] IsoFlo, Abbot Laboratories Queenborought, Kent, United Kindom.

[5] Vatamplius, FATRO, Ozzano dell'Emila (BO), Italy.

[6] Aagent, FATRO, Ozzano dell'Emila (BO), Italy.

[7] Meflosyl, Fortdodge, Aprilia (LT), Italy.

[8] Tanax, Intervet International BV, Boxmeer, NL.