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Combining a joint health supplement with tibial plateau leveling osteotomy in dogs with cranial cruciate ligament rupture. An exploratory controlled trial

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ABSTRACT

Canine cranial cruciate ligament rupture (CrCLR) is a very common pathology. Surgical stabilization is the first choice treatment, although it does not fully eliminate the increased risk of osteoarthritis. This preliminary study was carried out to explore whether a newly formulated joint health supplement would benefit metabolic, clinical and radiographic changes in dogs with CrCLR surgically treated with tibial plateau leveling osteotomy (TPLO). Besides chondroitin sulfate and glucosamine hydrochloride, the studied supplement contained anti-inflammatory and antioxidant ingredients, the main ones being N-palmitoyl-D-glucosamine (Glupamid®) and quercetin. It was thus intended to target not only chondrodegenerative components of osteoarthritis, but also post-injury inflammatory response and oxidative stress of joint tissues. Thirteen dogs underwent TPLO and were randomly allocated to treatment (n = 6) and control groups (n = 7), the former receiving the joint supplement for 90 days. Lameness and radiographic osteoarthritis changes were scored before (i.e., baseline) and at 30 and 90 days post-surgery. Synovial fluid samples were collected from injured stifles at the same time points. Levels of representative metabolites were measured by proton nuclear magnetic resonance spectroscopy in a blinded fashion. In the metabolomic analysis, special attention was paid to lactate, due to its emerging recognition as a key marker of inflammation. In the last time period (from the 30th to the 90th day), lameness improved by a factor of 2.3 compared to control dogs. No significant difference was observed in the radiographic osteoarthritis score between groups. In the first postoperative month, lactate and creatine levels significantly dropped in treated compared to control dogs. Compared to surgery alone, combining the joint supplement with TPLO resulted in a trend to a better clinical outcome in the later time interval but did not influence osteoarthritis radiographic progression. A significantly better rebalance of joint microenvironment in the early time interval (baseline – 30 days) was shown by metabolomic analysis, thus suggesting that the study supplement could limit ongoing inflammatory responses.

1. Introduction

Rupture of the cranial cruciate ligament (CrCLR) is one of the most common orthopaedic problems in dogs and an important cause of hindlimb lameness and osteoarthritis (OA) [1]. Surgery is the gold standard to stabilize the stifle [2]. Among several techniques used to provide joint stability, tibial plateau leveling osteotomy (TPLO) is currently the most preferred [2,3], especially in medium- to large-breed dogs. Nonetheless, and like other surgical procedures [4,5], TPLO neither reduces nor halts progression of OA [6–9]. Therefore, the recommended treatment for CrCLR should also target secondary OA. Although the mechanisms leading to OA following ligament injury have not been fully established, chondrodegeneration and inflammatory changes are both considered important contributors [9–11]. Dietary supplements for joint

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health have gained increased recognition as valuable management options within the combined therapy for OA [12]. The efficacy of joint supplements is clearly formulation-dependent, relying mainly on their respective composition (i.e., ingredients used, relative amounts, excipients, purity, etc). Since the 1990s, a wide variety of compounds with different chemical structures, bioavailability, mechanism of action and degree of purity have been introduced in the veterinary market as chondroprotective agents, i.e. substances specifically aimed to rebalance the metabolism of degenerating cartilage, by boosting the synthetic pathways (pro-anabolic effects) while inhibiting degradative responses (anti-catabolic effects) [13,14]. More recently, a new class of joint supplements has been developed, able not only to support or enhance the articular intrinsic repair capability (chondroprotection *sensu strictu*), but also exert anti-inflammatory and analgesic effects and improve the intrarticular oxidative status [12,15,16]. The joint supplement used in the present study as an add-on/complementary treatment belongs to this latter class. In this formulation, classical chondroprotective compounds (chondrointin sulfate and glucosamine) are combined with an antioxidant and anti-inflammatory agent to target both OA-associated inflammation and pain. N-palmitoyl-D-glucosamine and quercetin are the main constituents responsible for the latter activities, largely due to their anti-inflammatory, antioxidant and pain relieving effects [17–19].

Metabolomics is the "omic" technique aimed to study metabolic OA profiling (metabolome), and is considered a promising strategy to follow disease progression and evaluate the effect of disease-modifying therapies [20–22]. One of the most attractive high-throughput technologies for global screening of joint metabolites is high resolution proton nuclear magnetic resonance spectroscopy (^{1}H NMR) [23,24]. It is usually performed on synovial fluid samples, since metabolic joint products are most likely to accumulate earlier and at higher concentrations compared to other biological fluids (e.g., serum, urine) [25,26]. In evaluating the anti-inflammatory effects of the study supplement, particular attention was paid to synovial changes in lactate, since increased levels of this metabolite have been related to the degree of inflammation in metabolomic studies of joint disease [23,27].

This preliminary study was carried out to explore metabolic, clinical and radiographic outcomes in dogs with spontaneous ligament rupture surgically treated with TPLO and supplemented or not with a joint health supplement. Our working hypothesis is that addition of the study joint supplement to surgery may benefit joint metabolome, lameness and OA radiographic progression during the study period, in light of the purported chondroprotective and anti-inflammatory activities of said formulation.

2. Materials and methods

2.1. Animals

The study was designed as a 90-day, open-label, controlled preliminary trial, using an untreated group as control. Medium to large-breed client-owned dogs with naturally occurring unilateral CrCLR referred for TPLO were enrolled. The following inclusion criteria had to be met: (i) complete unilateral CrCLR, with no contralateral stifle problems as assessed clinically and radiographically; (ii) acute ligament injury, i.e., occurring less than 20 days before entering the study as determined by information provided by the owner or referring veterinarian; (iii) body weight between 15 and 55 kg; (iv) age between 12 months and 8 years; (v) mild to moderate stifle OA, corresponding to scores 1 and 2 on a 4-point radiographic grading system [28]. Exclusion criteria were any abnormality in the complete blood count and serum biochemical profile, any history or evidence of previous stifle joint surgery, and other orthopaedic and neurologic diseases. Additional exclusion criteria were concomitant meniscal lesions (as confirmed by arthroscopic meniscal inspection before TPLO procedure), and concurrent treatment with non-steroidal anti-inflammatory drugs and corticosteroids. All owners gave informed consent before enrolment of their dog and approval from the local ethical committee was obtained. Breed, gender, age, body weight and clinical history of each dog were recorded.

2.2. Surgery

All surgeries were performed by the same surgeon (F.M.M.) according to the TPLO technique as described by Slocum and Slocum [29] and modified by Pozzi *et al.* [30] without meniscal release. The hind limb was clipped and prepared for surgery in a routine manner. A skin incision was made medially from approximately the distal fourth of the femur to the proximal third of the tibia. Stifle arthroscopy was performed before TPLO in all procedures. The jig was not applied [31] and a tibial osteotomy was made using an 18 mm, 24 mm or 30 mm biradial saw blade. The proximal tibial segment was then rotated according to Slocum's recommendations on the basis of the tibial plateau angle calculated from radiographs obtained prior to surgery. All osteotomies were stabilized with one or two anti-rotational Kirschner wires placed from the tibial crest directed caudo-distally to exit the caudal cortex of the proximal fragment. The osteotomy was secured by either a 3.5-mm SLOCUM TPLO plate, 3.5-mm small SLOCUM TPLO plate, 3.5-mm TPLO SYNTHES locking compression plate, 3.5-mm broad TPLO SYNTHES locking compression plate, 3.5-mm SECUROS TPLO plate or 3.0/3.5-mm FIXIN T Support, based on the size and temperament of the dog. The K-wires were removed after application of the bone plate. Postoperative prophylaxis consisted of cefadroxil (20 mg/kg q12h PO for 5 days), carprofen (3 mg/kg q24h PO for 7 days) and tramadol if needed (3 mg/kg q6h PO days 1–3). A modified Robert Jones was applied for 48 h postoperatively. All owners received written instructions concerning postoperative care and follow-up examinations. Instructions for postoperative confinement and the 8-week rehabilitation period were based on TPLO course recommendations [32].

2.3. Treatment

After TPLO, dogs were randomly allocated into two groups according to a simple randomization procedure. Group C (control) received no further treatment. Group T (treated) received the joint health nutraceutical,[1] administered daily at a dose of 1 tablet/25 kg b.w./PO for 90 days, starting on the day after surgery. Each tablet had the following composition: low molecular weight normosulfated chondroitin sulfate (NSCS 5/20 patented fraction), 200 mg; glucosamine hydrochloride, 300 mg; N-palmitoyl-D-glucosamine (Glupamid®), 100 mg; quercetin, 75 mg; Vitamin E, 50 mg; ω3 essential fatty acids, 50 mg.

2.4. Synovial fluid sample collection and preparation

Synovial fluid samples (0.5 mL each) were aseptically collected by arthrocentesis from the affected stifle joints before surgery (baseline visit, V0), and on days 30 and 90 after TPLO (V30 and V90, respectively). Samples were filtered using membranes of 0.8 μm porosity, centrifuged at approximately 17,000 *g* for 15 min in order to separate the cells and stored at −80 °C. The main metabolites present in canine synovial fluid samples were studied

[1] Condrostress®, Innovet Italia Srl, Milan, Italy.

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