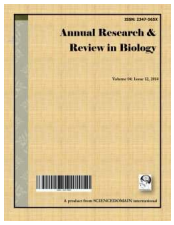

Annual Research & Review in Biology
9(3): 1-7, 2016, Article no.ARRB.23345
ISSN: 2347-565X, NLM ID: 101632869

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Intra-articular Injection of Ascorbic Acid and Dexamethasone for Management of Osteoarthritis in Dogs

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AB and BJW designed the study, wrote the protocol and interpreted the data. Authors AAP, RJ and RSG anchored the field study, gathered the initial data and performed preliminary data analysis. While authors TAK and MB managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2016/23345
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) David Sadigursky, Unifacs School of Medicine, Brazil.
(2) Heshu Sulaiman Rahman, Universiti Putra, Malaysia.
(3) Nitin Gupta, Nims Medical College, Jaipur, India.
Complete Peer review History: http://sciencedomain.org/review-history/13013

Original Research Article

Received 26th November 2015
Accepted 5th January 2016
Published 19th January 2016

ABSTRACT

Aims: The study was conducted to evaluate the beneficial effects and clinical outcome of intra-articular administration of ascorbic acid and dexamethasone for the management of osteoarthritis in dogs.
Study Design: The study was conducted on 6 clinical cases of dogs suffering from osteoarthritis in hip (n-2) and stifle (n=4). The study was conducted on dogs reported to Madras Veterinary College Teaching hospital as confirmed by clinical examination and x-ray.
Methodology: Ascorbic acid and dexamethasone in phosphate buffer mixture (ADM) was prepared using dexamethasone and ascorbic acid injection (Indian Pharmacopeia) each at the

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dose of 0.8 to 4 mg and 5 mg/ml concentration in one to two ml of sterile phosphate buffer solution, for intra-articular injection. The mixture was administered intra-articularly through ultrasound guidance. The clinical outcome and benefits were assessed by clinical improvements, x-ray and ultrasound.

Results: Significant improvement could be noticed in all cases after 14th day of administration as evident by clinical, radiographical and ultrasound.

Conclusion: The ascorbic acid-dexamethasone mixture in phosphate buffer solution injected intra-articularly for the management of osteoarthritis had exhibited promising results in the clinical cases and has emerged as an exciting and novel alternative to the conventional management modalities involving the use of non-steroidal anti-inflammatory agents, chondroprotectives, corticosteroids and surgical procedures.

Keywords: Osteoarthritis; ascorbic acid; dexamethasone; dog; intra-articular.

1. INTRODUCTION

Osteoarthritis (OA) is a slow progressive, low-grade inflammatory syndrome that affects all articular and periarticular tissues, including joint capsule, synovium, articular cartilage, and subchondral bone leading to severe pain and inflammation in joints [1]. Incidence of osteoarthritis is more than 20 per cent of all arthropathies in dogs and is characterized by degeneration of the articular cartilage surface, matrix loss, fibrillation and formation of fissures that can result in complete loss of cartilage surface and cause unbearable pain [2,3]. Partial or complete rupture of the cranial cruciate ligament (CrCL) results in stifle joint instability and contributes significantly to the development of osteoarthritis [4]. Most patients prefer treatments that are inexpensive, and have long-term efficacy, less painful, less invasive and more easily accessible with fewer side effects than with existing treatments. The current medical treatment for OA are the administration of non-steroidal anti-inflammatory drugs (NSAIDs) [5] and steroids, and the patient becomes refractory leading to salvage procedures like femoral head and neck ostectomy (FHO) and total or partial hip replacement (HR) prosthesis which are expensive. Therefore, given the needs of OA patients and the limitations of existing OA treatments, we proposed an intra-articular injection material that might confer greater therapeutic benefit in OA and fulfil patients' needs. Intra-articular injection of dexamethasone or ascorbic acid was conventionally practiced as medical options for the treatment of osteoarthritis considering their anti-inflammatory effect and nutritive support for cartilage regeneration respectively. Recent *in-vitro* studies strongly supports that a combination of dexamethasone and ascorbic acid at low concentrations induce chondrogenesis of adult mesenchymal stem cells in rabbits [6]. Based on the conventional medical practises and recent *in vitro* studies, a combination of dexamethasone and ascorbic acid at defined concentrations was formulated to study the efficiency for the management of osteoarthritis in canines.

2. MATERIALS AND METHODS

2.1 Drugs

Ascorbic acid and dexamethasone in phosphate buffer mixture (ADM) was prepared using dexamethasone (4% injectable solution, Lupidexa) and ascorbic acid injection IP (100mg/ml, Life care Pharmaceuticals Pvt. Ltd.) each at the dose of 0.8 to 4 mg and 5 mg/ml concentration in one to two ml of sterile PBS, for intra-articular injection. The dose rates of ascorbic acid and dexamethasone varied from 0.13 to 0.42 and 0.05 t0 0.11 mg/kg according to the volume of the mixture injected.

2.2 Study Population

Dogs reported to Orthopaedic Unit, Department of Veterinary Surgery & Radiology, Madras Veterinary College Teaching Hospital, Chennai-600 007 with osteoarthritis of joints with grade 3 and above as confirmed by clinical and radiographic evaluation, were utilized for the study. Among the dogs reported with hip and stifle osteoarthritis, dogs of either sex, irrespective of, age and breed were selected for the study. Based on an inclusion and exclusion criteria, six cases (n=6) were selected for the study, out of which two (n=2) were hip OA and four cases (n=4) were stifle OA. The inclusion criteria emphasized were radiographically confirmed diagnosis of osteoarthritis in either stifle or hip joint**,** demonstrated load-bearing

lameness referable to either the stifle or hip joint(s), free off all pain medications one week prior to appointment**,** weight was more than 11Kgs, age was between 4 to 12 years of age and otherwise medically healthy. The reasons for exclusion of cases were joint infections within two months of enrolment, surgery within three months on the affected or contralateral joint, previous systemic steroid administration within 2 months, bone on bone contact on radiograph and intra-bone fragments or fracture in the stifle or hip joint(s).

2.3 Intra-articular Injection

The dogs were premedicated with an intra-muscular injection of atropine sulphate (0.02 mg/kg) and xylazine hydrochloride (1 mg/kg). The skin over affected joint was prepared for aseptic intra-articular injection after clipping and scrubbing the injection site with 5% povidone iodine scrub solution. A 20 to 22 G sterile hypodermic or spinal needle was used for arthrocentesis depending on the size of the dog and the joint affected. Ultrasound guided intra-articular injection was performed over the hip or stifle joint using a 8-16 MHz curvilinear transducer [7]. Synovial fluid was collected and subjected to biochemical analysis. For intra-articular injection into the hip joint, the dog was placed on lateral recumbency. A 20 or 21 G hypodermic or spinal needle, based on the size of the dog was inserted immediately proximal to greater trochanter and directed perpendicular to the skin with a distal or ventral orientation of the limb held in slight abduction, to open up the joint, under ultrasound guidance, after confirmation of intra-articular position of needle, ADM was injected intra-articularly into the hip joint (Table 1). For intra-articular injection in the stifle joint, the needle was inserted midway between the centre of patella and tibial tuberosity, just lateral to the patellar tendon under ultrasound guidance, the needle placement was confirmed and ADM was injected intra-articularly into the stifle joint. Intra-operatively, pain was managed using Tramadol (2 mg/kg, i/v). Pre and post engraftment antibiotic therapy was done with cefotaxime (20 mg/kg).

2.4 Care after Intra-articular Injection

The owners were advised ice pack application over the affected joint post intra-articular injection to reduce local inflammation and pain. Tramadol 50 mg tablets were administered orally at 12 hours interval for 2 days. The owners were demonstrated therapeutic exercises for the joint involving active flexion and extension of the limb, to encourage limb usage and gradual return to normal activity levels. In required cases, intra-articular injection of ADM was repeated after 21 days.

2.5 Patient Assessment

The outcome of therapy was assessed based on the comparison of the following parameters before and after intra-articular therapy.

2.5.1 Owner's score

Owner's score was assessed by a six-point Likert scale was used to obtain owner's general impression of change in mobility, lameness, stiffness, behaviour at home and during different types of exercise and were scaled as 1 - large deterioration, 2 - mild deterioration, 3 - no change in signs, 4 - mild improvement, 5 - large improvement and 6 - without any signs of dysplasia [8].

2.5.2 Clinical evaluation

Lameness and clinical evaluation was performed by using a 15 point Olby pain score [9] comprised of five stages (1 to 5), with scoring ranged from 0 to 14 wherein the lowest score (0) indicated no pelvic limb movement and no deep

Table 1. Table showing the total quantity of ascorbic acid and dexamethasone and volume of PBS administered intra-articularly

Trial no.	Weight of the dog (Kg)	Joint affected	Total dose (mg)		Volume of PBS (ml)
			Ascorbic acid	Dexamethasone	
1	35	Stifle	5	4.0	2
2	15	Stifle	5	0.8	1
3	32	Hip	5	4.0	2
4	38	Hip	5	4.0	2
5	25	Stifle	5	2.0	2
6	12	Stifle	5	0.8	1

pain sensation and the highest score (14) indicated normal pelvic limb gait. The stage 1 (score 0 to 2) indicated no pelvic limb movement with varying degrees of sensation. Stage 2 (score 3 to 5) indicated minimal weight bearing protraction of pelvic limb less than 50% of time. Stage 3 (score 6 to 8) indicated weight bearing protraction less than 10% of time. Stage 4 (score 9 to 11) indicated weight bearing protraction 100% of time with reduced pelvic limb strength. Stage 5 (score 12 to 14) indicated ataxic pelvic limb gait with normal pelvic limb gait.

2.5.3 Lameness score

Lameness grading was done using a numerical rating scale of 1 to 10, where 1 had no lameness, 3 less than mild lameness upon rising and starting activity, 5 mild lameness when moving, 7 moderate lameness and dog does not want to play and 10 severe lameness with dog exhibiting high pitched cry when touched or moved [10].

2.5.4 Radiographic evaluation

Radiographic assessment of the joints was evaluated by grading system suggested by Impellizeri, 2000 (Table 2). All the dogs were in the grade of 3 and above.

2.5.5 Ultrasonographic evaluation

Ultrasonographic synovial fluid evaluation was performed on day 0, owing to the clinical nature of the study, to minimize trauma afflicted to the joint.

3. RESULTS

All cases of OA treated with ADM showed improvement as per owner's assessment score, clinical examination, radiography, ultrasonography and synovial fluid analysis.

3.1 Owner's Score

During pre-treatment stage, two cases (n=2) of Hip OA were accorded 1 and 2. On 14th post-injection day, the cases were accorded 3 and 5 respectively. On 28th day post-injection, the cases of hip OA were accorded 3 and 6 respectively. Among four (n=4) cases of stifle OA, during pre-treatment stages were accorded 1, 1, 1 and 2 respectively. During 14th day post-operatively, the cases were accorded 4, 4, 5 and 4 respectively.

3.2 Evaluation

The treated dogs exhibited increased pain tolerance as measured by Olby pain score [9,11,12]. The cases of stifle OA with complete or partial tear of Cranial Cruciate Ligament (CrCL) exhibiting cranial drawer movement on clinical examination pre-treatment, exhibited diminished to total absence of drawer movement within 2 weeks post injection suggesting partial tear. The weight bearing ability of the affected limb(s) and the gait of the animal showed improvement post-treatment with ADM. All the dogs were in the grade of 1 to 2 before start of treatment and improved to above 4 on 28th day of treatment.

3.3 Radiography

The radiographic signs of OA in the affected joint, progressively reduced post-treatment with ADM in 28 days. Pre-treatment pelvis ventrodorsal (VD) radiographs (CR-30X, Agfa) of hip OA exhibited grades 3 and 4 respectively. 14th day post-injection pelvis VD radiographs exhibited grades 2 and 3 respectively whereas 28th day post-injection pelvis VD radiographs showed grades 2 and 1 respectively signifying regeneration of osteoarthritic joint.

In four (n=4) cases of stifle OA, lateral views of osteoarthritic stifle joint exhibited grades 1, 1, 2 and 1 respectively with prominent "fat pad sign" indicating inflammatory changes in the joint. 14th day post-injection stifle lateral views exhibited diminished "fat pad sign" indicating reduced joint inflammation.

Table 2. Table showing radiographic assessment of osteoarthritis

Grade	Classification	Description
1	Slight	Periarticular osteophytes only
2	Mild	Periarticular osteophytes and femoral head remodelling
3	Moderate	Periarticular osteophytes and femoral head and neck remodelling and acetabular remodelling
4	Severe	Periarticular osteophytes and femoral head and neck remodelling and acetabular remodelling, subchondral sclerosis of femoral head and acetabulum

3.4 Ultrasonography

A 10 to 15 MHz linear probe detected joint effusion, disintegrity of articular cartilage, synovitis in osteoarthritic joint [13]. The visualization of the ruptured CCL in cases of stifle OA was visualized using a 8 to 15 MHz curvilinear transducer. The degree of joint effusion significantly reduced from 14^{th} day of administration.

4. DISCUSSION

The material for intra-articular use in management of osteoarthritis is an Ascorbic acid and Dexamethasone in Phosphate buffer (ADM) Mixture and it is formulated to supply nutrients to chondrocytes, which in turn synthesize collagen or proteoglycan (PG) to maintain the matrix network. Collagen fibres, especially type II collagen and PG, hold water to give tensile and compressive stiffness, and cartilage integrity depends on a successful symbiotic relationship between chondrocytes and interstitial matrix [14].

Corticosteroids injected intra-articularly, are frequently used in the treatment of OA, and it is presumed that the corticosteroid mechanism of pain reduction is via an effect on the synovial membrane lining the osteoarthritic joint capsule [15]. Corticosteroids also inhibit the production of pro-inflammatory cytokines interleukins (IL) 1 and 6 and TNF-α as well as decreasing the expression of COX-2 [16]. Steroids also inhibit the generation, proliferation and activation of T cells, which have been shown to infiltrate the synovium in OA joints. Dexamethasone exerts an anti-inflammatory and an analgesic effect when injected intra-articularly into the osteoarthritic joint [17]. Ascorbic acid is required for synthesis of type II collagen and proteoglycans as a cofactor in articular cartilage [18]. Ascorbic acid has been shown to stimulate *in-vitro* differentiation and production of matrix molecules by adipocytes. Vitamin C, b-glycerophosphate, and dexamethasone induced an increase of the mRNA levels for collagen type 1, osteocalcin, bone sialoprotein, and alkaline phosphatase in association with the development of bone nodules in an in vitro system. Ascorbic acid helps in synthesis and growth of collagen molecules [6]. Corticosteroid preparations can be either soluble or insoluble. Most corticosteroid preparations contain corticosteroid esters, which are highly insoluble in water and thus form microcrystalline suspensions. Dexamethasone preparations, however, are not esters and are freely soluble in water; hence, the preparation is clear (ie, nonparticulate). The potential advantage of corticosteroid ester preparations is that they require hydrolysis by cellular esterases to release the active moiety and consequently should last longer in the joint than do non-ester preparations. On the other hand, freely water soluble preparations such as dexamethasone sodium phosphate are taken up rapidly by cells and thus have a quicker onset of effect but with a concomitant reduced duration of action [17]. Dexamethasone also exerts differential effects on the chondrogenesis of mesenchymal stem cells (MSC) derived from various sources such as bone, fat, perisoteum, synovium and muscle. Dexamethasone significantly enhances TGF-β1 induced deposition of cartilaginous matrix in MSC aggregates derived from bone marrow [19]. Corticosteroids are sometimes administered after admixture with other agents in the same syringe. In addition to the use of ultrasound as a diagnostic modality for OA in dogs it also has a therapeutic effect when applied over the osteoarthritic joint. Application of 1 cm^2 transducer at 3 MHz frequency led to improvement in clinical status, muscle mass, mobility of affected limb without increase in circumference of osteoarthritic stifle joint in dogs [20]. Osteoarthritis is a complex syndrome and its diagnosis by clinical examination alone provides erroneous results. The clinical examination has to be correlated with radiographic views of affected joints, diagnostic ultrasound scan and synovial fluid analysis for diagnosing early cases of OA [2]. Drawer test and tibial compression test were used for detecting cruciate ligament instability in stifle joint of dogs. Drawer test alone or combined with tibial compression test had a poor sensitivity for correctly identifying cranial and caudal cruciate ligaments, or total cruciate ligament (TCL) rupture but exhibited a high sensitivity when examining normal limbs of dogs suspected to have a tear of cruciate ligaments, leading to the differential diagnosis of OA of the stifle joint from cruciate rupture due to direct trauma [21].

5. CONCLUSION

The use of ADM mixture injected intra-articularly for the management of OA has exhibited promising results in the clinical cases and has emerged as an exciting and novel alternative to the conventional management modalities involving the use of NSAIDs, chondroprotectives, corticosteroids and surgical procedures.

CONSENT

The present study was carried out after obtaining a written consent from the animal owners.

ETHICAL APPROVAL

The present study including the surgical protocol for intra articular injection was approved by the Institutional Ethical Committee for Stem Cell Research, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600051.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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