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LED Activated Mesenchymal Stem Cells in Management of Canine Hip Osteoarthritis

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ABSTRACT

Adipose-Derived Mesenchymal Stem and regenerative Cells (ADMSC) together with Platelet Rich Plasma (PRP) was used as an alternative method in improving mobility and reducing pain scores in canine hip osteoarthritis (OA) prior decision of surgical interference. In this study, ADMSC applied on five cases of osteoarthritic dogs. Osteoarthritis score chart (OAS) 1 and clinical investigations techniques were used to assess the therapeutic benefits. The aim of this study was to study the clinical efficacy of LED activation 2 prior to ADMSC administration in assessment of canine hip OA. In this pilot study, five osteoarthritic dogs of different breeds, ages and OA grads were uses. A significant results compared to the control were shown in OAS technique among treated dogs before and after ADMSC. The mean value of OAS and Weight showed significantly improved before and after ADMSC. Although, there are clear clinical improvements in mobility and pain scores were also shown. Post therapy, obvious improvements in hip osteoarthritic dogs were shown after ADMSC. Improvements were appeared in evaluating methods, mobility and reduced pain.

Keywords : Osteoarthritis, Canine, Hip, Adipose-derived mesenchymal stem cells, ADMSC.

I. INTRODUCTION

The field of adipose-derived mesenchymal stem cell (AD-MSC) therapy in regenerative medicine is a rapidly growing area of research, and stem cell therapy is being used to treat osteoarthritis (OA). Isolation of cells from adipose tissue entails mincing and washing followed by enzyme digestion, washing, and centrifugation. [1,2] The pellet formed from centrifugation, often termed the stromal vascular fraction, is a heterogenous mixture of cells including fibroblasts, pericytes, endothelial cells, blood cells, and AD-MSCs and is the treatment modality for these studies. Since 2003, veterinarians have used autologous ADMSCs to treat tendon and ligament injuries and joint disease in horses on a commercial basis. As a result of the "minimally manipulated" nature of the cells, this particular autologous stem cell therapy does not require FDA approval. [3]

Hip dysplasia (HD), a heritable condition that results in laxity of the coxofemoral joint, is one of the most common orthopedic diseases affecting dogs.1 In skeletally immature dogs, coxofemoral subluxation, joint capsule stretching, cartilage erosion, and subchondral bone fracture often produce pain and lameness. Disease progression can lead to degenerative and inflammatory changes within the joint and development of osteoarthritis in mature dogs. [4]

II. METHODS AND MATERIAL

According to *Black et al, 2007*

Study Population

Cases admitted to Animal Medical Centre had been involved in this study, which included 5 outpatient dogs with chronic OA of the hip joint. Before enrolment, investigators ensured that all dogs underwent routine clinical chemistry and hematology (complete blood cell count) evaluation (conducted at an outside laboratory) to ensure overall health. Study animals demonstrated gait changes characteristic of OA, including persistent lameness at a walk and trot, pain on passive manipulation of the affected joint(s), limited range of motion with pain at less than full range of passive motion, and functional disabilities as measured by willingness to walk and run. Each qualified case demonstrated pre-treatment radiographic evidence of degenerative joint disease, as evaluated by the investigator, of grade 2 or higher on the following radiographic scoring scale:

0 = Normal joint
1 = Radiographic evidence of instability; no degenerative change (no osteophytes)
2 = Mild degenerative change (occasional osteophytes)
3 = Moderate degenerative change (osteophytes, subchondral sclerosis)
4 = Severe degenerative change (osteophytes, subchondral sclerosis, bone remodelling) Dogs were excluded from the study if they had concurrent disease, such as a fungal, bacterial, or viral infection; malignant neoplasia; or any severe systemic disease that would confound interpretation of treatment effects. All enrolled dogs were deemed healthy with no systemic disease. Dogs that were on concomitant therapy, such as NSAIDs, were required to be on these medications for at least 60 days before enrolment in the study and to remain on the drugs at the same level throughout the study. Hyaluronic acid and polysulfated glycosaminoglycan injections, neutraceuticals, corticosteroids, and such alternative treatments as chiropractic and acupuncture, if used, were discontinued in all dogs beginning 10 days before enrolment in the study and were not administered during the study period. To be eligible, the dogs had to be cared for by attentive owners who agreed by informed consent to participate in this clinical study, to follow a set schedule of veterinary appointments, and to observe their dog for the entire study period.

Stem and Regenerative Cell Preparation

Adipose Tissue Collection

Adipose tissue was collected from either the abdominal, inguinal, falciform ligament, or thoracic wall regions of the dogs. A small (5 cm) surgical incision was made aseptically after the patient was anesthetized. The adipose tissue was resected by scalpel or surgical scissors and placed into a labeled sterile tube containing 15 ml of PBS. The sample tube was placed in a validated, temperature-controlled 2°C to 8°C transport box specially fitted with a frozen cold pack and shipped overnight to the laboratory for processing. Tissue Processing for Stem and Regenerative Cell Isolation Adipose tissue was washed with PBS, minced, washed several more times with PBS to remove debris and excess blood, and centrifuged as previously reported.9 An aliquot of the final cell suspension was assessed for viability (trypan blue exclusion method) and total nucleated cell yield. This constitutes the stromal vascular fraction preparation, which consists of a heterogenous mixture of cells including AD-MSCs, hematopoietic stem cells, preendothelial cells, fibroblasts, pericytes, and endothelial cells. Evaluations Veterinary evaluation incorporated history, physical examination, and lameness examination including joint mobility, notation of pain on manipulation, and functional disability. Clinical outcome measures were based on veterinary orthopaedic examination evaluation by a single investigator using the following numeric rating scale:

- Lameness at walk and trot: 1 (normal), 2 (intermittent), 3 (persistent), 4 (non–weight bearing), 5 (ambulatory only with assistance), 6 (no ambulatory)
- Pain on manipulation: 1 (no pain), 2 (mild pain; attempts to withdraw limb), 3 (severe; immediately withdraws limb)
- Range of motion: 1 (normal), 2 (pain only at full range of motion), 3 (pain at less than full range of motion), 4 (pain on any joint manipulation)
- Functional disability: 1 (normal; no stiffness), 2 (slightly stiff gait noticeable only on running), 3 (stiff; dog has noticeable stiffness while walking and running), 4 (very stiff; dog does not want to walk or run without being coaxed), 5 (does not want to walk; will not run; must be helped up) Baseline results for both owner and veterinary evaluations were recorded before adipose tissue harvest and between 2 and 14 days before the dogs received AD-MSC therapy by intraarticular injection. Follow-up visits to the veterinary clinic were required at 30, 60, 90, and 180 days after the dog's intraarticular injection. At each visit, owners were also asked to complete a numeric rating scale (1 [best] to 5 [worst]) as part of a standard questionnaire adapted from the Cincinnati

Orthopedic Disability Index (CODI), which included evaluation of the following 13 parameters: walk, run, jump, turning suddenly, getting up from lying down, lying down from standing, climbing stairs, descending stairs, squatting to urinate or defecate, stiffness in the morning, stiffness in the evening, difficulty walking on slippery floors, and willingness to play voluntarily.

Statistical Evaluation

The statistical significance of changes in veterinarian and owner clinical scores over time from baseline values was analyzed separately by repeated measures analysis of variance on ranks. All comparisons were made at the nominal .05 level of significance. To provide an estimate of the practical relevance of the apparent effects of treatment, the standardized treatment effect defined as the mean change from baseline divided by the standard deviation of the change, was calculated for each outcome variable at each post treatment evaluation. The significance of correlations between veterinarian and owner scores was determined by the Spearman rank order correlation method.

III. RESULTS AND DISCUSSION

In this pilot study, five osteoarthritic dogs of different breeds, ages and OA grads were uses. A significant results compared to the control were shown in OAS technique among treated dogs before and after ADMSC. The mean value of OAS and Weight showed significantly improved before and after ADMSC. Although, there are clear clinical improvements in mobility and pain scores were also shown.

Of the cases admitted to animal medical centre (AMC) for lame and hip dysplasia, five dogs with different breeds, Ages, sex and OA degrees. Dog's breeds are including golden retriever, Rottweiler, Siberian husky, Great Dane, GSD, and Pomeranian. Age ranges from 6 months to 5 years. OA score degrees are ranging from (3) moderate to (5) sever. All the cases have evidence of joint laxity. A distinguishable significant improvement in OES is clear in all OES criteria compared with baseline along period of treatment follow up till 12 months. The improvement progression becomes constant after 12 months of treatment (table 1) (Figure 1and 2).

Table (1) comparison of mean difference of OES baseline to OES among different periods of time (n=5)

Criteria	1 Month	2 month	3 Month	12 Month	24 Month	SEM ±
Lame (Walk)	1.0*	1.9*	2.13*	2.3*	2.3*	0.24
Lame (Trot)	1.0*	1.9*	2.13*	2.3*	2.3*	0.24
Pain at manipulations	1.13*	1.8*	1.5*	1.9*	1.13*	0.18
Range of motion	0.9*	1.6*	1.6*	1.9*	1.9*	0.24
Functional disability	1.5*	2.4*	2.4*	2.5*	2.5*	0.19

*P value vs baseline; one-way analysis of variance was applies followed by Post-hoc multiple comparison test using Tukey's procedures. F(dl)= 28.36(5),28.36(5),34.18(5),19.04(5),57.7(5) respectively, $p \leq 0.001$

Evaluation of treatment through DI is shown highly significant in comparing with baseline. While, in OAS is shown also significant results in comparing with baseline. In belongs of weight before and after treatment, It's shown that there are increasing of body weight. Among different methods of evaluation criteria, the results of treatment of ADMSC are significant improve in DI, OES, OAS and Wt. OES evaluation are made by professional veterinarian along the expanded duration of this study that reached to 24 months of follow up the cases under study. The dependable evaluating methods are selected to measure clinical efficacy of ADMSC treatment and did not include force plat analysis due to impractical at time of study and some cases were in severing conditions[1].

Table (2): Comparison of mean DI, OFA and Wt between baseline and after treatment among osteoarthritic dogs (n=5)

	DI		**OAS**		**Weight**	
	Baseline	After	Baseline	After	Baseline	After

Mean (SD)	0.775 (0.17)	0.562 (0.56)	4.50 (0.93)	3.50 (0.53)	19.67 (8.34)	27.52 (13.49)
MD (95% CI)	0.212(0.182,0.242)		1.00 (0.37,1.63)		-8.22 (-16.814,0.364)	
t-statistics* (df)	17.00 (7)		3.74 (7)		-2.26 (7)	
p-value*	0.000		0.007		0.056	

*Paired t-test

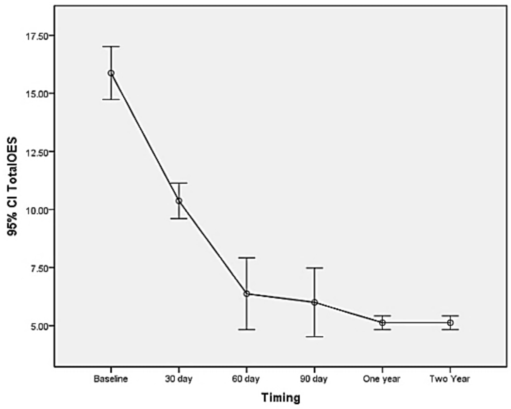

Figure 1: Mean of improvement (±SEM) in over whole OES among period of time.

Overall mean of OES appear to be significant improve consciously along time till 12 months after treatment then become constant till 24 months after treatment. Distinguishable significant improvements that appear in DI, OES, OAS and weight are clear that continued till one year (Figure 1 and 2). Although, there are no clinical improvements or change in mobility or pain scores were showed for the next 12 months. This improvement might be derived from MSC [2-6]. The role of MSC in secretion of cytokines, interleukin-1 (IL-1) receptor antagonist (IL-1ra) and growth factors as mentioned by Ortiz et al., 2007 thought to be reduce inflammation and fibrosis the reason to these improvements[4, 6-8].

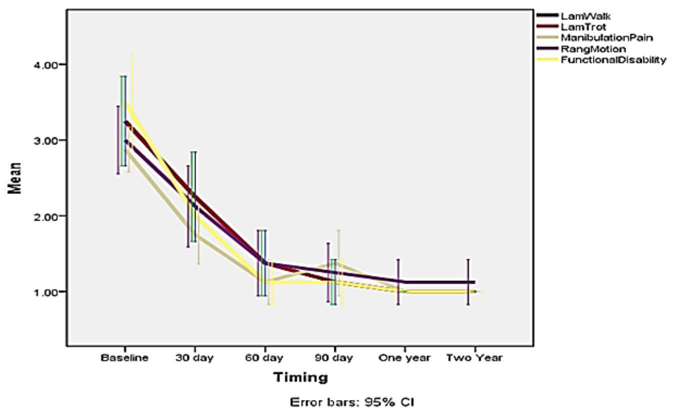

Figure 2: Mean of improvement (±SEM) in scores of lameness at the walk, lameness at the trot, pain on manipulation, range of motion and functional disability among period of time.

From the significant improved results of this study, it's possible of multiple mechanism of MSC to improve OA, not only by interleukin and growth factor secretions but also cellular differentiation of MSC into chondrocytes[9] as mentioned by Nathan and associates. The high acting and long lasting effect of ADMSC in this study (table1 and 2)(Figure 1 and 2) till 12 month-that appeared superior to other studies[2, 3, 10] evaluated ADMSC-might due to duplication of administration way of ADMSC into dogs through intraarticular and intravenous injections. One more possible cause for persistency of significant improvement is LED activation of ADMSC[11, 12]. In comparing P values of all evaluating methods used in this study (Table 3), the highest significant level found in OES and DI ≤ 0.001 followed by OAS ≤ 0.01 then ≤ 0.05 for Wt. respectively. Power and associates refer that to superiority of DI on OAS in investigation of OA and detection of susceptible cases[13]. P vale of weight has the lowest level that may be poor relation to OA[24] as mentioned by Impellizeri and associates or may not be useful as an evaluating methods.

IV. CONCLUSION

Over of two years of monitored investigation, follow up of osteoarthritic dogs through OAS, DI, OES and weight. Intraarticular and intravenous administration of ADMSC was seen as a significant improvement in hip osteoarthritic dogs. The duration of maximal effect might be extended up to 12 months. Post therapy, obvious improvements in hip osteoarthritic dogs were shown after ADMSC. Improvements were appeared in evaluating methods, mobility and reduced pain.

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