Augmentation of Carpal Arthrodesis Using Mesenchymal Stem Cells Derived Microvesicles and Cancellous Bone Graft in Dogs

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1. Abstract

A novel application of mesenchymal stem cell-derived microvesicles (MSC-derived MVs) with arthrodesis in dogs is described, and radiographic osseous union, lameness score, histological findings, and complications following surgery are assessed. This study compared the procedure type in 20 cases of pan carpal arthrodesis in dogs treated with (MSCs derived MVs) and cancellous bone autografts (mixed group) and cancellous bone autografts alone (BG group). Radiographs were compared at 4, 8 and 12 weeks after surgery to find that the (MSCs derived MVs) group had greater scores than others in terms of radiographic osseous union and histopathological evidence of bone formation. When compared to the (MSCs derived MVs) group, the BG group had considerably more serious problems that necessitated re-operation for implant removal or treatment of a deep infection. Overall, MSC-derived microvesicles (MSC-derived MVs) can be used to complement arthrodesis in dogs without causing considerable morbidity.

Key words: *arthrodesis, pan carpal, micro vesicle, stem cells.*

2. Introduction

Canine carpal joint was a complex diarthrodial joint consisting of multiple smaller joints and supportive ligaments. Palmar carpal ligaments support the carpal joints at its three levels and permit 10 to 12 degrees of extension at the antebrachiocarpal joint in standing animal [1].

Canine carpal arthrodesis was defined as surgical induction of osseous union of the carpal joints. Pan carpal arthrodesis is performed to salvage fore limb function in various diseases that result in canine carpal instability included sever carpal bone fractures, chronic arthropathies, hyperextension injures and complex distal antebrachial fracture [2, 3]. Materials reported to stimulate bone bridging with arthrodesis as demineralized bone matrix, [4] recombinant bone morphogenic protein, [5] new biomaterials (hydroxyapatite and collagen), [6], bioartificial composite bone grafts composed of b-tricalciumphosphate and platelet rich plasma [7] and free autogenous omental graft [8] have been reported to enhance bone bridging with arthrodesis.

Microvesicles act as a message transferred from one cell to another. They contain many potential regulatory components including proteins, growth factors, cytokine, mRNA, and lipid that initiate tissue repair and suppress the inflammatory response [9]. The aim of the study is to investigate and compare the therapeutic effect, healing enhancement and reducing post operative complications of mesenchymal stem cell derived Microvesicles (MVs) and cancellous bone graft during experimental pan carpal arthrodesis in canine model.

3. Materials and Methods

3. 1. Experimental animal

The study was approved by the institutional animal care and use committee of Cairo university. The present study was conducted on twenty apparently healthy mongrel dogs (1- 2 years, 12-18 Kg weight) with normal orthopaedic and radiographic examinations. Dogs were grouped randomly and equally allocated into four groups (five in each). Group1 (cancellous bone graft) including dogs undergo dorsal pan carpal arthrodesis using dynamic compression bone plate and are treated by autologous cancellous bone grafting. Group2 (stem cells derived Microvesicles group MVs) including dogs undergo dorsal pan carpal arthrodesis and received stem cell derived Microvesicles. Group3 (mixed group) including dogs undergo dorsal pan carpal arthrodesis treated with cancellous bone grafting and stem cells derived microvesicles. Group4 (Control) treated only by pan carpal arthrodesis.

3. 2. Isolation and Preparation MVs

The mesenchymal stem cell was isolated from bone marrow sample then cultivated in DMEM media deprived of fetal bovine serum and supplemented with fetal bovine albumin then ultracentrifugation of the media was performed to collect the exosomes which would be used for treatment [10].

3. 3. Isolation of cancellous bone graft

Autologous cancellous bone graft was harvested from the iliac crest during surgical operation then applied to surgical sites.

3. 4. Surgical technique

Experimental dogs were anesthetized after overnight fasting. Aseptic preparation at the surgical site and application of tourniquet above elbow joint were done as pre-operative procedures. Dogs were positioned (ventral recumbency). A dorsal skin incision over the dorsal aspect of the distal third of radius and extending laterally to Accessory cephalic vein to the distal metacarpal bone. the abductor policies longus muscle was identified and then divided as it passes over the extensor Carpi radials, both tendon sheath of extensor Carpi radials and common digital extensor were opened along its entire the length cephalic vein and adductor pollicus longus muscle were carefully preserved during the dissection. Once the joint capsule incised, the surgical site was exposed showing distal radius, carpal bone and metacarpal carpal bone. All articular cartilages were removed using an oscillating saw and a pneumatic drill and lavaged with adequate amount of sterile saline solution to reduce risk of thermal necrosis. The subchondral bone of the distal radius was penetrated till bleeding occurred to create vascular channels. Then, the stainless-steel plate (Dynamic compression plate DCP 6 holes 3.5mm) was positioned dorsally over the distal radius, carpal bone and third metacarpal bone (Figure 1). The plate was contoured to allow approximately 10-15 degree of carpal extension. Muscles, subcutaneous and inter dermal were sutured simple continuous pattern using vicryl 2/0. Skin was sutured simple interrupted pattern using vicryl 0.

3. 5. Post-operative management and follow up

All dogs were received antibiotic (ceftriaxone®, Elkahira pharm, Cairo, Egypt) with dose 22 mg/kg intramuscular day before operation and 5 days after the surgical intervention. The limb was supported with a bandage during the first 3days. Postoperative orthogonal radiographic evaluation of operated limbs was obtained to assess the limb alignment,

plate position, screw placement, carpal angulation and also the width of the joint spaces.

Evaluation of outcome of different groups included lameness score, radiographic scoring and histopathological evaluation after euthanasia at 4, 8 and 12 weeks post operative.

1. Orthopedic examination

a) Lameness score

Lameness grading was done according to [11] into 6 grades.

b) Radiographic evaluation

The radiographic evaluation was done according to [12] into scores 0,1,2 and 3.

2. Histopathological examination:

The specimens were collected after euthanasia then fixed in a 10% neutral buffered formalin solution. Specimens then trimmed, washed, and dehydrated in ascending grades of alcohols, cleared in xylol, embedded in paraffin then sectioned (4-6µ), and finally had been stained with hematoxylin and eosin [13].

4. Results

Pancarpal arthrodesis was assessed in all groups of dogs clinically, radiographically and histopathologically at fixed intervals to compare the therapeutic effect (enhancement healing process) and post operative complication of augmentation of pancarpal arthrodesis alone or combined with either cancellous bone autograft or MVs or both.

It was noted that the MVs group was the fastest one by placing the foot on the ground and improved movement throughout the length of the experiment. Control and cancellous bone graft groups showed 6th grade lameness after 4 weeks and 4th grade lameness at 8 weeks. Lameness score was recorded for all groups at 4 weeks, 8 weeks and 12 weeks (table 1) Radiographic evaluation revealed faster bony union (score 2) at the MVs followed by the mixed group (cancellous and MVs). Control group showed no bony bridging and the joint spaces appeared wide throughout the length of the experiment.

Radiographic scoring and findings for the different groups at 4, 8 and 12 weeks were documented in (table 2) and Figure 2.

Minor and major post operative complications were documented in Table 3. Minor complications included stop feeding, swelling, and bandage sore. These complications were present in all animals in all groups. Minor complications disappeared in MSCs derived MVs at the end of the 4th week but were still present in other groups (table 3). Major complications included implant loosing, sinus formation and osteomyelitis) were not happened in MSCs derived MVs group and present mainly in cancellous bone graft group (table 4).

Histopathological evaluation of all groups throughout the length of the experiment (Figure 3). The findings revealed that MVs and MVs mixed cancellous graft group showed cartilage conversion to bone by organisation of fibrous connective tissue with osteocytes homing after 8 weeks. While, there was no bone formation in both cancellous and control groups.

5. Discussion

The present study showed a novel application of mesenchymal stem cells derived microvesicles to stimulate the healing process, bony fusion and decreased post operative complications after pancarpal arthrodesis in dogs. In the concurrent study, radiographic and histopathological evaluation was used to compare between cancellous bone graft, MVs and mixed groups.

Arthrodesis is a protective procedure that results in a complete loss of motion of the

arthrodesed joints resulting in lameness [14]. In the present study, augmentation of internally stabilized pan arthrodesis of the carpal joint with MVs and mixed MVs with cancellous bone autograft resulted in improved lameness score, radiographic score of osseous union with reduced both minor and major complications compared to control and cancellous bone autograft groups.

The paracrine effects of the MSC cytokines and growth factors decreased inflammation, enhanced progenitor cell proliferation and improved tissue repair [15]. Previous studies revealed that MVs support skeletal muscle repair and bone fracture healing through acceleration of biological function such as angiogenesis and cell differentiation [16].

Radiographic correct scoring of the joint spaces depended mainly on correct positioning of the limb during radiographic procedure. Lateromedial projections were mainly used for evaluation of the healing process, the area between the dorsal aspect of the carpus and the plate and the carpal joint spaces. The same findings were documented by [12].

Complete radiographic osseous union following arthrodesis have been documented to take 12 weeks [17]. In the present study, complete osseous union (score 3) of carpal arthrodesis hasn't been reported at 12 weeks but the MVs group showed higher radiographic score close to full osseous union. A retrospective study found that 40% to 46% of cases were healed at a mean of 30 to 41 weeks [18]. The healing process and bone modelling slowed down after eight weeks; it may be due to the stress of the decompression plate which may be the main reason for not reaching full radiographic osseous union in the first 12 weeks. Similar findings were observed by [12].

The minor complications appeared in all groups including swelling, serous incisional discharge and were treated with daily dressing with antiseptic solution and limb bandage. These complications resolved within first week after surgery in both MVs and mixed group while, they persist up to 8 weeks in cancellous group. There were two major complications in the cancellous bone group only included implant loosening and radiographic signs of osteomyelitis. Autologous cancellous bone graft had increased risk of infection [19].

Histopathological bone formation in both MVs group and the mixed group started at 8 weeks which earlier than that of both cancellous and control groups. These findings support the crucial role of the stem cell derived microvesicles for stimulation and enhancing the rate and quality of the healing process. This result may be due to the role of exosomes to carry out various functions in organisms that include tissue, immune response regulation, and reduce the inflammatory process [20].

6. Conclusion

Microvesicles stem cells alone or mixed with cancellous bone graft had been successfully used to augment carpal arthrodesis in dogs. They improve radiographic signs of osseous union and reduce the incidence of both minor and major complications.

7. References

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Table 1. shows the lameness score in animals' groups:

Groups	**4 weeks**	**8 weeks**	**12 weeks**
Cancellous bone graft	6	4	2
MSCs derived MVs	4	2	1
Mixed (Cancellous + MSCs derived MVs)	5	3	2
Control	6	4	4

Table 2. shows the mean average of the radiographic score in animals' groups:

Groups	**4 weeks**	**8 weeks**	**12 weeks**
Cancellous bone graft	0.4	1.4	1.6
MSCs derived MVs	0.8	1.8	2.4
Mixed (Cancellous + MSCs derived MVs)	0.6	1.6	1.8
Control	0.2	0.8	1.2

Table 3. shows the average percentage of the presence of minor complications

Groups	**4 weeks**	**8 weeks**	**12 weeks**
Cancellous bone graft	100%	20%	0%
MSCs derived MVs	100%	0%	0%
Mixed (Cancellous + MSCs derived MVs)	100%	0 %	0%
Control	100%	20%	0%

Table 4. shows the average percentage of the major complications

Groups	**4 weeks**	**8 weeks**	**12 weeks**
Cancellous bone graft	20%	40%	60%
MSCs derived MVs	0%	0%	0%
Mixed (Cancellous + MSCs derived MVs)	0%	20%	20%
Control	20%	20%	40%

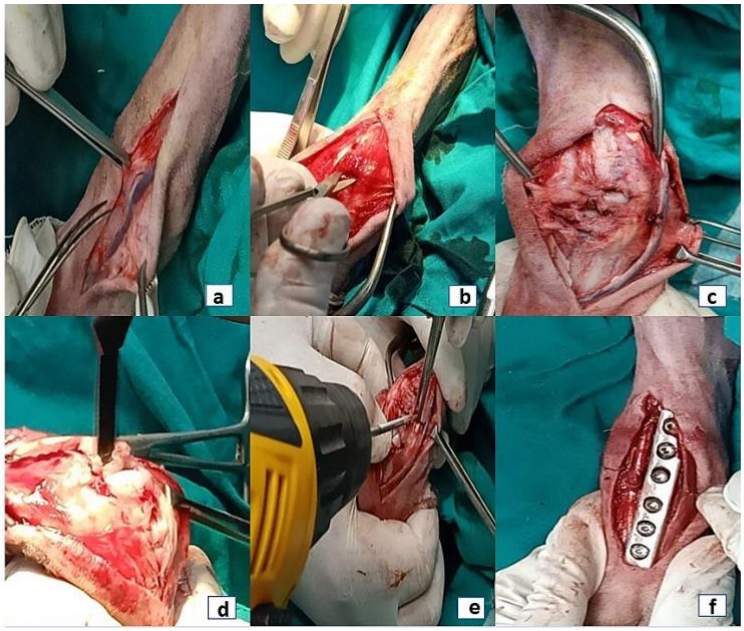

Fig .1. Surgical procedure of pan carpal arthrodesis. (a) A dorsal skin incision (b) incision of the joint capsules (c) exposure of the carpal joints using gelpi retractor (d) all the articular cartilage was removed with using an oscillating saw (e) The subchondral bone of the distal radius was penetrated using a pneumatic drill. (f) DCP 6 holes 3.5mm was contoured and positioned dorsally over the distal radius, carpal bone, and third metacarpal bone.

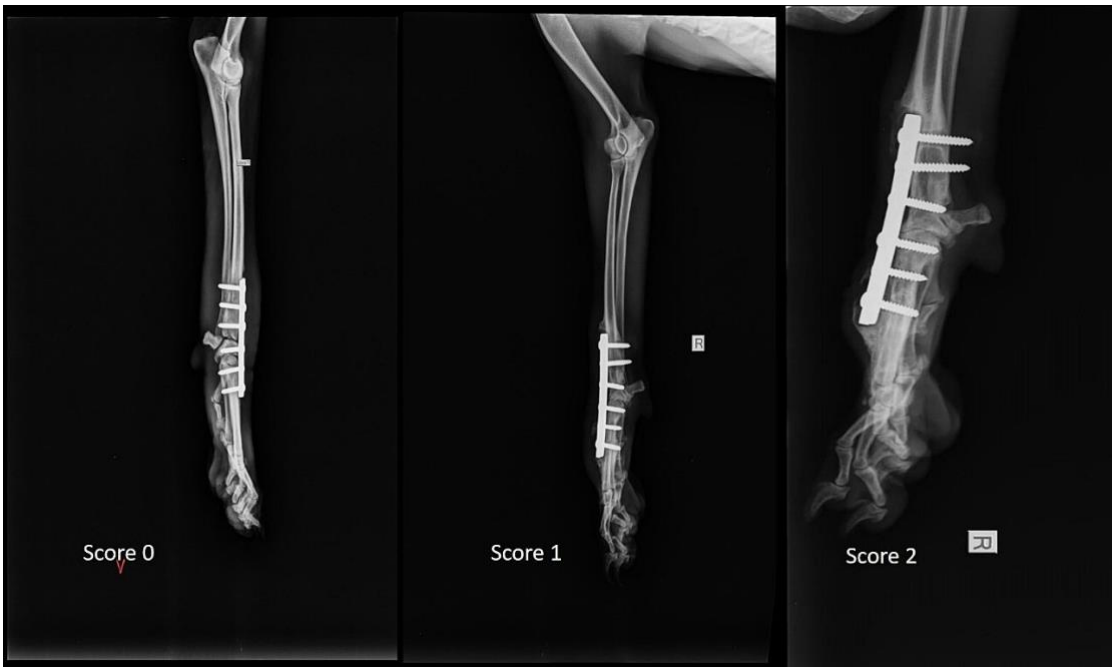

Fig .2. Radiographic scoring of pan carpal arthrodesis showing score (0) No mineralized(bone) tissue seen in the joint space. score (1) shows cancellous bone bridging the joint space, but the space of the joint is still clearly visible. score (2) shows bony bridging of joint space but subchondral bone is still clearly visible.

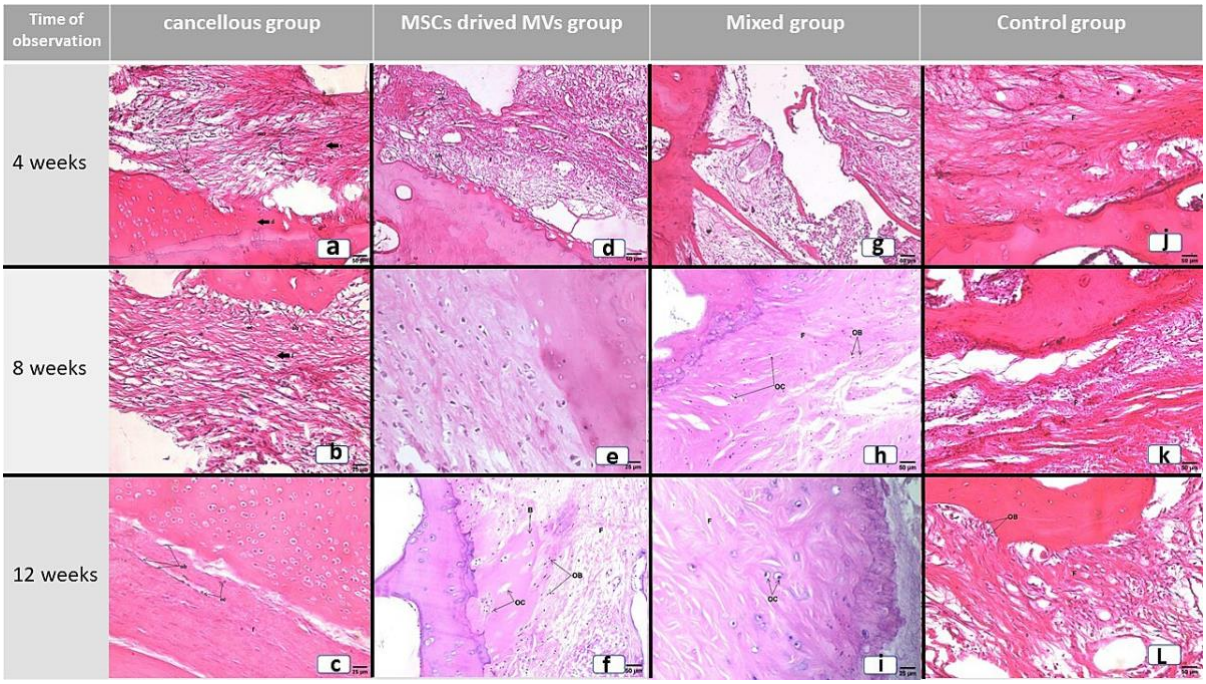

Fig .3. Histopathological section stained by H&E. cancellous group at 4 weeks (a) showing degenerative articular surface with organized fibrous connective which containing osteoclasts and osteoblasts, at 8 weeks (b) showing osteoclasts and osteoblasts at the margin of bone with organized fibrous connective tissue, at 12 weeks (c) showing articular degeneration with fibrous connective tissue formation and presence of osteoblasts and few numbers of osteoclasts embedded in connective tissue. MSCs derived MVs group at 4 weeks (d) showing the presence of osteoblasts at the margin of bone and embedded in organized fibrous connective tissue. at 8 weeks (e) showing the presence of osteoblasts at the margin of compact bone and at the margin of the central canal with the formation of organized fibrous connective tissue. at 12 weeks. (f) showing the formation of organized fibrous tissue with homing of osteoblasts and formation of newly formed bone containing mature osteocytes. Mixed group show at 4 weeks (g) showing severe degeneration in articular surface with formation of some bony streaks and fibrous connective tissue. at 8 weeks (h) showing the formation of organized fibrous tissue with homing of osteoblasts and mature osteocytes. At 12 weeks shows the formation of highly organized fibrous connective tissue with homing of mature osteocytes. Control group show at 4 weeks (j) shows the formation of organized fibrous connective tissue in one month control positive.at 8 weeks shows the formation of organized fibrous connective tissue infiltrated with mononuclear inflammatory cells in two-month control positive. at 12 weeks showing the formation of organized fibrous connective tissue with presence of osteoblasts in three-month control positive.