Vascularized Bone Grafting in a Canine Carpal Avascular Necrosis Model

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Abstract

Background Limited experimental research has been performed on the treatment of avascular necrosis (AVN) by vascularized bone grafting.

Questions/purposes A new model simulating carpal AVN was created to investigate surgical revascularization of necrotic bone.

Methods In seven mongrel dogs, AVN was induced by removal of the radial carpal bones bilaterally, deep-freezing, coating in cyanoacrylate, and reimplantation. A reverse-flow vascularized bone graft from the distal radius was implanted in the avascular radial carpal bone. The contralateral side served as an untreated ischemic control. Bone blood flow, bone volume, radiography, histomorphometry, histology, and MRI were analyzed at 4 weeks.

Results Blood flow was substantially higher in grafted bones when compared with controls (14.68 ± 15.43 versus 0.27 ± 0.28 mL/minute/100 g). Blood flow correlated with increased osteoid formation and higher levels of bone turnover. T1 and T2 signals on MRI did not correlate with quantitative bone blood flow measurements. Necrotic bones with no blood flow had normal T1 and T2 signals, whereas revascularized bones had signal changes when

compared with adjacent carpal bones. No major collapse occurred in any radiocarpal bone.

Conclusion In a canine experimental model, investigation of carpal AVN shows the ability of vascularized bone grafting to revascularize and remodel avascular bone.

Clinical Relevance Surgical revascularization of necrotic bone induced by vascularized bone grafting results in increased bone perfusion and bone remodeling as compared with untreated necrotic bone. MRI T1 and T2 signals can be normal in necrotic avascular bone.

Introduction

Aseptic necrosis of bone was first reported in the femur by Paget in 1870 [25]. Peste described collapse of the carpal lunate in cadaveric specimens, which he believed to be posttraumatic changes secondary to fracture [26]. After the development of radiographic imaging, Preiser identified avascular necrosis of the scaphoid and Kienböck published his report of lunatomalacia in 1910 [14]. The etiology of AVN in any bone is likely multifactorial, including factors extrinsic and intrinsic to the involved bone. Factors cited include abnormal loading, trauma (acute or repeated repetitive), morphologic factors, and vascular predisposition or injury (limited number and/or anastomotic connections of nutrient vessels, vasculitis, trauma-related extraosseous and/or intraosseous vascular injury, intraosseous venous stasis, and increased intraosseous pressure) [1, 8, 16, 18, 30]. Histologic studies show diminished vascularity, cellular necrosis, and fibrous proliferation with some evidence of new bone formation. Carpal AVN ultimately may progress to end-stage degenerative arthritis [6, 18].

No universally accepted treatment has been established for AVN [30]. In early stages, conservative treatment may

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be successful. Surgical interventions that alter wrist mechanics and reduce forces to the lunate such as joint leveling or intracarpal fusion are popular treatment options for AVN of the lunate. Furthermore, surgical revascularization is an attractive alternative for any bone affected by AVN [2, 3, 7, 10, 13, 20, 31]. It may increase the rate and extent of revascularization and induce new bone formation. Revascularization may be accomplished by implanting vascularized bone grafts or arteriovenous pedicles [11, 32, 36, 41]. Although clinical results of these procedures have been encouraging, basic research of surgical revascularization is limited, and controlled experimental validation for the treatment of carpal AVN is lacking [4, 12, 40]. Sheetz et al. investigated the extraosseous and intraosseous blood supply of the distal radius and ulna and defined potential vascularized pedicled bone grafts to the carpal bones [31]. A useful graft for implanting vascularized bone in the lunate is the pedicled bone graft based on the fourth extensor compartment artery with retrograde flow through the fifth extensor compartment artery [23]. Tu et al. described the extraosseous and intraosseous blood supply to the canine distal radius and ulna and found many anatomic similarities to humans [38]. The 2,3 intercompartmental supraretinacular artery (2,3 ICSRA) provides consistent nutrient arteries penetrating the dorsal radial cortex to enter the cancellous bone. Concomitant veins always can be identified with the artery. This, and the distal anastomotic connection of the 3,4 intercompartmental artery (3,4 ICA) to the dorsal carpal rete, allow creation of a retrograde-flow pedicled vascularized bone graft from the dorsal canine radius, which can be implanted in the carpus (Fig. 1).

We investigated revascularization of avascular, necrotic bone in the canine carpal model. The effect of surgical revascularization was analyzed by determining bone blood flow, volumetric and dimensional changes, histologic and histomorphometric differences, and signal changes on MRI.

Materials and Methods

We used seven adult male mongrel dogs (19–25 kg). The study was approved by the Institutional Animal Care and Use Committee.

With the animal under anesthesia (intravenous pentobarbital injection, 30 mg/kg body weight), we induced AVN by removing the scapholunate (radial carpal) bone bilaterally followed by deep freezing in liquid nitrogen for 3 minutes. Before orthotopic reimplantation, we measured the radial carpal bone volume by fluid displacement to the nearest 1/10th mL and anterior to posterior and lateral radiographs were obtained. Additionally, experimental and control bones were coated with a thin layer of

Fig. 1 The reverse-flow vascularized bone graft based on the 2,3 intercompartmental supraretinacular artery with retrograde flow through the 3,4 intercompartmental artery in a canine is shown. 3 ECA = third extensor compartment artery; 3,4 ICA = 3,4 intercompartmental artery; 2,3 ICSRA = 2,3 intercompartmental supraretinacular arteries; 1,2 ICSRA = 1,2 intercompartmental supraretinacular artery; CIA = caudal interosseous artery.

cyanoacrylate (Pfizer, Rutherford, NJ, USA) by immersion to prevent extraosseous vascular invasion.

We randomized sides and in one side a dorsal radial vascularized bone graft based on the 2,3 ICSRA and the 3,4 ICA was elevated measuring $5 \times 5 \times 10$ mm. It was placed with the radial carpal bone on the randomly selected side, drilling a 5-mm dorsal-to-palmar hole through which it was passed with the vascular pedicle oriented vertically into the bone. Care was taken to ensure the pedicle was free of kinks and compression. The contralateral carpus served as a negative control in which the graft was replaced after deep freezing without violation of its coated surface. Immediate postoperative radiographs (AP and lateral views) confirmed proper radial carpal bone and vascularized bone graft position.

Survival time was 4 weeks. The survival time and decision to use a cyanoacrylate coating was based on our earlier unpublished data. These data showed substantial spontaneous revascularization in the same model, but without a cyanoacrylate barrier, at 2.5 weeks. A longer period with addition of a barrier to vascular ingrowth from other sources provides an experimental model permitting analysis of the effect of vascularized bone grafting without being masked by the canine's robust spontaneous

angiogenic response. Both wrists were immobilized in snugly fitted casts. Dogs were allowed unrestricted weight-bearing in casts until euthanasia.

We conducted quantitative assessment of blood flow in a nonsurvival procedure using the radioactive microsphere entrapment method described previously [19, 39]. ^{141}Ce -labeled microspheres (New England Nuclear, Boston, MA, USA) were used for this purpose, administered at a dose of 0.5 million/kg. Dissection of the wrist at this point, considered to assess vessel patency, was not performed to permit MRI scanning without compromised anatomy. Tu et al. reported that the same reverse-flow vascularized grafts from the canine distal radius maintain patency long-term and in fact become hyperemic with a fourfold increase in blood flow over 2 weeks' survival [39]. After microsphere injection, the dogs were euthanized with an intravenous overdose injection of sodium pentobarbital.

Within 1 hour of euthanasia, MR images of both wrists were obtained using a 1.5-T Signa System scanner (General Electric, Milwaukee, WI, USA) with a transmit-receive extremity coil. Images were acquired with two-dimensional spin-echo T1-weighted (repetition time/echo time [TR/TE], 500/14 ms) and double-echo T2 weighted (TR/TE, 2000/30, 2000/60 ms) pulse sequences. Fat saturation was used on the T2-weighted sequences. Images were acquired in the coronal and sagittal planes with a 12-cm field of view, two excitations, and 3-mm slice thickness with a 1.5-mm interslice gap. An image matrix size of 256×256 was used for the T1- and 256×192 for the T2-weighted images. We analyzed the control and revascularized radial carpal bone images for their signal characteristics. Additionally, both wrists of one normal dog (no wrist surgery) were evaluated using the same technique to allow for comparison.

Immediately after MRI, we removed the grafted and control radial carpal bones from the wrists and stripped them of all soft tissue. The radial carpal bones then were inspected for visual signs of collapse and integrity of the cyanoacrylate coating. Bone volume then was remeasured. We obtained AP and lateral radiographs.

Collapse of the radial carpal bone was assessed by comparison of volumetric and radiographic measurements taken at the index surgery and at the time of euthanasia. Radiographic evaluation was performed by one author (CA) in a blinded fashion and included AP height-to-width ratios and lateral height-to-width ratios. Use of these ratios effectively standardized the radiographic measurements, a method used clinically to discern change in morphologic features resulting from AVN in the human lunate [34].

Bone blood flow then was measured in the treated and untreated radiocarpal bone specimens and for humerus control subjects using the radioactive microsphere entrapment method described previously [19, 39].

Preparation for histologic analysis required dehydration in a series of increasing concentrations of alcohol followed by embedding without demineralization in a mixture of methylmethacrylate-2-hydroxyethyl-methacrylate (12.5:1) and sectioning at a thickness of 5 μm . Histologic analysis was performed in a blinded fashion by one author (CA) on the prepared slides, which included unstained, toluidine blue-stained, and Masson-Goldner trichrome-stained tissue. Both stains were prepared according to the methods of Recker [27].

For quantitative histomorphometry, fluorochrome labels were administered as follows throughout the survival period: Day 1, oxytetracycline (1000 mg orally); Day 11, alizarin (30 mg/kg subcutaneously); Day 18, demeclocycline (250 mg orally); and Day 24, calcein (20 mg/kg subcutaneously). Alizarin and calcein were prepared for injection according to the methods described by Paddock et al. [24]. The resulting fluorescence labels were evaluated under ultraviolet light and were used for histomorphometric measurements of bone remodeling by fluorescence microscopy on unstained sections. The digitalized analysis was performed using a semiautomatic image analysis system (Osteomeasure®; Osteometrics Inc, Atlanta, GA, USA). Every other adjacent $\times 10$ square field in the implanted bone graft (graft area) and immediately outside the entire perimeter of the graft (perigraft area) was analyzed. Corresponding regions were studied in the control bone. The perimeter of fluorescent label on trabecular bone was calculated as a percentage of the total trabecular bone perimeter representing active bone formation surface to total bone surface. Samples of humeral cortex from each animal also were observed to confirm the presence of all labels administered.

Stained sections were evaluated in a blinded fashion (CA) for the presence of osteoclasts (bone resorption), osteoblasts (bone formation), fatty bone marrow, and fibrous tissue. Grafted and control radial carpal bones were examined at $\times 100$ magnification.

Means of the results for grafted sides versus control sides were analyzed statistically using the Mann-Whitney U test. Repeated measures for ANOVA were performed with the Kruskal-Wallis test for variables that were determined at the time of surgery and at euthanasia (height-to-width ratio and bone volume). Significance was set at $p < 0.05$. Results are presented as mean $\pm$ SD.

Results

Two animals were excluded from analysis as a result of failure to thrive and wound infection. One animal (Number 2) was excluded for comparative analysis as a result of disruption of the coating of the control carpal bone confirmed

by a substantially higher resultant blood flow in the control carpal bone (8.32 mL/minute/100 g) as compared with overall humeral control blood flow (mean, 3.14 ± 1.7 mL/minute/100 g) and the other control carpal bones (mean, 0.27 ± 0.28 mL/minute/100 g). However, we did include the contralateral (grafted) bone in Animal 2 for descriptive histologic and histomorphometric analyses only.

Blood flow was significantly higher at euthanasia in the grafted side compared with the control carpal bones ($p < 0.05$). Mean absolute blood flow was 14.68 ± 15.43 mL/minute/100 g in the grafted side and 0.27 ± 0.28 mL/minute/100 g in the control (no revascularization) side. When normalized for concomitant humeral blood flow, the mean blood flow was 4.26 ± 2.81 mL/minute/100 g and 0.83 ± 1.65 mL/minute/100 g on the revascularized and control sides, respectively. Differences between sides were significant after normalization ($p < 0.05$).

Bone volume did not change with time in revascularized and control bones (graft bones, 2.3 ± 0.45 cm³ [index surgery] to 2.5 ± 0.46 cm³ [euthanasia]; control bones, 2.1 ± 0.34 cm³ [index surgery] to 2.5 ± 0.46 cm³ [euthanasia]). Differences with time and between sides were not major. The height-to-width ratio measured from lateral radiographs remained equal with time (graft bones, 1.52 ± 0.05 [index surgery] to 1.62 ± 0.17 [euthanasia]; control bones, 1.51 ± 0.15 [index surgery] to 1.63 ± 0.11 [euthanasia]). The height-to-width ratio in AP views decreased slightly but not substantially with time in both groups (graft bones, 0.39 ± 0.02 [index surgery] to 0.36 ± 0.03 [euthanasia]; control bones, 0.40 ± 0.05 [index surgery] to 0.34 ± 0.02 [euthanasia]). Differences between sides in radiographic ratios were not substantial.

All four fluorochrome labels were found in every humeral cortical specimen confirming successful administration. Extensive bone formation was evident in the grafted sides as opposed to control carpal bones. Large areas of empty lacunae were observed in the control bone, consistent with necrosis (Fig. 2A). No fluorochrome uptake was observed in the control bones, whereas three of five grafted radial carpal bones had substantial osteoid formation (Fig. 2B). Osteoid deposition in these animals occurred in an average of 38.36% (range, 35%–43%) of perigraft trabeculae and in 45.67% (range, 37%–54%) of graft trabeculae. These three animals observed with fluorochrome labeling were found concomitantly to have the highest blood flows (range, 7.23–40.97 mL/minute/100 g). Additionally, osteoid formation in the latter dogs was accompanied by increased bone turnover reflected by osteoclastic and osteoblastic activity. This was not observed in the remaining two grafted samples without osteoid formation and in the control bones. A possible explanation of poor flow and absent osteoid formation in these two samples may be pedicle thrombosis. These data

Fig. 2A–B (A) Necrotic radiocarpal control bone is characterized by empty lacunae and no viable marrow at 4 weeks. (B) Revascularized radiocarpal bone depicts active bone remodeling and viable marrow (Stain, Masson-Goldner trichrome; original magnification, $\times 100$).

clearly show osteoid formation correlates with blood flow in the revascularized canine radial carpal bones.

MR images of the ischemic nonrevascularized control radial carpal bones (deep frozen, coated with cyanoacrylate, and replaced without revascularization) showed normal T1 and T2 signals in all but one specimen. This specimen, whose cyanoacrylate coating was disrupted and had high blood flow measurements, and all revascularized radial carpal bones showed decreased T1 signals and increased T2 signals, which likely represents early revascularization (Fig. 3).

Discussion

This study aimed to create a reproducible canine model that simulates AVN for experimental purposes. Using this model, we also wished to determine the effects of surgical revascularization with vascularized bone grafts by determining bone blood flow, new bone formation, and assessing MR images at euthanasia. The histology and

Fig. 3A–B Representative (A) T1- and (B) T2-weighted MR images of a dog wrist after removal of the radiocarpal bone and coating with cyanoacrylate are shown. Bones were either revascularized with a vascularized bone graft from the radius or were not treated (control). There is high T1 signal and intermediate T2 signal in the control scapholunate (right) and low T1 signal and high T2 signal in the grafted radial carpal (left).

bone blood flow values obtained showed the bone to be largely necrotic with minimal blood flow when coated with cyanoacrylate unless surgically revascularized. Processing the radial carpal bone with liquid nitrogen and limiting rapid spontaneous revascularization by means of a cyanoacrylate coating provides a useful model. Without a physical barrier, robust angiogenesis in the canine rapidly restores blood flow, masking differences between experimental and control carpal bones after a brief (2.5 weeks) survival period (authors' unpublished data). Cyanoacrylate coating successfully blocks revascularization, as observed in four of five control samples, whose blood flow values were no greater than 0.59 mL/100 g/minute at euthanasia.

We recognize several limitations of our model and study. Radiographic dimensions were measured accurately to the second decimal in a blinded fashion and one author (CA) performed histologic evaluation, but no interobserver reliability was tested. We were unable to reproduce the progressive collapse seen in some cases of Kienböck's

disease in this animal model. It is possible that it is the gradual revascularization process occurring over a period of several months to several years that leads to the observed collapse in Kienböck's disease and not in our canine radiocarpal bones. For example, Bochud and Büchler reported 42% of lunates to be at least partly viable and only 15% of lunates necrotic based on MRI data for 26 patients obtained at an average of 6.7 years (range, 2.5–9.3 years) after core revascularization of the lunate in Stage IIIA Kienböck's disease [9]. Sowa et al. reported MRI evidence for revascularization was observed at 12 months in three of five lunates with Kienböck's disease treated with radial shortening osteotomy [33]. A longer-term survival period might result in the classic bony collapse seen in the human carpus. Nevertheless, we believe this model has value in studying avascular necrosis of the carpal bones. The histologic and quantitative blood flow changes observed in untreated, coated radiocarpal bones allows direct comparison of this untreated wrist with the contralateral radiocarpal bone in which a specific experimental variable is introduced.

The second aim of this study was to evaluate the effects of vascularized bone grafting to treat carpal AVN. We used a direct comparison to untreated necrotic carpal bones with bilateral surgery. We found bone blood flow to be significantly higher in the treated carpal bones. Moreover, histologic and histomorphometric differences were observed between revascularized carpal bones and the control group. A relative high blood flow was measured in animals with active osteoblastic and osteoclastic activity, indicating revascularization is required to initiate bone turnover. No substantial carpal collapse was seen in any bone. Aspenberg et al. posed that avascular necrotic bone collapse is initiated by revascularization [4]. We did not find that carpal bone revascularization or increased bone turnover affected measures of radial carpal bone collapse at 4 weeks. However, the relatively short survival time and casting of the dogs' wrists might have prevented carpal collapse. A more extended survival period with measurement of long-term biomechanical properties may help prove or disprove this common but unproven assumption. A clear relation between bone revascularization and subsequent active bone formation was detected. Thus, osteogenesis is accelerated by the use of vascularized grafts, likely as a result of transplantation of viable marrow cells from the vital graft while additionally serving as a strut to induce neoangiogenesis throughout the necrotic bone and increase the supply of nutrients and osteoprogenitor cells.

MRI is reported to be a sensitive imaging tool for the diagnosis and staging of AVN [4, 15, 21, 35, 42]. The early detection of AVN with MRI is based on its assumed sensitivity to detect differences between ischemic, necrotic,

and normal bone using T1- and T2-weighted images. In the clinical setting of AVN, the combination of loss of T1 signal and normal or increased signal on T2-weighted images has been suggested to represent an earlier stage of AVN or possibly early revascularization with viable granulation tissue [22, 33]. Normalization of the MRI marrow signal theoretically should occur with return of blood flow, fat cells, and hematopoietic elements [33].

Our study directly compares measured bone blood flow with MRI signal, unlike other studies. The presence of normal T1 and T2 signals in completely avascular control bones is an important finding in this study. Abnormal signals were found only as the process of revascularization began. Thus, although the high specificity of MRI signal changes in AVN have been reported [37], it would appear that something other than avascularity is observed. As with our results, Lang et al. noted preservation of the normal marrow fat signal in histologically necrotic bone not yet reached by invading capillaries and mesenchymal tissue [15]. In addition, Mitchell et al. examined three available femoral heads ex vivo whose central MRI signal intensity was similar to fat on T1-weighted and T2-weighted images [22]. Only one of the three specimens contained fat. The other two were composed primarily of aqueous debris. They believed the apparently normal MRI findings observed could be the result of “mummified” fat, as described in a case report by Bassett et al. [5], or other material with signal characteristics similar to those of fat. Moreover, Ruland et al. found that MRI was only sensitive for detecting (histologically confirmed) AVN once the process of replacing nonviable fatty marrow was induced [28].

Our study directly compares in vivo blood flow with MRI signal. Our findings show MRI signal may appear normal in bone with no or minimal blood flow and abnormal in bone with substantial flow, and that MRI does not correlate directly with bone blood flow in an early stage of the disease. Thus, these results confirm the need for some caution in the interpretation of apparently normal T1 and T2 signal areas in human AVN conditions such as Kienböck’s disease, particularly in correlating signal changes in conventional MRI to the presence of blood flow. The use of contrast-enhanced MRI may offer more reliable information on bone perfusion [17, 29].

A canine model of carpal AVN has been developed and applied to the study of surgical revascularization. Bone blood flow and new bone formation are enhanced by vascularized bone grafting into avascular necrotic bone. Noncontrast-enhanced MRI does not reliably correlate with bone viability or quantitative measures of bone blood flow. This study supports the concept of surgically revascularizing necrotic bones such as is found in Kienböck’s disease.

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