Biologic Model of Bone Transport Distraction Osteogenesis and Vascular Response

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Summary: We developed an experimental model in the rabbit of distraction osteogenesis through bone transport that closely corresponds to the clinical use of bone transport in humans. We also applied injection angiography to study the arterial response of a limb undergoing bone transport. This model includes a proximal osteotomy and bone transport to fill in a segmental tibial diaphyseal defect. Regenerate bone formed well in the gap that was created that trailed the transport segment, and slow healing at the docking site was observed, as seen in humans. The angiographic techniques clearly revealed, by radiography and anatomic dissection, the arterial response to bone transport. The results showed that the transport segment had an arterial supply after osteotomy and after transport. They also demonstrated an extensive increase in vessels in limbs that had undergone distraction osteogenesis, an observation made clinically in humans but not well demonstrated experimentally. Furthermore, angiography showed proximal stretching and distal kinking of the major artery of the leg. This model closely resembles distraction osteogenesis through bone transport in humans and definitively demonstrates that the transport segment can maintain blood supply and remain viable during the transport process. The results of this study provide a basis for further work on factors that enhance and interfere with successful bone transport in humans.

Distraction osteogenesis has proved a powerful clinical tool in treating patients with a variety of skeletal problems (2,5,7,9,11,12,17-19,26). The basic science of distraction osteogenesis has been the focus of increasing experimental investigation during the past 5 years (1,3,10,13,14,21,27). Two techniques of distraction osteogenesis are in common clinical use: lengthening and bone transport. Virtually all of the basic science of distraction osteogenesis has been based on lengthening models (1,3,10,13,14,21,23,27), despite the fact that bone transport has become the most commonly used and clinically efficacious technique for treating segmental defects (2,5,9,11,12,17-19,26). For bone transport, a segmental diaphyseal bone defect (Fig. 1, D) is filled by creating a bone segment (Fig. 1, C) through an osteotomy (Fig. 1, B) and gradually pulling it into the defect, resulting in a docking site (Fig. 1, H) with the distal segment (Fig. 1, F). This creates a new defect trailing the segment (Fig. 1, G), and this trailing defect ossifies by the process of distraction osteogenesis. Although a commonly used clinical technique, bone transport is associated with most of the complications encountered with lengthening (6) as well as with new complications. Complications associated specifically with bone transport include delayed union or nonunion at the docking site and devascularization of the transport segment (5,18,26). A good model is needed to investigate this technique. The bone transport models that have been reported are modified lengthening models that do not closely simulate the clinical technique for bone transport in humans (28,29). Recent development of a suitable external fixator has made it possible to develop in the rabbit a model of distraction osteogenesis through bone transport.

Literature on bone transport suggests that the process of osteotomy necessary to create a transport segment may devitalize the segment entirely, although formation of regenerate bone is still possible (28). This observation contrasts with the clinically observed apparent viability of the transport segment (26).

Ilizarov and others have suggested that distraction osteogenesis is associated with increased blood flow to the limb, which is helpful in healing various pathological conditions indirectly (10,12,16). Although this phenomenon has been observed, only limited scien-

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FIG. 1. Schematic illustration of a model of bone transport illustrating relationships before and after transport. A = External fixator, B = osteotomy in the proximal one-third of the tibia and fibula, C = transport segment, D = segmental defect in the diaphysis of the tibia, E = proximal segment of the tibia, F = distal segment of the tibia, G = regenerate bone trailing the transport segment, and H = docking site of the transport segment to the distal tibia.

tific data exist to support it in a lengthening model and none exist to support it in a model of bone transport. The effects of bone transport on the vascularity of the limb have not been extensively reported.

The purpose of this study was to develop in an animal a model similar to bone transport in humans and to determine the arterial response of a limb undergoing bone transport with particular attention to the viability of the transport segment.

MATERIALS AND METHODS

The experimental protocol was carried out under the control of the guidelines for animal experimentation of the Faculty of Medicine at the University of Oxford and was performed under the animal license and supervision of the British Home Office.

A model for bone transport was developed in the rabbit tibia from an established leg-lengthening model (Fig. 1) (13,14) with use of 12 mature female New Zealand White rabbits with a mean weight of 4.5 kg (3.6-5.1 kg). The animals were randomly divided into control group A (n = 6) and experimental group B (n = 6). The experimental group underwent osteotomy and transport, whereas the control group underwent only osteotomy. For the surgical technique, the animals in both groups were premedicated with an intramuscular injection of fentanyl citrate (0.2 ml/kg) (Hypnorm; Janssen Animal Heath, High Wycombe, England) and an intravenous injection of midazolam (1 mg/kg) (Hyponovel; Roche Products, Welwyn Garden City, England) and locally infiltrated with 0.25% bupivacaine (Marcain; Astra Pharmaceutical, Kings Langley, England). The procedure was performed under aseptic conditions. The medial side of the leg was shaved, cleansed, and draped. A 2.5-cm incision was made over the medial aspect of the distal tibia shaft, and a 1-cm incision was made over the medial aspect of the proximal tibia. A 2-cm segment of bone was removed (Fig. 1, D) with a handsaw from the midshaft of the tibia, which corresponded to three times the width of the bone. Surrounding muscles and tendons were protected, but the periosteum was re-

moved to create a bone defect similar to that commonly encountered in posttraumatic reconstruction cases. Three pairs of stainless-steel, 2.5-mm threaded-tip pins were inserted into the proximal tibia (Fig. 1, E), transport segment (Fig. 1, C), and distal tibia (Fig. 1, F) after previous drilling. The minifixator transport frame was attached to the pins (EBI Medical Systems, Parsippany, NJ, U.S.A.), maintaining the limb at normal length. The 2-cm transport segment was created in a manner similar to the corticotomy of Ilizarov (11). A flexible 0.5-mm saw blade was passed posterior to bone through small medial and lateral incisions to minimize injury to surrounding muscle. The bone and periosteum were divided by hand with protection of the anterior skin. The tibia was transected at the junction of the proximal and middle thirds to simulate the usual clinical situation of bone transport in humans (Fig. 1). Fibular osteotomy was required because the fibula fuses with the tibia above the ankle in rabbits. The wounds were irrigated and closed with suture. A sterile compressive dressing was applied, and the rabbits recovered from the anesthesia. They were allowed free ambulation in their cages immediately after the procedure.

A 7-day waiting period was allowed for the formation of suitable tissue for distraction as in the clinical use of bone transport in humans (27). Control group A consisted of six rabbits that underwent triple osteotomy with removal of the bone segment and application of external fixation but not transport. These animals were handled and fixator tightness was checked twice daily, but no transport was performed. In the experimental group B of six rabbits, the triple osteotomy was performed, creating the scgmental defect and transport segment, the fixator was applied, and transport was performed at 1 mm/day in divided doses for 3 weeks until docking of the transport segment occurred. This rate and rhythm were chosen to match the clinical situation in humans as closely as possible (18,26). Radiographs were obtained at weekly intervals (Fig. 2). Docking of the transport segment to the distal tibia was achieved after 4 weeks in all six animals. Two rabbits were studied at the time of docking. After docking, 2 weeks were allowed for consolidation of the regenerate bone and healing at the docking site in two rabbits. The other two rabbits were studied 8 weeks



FIG. 2. Radiographs demonstrating a segmental tibial diaphyseal defect and proximal osteotomy stabilized by external fixation. A: Before transport, time = 0; **B**: during transport, time = 2 weeks; **C**: at docking, time = 4 weeks; and **D**: after healing and consolidation, time = 6 weeks.

after the operation (4 weeks after docking). At death, each animal was sedated with Hypnorm and given 10,000 U heparin for anticoagulation. Each was then given a lethal overdose of anesthetic agent (pentobarbital) (Euthalal; Roche Products). The transport segment, docking site, and regenerate bone were examined by anatomic dissection and histology.

Lead angiography was performed on each rabbit after death. For angiography (4,8,15,20), the abdomen was shaved and opened in the midline and the lower intestinal tract was eviscerated to allow exposure of the inferior vena cava and aorta proximal to the inferior mesenteric vessels. A segment of aorta was dissected below the inferior mesenteric artery, and a V-shaped aortotomy was made in the proximal segment. A 14-gauge blunt cannula was introduced above the aortic bifurcation and secured by two ligatures. A venotomy was made in the inferior vena cava. Arterial vessels to the lower intestinal tract were ligated. One hundred milliliters of warm (40°C) saline solution was used to irrigate blood from the vascular system. Any leaks in the vessels of the peritoneal cavity were identified and ligated. Contrast material for injection was a 160% solution of lead oxide (Pb₃O₄) and 8% gelatin in normal saline solution. The gelatin was dissolved in a saline solution at 50°C, and lead oxide was added slowly during stirring to make a smooth suspension. The contrast material was cooled to 40°, and 8 ml was injected into the aorta in a pulsatile manner (15). The rabbit was left undisturbed for 30 minutes to allow the gelatin to set into a rubbery elastic cast inside the arteries of the leg. Radiographs were obtained, and the limbs were subsequently dissected (22). The orange rubbery contrast material in the arteries greatly facilitated dissection and identification of blood supply to the transport segment. We found freezing, recommended by Mc-Nally et al. (15) to expand the vascular structures, unnecessary in this application.

In addition to the 12 rabbits already described (groups A and B), eight rabbits were used to study the acute effect of the operation on the vascularity of the limb (groups C1 and C2). Four animals (group C1) underwent angiography immediately after the operation (triple osteotomy and external fixation), and four others underwent osteotomy, rapid transport, and then angiography in

	Osteotomy					X-ray and
Group	Ν	and XF	Transport	Time ^a	Angiography	histology
A	6	Yes	No	4, 6, and 8	Yes	Yes
В	6	Yes	\mathbf{Yes}^h	4, 6, and 8	Yes	Yes
C1	4	Yes	No	0 ^c	Yes	No
C2	4	Yes	Yes ^d	0°	Yes	No

TABLE 1. Distribution of the four groups by number (N), interventions performed, time course, and outcome measures

XF = external fixation

"Time after the operation to death is given in weeks, with two rabbits from each group killed at each time interval.

^b Transport in group B was physiologic.

^cThe rabbits in groups C1 and C2 were killed immediately after the operation.

^dTransport in group C2 was not physiologic.



FIG. 3. Results from lead injection demonstrate vascularity of the transport segment. A: A longitudinal section of the transport segment shows a small endostcal artery filled with lead. B: Radiograph of the cross section of the transport segment after removal of soft tissue illustrates endosteal lead, indicating vascularity of the transport segment.



FIG. 4. Radiographs showing endosteal callus from the transport segment, which suggests physiologic viability of the transport segment. A: Distal end of the control tibia, and B: proximal end of the transported segment.



FIG. 5. A: Histology of the transport segment after ostcotomy without transport reveals a 1-mm area of dead bone adjacent to the cut surface and viable bone elsewhere in the segment. Hematoxylin and eosin; magnification: $\times 100$. B: Histology of the transport segment after osteotomy with transport demonstrates viable osteocytes within lacunae. Hematoxylin and eosin; magnification: $\times 400$.

a single surgical setting (group C2). None of these eight animals had physiologic bone transport, and all were killed prior to angiography. A summary that identifies the procedures, time course, and outcome measures for each of the four groups is provided in Table 1.

RESULTS

The results were primarily observational as opposed to quantitative. This model successfully simulated the clinical experience through distraction osteogenesis by bone transport. The frame could be applied and triple osteotomies could be performed with a surgical exposure analogous to the technique used in humans. All 12 rabbits tolerated the frame and surgery well and returned to normal activity. The 2-cm gap did not heal spontaneously in any of the six control rabbits; however, all showed healing of the proximal osteotomy as expected, and the segment appeared viable radiographically in all control rabbits. Control group A showed this model to be appropriate

for tibial nonunion with segmental defect. Bone transport proceeded in a manner similar to that experienced in humans, with good tolerance of transport at a rate of 1 mm/day in divided doses. Transport proceeded in an orderly manner with accurate docking and reliable formation of regenerate bone in all six rabbits in group B. Healing at the docking site was slow as observed in humans. Incomplete healing was observed radiographically and histologically in both rabbits killed 2 weeks after docking, and union was seen radiographically in the two animals observed 4 weeks after docking. The docking sites in group B healed at a slower rate than the healing observed at the osteotomy without bone transport in group A. Regenerate bone formed and matured in all six rabbits as observed radiographically and by dissection. Overall, the model achieved successful healing of a segmental defect with reliable formation of regenerate bone in a manner and with a technique analogous



FIG. 6. Angiography after bone transport demonstrates a generalized increase in the vascularity of the limb (\mathbf{A}). In addition, the main artery of the leg is stretched proximally and redundant distally (\mathbf{B}).

to the technique used in orthopaedic practice.

The angiographic technique was very successful in demonstrating, by radiography and anatomical dissection, the arterial supply of the limb (Figs. 3 and 6). When angiography was performed immediately after triple osteotomy and application of the external fixator (group C1), contrast material was seen grossly and radiographically in the transport segment in all four animals. This demonstrates the ability to maintain vascularity of the transport segment during the initial surgical procedure. The main nutrient artery was cut at the proximal osteotomy. However, collateral flow by the periosteum was sufficient to provide intraosseous blood supply to the transport segment and even backfill the distal end of the main nutrient artery. When angiography was performed immediately after triple osteotomy and acute transport (group C2), there was no contrast material in the transport segment in any of the rabbits, indicating that immediate transport may cause arterial disruption. Angiography of group A (control) demonstrated contrast material in the segment that was not transported, indicating that the osteotomy and external fixator application does not result in a devascularized segment. Contrast material was observed grossly and radiographically in the transport segment when angiography was performed after slow bone transport (Fig. 3), demonstrating that the transported bone segment remains vascular.

Radiographically, the control (osteotomy, no transport) tibial segment appeared viable by the appearance of endosteal new bone at the distal end of the segment (Fig. 4A). The transported segment also appeared viable by the presence of ossifying callus at its trailing endosteal surface (Fig. 4B).

Viable osteocytes were seen histologically in the

bone transport segment in samples obtained from group A (osteotomy, no transport) (Fig. 5A), after transport at the time of docking (Fig. 5B), and 2 and 4 weeks after docking. This is direct evidence of the viability of the transport segment before, during, and after transport.

A diffuse increase in angiographic contrast material was observed in limbs undergoing distraction osteogenesis (Fig. 6A). This increase was not observed when ostcotomy was performed without transport, suggesting that distraction, and not osteotomy alone, is important in triggering this increased vascularity. Proximal stretching and distal redundancy (kinking) of the main artery of the leg resulted from the bone transport (Fig. 6B).

Complications that occurred included a nondisplaced fracture through a pin track in the proximal tibia that did not result in pin loosening. One rabbit in the group that underwent bone transport ate poorly for 2 weeks and lost 200 g, but it regained its preoperative weight by 6 weeks and good regenerate bone formed.

DISCUSSION

This study was designed specifically to develop a model of distraction osteogenesis through bone transport that closely simulates the technique used in orthopaedic practice (2,5,9,19). We created a successful model that was much more similar to the clinically used techniques of bone transport than are others in the literature (28,29).

Most studies of distraction osteogenesis have used lengthening models and extrapolated the findings to bone transport (1,10,13,14,21,23). One model of bone transport by Windhager et al. utilized a two-pin-clamp

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fixator designed for lengthening and not for bone transport (28). This required simulated bone transport by acute shortening at one site and gradual lengthening at another, creating a soft-tissue redundancy not typically encountered clinically. The relative motion between the transport segment and surrounding tissue is lost in this model. This model also required the use of interposed polytetrafluoroethylene to prevent union. The presence of this foreign body causes many undesirable effects and precluded meaningful analysis of the docking site. The findings reported by Windhager et al. (28) of complete devascularization of the transport segment were perhaps due to the particular model and may not represent the usual clinical situation in humans. Our findings are more representative of the clinical experience in which the transport segment does not appear completely avascular. Another report by the same author (29) used sliding plates, a technique not commonly used in humans. The model we report uses a three-pin-clamp unilateral external fixator applied from the medial side in a manner directly analogous to human bone (7,23). The fixator has

recently been manufactured in a size applicable to

Windhager et al. (28) found the process of osteotomy to completely devascularize and devitalize the transport segment although it still resulted in the formation of solid regenerate bone. The complete necrosis of both short and long-bone transport cylinders as seen histologically was followed by revitalization through newly formed vascular channels from the surrounding soft tissue (28). Our results are considerably different from those reported by Windhager et al. but are more similar to the phenomena observed clinically in humans. The pattern of callus on radiographs suggested, but did not prove, the viability of the transport segment. The presence of angiographic dye in the transport segment after osteotomy confirmed that creation of a vascular transport segment is possible. There are differences in the model that could contribute to the results. The amount of soft-tissue dissection of any osteotomy is always variable, and it is certainly possible to devascularize the transport segment by dissection of soft tissue. Ilizarov (11) emphasized the preservation of soft tissue during corticotomy to minimize injury to the tissue. We used a different technique for performing the osteotomies (handsaw compared with oscillating power saw). The fixators were applied from the medial side, which is subcutaneous, rather than from the lateral side, which requires further muscular dissection. We maintained limb length and soft-tissue tension rather than acute shortening with subsequent lengthening, which might have had a deleterious effect on the blood supply to the transport segment. Our proximal osteotomy was performed more proximally; this is more similar to the clinical technique of proximal corticotomy in humans. The results of this study demonstrate that it is at least possible to maintain vascularity and viability of the transport segment in a tibial model of bone transport.

The angiographic technique successfully demonstrated blood supply to the transport segment. It also showed an increased vascularity of the limb undergoing distraction osteogenesis by bone transport. The qualitative increase is similar to the clinical and scintigraphic observations of Ilizarov and others (10,12,16). This increased blood supply may be important in the formation of regenerate bone, healing at the docking site, and healing of other lesions on the affected limb (10,24,25). A qualitative analysis of the increase in vascularity is beyond the scope of this report but is planned for the future (16).

These results may provide the basis for other studies, including the utilization of this model to determine the viability of the transport segment. Various rates of distraction are possible with bone transport because it is not generally limited by lengthening of arteries and nerves. However, fast rates may have a deleterious effect on the attachment of soft tissue and the viability of the transport segment. The size of the transport segment is also important clinically. This model could be used to study the effect on cylinder length as a function of gap size. The effect on the soft-tissue sleeve during bone transport is interesting because an area of stretching appears proximally and an area of compression appears distally, in contrast to the lengthening process during which all the tissue is under stretch. The docking site is a common source of problems, particularly that of slow union. This model could be utilized to investigate the effects on the docking site that may facilitate union.

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