Mechanical Induction of Osteogenesis*

Preliminary Studies

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ABSTRACT

The animal model developed in the Soviet Union by Ilizarov7,15 has been reliably reproduced by us for the mechanical induction of osteogenesis using slow distraction. Our preliminary studies in six adult dogs indicate that this osteogenesis originates from well-structured intramembranous ossification, with rapid maturation to lamellar bone, indistinguishable from surrounding host bone. Mineralization increases steadily, reaching critical levels for radiographic visualization between Days 21 and 28. In this model, the osteogenic area then exceeds normal bone density temporarily but returns to normal density within three months.3 Distraction for 28 consecutive days (at 0.25 millimeters every six hours using rigid transfixion wires, as Ilizarov describes) reliably lengthened the tibiae by 12 percent, increasing mass by 27 percent, and volume by 26 percent with only a one percent change in overall density. The process required four months to add 24 millimeters of mature, lamellar bone capable of full weight-bearing by the dogs. This rate of osteogenesis, estimated at 202 microns per day, is four times faster than a human’s fastest growth plate (child’s distal femur at 50 microns per day).3 Calcium/collagen ratios did not differ significantly from normal bone controls.

Introduction

Natural osteogenesis occurs at maximum rates (2) (50 to 100 microns per day) within growing bones while continuing at a much diminished rate (one to two microns per day) as a remodeling phenomenon in mature bones.16,21,22 Bone function depends upon exquisite macro- to ultra-structural organization of the bone crystal lending itself to load-bearing, calcium-phosphate homeostasis and hematopoiesis.29 That localized

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Osteogenesis can be re-induced by mechanical perturbations of bone is well established in studies of fracture healing. The latter form of osteogenesis, however, occurs in a haphazard pattern that often requires months to years to remodel to an organized structure.

The technique of slow distraction (0.25 millimeters every six hours) following a seven-day latency period to induce osteogenesis across a metaphyseal osteotomy site using a simple ring external fixator is ascribed to Ilizarov. Smooth steel wires (1.6 millimeters in diameter) drilled percutaneously through the bone on either side of the osteotomy site are affixed under tension to circumferential rings. Threaded rods connecting the rings can transmit controlled distraction force across the osteotomy. Osteogenesis is reliably produced, even in skeletonally mature bones.

The metaphysis is an ideal location for osteogenesis as it maintains the best collateral circulation and the highest blood flow measured within a long bone. Its thin cortex and large trabecular surface area facilitate a low energy osteotomy for rapid endosteal repair. A seven-day latency period prior to distracting the osteotomy seems logical when considering prior studies of fracture healing. At seven days, a fibroblastic-collagenous interface between fracture surfaces creates a biomechanically "rubbery" phase in healing. This elastic interface between rigid bone segments seems ideally suited for mechanical distraction.

Since the turn of the century, external fixation devices have been used to lengthen children's bones. Besides the limitations of soft tissues (i.e., nerves and vessels), previous techniques often resulted in large, unbridged bone gaps that required subsequent bone graft operations. The Ilizarov technique must be clearly distinguished from conventional methods by three unique qualities: (1) osteogenesis spontaneously bridges the distraction gap, (2) the new bone remodels rapidly to normal structure, and (3) this osteogenic potential exists in skeletonally mature bone.

Our preliminary investigations concern reproducing the animal model described in Ilizarov's early work utilizing reliable methods to analyze our results. Mature bone formation requires well-organized deposition of calcium as hydroxyapatite crystal in and around parallel bundles of collagen. The actual maturation process (mineralization) can be quantitated in vivo by radiodensity changes since calcium, the primary mineral, absorbs x-rays at characteristic wavelengths. Actual quantification of calcium and collagen, the two most prevalent constituents of bone by weight, provides direct verification of bone composition. Special histology confirms the microstructure of the newly formed bone.

Methods

Large dogs were selected for our animal trials since their bone structure and biology closely resembles that of man. All dogs were skeletonally mature at the outset of the study to eliminate the osteogenic potential of adjacent growth plates.

The experimental sequence originally reported by Ilizarov was carried out on the left tibia of all dogs, the right tibia serving as the control. On day 0, under general oro-tracheal anesthesia, a low energy osteotomy of the proximal tibial metaphysis (subperiosteal osteotomy) was then held in stable alignment by two percutaneous steel wires (1.6 mm diameter) above and two below the osteotomy fixed under tension to aluminum rings. The rings were connected by three threaded rods designed to distract the proximal wires from the distal wires in controlled fashion (figure 1). A partial fibulectomy through a separate incision isolated the tibia from adjacent bony
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Figure 1. This scaled replica of Ilizarov’s device is fabricated of aluminum (rings) and steel (threaded connecting rods, nuts, bolts and transosseous 1.6 millimeter wires). The upper ring must be incomplete to avoid pressure sores behind the dogs knee, where soft tissues cross posteriorly; a similar incomplete ring is used in humans by Ilizarov near the hip joint for the same reason. The transosseous wires are drilled through skin and bone at right angles if soft tissues permit to provide stability in both coronal and sagittal planes. These “smooth” wires are “tensioned” by twisting their connecting bolts, attached to the rings, thus stretching the wire like those in bicycle wheels for increased rigidity. The three threaded connecting rods, between rings, are used to distract the osteotomy site (located about three centimeters distal to the knee) by turning the appropriate lock nuts.

tether. The dog recovered with analgesia as required and resumed normal activities in his 4’ × 8’ cage.

On Day 7, following a seven-day latency period for soft tissue healing, distraction was begun at 0.25 millimeters every six hours until an osteotomy gap of 28 millimeters (approximately 15 percent of the initial tibial length) was achieved. On Day 35, relative compression was applied across the gap by reversing the fixator by two millimeters. On Day 77, the fixator and pins were removed under anesthesia allowing the dog full weight bearing as tolerated. If adequate bony union had occurred, the dog was sacrificed on Day 119 (four months); if the gap failed to bridge, the dog was sacrificed immediately on Day 77.

Of the six dogs studied, two underwent open biopsies following the distraction phase (Day 35) and following the compression phase (Day 77) while the other four dogs completed the 119-day schedule without further operative intervention.

All dogs had weekly standardized lateral radiographs made of the osteogenic area with an adjacent aluminum step-wedge. By the methods of Vose and Colbert, variables in film emulsion, technique, and equipment were controlled by reference to midportion of the dose-response of the step-wedge so that each subsequent radiograph could be corrected to the original. Optical density of the osteogenic area, adjacent diaphyseal cortex, and the control metaphysis was measured as the average of five random readings of light transmission (X-Rite Model 301 Photodensitometer) at each site and converted to bone density by the reciprocal of the antilogarithm. All values of bone density were then expressed as a percentage of the diaphyseal cortex. Each week the readings were made in the same coronal plane for consistency, since these linear transmissions are exquisitely sensitive to the depth of tissue.

At sacrifice, the left and right tibiae of each dog were macerated for whole bone gravimetrics by the method of Robinson. Increases in length, mass, and volume were noted for each dog along with calculated changes in density.

Fresh specimens cut with the Bronwill saw into one millimeter-thick coronal and transverse slabs from the osteogenic area and control side metaphysis were sequentially analyzed for calcium and collagen content (figure 2). Calcium was serially extracted with 10 percent disodium EDTA in 0.1M TRIS at pH 7.0 and quantitated on an atomic absorption spectrometer.* The decalcified specimens then underwent collagen extraction by the methods of Lansing and Hollander for hydroxyproline assay.5

* Perkin-Elmer, Model 5000.
Calcium and collagen were expressed as micrograms per milligram dry bone. The calcium/collagen ratio provided an indication of bone maturity. Both decalcified (5 to 10 micron) and non-decalcified (50 to 100 micron) histological preparations provided for qualitative microscopic analysis of the specimens. Hematoxylin and eosin stains highlighted the cellular activity while von Kossa stain delineated sites of mineral deposition. Collagen orientation within bone crystal was visualized by polarized light microscopy. Areas of new bone formation were confirmed by tetracycline labeling and fluorescence microscopy.

Results

Each of the six mongrel dogs initially weighed between 20 and 30 kilograms and demonstrated closure of bony growth plates by preoperative radiographs. The operations and external fixation devices were well-tolerated without infections and with minimal pain. The actual fixators were fabricated to match the specifications of the authentic Ilizarov device to a scale matching the size and shape of each dog's hindlimb. Weekly radiography required parenteral sedation and a special plexiglass frame to hold the x-ray cassette, aluminum step-wedge and limb for standardized anteroposterior and lateral views. Radiography confirmed the location of the osteotomy within the proximal tibial metaphysis and subsequent linear distraction gap (figure 3). In all six dogs, radiodense columns projected from each osteotomy surface separated centrally by a two- to three-millimeter radiolucent band. Although the radiodensity within the gap steadily increased from Days 14 through 35 by photodensitometry, actual visual changes occurred suddenly between Days 21 and 28 after a 40 percent increase in density. The macrostruc-
Figure 3. This sequence of three radiographs comes from Dog D on Days 21, 28, and 77. Seen in the lateral projection on Day 21, a distraction gap of 14 millimeters has been created, appearing empty (radiolucent). Histologically, the bone ends are bridged by parallel, longitudinally oriented collagen fibrils and interspersed fibroblasts (see figure 5). By Day 28, radiodense projections extend from each end of the original osteotomy surface toward a central radiolucent zone. The measured increase in radiodensity now exceeds 40 percent; the gap, still undergoing daily distraction, is now 21 millimeters. By Day 77, the 28 millimeter gap has been reached (Day 35) and the gap has been placed under compression by reversing the threaded rods by two millimeters for six weeks. Complete osteogenic bridging has occurred and early cortex formation is seen posteriorly.

Figure 4. This graph demonstrates the progressive change in radiodensity of the osteogenic gap, created during distraction, as a percentage of that measured in diaphyseal cortex. Since diaphyseal cortical bone is known to change its mineral content very slowly, it serves as a good control. The control side metaphysis (opposite tibia) measured on Day 0, serves as the lower end control. All measurements were standardized to an aluminum step-wedge to control for changes in technique and materials. Visible radiodense projections are noted only after the osteogenic area increases from 25 percent to 75 percent of cortical density on Day 28 (see figure 3).
form bridge of dense fibrous tissue arranged parallel to the longitudinal distraction force. The interface between fibrous tissue and bony columns contained a transition zone of eosinophilic matrix and swollen fibroblasts and early calcium deposition by von Kossa stain. A few islands of cells resembled chondroblasts but overall the process resembled intramembranous ossification (figure 5).

By Day 77, the bony columns appeared to be more mature with less osteoblastic activity on the surfaces and normal bone marrow filling the longitudinal spaces. By Day 119, the bone had assumed lamellar structure with Haversian systems of alternating collagen orientation by polarized light. Tetracycline fluorescence confirmed the entire osteogenic area as new bone.

**Figure 5A.** This specimen was taken as a biopsy from the osteogenic area from one dog on Day 35. Spindle-shaped fibroblasts are interspersed with highly organized, parallel (collagen-stain positive) fibrils at the bottom of the microscopic field (magnification 20 X). This fibrous tissue corresponds to the radiolucent zone seen in figure 3, Day 28. As the fibroblasts swell (center), the darker matrix surrounding them indicates transformation into bone. Multiple large vascular beds are seen in the vicinity of new bone formation.

**Figure 5B.** This specimen, also taken on Day 35, is closer to the original osteotomy site, at the same magnification. Longitudinal spaces (radiolucent) are filled with loose fibrovascular tissue. The swollen cells, when completely surrounded by more mature bony matrix, shrink to less than half their former size, taking on the appearance of normal osteocytes. By polarized light microscopy, this highly organized bone approaches the appearance of mature lamellar bone. Note the rows of osteoblasts lining the bone within the fibrovascular spaces, indicating intense biological activity.
Gravimetric analysis of the macerated whole bones demonstrated the experimental tibiae to have significant gains in length, mass and volume with minimal change in overall density when compared to the opposite control tibiae. The experimental tibiae were reliably lengthened by 12 percent (24 mm ± 4), increasing mass by 27 percent (18 g ± 2) and volume by 26 percent (13 cc ± 2) with only a one percent change in overall whole bone density.

The transverse slabs of bone taken from the osteogenic areas contained an average 29 percent calcium and 24 percent collagen by dry weight while the bone specimens taken from the control sides contained an average 32 percent calcium and 27 percent collagen. These values were not significantly different by a Wilcoxon two-sample test. The calcium-collagen ratio of 1.25 within the osteogenic areas (average) compared closely to the 1.20 ratio in the control sides (average) consistent with reported ratios in mature bone.

Discussion

Our histological sequence resembling intramembranous ossification closely duplicates the work of Ilizarov. A growing body of experimental evidence links periosteal new bone formation, which is well known to occur from intramembranous ossification, to tension forces across the periosteal membrane itself. Since the early phase (Days 7 to 35) of the mechanical induction of osteogenesis involves tension forces created by distracting the fibrous interface bridging the surfaces of the osteotomy, intramembranous ossification would have solid biological precedent.

Rapid remodeling to lamellar bone structure when subjected to a compression force later in the experimental sequence (Days 35 to 77) must have something to do with the well-organized and highly vascular structure of the bone columns created by intramembranous ossification. Fracture-gap healing, which is highly disorganized in comparison, with large areas of enchondral ossification, requires much longer to remodel to lamellar bone. Longitudinally-oriented support columns are biomechanically most stable under axial compression force; the osteogenic area withstands early weight-bearing without loss of length.

The added mass and volume without significant change in whole bone density verifies that true osteogenesis has occurred within each bone. The major constituents, calcium and collagen, compare closely to control bone measurements. Calcium to collagen ratios indicate the maturity of bone organization. A strong body of experimental evidence supports the hypothesis that phosphate groups within stereochemical pores of the collagen macromolecule not only seed hydroxyapatite \((\text{Ca}_{10}\text{P}_6\text{O}_{26}\text{H}_2)\) crystallization. Binding sites eventually limit crystal size as the collagen molecules become saturated. Bone crystal remains undersaturated during early phases of formation reflected by lower calcium-collagen ratios and eventually attains a ratio consistent with the "mature" crystal-collagen state.

Radiodensity changes do not exactly parallel Ilizarov's original graph. He indicated that the osteogenic area attains 60 percent of cortical bone density by the first week after the osteotomy, maintaining a steady state during distraction, then rising slightly to over 70 percent during compression and reaching normal levels of about 80 percent by Day 119. Our data indicate that the osteogenic area gradually increases in radiodensity at 20 percent of cortical bone by the first week, peaking at 70 percent by the fourth week and decreasing to normal metaphyseal levels of 40 percent by Day 77. It is unclear from the available literature how Ilizarov actually quantitated mineral content in his own canine exper-
iments. If he used ashed mineral weight rather than \textit{in vivo} photodensitometry, then some variations could exist. Our sampling techniques required averaging random samples from the osteogenic area which necessarily combined fibrous with calcified areas. Ilizarov might have been measuring only the density of calcified areas.

Our photodensitometry graph bears strong resemblance to a graph published by Rubin and Lanyon\textsuperscript{27} regarding the radiodensity changes in bone subjected to cyclic loading. The 40 percent rise during the first four weeks followed by a slight fall off bore striking similarity to our graph. It has also been well-documented that a visible change in radiodensity requires a change of 40 percent in actual bone density.

This animal model for the study of osteogenesis by mechanical induction provides large areas of new bone formation, isolated both temporally and spatially from host bone. The clinical application for such a biological process has obvious potential for healing problems of bone deficiency in both children and adults.

Acknowledgments

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References

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