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The effects of PTH, loading and surgical insult on cancellous bone at the bone–implant interface in the rabbit

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ABSTRACT

Enhancing the quantity and quality of cancellous bone with anabolic pharmacologic agents may lead to more successful outcomes of non-cemented joint replacements. Using a novel rabbit model of cancellous bone loading, we examined two specific questions regarding bone formation at the bone–implant interface: (1) does the administration of intermittent PTH, a potent anabolic agent, and mechanical loading individually and combined enhance the peri-implant cancellous bone volume fraction; and, (2) does surgical trauma enhance the anabolic effect of PTH on peri-implant bone volume fraction. In this model, PTH enhanced peri-implant bone volume fraction by 30% in loaded bone, while mechanical loading alone increased bone volume fraction modestly (+10%). Combined mechanical loading and PTH treatment had no synergistic effect on any cancellous parameters. However, a strong combined effect was found in bone volume fraction with combined surgery and PTH treatment (+34%) compared to intact control limbs. Adaptive changes in the cancellous bone tissue included increased ultimate stress and enhanced remodeling activity. The number of proliferative osteoblasts increased as did their expression of pro-collagen 1 and PTH receptor 1, and the number of TRAP positive osteoclasts also increased. In summary, both loading and intermittent PTH treatment enhanced peri-implant bone volume, and surgery and PTH treatment had a strong combined effect. This finding is of clinical importance since enhancing early osseointegration in the post-surgical period has numerous potential benefits.

1. Introduction

Fixation of total joint replacements without the use of cement requires a viable and biomechanically robust cancellous bone bed for a total joint implant to osseointegrate properly. A variety of host factors, such as cancellous bone quantity and quality, determine if the cancellous bone is sufficient for the successful osseointegration of an implant. Bone quality and quantity can be modulated by a number of pharmacological agents and by mechanical loading of peri-implant bone tissue. In the United States intermittent PTH is currently the only FDA-approved anabolic pharmacological agent for enhancing bone formation and improving bone strength in osteoporotic and osteopenic individuals. The anabolic effect is site specific and occurs primarily at cortico-cancellous sites. In addition, PTH enhances early screw fixation through increased bone apposition [1,2], suggesting that similar effects may be present at the bone–implant interface.

In a variety of animal models, applied mechanical loading can directly enhance cancellous bone formation and inhibit bone loss by increasing bone formation and bone mass [3–6]. Our group previously demonstrated increased bone volume fraction and altered bone architecture when loading was applied directly to healthy cancellous tissue in a rabbit model [7,8]. In further studies, when an implant was included in the model, loading increased the amount of bone tissue and decreased the amount of fibrous tissue in the loaded porous implant [9].

While surgical trauma may be linked to enhancing tissue quality surrounding implants with intermittent PTH administration, the exact mechanism is unclear [10–12]. In a canine model with loaded implants, the surgical insult dominated the early healing response [13]. One possible explanation is that the particulate bone debris generated during preparation of the bony bed prior to the insertion of an implant may contain growth factors that stimulate osteogenesis. A second potential explanation is that the inflammatory reaction induced by the surgical trauma to the tissues results in stimulatory cytokine and chemokine expression [14].

We have developed a rabbit model for the study of functional adaptation to an implant in cancellous bone under a well-controlled mechanical loading. Increased bone volume fraction, altered trabecular architecture, and enhanced mineral apposition rate were seen after

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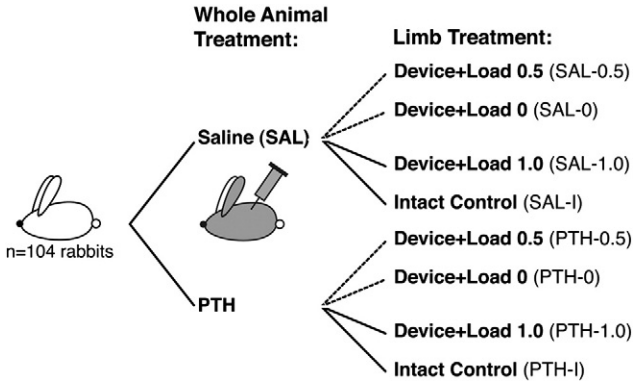

Fig. 1. Study design with treatment groups (PTH or saline) and pair-wise design with loaded, non-loaded and intact limbs.

4 weeks of cyclic loading at 1 MPa [7–9]. On the other hand, how different anabolic treatments can be combined with loading to enhance the cancellous bone bed for implant surgery is still unclear. In this current study, we used our rabbit loading model to examine two specific hypotheses about tissue formed at the bone–implant interface: (1) PTH and mechanical loading individually and combined will enhance the peri-implant cancellous bone volume fraction; and (2) surgical trauma will enhance the anabolic effect of PTH on peri-implant bone volume fraction.

2. Materials and methods

A previously validated rabbit model for adaptation in surgically-exposed cancellous bone was used to explore (1) the individual and combined effects of loading and PTH and (2) the effects of PTH and surgery. We characterized the tissue microarchitecture, histology and biomechanical properties, and cellular activity and localization of expression. All experiments were carried out with IACUC approval at the Hospital for Special Surgery.

2.1. Study design

One hundred and four New Zealand White rabbits (minimum age 7 months old, 3.81 ± 0.15 kg body mass) underwent surgical implantation of a custom loading device on the lateral aspect of the distal femur. Half of the rabbits were treated with PTH (20 μg/kg, SQ, 5 days/week, Lilly, Indianapolis, Indiana), and the other half received saline injections for 4 weeks starting the day after surgery (Fig. 1). In total eight experimental groups were examined (n = 26 limbs/group): saline-treated non-operated, intact control limbs (SAL-I), and saline-treated limbs implanted with the loading device receiving three different load levels: no load (SAL-0), 0.5 MPa load (SAL-0.5) and 1.0 MPa load (SAL-1.0); and, PTH-treated non-operated intact control limbs (PTH-I) and PTH-treated limbs implanted with the loading device receiving three different load levels: no load (PTH-0), 0.5 MPa load (PTH-0.5) and 1.0 MPa load (PTH-1.0).

2.2. Cancellous loading device and in vivo protocol

The loading device was inserted into the distal femurs of skeletally mature male New Zealand white rabbits, as detailed previously (van der Meulen et al. [7,8]; Willie et al. [9]). The device consisted of a stationary base mounted on the lateral femoral condyle with two bicortical screws, a movable loading core (5 mm diameter), and a top (Fig. 2). During surgery to place the device, the surface of the cortical bone was milled down to ensure direct contact of the core with the underlying cancellous bone prior to attaching the base to the cortex. Transcutaneous loading was applied under isoflurane anesthesia (2%, 1 l/min). During loading, the core slid within the base to compress the underlying bone with a load of known magnitude. The loading device included feedback control (National Instruments, Labview v8.2) using an in-line force transducer (Sensotec Precision Miniature Load Cell, Model 31) to control the actuator [32]. Mechanical loading of the right limb was initiated immediately post-operatively at either 0.5 or 1.0 MPa for 50 cycles at 1 Hz per day, 5 days/week for 4 weeks. The left limb was not loaded but had the same device implanted in half the rabbits (no load groups: SAL-0 and PTH-0).

To label active bone formation, fluorochrome labels were administered 14 (Xylenol orange, 30 mg/kg, IV) and 3 (0.1% Calcein, 15 mg/kg, IV) days pre-euthanasia. Upon completion of the 4-week experiment, animals were euthanized by barbiturate overdose (Sodium Pentobarbital 26%/Isopropyl alcohol 10% euthanasia solution, 2.0 ml, IV).

2.3. Sample distribution and analyses

Immediately after euthanasia, a 5 mm × 5 mm (D × H) cylindrical core of cancellous bone was removed directly below the loading device in the lateral distal femur (Starlite Industries, Core Drill 101055). In intact limbs, cores were taken from the same location on the femur and included the cortex. All cancellous cores (n = 208) were first scanned by microcomputed tomography (microCT). Thereafter, cores were prepped for subsequent tissue or cellular analyses. Samples for dynamic histomorphometry were dehydrated and embedded in polymethyl methacrylate (PMMA). Samples for cellular analyses were fixed, decalcified and embedded in paraffin for cellular analyses.

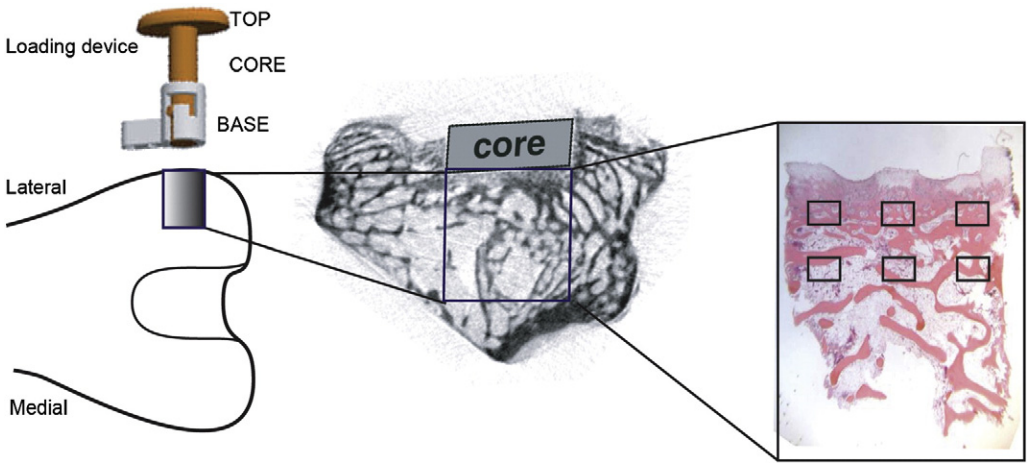

Fig. 2. Schematic of loading device (left) on femur and lateral view of distal femur showing device position and core location (middle) and location of the underlying cancellous bone (right) with histological areas of interests.