Available online at www.sciencedirect.com
ScienceDirect
Medical Engineering & Physics 31 (2009) 42–47
Medical Engineering & Physics
www.elsevier.com/locate/medengphy

Ultrasound mimics the effect of mechanical loading on bone formation *in vivo* on rat ulnae

Mark J. Perry [a,*,1], Laura K. Parry [b,1,2], Victoria J. Burton [a], Sabina Gheduzzi [c], Jon N. Beresford [d], Victor F. Humphrey [e], Tim M. Skerry [b,3]

[a] *Department of Anatomy, University of Bristol Vet School, Southwell Street, Bristol BS2 8EJ, UK*
[b] *Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU, UK*
[c] *Centre for Orthopaedic Biomechanics, Department of Mechanical Engineering, University of Bath, Bath BA2 7AY, UK*
[d] *Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK*
[e] *Institute of Sound and Vibration Research, University of Southampton, University Road, Southampton SO17 1BJ, UK*

Received 12 January 2007; received in revised form 8 April 2008; accepted 11 April 2008

Abstract

While the effect of ultrasound as an extreme example of low-magnitude high-frequency stimulation has been explored in the response of bone to injury, little is known about its effect on normal bone. This experiment was designed to test the hypothesis that ultrasound exerts a similar influence on bone as mechanical stimulation at a physiological level.

Three groups of female Wistar rats were anaesthetised (6 per group). In one group, the left ulna was loaded cyclically *in vivo* 40 times, repeated on a further 5 occasions on alternate days. In a second group, transcutaneous low-intensity pulsed ultrasound stimulation was applied to the left ulnae for the same duration as the period of loading. In a third group, loading and ultrasound stimulation were applied concurrently. The right ulna served as non-loaded control in each animal. At the end of the experiment after 14 days, both ulnae were removed. Induced bone formation was assessed by measuring the proportion of medial periosteal bone surface with double label (dLS/BS, %) and by calculation of mineral apposition rate (MAR) from the inter-label distance. All three treatments induced a significant periosteal response, increasing dLS/BS values from <10% in control limbs to >80% in treated limbs. Increases in MAR of experimental ulnae versus contralateral control ulnae were 2.9 (±0.9), 8.6 (±2.4) and 8.7 μm (±3.2) for the ultrasound only, ultrasound and load, and load only groups, respectively. The effects of loading plus ultrasound were not significantly different from ultrasound alone. These data suggest that ultrasound is able to induce changes in bone that share at least some features with mechanical loading.

Keywords: Ultrasound; Mechanical loading; Rat; Bone formation; Periosteum

1. Introduction

The term ultrasound is used to describe sound waves with a frequency above the limit of human hearing, 20,000 Hz [1]. Ultrasound consists of mechanical energy propagating in a material by means of pressure waves [2]. In medical applications, the frequency range of ultrasound typically varies between 500 kHz and 30 MHz [1]. In clinical musculoskeletal applications, low-intensity (<150 mW/cm^2) pulsed ultrasound (LIPUS) is characterised by a relatively low-frequency (1.5 MHz) pulsed signal [3,4].

The pressure waves produced by ultrasound in tissues can cause effects at the cellular level [5]. Ultrasound represents a very mild mechanical stimulation of bone, and its effect has almost exclusively been studied in models and clinical circumstances involving fracture and other healing responses [3,4,6–9] rather than in intact bone.

* Corresponding author. Tel.: +44 117 9288524; fax: +44 117 9254794.
E-mail address: Mark.J.Perry@bristol.ac.uk (M.J. Perry).
[1] These authors contributed equally to this article.
[2] Present address: Genzyme Europe Research, 310 Science Park, Milton Road, Cambridge CB4 0WG, UK.
[3] Present address: Academic Unit of Bone Biology, Division of Clinical Sciences, School of Medicine & Biomedical Sciences, Beech Hill Road, Sheffield S10 2RX, UK.

1350-4533/$ – see front matter © 2008 IPEM. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.medengphy.2008.04.003

It has been suggested that LIPUS accelerates fracture healing by acting on cellular mechanisms involved in the healing process such as inflammatory responses [10]. Although most studies have applied LIPUS daily over longer periods of time [3,4,6–9], one study using a rat fracture model revealed that a treatment regimen of only one week in duration was able to accelerate fracture healing [10].

Cell culture studies using primary osteoblasts or osteoblast-like cell lines have shown that LIPUS enhances osteoblast activity through a number of mechanisms including stimulation of prostaglandin E2 [11] and ATP release [12], elevation in Runx2, osteocalcin, IGF-I and VEGF expression [11,13–16] and increases in integrin and alkaline phosphatase expression [17]. All of these changes would be consistent with increased levels of bone formation that would lead to accelerated repair of a bone fracture. However, the principles illustrated by these *in vitro* studies are somewhat simplistic in that they lack the ability to determine architectural responses of the skeleton to loading including the initiation of both formation and resorption at different sites so that the mechanical properties of the tissue are appropriate to the loads applied [18].

The purpose of this study was to determine whether LIPUS was able to stimulate the growth of intact non-fracture bone in a whole animal. We used a well-established model of mechanical loading *in vivo*, to compare the ability of load and/or LIPUS to stimulate periosteal bone formation. The study intended to replicate the ultrasound parameters characteristic of the EXOGEN 2000+ low-intensity ultrasound fracture healing system (Smith & Nephew), the only commercially available device which has been proven to accelerate healing in two independent multi-centre, double blind and placebo controlled, randomised studies [3,4]. We demonstrate here that LIPUS mimics the ability of physiological mechanical loading on bone albeit at a lower level with current ultrasound parameters used clinically. LIPUS activated a similar proportion of periosteal bone surface as mechanical loading alone or in combination with LIPUS, but overall bone formation with LIPUS was only a third of that of combined mechanical loading and LIPUS or loading alone.

2. Materials and methods

2.1. Animals and loading regimen

All experiments were performed according to standard procedures approved by the Local Ethical Review Committee and the UK Home Office.

Three groups of adult female Wistar rats (220 g) were used with 6 rats per group as used previously [18]. Rats were anaesthetised using a mixture of fentanyl, fluanizone and diazepam (240, 7.5, and 3.75 mg/kg, respectively) on alternate days for two weeks. Animals from the ultrasound only group (group 1) had an ultrasound transducer placed against the left ulna for the same duration as the application of loading in the other groups (420 s). The transducer was placed over the midshaft of the forelimb bones. The size of the transducer head was such that it covered approximately the middle third of the forearm's length, thus covering the whole of the length that was analysed. In group 2, mechanical loading and ultrasound were applied concurrently and in group 3, mechanical loading alone was applied. The loading methods and model are those used extensively by ourselves and others [18,19]. Briefly, 40 cycles of axial compression were applied between the elbow and flexed carpus at a magnitude that induced peak strains of 0.003 at 0.12/s, with a 0.46 s rest period at peak strain and a 10 s rest period between each cycle. The left ulnae from groups 2 and 3 were loaded on Monday, Wednesday and Friday for two consecutive weeks, a regimen that we and others have shown to be effective in initiating adaptive modelling changes [18,20–22].

In order to apply ultrasound to the forelimb, the hair was shaved from the medial aspect of the forearm and a custom-made 1 MHz transducer coupled to the skin of each left rat ulna with an intervening layer of coupling gel (Diagnostic Sonar Ltd., UK) (Fig. 1).

The ultrasonic transducer was excited using a function generator (HP 33120A, US) programmed to produce sinusoidal voltage pulses having a burst width of 200 μs containing 1 MHz sine waves. The repetition rate was set at 1 kHz. The voltage signal was amplified (ENI power amplifier model 310L, US) in order to attain a spatial average-temporal average intensity of 150 mW/cm^2 at the face of the transducer. Since only a proportion of the transducer

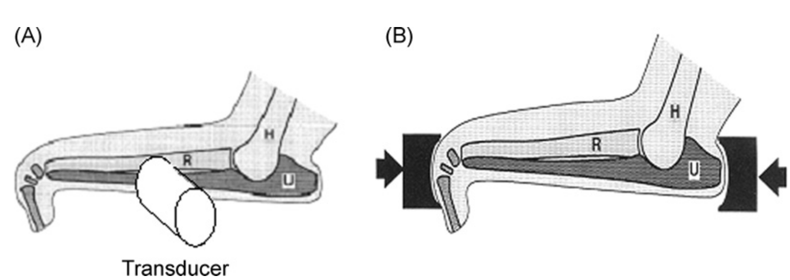

Fig. 1. Diagram showing (A) the application of ultrasound via a transducer to the left ulna and (B) the direction of the applied mechanical load. (R = radius, U = ulna and H = humerus).