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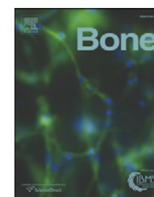

Original Full Length Article

Bone growth resumption following in vivo static and dynamic compression removals on rats

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ABSTRACT

Mechanical loadings influence bone growth and are used in pediatric treatments of musculoskeletal deformities. This in vivo study aimed at evaluating the effects of static and dynamic compression application and subsequent removal on bone growth, mineralization and neuropathic pain markers in growing rats. Forty-eight immature rats (28 days old) were assigned in two groups (2- and 4 weeks experiment duration) and four subgroups: control, sham, static, and dynamic. Controls had no surgery. A micro-loading device was implanted on the 6th and 8th caudal vertebrae of shams without loading, static loading at 0.2 MPa or dynamic loading at 0.2 MPa ± 30% and 0.1 Hz. In 2-week subgroups, compression was maintained for 15 days prior to euthanasia, while in 4-week subgroups, compression was removed for 10 additional days. Growth rates, histomorphometric parameters and mineralization intensity were quantified and compared. At 2 weeks, growth rates and growth plate heights of loaded groups (static/dynamic) were significantly lower than shams ($p < 0.01$). However, at 4 weeks, both growth rates and growth plate heights of loaded groups were similar to shams. At 4 weeks, alizarin red intensity was significantly higher in dynamics compared to shams ($p < 0.05$) and controls ($p < 0.01$). Both static and dynamic compressions enable growth resumption after loading removal, while preserving growth plate histomorphometric integrity. However, mineralization was enhanced after dynamic loading removal only. Dynamic loading showed promising results for fusionless treatment approaches for musculoskeletal deformities.

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1. Introduction

Mechanical loadings are essential for normal bone growth and tissue health. In growing individuals, asymmetric loading or overloading contributes to the progression of musculoskeletal deformities such as adolescent idiopathic scoliosis [1], early onset scoliosis [2], limb asymmetries [3], genu varum [4] or Blount's disease [4,5]. Several in vivo animal studies [6–9] provide evidence that compressive loading reduces bone growth rate while releasing compression accelerates it, according to the Hueter–Volkmann principle [10,11]. Fusionless or growth friendly [2,12] devices use the patient's remaining growth potential to apply appropriate loadings to reverse or halt the progression of the deformity [12]. In vivo studies in rats and rabbits reported bone growth rate reduction due to both static and dynamic compressions [7,9,13] along with decreases in growth plate height [6,9], proliferation [6], and hypertrophic cell height as a marker of hypertrophy [9]. Studies showed that dynamic loading better preserved growth plate integrity causing fewer morphologic changes to the growth plate [9]. However, loading duration [6,14] and magnitudes [7,13] were found to influence growth plate function.

Upon correction of the deformity, fusionless implants need to be removed, possibly with a remaining growth potential. However, it is not known if the physis would preserve its integrity and functionality. The in vivo study by Mente et al. investigated long-term effects of static compression application and removal on bone growth and growth plate integrity, and found growth resumption in rats [11]. Dynamic loading removal was investigated by Ohashi et al. on a rat ulna model [13]. They observed recovery of longitudinal bone growth in two groups with magnitudes within physiological ranges. Animals with supra-physiological dynamic magnitude still had reduced growth, as well as greater growth plate heights, and impaired mineralization

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[13]. Mineralization and newly formed bone can be affected by mechanical loadings through the bone remodeling process referred to as Wolff's law [15,16]. In growing bones, no studies report in vivo effects of physiological mechanical loading on early stages of mineralization in the hypertrophic growth plate zone and subsequently its relation to bone quality.

The in vivo rat tail model is used to study growth plate mechanobiology, through minimally invasive surgery of an external device implantation [11,17]. However, implantation surgery may lead to central neurogenic changes, modifying nociceptive signal transmission and modulation. In particular, substance P (SP) and calcitonin gene-related peptide (CGRP) are nociceptive peptides, which play key roles in pain transmission and sensitization [18]. Both peptides can be found in the spinal cord dorsal horn [19]. Following lesions in articulations [20–22] and tendons [23], these peptide concentrations increase and are associated with pain-related behaviors in rats.

The objective of this in vivo study using the rat tail model was to assess the effects of sequential full-time loading application and removal on bone growth, more specifically growth rate, histomorphometry, mineralization and neuropathic pain. The research hypotheses stated that (1) dynamic as opposed to static compression removal restores bone growth rate, (2) static loading affects growth plate histomorphometry and mineralization, while dynamic loading preserves it, and finally (3) both static and dynamic loadings cause neuropathic pain.

2. Materials and methods

2.1. In vivo experimental conditions

The Institutional Animal Care Committee approved the protocol. Forty-eight male Sprague–Dawley rats (28 days old) were divided into two groups by experiment duration (2-week and 4-week), each containing four subgroups: control, sham, static, and dynamic (Table 1). In 2-week groups, compression was applied full time for 15 days before euthanasia (43 days old). In 4-week groups, compression was removed for 10 additional days before euthanasia (53 days old) (Fig. 1-A, Table 1). In both 2- and 4 -week groups, control subgroups had no surgery and sham subgroups had the device implanted without any loading. The device applied static (sustained) compression of 0.2 MPa or dynamic compression ranging from 0.14 MPa to 0.26 MPa (0.2 MPa ± 30%) oscillating at 0.1 Hz.

2.2. Compression device

The micro-loading device, inspired by Walsh et al. [24], was implanted on the 6th and 8th caudal vertebrae to load the 7th caudal vertebra (Cd7). A bladder support was added at the distal extremity of the rat tail. The bladder was the force generator for the micro-loading device. Static and dynamic compressions were transmitted through bladder (DipTech Systems Inc., Kent, Ohio) inflation and/or deflation controlled by a pneumatic system, as described in previous studies [9, 25]. Prior to in vivo experimentation, bladders were calibrated using a micro-mechanical loading machine (MACH-1, Biomomentum, Laval, QC, Canada) to determine force/voltage numerical relationship to adjust for transversal growth of caudal vertebra [17].

2.3. Surgical procedure and animal care

Surgery was similar to previously published papers [9,25]. Rats were anesthetized with isoflurane during the entire implantation procedure and had analgesic (Buprenorphine, 50 μg/kg, sc) and antibiotic (Baytril, 5 mg/kg, sc) injections [9,25]. Vertebrae localization was performed with fluoroscopic images (GE OEC 9900 Elite) (Fig. 1-C) [9,25]. Rats recovered quickly following surgery, and were given ad libitum food and water (Fig. 1-B). Compression was adjusted daily according to force/voltage relationship with use of a calibration guide to maintain fixations in parallel positions (Fig. 1-B and D). For growth measurements, two calcein injections (Sigma-Aldrich, St.-Louis, MO, USA; 15 mg/kg, ip) were performed five and two days prior to euthanasia. Rats were anesthetized with isoflurane before decapitation [26].

2.4. Spinal cord collection and analysis

Immediately following euthanasia, lumbar enlargement and first sacral segments of rat spinal cords were collected and preserved at −80 °C until peptide analysis. Quantitative analysis was performed once all subgroups of rats within the same group were completed. SP and CGRP concentrations were obtained using high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS), similarly to literature parameters described by Ferland et al. [20,21]. SP and CGRP concentrations were measured individually in spinal cord samples at 4 weeks, and the final subgroup value is reported as the average of six rats per subgroup.

2.5. Vertebra collection

Two caudal vertebrae were extracted: Cd5 as within-animal control and Cd7 loaded vertebra. Vertebrae were fixed in formalin, dehydrated and clarified before methylmetacrylate embedding (Fisher Scientific Canada, Nepean, ON, Canada). Both vertebrae were cut along their longitudinal axis in 6 μm sections using a microtome (Leica SM2500), at vertebra center (Fig. 2-A). The first slide of each series was stained with toluidine blue for histomorphometric measurements (growth plate heights, zone heights, hypertrophic cell heights). The second slide was mounted for growth rate measurements. The third slide was stained with alizarin red for calcium mineral deposit assessment [27] and quantification using a standalone Matlab program developed by Kaviani et al. [28]. Alizarin red staining was performed with all experimental groups in one batch to reduce error from pigmentation and two slides were analyzed for each group. Slices were observed under a microscope (Leica DMR with RetigaQimaging Camera) using 5× short wavelength light to produce fluorescence for growth rates (calcein labeling), 10× bright light for total growth plate heights, zone heights and calcification (pixel size of 1.0652 μm), and 20× for hypertrophic cell heights.

Table 1
Experimental groups/subgroups: number of rats, age and weight at surgery and euthanasia (mean value ± SD) with [a]$p < 0.05$ compared to controls.

Groups	Subgroups	Number of rats	Age at surgery [days]	Age at euthanasia [days]	Weight at surgery [g]	Weight at euthanasia [g]
2-week (15 days loading)	Control	n = 6	28	43	91.8 ± 5.6	242.3 ± 19.2
	Sham	n = 6	28	43	83.7 ± 9.2	212.5 ± 16.8[a]
	Static	n = 6	28	43	89.0 ± 8.2	221.5 ± 11.0
	Dynamic	n = 6	28	43	89.2 ± 3.4	217.5 ± 15.1
4-week (15 days loading + 10 days unloading)	Control	n = 6	28	53	92.2 ± 9.9	348.8 ± 45.5
	Sham	n = 6	28	53	84.3 ± 12.2	300.0 ± 37.0
	Static	n = 6	28	53	83.5 ± 18.0	292.2 ± 29.7
	Dynamic	n = 6	28	53	81.3 ± 6.0	328.0 ± 39.9

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