

Nitric oxide production by bone cells in response to mechanical vibration

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INTRODUCTION

Bones are able to adapt their mass and structure to the demands of mechanical loading. Furthermore, it has been suggested that the rate rather than the magnitude alone of the applied loading stimulus correlates to bone formation *in vivo* [1]. In bone, mechanical loading is likely transferred to the cells by strain-induced fluid flow through the lacuno-canalicular system [2]. We have recently found in another study that the NO production by MC3T3-E1 cells was linearly dependent to the rate of fluid shear stress, which depended on both magnitude and frequency [3]. In that study however, the applied fluid shear stress was only up to a maximum of 9 Hz. Using animal models, low magnitude ($< 10 \mu\epsilon$) high-frequency (10 - 100 Hz) mechanical stimuli have been shown to be capable of stimulating bone growth by doubling bone formation rates and inhibiting disuse osteoporosis [4]. Thus, it would seem that higher frequencies are also stimulatory to bone cells. Regardless of frequency range, the applied rate of loading seems to be a decisive factor in bone formation and maintenance. However, the nature by which bone cells respond to mechanical vibration is unknown. We studied the response of bone cells to mechanical vibration at a wide frequency range (5 Hz upto 100 Hz), at different magnitudes. We tested whether vibration stress applied with varying frequencies and amplitudes affects the nitric oxide (NO) production by MC3T3-E1 osteoblast-like cells.

MATERIALS AND METHODS:

Bone cell culture: For vibration stress treatment, MC3T3-E1 cells were plated in 24-wells plates, at 4×10^4 cells/cm², in CO₂-independent α -MEM medium with 2% FBS, and incubated for 5 min in the presence of mechanical vibration at varying frequencies (5Hz, 30Hz, 60Hz, 100Hz) and amplitudes (see Table 1). Vibration stress was implemented on attached cells by sinusoidal displacement of the 24-wells plate along the cells' plane of attachment using a voltage controlled linear actuator. Conditioned medium was sampled after 5 min of vibration stress treatment to measure accumulated NO in medium produced by MC3T3-E1 cells.

NO: The conditioned medium was assayed for NO, which was measured as nitrite (NO₂⁻) accumulation in the conditioned medium, using Griess reagent. Data from separate experiments were collected and expressed as mean total amount of NO in nmol units.

Statistics: All results were analyzed by linear regression of the S-Plus 2000 package (release 1).

RESULTS:

NO production in rapid response (within 5 min) to treatment with vibration stress linearly correlated with the applied maximum acceleration rate, which is third order in time-dimension (Figure 1A; $p < 0.027$, $R = 0.97$). However, NO production did not linearly correlate with the applied maximum velocity, which is first order in time-dimension (Figure 1B).

DISCUSSION:

NO production is an essential step for mechanical loading-induced bone formation as observed in rats *in vivo* [5]. Hence, NO production in response to mechanical vibration is a meaningful parameter for measuring bone cell activation. NO production by bone cells linearly correlated with the maximum applied acceleration rate, which is third order or cubic in time dimension (see Table 1). This suggests that the bone cell response to vibration stress treatment is highly dependent on the applied frequency of loading regardless of the applied amplitude. The response however, did not linearly correlate with the applied maximum velocity, which is a joint effect of the applied amplitude and frequency, both at first order. This corresponds to our earlier finding that bone cell response is linear to the applied fluid shear stress rate (also third order in time dimension) [3]. Interestingly, bone cells do respond to high frequency vibration stress (i.e., 100 Hz) although fluid shear stresses *in vivo* might involve lower frequencies [3]. In this study we have not considered in detail the physical differences of the effects of fluid shear stress or vibration stress on bone cell deformation. However complicated the transfer of forces at the cellular level might be, in terms of fluid shear stress or vibration stress, our results imply that the joint effect of the frequency and amplitude of loading might play similar roles for different types of stresses on bone cells. Our results suggest that the joint effect of the frequency (at third order) and amplitude (at first order) of loading correlates to the biochemical response of bone cells that contribute to sustained bone metabolism. Furthermore, this response might involve mechanisms that contribute

to a specific behavior of bone cells in response to vibration stress enabling recognition of high frequency loading.

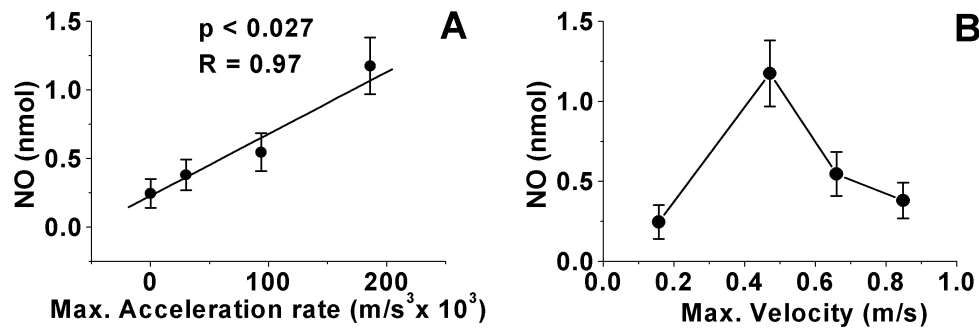
Table 1. Data of applied vibration stress

| Regime | 5 Hz | 30 Hz | 60 Hz | 100 Hz |
|--|------|-------|-------|--------|
| Amplitude (mm) | 5 | 4.5 | 1.75 | 0.75 |
| Maximum acceleration rate ($\text{m/s}^3 \times 10^3$) | 0.15 | 30.1 | 93.8 | 186 |
| Maximum velocity (m/s) | 0.15 | 0.85 | 0.66 | 0.47 |

Maximum velocity = amplitude x frequency x (2π)

Maximum acceleration = amplitude x frequency³ x $(2\pi)^3$

Figure 1. Effect of vibration stress on NO production by bone cells. A. Bone cells respond linearly to the applied maximum acceleration rate of vibration stress immediately after 5 min. B. The response to mechanical vibration does not correlate linearly to the applied maximum velocity (see also Table 1). Values are mean total amount \pm SEM.



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