INTERRACELLULAR SIGNALING RESPONSES TO CONTROLLED MECHANICAL STIMULATION IN AN IN VIVO MODEL OF TRABECULAR BONE FORMATION

+*Moalli, M R; +*Wang, S; +*Caldwell, N J; +*Patil, P V; +*Maynard, C R; +*Goldstein, S A
+*Orthopaedic Research Labs, University of Michigan, Ann Arbor, MI 400 N. Ingalls, Room G161, Ann Arbor, MI 48109-0486, 734-763-9674, Fax: 734-647-0003, mmoalli@umich.edu

Introduction: Mechanical signal transduction involves the conversion of a biophysical force into a cellular/molecular response leading to rapid changes in kinase-mediated gene expression, as well as later adaptive changes in cytoskeletal arrangement. This research project is based on the assumption that mechanical signals are transmitted from the extracellular matrix to the interior of the cell by integrins which results in the formation of focal adhesion complexes and the phosphorylation of focal adhesion kinase (FAK), which then initiates a variety of signaling cascades. Previous work in our laboratory has demonstrated that a 30 minute, 1800 cycle load stimulus will elicit FAK activation.(1) The purpose of this study was to determine the mechanical stimulus threshold that elicits FAK activity.

Methods: Bone chambers equipped with hydraulic actuators(2) were aseptically inserted into each proximal tibial metaphysis of eight adult, male dogs. After 8 weeks of bone infiltration the dogs were returned to the operating room, and the servohydraulic loading mechanism was activated to apply a load stimulus to the woven trabecular bone that had formed in one chamber, while the contralateral chamber served as the unloaded control. After loading, a specially designed coring tool was used to extract a 6 mm cylindrical specimen of bone. The empty chambers were then sealed with a hemispherical cap, and the dogs were allowed normal cage activity while a second volume of trabecular bone infiltrated the chamber for another 8 week period. This load/extraction sequence was repeated for up to 7 biopsies per dog.

The load stimulus consisted of a trapezoidal waveform, with a maximum compressive load of 17.8 N, loading rate of 89 N/sec, at 1Hz frequency. For the experiments, the loading duration was either 30 minutes (1800 cycles), 15 minutes (900 cycles) or 2 minutes (120 cycles). In order to determine the temporal activation of FAK, a series of specimens were extracted from 4 dogs immediately, 15 minutes, or 45 minutes following a single load stimulus of 1800 cycles. In another series of experiments, designed to determine if the timepoints, but it was not sensitive to mechanical load. A 15 minute load stimulus (900 cycles) also elicited FAK phosphorylation above baseline levels regardless of whether the specimens were collected immediately (3 out of 4 experiments), or following a 15 minute delay (2/3 experiments) from the end of loading to extraction (Figure 1). A load stimulus of only 2 minutes (120 cycles) did not result in an increase in FAK phosphorylation when specimens were collected either immediately (0/3 experiments), or 28 minutes (0/1 experiments) after loading (Figure 1). Evaluation of cryosections revealed prominent FAK immunoreactivity among marrow fibroblasts and stromal cells. However, there was no immunostaining associated with osteoblastic cells on trabecular surfaces or within osteocytic lacunae (Figure 2).

Results: In 6 out of 8 experiments where a specimen was extracted immediately following a 30 minute load stimulus (17.8N @ 89N/sec, 1800 cycles @ 1Hz), the Western blot analysis demonstrated increased FAK phosphorylation as compared to the contralateral controls (Figure 1). Tyrosine phosphorylation of FAK was demonstrated at the 15 and 45 minute timepoints, but it was not sensitive to mechanical load. A 15 minute load stimulus (900 cycles) also elicited FAK phosphorylation above baseline levels regardless of whether the specimens were collected immediately (3 out of 4 experiments), or following a 15 minute delay (2/3 experiments) from the end of loading to extraction (Figure 1). A load stimulus of only 2 minutes (120 cycles) did not result in an increase in FAK phosphorylation when specimens were collected either immediately (0/3 experiments), or 28 minutes (0/1 experiments) after loading (Figure 1). Evaluation of cryosections revealed prominent FAK immunoreactivity among marrow fibroblasts and stromal cells. However, there was no immunostaining associated with osteoblastic cells on trabecular surfaces or within osteocytic lacunae (Figure 2).

Discussion: These results demonstrate that induction of tyrosine phosphorylation of FAK occurs in response to specific mechanical load stimuli. The threshold for mechanical stimulation of FAK activity appears to lie between 120 and 900 cycles of an applied load of 17.8 N. The persistent activation of FAK 15 minutes following a load stimulus of 900 cycles could be due to the recruitment of other signaling molecules to the focal adhesion complex and subsequent transphosphorylation of FAK at additional tyrosine residues. The decline in FAK mechanosensitivity 15 minutes after an 1800 cycle load stimulus may correspond to activation of structural proteins and cytoskeletal changes.

Interestingly, FAK immunolocalization was limited to the marrow stromal tissue, an area in which the load stimulus could potentially engender high strains due to the somewhat disconnected nature of the woven trabecular bone. In addition, the loading could have induced a significant amount of interstitial fluid flow through the marrow microenvironment. These local mechanical signals appear to have been perceived by the stromal fibroblasts, which subsequently increased their tyrosine kinase activity in order to propagate the process of mechanotransduction.

References:
2. Guldberg RE, et al., 1997, JBMR, 12, 8;1295-1302.

Acknowledgements: The authors thank D Kayner, B Nolan, M Stock, and K Sweet for their contributions to this work. This work was supported by the National Institutes of Health (AG00713 and AR31793) and the Whitaker Foundation.