MECHANICAL STRESS DRIVEN RELEASE OF TGF BETAP2 FROM MINERALIZED CANCELLOUS BONE

ABSTRACT: Maintenance of cancellous bone integrity through remodeling is a coordinated process that controls the accumulation of damage in the tissue and renews the matrix. The mechanisms for signaling the presence of damage, that provide for targeting of the damaged site and also for initiating the repair process are of significant interest. We have discovered a novel mechanism for the signaling of mechanical loading in bone—damaging mechanical loads release biologically significant amounts of sequestered transforming growth factor beta (TGF \(\beta_2\)) from devitalized bone matrix.

BACKGROUND: The remodeling process is intimately associated with angiogenesis and can be initiated and is regulated by mechanical loading (2,7). Parfitt (7) has recently presented a compelling hypothesis that monocytes fated to merge and form osteoclasts are targeted to their site of action using a system of adhesion and transmigration similar to that used by leukocytes. In his concept, the monocytes transmigrate at the appropriate site of the sinusoid even though it is continuously lengthening because growth factors cause the endothelial cells to present the proper binding sites only near the site to be remodeled.

Bone matrix is the largest repository of TGF\(\beta_1\) (largely TGF\(\beta_1\)) in the body (1, 10). During resorption it has been proposed that TGF\(\beta_1\) is activated and released after transcytosis along with the other material removed from the resorption site (5). Potential regulatory activities of TGF\(\beta_2\) in bone remodeling are very broad, including: 1) Increase vascular endothelial growth factor (VEGF) production by fibroblasts and endothelial cells (9). 2) Biphasically increase then decrease the rate of vascular growth (8). 3) Act as a chemotactic signal for osteoclasts, causing them to migrate to the appropriate site for formation (4, 11). 4) Promote osteoclast apoptosis (12).

As a result of the large number of biological targets for TGF\(\beta_2\) in bone, we performed experiments to test whether this growth factor could be released from devitalized bone matrix by mechanical loading.

METHODS: Four 1cm cubes of cancellous bone, two each from two femoral heads resected for total hip arthroplasty from a 31 year old and a 54 year old female were cut using a diamond blade saw (EXAKT). The cubes were cleaned of all marrow using a tap water jet. The cubes were washed sequentially five times. Wash solutions were either normal saline (PBS) or calcium buffered saline. Release of TGF \(\beta_2\) from the specimens was measured by ELISA after concentration of the wash solution using a centrifugal concentrator. Bone cubes were compressed to 50% strain to create massive matrix damage after the second and fourth washes (Fig. 1).

RESULTS: TGF \(\beta_2\) was released from all specimens in a physiologically significant amount first from the freshly cut specimens and again in response to both the first and second mechanical loads (Fig. 2). TGF \(\beta_2\) release increased with mechanical damage. In the first assay, we attribute the release of TGF \(\beta_2\) to the freshly cut surfaces of the specimen. The second assays of the unloaded specimens showed a decreased release of TGF \(\beta_2\), consistent with the new surface being washed clear of that growth factor. In both of the loading cycles damage to the matrix (new surface) was caused (50% strain certainly makes many microcracks in cancellous bone both in the initial and the second loading, 3) and a statistically significant release of TGF \(\beta_2\) occurred. The TGF \(\beta_2\) release was less in the second assays after each loading. This pattern of growth factor release is consistent with a mechanism where each loading cycle exposed fresh matrix (new microcracks) that were depleted of TGF \(\beta_2\) by the initial assay.

CONCLUSION: Our discovery of TGF \(\beta_2\) release from bone matrix driven by mechanical loading is one-hundred percent in line with what is already known about bone matrix sequestered growth factors. Demineralized bone matrix is osteogenic and the osteogenic character is, in part, caused by the release of growth factors such as TGF \(\beta_2\). What was not appreciated until our data is that mechanical loading accelerates release of TGF \(\beta_2\) even from mineralized matrix. It is virtually certain that mechanical loading will also accelerate the release of other growth factors from mineralized bone matrix. Mechanically accelerated release of growth factors from bone matrix may have profound implications for understanding how applied loading regulates bone remodeling during aging.