DEFINING THE UPPER PERMEABILITY LIMIT OF LARGE MOLECULAR WEIGHT MOLECULES IN CORTICAL BONE

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INTRODUCTION

As an organ, bone provides the largest porous surface for ion-exchange, filtration, and molecular sieving in the body; however relatively little work has been conducted to understand this function of bone. With its complex hierarchical structure, bone has various levels of porosity from its haversian capillaries (50-70 µm in diameter), canalicual channels (0.10-1 µm in diameter), pericellular, proteoglycan meshwork-filled fluid spaces (15-50 nm), and matrix microporosity (5-12nm) through which various molecules may pass [1]. Because of its nanoporous structure, cortical bone displays size exclusion properties that restrict the free diffusion of various molecules.

An understanding the permeability and molecular transport pathways in bone is integral for tissue engineering and drug delivery applications. The bone cells, i.e. osteocytes, embedded within the bone matrix can be at distances up to 250 µm away from the nearest blood supply; therefore transport through the porous network of cortical bone is vital for cell survival. Elucidation of the permeability and molecular transport pathways in bone could provide a foundation for new strategies to deliver nutrients and growth factors to promote bone regeneration and develop new treatment modalities for bone disease.

As a continuation of previous work conducted by our group, permeability of large molecular weight molecules was studied in vivo under both diffusive and convective molecular transport mechanisms in a previously developed end load model of the rat ulna [1]. In contrast to previous studies, globular fluorescent proteins including Bovine Serum Albumin (BSA, 66 kDa) and Ovalbumin (Oval, 45 kDa) were chosen as tracer molecules to exclude effects of microextrusion that may occur, e.g. in the case of polydisperse dextran molecules [3]. Hence, the goal of this study is to define the upper permeability limit of cortical bone using globular tracers of well defined size.

METHODS

To assess the permeability of cortical bone, we selected fluorescent globular protein probes with molecular weights of 45 and 66 kDa. In vivo experiments were conducted using 290-310 g Sprague-Dawley rats (5 rats per tracer group). They were anesthetized using 1.5-3% isoflurane, and 0.1 ml of tracer per gram body weight was injected into the lateral tail vein. After tracer injection, the left forelimb was loaded sinusoidally under compression from 2-14N at 2Hz using an EduraTec Elf 3200 load cell (Enduratec, Minnetonka, Minnesota). Immediately after loading, the loaded left limb and the control right limb were dissected and fixed in formalin.

After embedding in polymethylmethacrylate, 250 µm, transverse cross-sections were cut from the mid-diaphysis region of the limb using a Leica SP1600 diamond wafer saw (Leica Microsystems, Mannheim, Germany). The slices were then polished down to 100 µm and mounted. Laser scanning confocal micrographs were obtained for cross sections of the ulnae using constant settings for gain, offset, exposure (Leica Microsystems, Mannheim, Germany). Permeability of tracer at the tissue level was analyzed qualitatively, while pericellular permeability was quantified by counting pericellular spaces exhibiting fluorescence in micrographs of ulnae cross-sections.

RESULTS

Based on qualitative image analysis, large concentrations of tracer are present within the vascular regions of ulnae cross-sections and the medullary canal space. However it was observed that a greater concentration of the 45 kDa tracer was seen in mineralized matrix region of the bone than the 66 kDa, under both loaded and unloaded conditions. Most matrix permeation was seen in the anterior and posterior regions of the ulnae cross-sections. Without loading, the 66 kDa tracer showed limited to no penetration into the matrix and pericellular space. With loading, the 66 kDa tracer showed limited penetration into small areas of the posterior aspect.

To assess pericellular permeability, the percent change in pericellular spaces exhibiting tracer was measured. Loading resulted in an increase in pericellular permeability to the 45 kDa tracer (41.4% increase ± 21). Loading resulted in an increase in pericellular permeability for the 66 kDa tracer as well, however the relative difference between the loaded and unloaded sides was smaller (22% increase ± 16).

DISCUSSION

Based on the results of this study, bone exhibits an upper permeability limit virtually excluding molecules of approximately 66 kDa or larger. The results of this study corroborate those of previous studies conducted by our group and show the increased permeation of bone with load-induced convective transport [1,2]. It was of particular interest that the 66 kDa tracer did not penetrate the pericellular space, even after with mechanical loading. This study implicates the bone microporosity as the restricting factor for permeability rather than the capillary membrane porosity. Although greater penetration of the 45 kDa tracer was seen compared to the 66 kDa tracer, these data suggest that load-induced convective transport is necessary for any significant penetration of these large molecular weight tracers through the pericellular spaces within the bone matrix. Hence, the upper permeability limit for biomolecules under convective transport is in the 40-55 kDa range. This has profound implications for targeted drug delivery and tissue engineering in bone.

REFERENCES