Sulforaphane Influences Bone Microarchitecture in a Site-Specific Manner in Both Hypertensive and Normotensive Rats

Erika Kristensen1, Rochelle Nieuwenhuis1, Ali Banigesh2, Tehereh Talaieit Kozani3, Vijitha Senanayake3, Bernhard H. Juurlink2, Steven K. Boyd1

1Department of Mechanical and Manufacturing Engineering, University of Calgary, Calgary, AB, Canada; 2Department of Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, SK, Canada

ekristensen@gmail.com

Introduction: Osteoporosis is a disease in which bone mass and architecture deteriorate, leading to a greater risk of fractures. Inflammation is believed to be a factor in the development of osteoporosis through a maladaptation of the link between inflammation and bone turnover [1]. Inflammation is also associated with oxidative stress, which is an increase in intracellular reactive oxygen species (ROS)[2]. ROS cause tissue damage by initiating free radical chain reactions [3]. Sulforaphane, a phase 2 protein inducer, decreases oxidative stress by promoting antioxidant scavenging [4]. We will test the hypotheses that 1) animals undergoing oxidative stress will have osteoporotic bone, and 2) if sulforaphane inhibits oxidative stress, then the ingestion of sulforaphane will prevent the development of osteoporosis in individuals undergoing oxidative stress by mitigating inflammation causing the bone loss. The purpose of this study was to investigate the effects of sulforaphane on bone microarchitecture using a rat model of oxidative stress.

Materials and Methods: Spontaneously hypertensive stroke-prone rats (SHRsp) undergo oxidative stress, and thus are hypothesized to have osteoporotic bone. SHRsp and Sprague Dawley (SD) rats (n=5/group) were fed an experimental diet of 20μMol/kg sulforaphane mixed in corn oil by gavage. Control rats of SHRsp and SD (n=5/group) were fed corn oil by gavage. All rats were fed daily from age seven weeks to 24 weeks, at which point the rats were sacrificed and the left tibia and L4 vertebra were removed. The bones were imaged using a micro-computed tomography (micro-CT) scanner (μCT40, Scanco Medical AG, Switzerland). For the tibia, a 3D volume 3.2mm thick (200 slices) was acquired directly below the growth plate, and for the vertebra, the entire vertebral body was scanned. An isotropic voxel size of 16μm was used for both the tibiae and vertebrae. Cortical and trabecular bone were separated with user-defined contours drawn every 15 slices, and interpolated to obtain a contour for every slice. For the tibia, 200 slices directly below the growth plate were contoured, and for the vertebrae, the entire vertebral body 0.16mm (10 slices) from the growth plates was contoured. The extracted trabecular regions were Gaussian filtered (σ=1.2, supp=2) and thresholded (27% of maximal grayscale value) to extract the mineralized bone structure. To segment the cortical bone, the tibia whole bone was Gaussian filtered and thresholded in the same manner as the trabecular bone, and the trabecular bone was subtracted leaving the cortical bone. Bone architectural parameters were calculated (Image Processing Language v. 4.29d, Scanco) including bone volume ratio (BV/TV), trabecular thickness (Th.Th), trabecular number (Th.N.), trabecular separation (Th.Sp), and structure model index (SMI). Cortical thickness (Ct.Th) was calculated for the tibiae. Statistical analysis was performed using Mann-Whitney U tests and p-value of 0.01 to determine significant differences.

Results: BV/TV in the left proximal tibiae (Fig 1) and L4 vertebrae (Fig 2) are presented. A significant decrease in BV/TV was identified in the vertebrae of SHRsp rats treated with sulforaphane. However, sulforaphane treatment significantly decreased BV/TV in the tibia of the experimental SD rats (p=0.01). These results contradicted the stated hypotheses. In the tibia, BV/TV of the SHRsp rats was less than that of the SD rats, supporting the hypothesis. (IMAGE 1)(IMAGE 2)

Discussion: The results indicate site-specific differences of the effects of sulforaphane. A reduction in BV/TV was observed for both the SD and the SHRsp rats treated with sulforaphane, contrary to the expected results. Thus, sulforaphane must affect bone metabolism by a process other than the reduction of oxidative stress and inflammation. Sulforaphane is known to inhibit osteoclastogenesis [5]; it is unknown what effect sulforaphane has on osteoblastic activity.

Since genetics play an important role in bone quantity and quality in mice [6,7] it is likely that the same is true for SHRsp and SD rats, but the differences are site-specific (e.g., tibia is different, but not vertebra; Fig 1&2). The results of this study should be verified with larger groups sizes (>5/grp), or through the use of in vivo micro-CT to give insight into the patterns of bone loss and increased statistical power. Sulforaphane causes bone loss in both the normal SD and the experimental SHRsp rat in a site-specific manner, although the pathway leading to the bone loss is not yet understood.


Figure 1: BV/TV for left proximal tibiae (p<0.01)

Figure 2: BV/TV for L4 vertebrae (p<0.01)