Comparison of the Effects of Alendronate and Raloxifene in Osteopenic Sheep Using Fourier Transform Infrared Spectroscopy

INTRODUCTION
Osteoporosis is a progressive disease of bone loss resulting in fracture that is not necessarily a normal consequence of aging. This disease can affect anyone, but it primarily affects Caucasian and Asian women. Osteoporosis is commonly diagnosed after a medical history and bone mineral density scans by DXA at sites such as the hip and wrist.

Common therapies used to treat osteoporosis—and potentially stop bone loss—include bisphosphonates (such as alendronate, ALN; brand name Fosamax, Merck) and newer drugs, such as selective estrogen receptor modulators or SERMs (such as raloxifene, RLX; brand name Evista, Eli Lilly). Clinically, there is some controversy over which drug provides better results and how best to prescribe these drugs. This study addresses the hypothesis that RLX provides better results (restoring bone quality as defined by Fourier Transform Infrared spectroscopy) than ALN.

Bone mineral density (BMD) measurements do not provide sensitive information about “bone quality,” which we propose can be measured by Fourier Transform Infrared Imaging (FTIRI). We are currently investigating a sheep model of human osteoporosis to examine the effects of drug treatments on bone quality. We investigated bone changes in iliac crest biopsies (1) to allow comparison with patient data and (2) to provide sufficient cancellous bone for analysis.

FTIR parameters developed in our laboratory that are associated with bone quality include: (1) mineral-to-matrix ratio, which corresponds to the ash weight of bone [Pienkowski et al. (1997) JBMR 12:1936-1943], (2) carbonate-to-mineral ratio, which represents the carbonate portion of the mineral content, (3) crystallinity, which correlates to the crystal length in the c-axis direction [Gadalea et al. (1996) Calcif Tissue Int 58:9-16], and (4) cross-link ratio, which represents collagen maturity and is the relative ratio of nonreducible to reducible collagen crosslinks [Atti et al. (2002) Bone 31:675-684; Paschalis et al. (2001) JBMR 16:1821-8].

MATERIALS AND METHODS
Skeletally mature (4-7 yo; 169±29 lb) Ramboullet-Columbia crossbreeds (n=18) were administered a diet to induce MA and create bone loss similar to that seen in human osteoporosis [MacLeay et al. (2004) J Bone Miner Metab 22: 561-8]. At t=6 months, daily treatment (vehicle, RLX, or ALN) was administered for 6 months by a permanent cannula and similar to that seen in human osteoporosis [MacLeay et al. (2004) J Bone Miner Metab 22: 561-8]. At t=6 months, daily treatment (vehicle, RLX, or ALN) was administered for 6 months by a permanent cannula and similar to that seen in human osteoporosis [MacLeay et al. (2004) J Bone Miner Metab 22: 561-8].

TRANSILIAC crest biopsies (8 mm dia.) were removed at 6 months and at 12 months from bilateral sites, cleansed, and placed in airtight containers with 70% ethanol under an IACUC-approved protocol (CSU). Biopsies were shipped to New York and bisected axially using an ISOMET low speed saw. The cortical ends were removed from each half with the low speed saw and the cancellous bone portion was prepared for IR analysis. Samples were defatted, lyophilized, pulverized, ground with a mortar and pestle, and then formed into KBr pellets (1:20wt/wt) using a 10 ton press. A single absorbance spectrum was collected for each specimen. 2-mm thick sections were placed on BaF2 windows (25 mm x 2 mm) for infrared imaging in the mid infrared range (4000 cm⁻¹ to 700 cm⁻¹) at 4 cm⁻¹ spectral resolution and ∼7 μm spatial resolution (Perkin Elmer Spotlight). Absorbance spectra were collected for three random trabeculae on each section, for a total of 9 trabeculae per specimen. Spectra were processed as described elsewhere [Gourion-Arsiquaud et al. (2007) Curr Opin Orth 18:499-504]. Histograms for the FTIRI data were prepared so that the distribution of these parameters within each image could be assessed.

RESULTS

![Figure 1. DXA data of sheep lumbar vertebra after 12 months on MA diet, with (RLX or ALN) therapeutic intervention or without (vehicle). *P<0.001, comp. to baseline (t=0); ***P<0.05, comp. to all other treatment groups at 12 months.](image)

![Figure 2. Representative histogram of FTIRI section from sheep iliac crest biopsy taken at 12 months from sheep on MA diet with (RLX and ALN) and without therapeutic intervention (vehicle). The y-axis represents number of pixels.](image)

DISCUSSION
The DXA data shows statistically significant mineral loss at 12 months in the sheep that was given no treatment (vehicle), but this loss appears to recover in the sheep that were given RLX and ALN (Figure 1). The FTIRI data and the homogenized FTIR data show similar trends and similar magnitudes for the vehicle, RLX, and ALN data; however, histograms of the imaging data show distribution changes between these three groups (Figure 2). Histograms of mineral/matrix and crystallinity in the non-treated sheep (veh) show broad distributions of these parameters, with two populations that may reflect bone growth/turnover. These results show that BMD alone is not the best indicator of bone “quality.”

In the histograms for the treated groups, the mineral/matrix peak appears to get sharper for both groups and is noticeably sharper for the RLX group. Additionally, the crystallinity peak appears to sharpen with treatment, but the histogram peak for RLX noticeably shifts towards higher intensity values, reflecting larger crystal sizes and an absence of the second, smaller crystal size population. These results validate our hypothesis; they suggest that both treatments may restore some bone mass after bone loss and indicate that RLX may provide a unique type of treatment not typically seen with bisphosphonate treatment.

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