Sevelamer Restores Bone Volume and Improves Bone Microarchitecture and Strength in Aged Ovariectomized Rats

T. Kuber Sampath, Petra Simic, Sarah Moreno, Nikolay Bukanov, Natasa Draca, Vera Kufner, Ana Tikvica, Andrew Blair, Damir Semenski, Mladen Brncic, Steven K. Burke, and Slobodan Vukicevic

Genzyme Corporation (T.K.S., S.M., N.B., A.B.), Framingham, Massachusetts 01701; and Laboratory for Mineralized Tissues (P.S., N.D., V.K., A.T., S.V.), Medical School, University of Zagreb, Faculty of Mechanical Engineering and Naval Architecture (D.S.), and Faculty of Food Technology and Biotechnology (M.B.), HR-10000 Zagreb, Croatia

Sevelamer hydrochloride, a noncalcium phosphate binder, has been shown to reduce coronary artery and aortic calcification, and to improve trabecular bone mineral density in hemodialysis patients with chronic kidney disease. Here, we examined whether sevelamer given orally for 12 wk with normal food could restore bone volume (BV) and strength in aged ovariectomized (OVX) rats starting at 4 wk after OVX. Dual-energy x-ray absorptiometry, microcomputerized tomography, and bone histomorphometry analyses showed that OVX animals receiving sevelamer had increased trabecular BV (51%), trabecular number (43%), trabecular thickness (9%), cortical thickness (16%), mineral apposition rate (103%), bone formation rate (25%), and enhanced cortical and trabecular bone mechanical strength as compared with OVX rats. Sevelamer decreased collagen C telopeptide, increased osteocalcin levels, and decreased phosphate and magnesium levels without affecting calcium levels in the blood. Although sevelamer was not absorbed systemically, it stimulated osteoblast differentiation in BM-derived mesenchymal stem cell cultures, as evaluated by alkaline phosphatase positive colony-forming units, and inhibited recombinant human soluble receptor activator of nuclear factor-κB ligand-induced osteoclast differentiation, as evaluated by tartrate-resistant acid phosphatase positive cells in bone mineral-hematopoietic stem cell cultures. Surface enhanced laser desorption/ionization time-of-flight mass spectrometry analysis revealed that 69 proteins were differently expressed after OVX, of which 30% (20 of 69) were reversed to sham activity after sevelamer intake. PTH, fibroblast growth factor-23, and cytokine profile in serum were not significantly changed. Together, these results suggest that sevelamer in food increases the BV and improves biomechanical properties of bone in OVX rats. (Endocrinology 149: 6092–6102, 2008)
In the present study, to explore the role of sevelamer treatment on bone remodeling, we evaluated its effect in an ovariectomized (OVX) osteoporosis rat model with high bone turnover due to estrogen deficiency but with an intact kidney function. The results demonstrate that sevelamer given with food to OVX rats increased bone volume (BV) and improved bone microarchitecture and strength, suggesting a positive effect on bone remodeling.

Materials and Methods

Animals
Six-month-old Sprague Dawley rats were subjected to ovariectomy. Animals were anesthetized with an ip injection of thiopental at doses of 4 mg/kg body weight. There were 15 animals per experiment subjected to sham surgery during which the ovaries were exteriorized but replaced intact. Bilateral ovariectomies were performed in the remaining rats from the dorsal approach, and the sevelamer therapy was initiated 1 month after OVX to await the development of osteopenia. Of sevelamer, 3% was mixed with the standard rodent diet Mucedola 4RF21 (Mucedola, Settimo Milanese, Italy) and pelleted before use. Control animals received standard rodent diet Mucedola 4RF21 only. Food was given ad libitum to the animals. In total three experiments were performed as follows. Experiment 1 contained the sham (n = 15), OVX (n = 15), and OVX plus sevelamer 3% (n = 15) animals that were treated for 12 wk, starting at 4 wk after OVX. In experiment 2, to examine whether sevelamer influences bone remodeling at an earlier time point, we treated shams (n = 10), OVX (n = 10), and OVX plus sevelamer 3% (n = 10) animals 1 month after OVX for 4 wk. For experiment 3, sevelamer was given to sham (n = 10) and sham plus sevelamer (n = 10) animals for 25 wk. All experiments and protocols were approved by the School of Medicine, University of Zagreb Animal Care Committee.

In vivo and ex vivo BMD measurement by dual-energy x-ray absorptiometry (DXA)

At 4 wk intervals, the animals were scanned for bone density measurements by DXA (Hologic QDR-4000; Hologic, Waltham, MA) (9). At the end of each experiment, animals were anesthetized, weighed, and killed by cervical dislocation. The hind limbs and lumbar vertebrae were removed and fixed in 70% ethanol. The right femur and tibia, and the lumbar vertebrae (L1–L5) were used for determination of the BM content and BMD by DXA (10) equipped with Regional High Resolution Scan software (Hologic QDR-4000). The scan field size was 5.08 × 1.902 cm, resolution was 0.0254 × 0.0127 cm, and the speed was 7.25 mm/sec. The scan images were analyzed, and the bone area, BM content, and bone density of whole bones, proximal and distal metaphyses, and the shaft of femora and tibiae were determined.

Peripheral quantitative computerized tomography

Isolated femora were scanned by a peripheral quantitative computerized tomography x-ray machine (Stratec XCT Research M; Norland Medical Systems, Fort Atkinson, WI) with the software version 5.40. Volumetric content, density, and area of the total bone, trabecular, and cortical regions were determined as previously described (11, 12).

Microcomputerized tomography (microCT)

The microCT apparatus (μCT 40) and the analyzing software used in these experiments were obtained from SCANCO Medical AG (Bassersdorf, Switzerland) (13). The distal femur and fifth lumbar vertebra were scanned in 250 slices, each 13-μm thick in the doors-ventral direction (14). Three-dimensional reconstruction of bone was performed using the triangulation algorithm. The trabecular BV (mm³), trabecular thickness (μm), and trabecular separation (μm) were directly measured on three-dimensional images using the method described by Hildebrand et al. (15). The trabecular bone pattern factors and the structure model index were computed using software provided with the microCT machine (16, 17).

Histology and histomorphometry

Animals were given a sc injection of the fluorochrome calcein at 10 mg/kg (Sigma Chemical Co., St. Louis, MO) at 14 and 4 d before death. The femora were removed at death, prepared for histomorphometric analysis, and quantified using a computer-aided image analysis system (Bioquant II; R and M Biometrics, Inc., Nashville, TN) as previously described (18–20). Statistical analyses were performed using StatView 4.0 packages (Abacus Concepts, Berkeley, CA). Organs were taken for histological analyses, embedded in paraffin, cut into 10-μm thick sections, and stained with hemalaun-eosin and toluidine blue to reveal potential therapeutic adverse effects.

Biomechanical testing

Using a materials testing system (TA.HD plus Texture Analyzer; Stable Micro Systems Ltd., Surrey, UK), two types of mechanical testing were performed on the femur. A three-point bending test of the femoral shaft was used to determine the mechanical properties of the midshaft femur. The midshaft of the femur was cut into polypropylene tubes and subjected to failure at a displacement rate of 0.1 mm/sec, as described by Turner and Burr (21), using a 30-kg load cell (TA HDplus Load Cell 30 kg; Stable Micro Systems). The maximal load and stiffness were calculated from the load-displacement curve. Indentation test of the distal femoral metaphysis was used to determine the mechanical properties of cancellous bone in the marrow cavity of the distal femoral metaphysis, as described previously (22, 23).

Cell culture experiments

Bone marrow cells were harvested from femora and tibiae of rats after 4 wk sevelamer treatment, pooled, and plated at 24 × 10⁶ per well on 24-well plates in α-MEM [Life Technologies, Inc., Gaithersburg, MD, containing 10% fetal calf serum (FCS)]. Half of the volume of the media was changed on d 3. Differentiation media for osteoblasts containing α-MEM, 10% FCS, 8 mM β-glycerophosphate, 50 μg/ml ascorbic acid, and 10⁻⁸ M dexamethasone was added at d 7, and media were subsequently changed every 2 d until the termination of the culture. At d 14, single cell suspensions were washed twice with PBS and resuspended in 1 × binding buffer [0.01 M HEPES/NaOH (pH 7.4), 140 mM NaCl, and 2.5 mM CaCl₂] at a concentration of 1 × 10⁶ cells per ml. The cells were all plated (100 μl) into polypropylene tubes and coated with annexin V fluorescein isothiocyanate (5 mM), and 50 μg/ml of d 14 of the cell culture, cells from three wells per each group, was analyzed by flow cytometry. For fluorescence-activated cell sorter analyses, cells were stained with annexin V/propidium iodide (10 μl) for 15 min at 4 C in the dark. Flow cytometric analysis was performed on a FACScalibur instrument (CELLQuest software; Becton Dickinson, Mountain View, CA) by analyzing 5–10 × 10⁶ cells per sample. Cells from the remaining wells were fixed with 4% paraformaldehyde, and adherent osteoblast progenitor cells were identified by alkaline phosphatase staining (cell viability, formation of alkaline phosphatase-positive) using a commercially available kit (86-R ALP staining kit; Sigma Chemical) (24, 25). Differentiation media for osteoclasts contained α-MEM, 10% FCS, recombinant murine macrophage colony-stimulating factor (CSF) (50 ng/ml; Sigma Chemical), and recombinant human soluble receptor activator of nuclear factor-κB ligand (RANKL) (50 ng/ml; Sigma Chemical). The media and factors were added on d 1 and replaced every second day until termination at d 6. Cells from three wells per each group were analyzed by flow cytometry as described for osteoblast analysis. The remaining wells were fixed with 4% paraformaldehyde, and adherent osteoclasts were identified by tartrate-resistant acid phosphatase (TRAP) staining using a commercially available kit (Sigma Chemical). Data presented correspond to three independent experiments with three replicate wells within each experiment.

Biochemical serum and urine parameters

Serum bone formation and resorption markers were measured by commercially available kits. Serum concentration of osteocalcin was measured by ELISA using rat osteocalcin EIA kits (Biomedical Technologies Inc., Stoughton, MA). Serum concentration of C telopeptide (CTxs) was measured by ELISA using RatLaps ELISA kits (Nordic Bio-
The cytokine profiles in serum were analyzed using Rat Cytokine 24-Plex premixed kit (Millipore Corp., Billerica, MA) according to the manufacturer’s instructions. Analyzed cytokines included: eosin, granulocyte-macrophage-CSF, granulocyte-CSF, IL-1α, monocyte chemoattractant protein-1, macrophage inflammatory protein 1α, IL-4, IL-1β, IL-2, IL-6, IL-9, IL-10, IL-12p70, IL-5-5, interferon γ, IL-17, IL-18, inducible protein-10, growth regulated oncogene KC, RANTES, TNF-α, and vascular endothelial growth factor. For each sample, duplicate wells were performed using appropriate dilution. The fluorescent intensity value from each sample was calculated using standard curves to determine the cytokine concentration. Serum values of PTH and FGF-23 were measured by Rat intact PTH ELISA kits (Immutopics, San Clemente, CA) and FGF-23 ELISA kit (Kainos Laboratories, Tokyo, Japan), respectively, according to the manufacturers’ instructions.

### Proteomics

**Surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF) profiling of serum.** We used SELDI-TOF mass spectrometry (MS) (26, 27) to identify serum protein signatures to assess the changes in OVX rats compared with sham and OVX plus sevelamer-treated animals. To decrease the complexity of samples and increase the resolution, serum samples were fractionated before loading on the chip. The fractionation was performed by using strong anion exchange chromatography with the ProteinChip Q Filtration Plate provided in the Serum Fractionation Kit (Bio-Rad Laboratories, Inc., Hercules, CA) according to the manufacturer’s instructions. Three different protein chips were used for protein profiling of fractionated serum samples: CM10 (weak cation exchanger) under low stringency conditions, IMAC30 (metal binding proteins), and H50 (hydrophobic binding). The corresponding binding buffers were as follows: CM10 low-stringency buffer (0.1 M sodium acetate (pH 4.0)); IMAC30 buffer (surface was charged with 0.1 M cupric sulfate, neutralized with 0.1 M sodium acetate (pH 4.0), and 0.1 M sodium phosphate, 0.5 M sodium chloride (pH 7.0) was used for binding); and H50 buffer (surface was prewashed with 50% acrylonitrile (ACN), and binding was completed in 10% ACN, 0.1% trifluoroacetic acid). Chips

**Fig. 1.** In vivo BMD measurement of total body (A), hind limbs (B), and lumbar spine (C) in aged OVX rats treated with 3% sevelamer in normal food for 12 wk, 4 wk after OVX. Data are mean ± SEM. *, Significantly different from OVX (ANOVA Dunnett test, P < 0.05) (n = 15 in all groups) Ex, Ex vivo.

**Fig. 2.** Ex vivo BMD measurement of bones in aged rats treated with 3% sevelamer in normal food for 12 wk, 4 wk after OVX. Ex vivo total femur (A), total tibia (B), and lumbar spine BMD (C) revealed that 3% sevelamer was effective in increasing BMD. Data are mean ± SEM. *, Significantly different from OVX (ANOVA Dunnett test, P < 0.05) (n = 15 in all groups).
were equilibrated twice with 150 µl of the binding buffer and loaded twice with 25 µl fractionated serum. After incubation at room temperature for 30 min, unbound proteins were removed by washing three times with 150 µl binding buffer and three times with 150 µl deionized water. After the chip surface was dried, 1 µl sinapinic acid (Bio-Rad Laboratories) dissolved in 50% ACN, 0.5% trifluoroacetic acid was applied twice to the loaded surface and then dried. The ProteinChip System, Series 400 (Bio-Rad Laboratories), was used to analyze bound proteins by SELDI-TOF. Spectra were collected between 2 and 100 kDa with a focus mass of 8 kDa. There were 530 shots collected with a laser energy of 3000 nJ. Mass calibration was performed with ProteinChip All-in-One Protein Standard II (Bio-Rad Laboratories). All spectra were analyzed using the CiphergenExpress Software 3.0 (Bio-Rad Laboratories). Spectra were corrected for baseline and noise between 2 and 100 kDa. Total ion current was used to normalize between 2,000 and 100,000 m/z. Peak detection was performed in automatic mode with first pass.
signal-to-noise greater than three with a minimum peak threshold of 10% of total spectra. Second pass had an signal-to-noise greater than two between the m/z ranges of 2,000 and 100,000. Three groups of serum samples from rats treated for 4 wk with sevelamer were profiled using SELDI-TOF technology: 1) sham (n = 10), 2) OVX (n = 10), and 3) OVX treated with sevelamer 3% (n = 8). All fractions for each serum sample were analyzed independently. Analysis of individual spectra was performed with the CiphergenExpress Software 3.0.

Two-dimensional (2-D) electrophoresis and HPLC/MS/MS analysis. Serum was depleted of highly abundant proteins (transferrin, albumin, and IgG) by using a mouse antibody based affinity spin cartridge (Agilent Technologies, Palo Alto, CA). After depletion, samples were precipitated with trichloroacetic acid/acetone and analyzed by 2-D electrophoresis, first by isoelectric focusing and then by SDS-PAGE. Gels were stained with Deep Purple (Amersham Biosciences, Piscataway, NJ) and scanned with a Typhoon 9200 Variable Mode Imager (Amersham Biosciences), and subsequently analyzed using Phoretix software (Kendrick Labs, Madison, WI). To identify proteins of interest, relevant spots were excised from a representative gel and sent for analysis via HPLC/MS/MS on a Thermo LCQ Deca ion trap mass spectrometer (Tufts University Core Facility, Boston, MA).

Statistical analysis. Distributions of quantitative data were tested with the Kolmogorov-Smirnov test. All data measurements within the same time point were analyzed with one-way ANOVA and the one-sided Dunnett t post hoc test against OVX and sham animals.

Results
Sevelamer restores lost bone in OVX rats

To assess the therapeutic effect of sevelamer, OVX rats were left untreated for 4 wk to allow the development of osteopenia before the initiation of a 12-wk sevelamer treatment regimen. By 12 wk after the initiation of sevelamer treatment, in vivo BMD measurements of the hind limbs and the lumbar spine were elevated 4%, and the total body BMD was elevated 3% in rats treated with sevelamer compared with untreated OVX animals (Fig. 1). Ex vivo DXA measurements showed an increase in femur BMD of 7%, tibia of 10%, and the lumbar spine of 9% (Fig. 2). microCT measurements of the distal femur demonstrated that sevelamer treatment increased BV/trabecular volume by 51%, and the trabecular number by 43%, with a modest change in the trabecular thickness of 9% (Fig. 3). Decreased trabecular pattern formation and structural model index further suggested that sevelamer improved the trabecular bone structure and connectivity. Importantly, a significant increase in the cortical thickness of 16% was achieved in animals receiving 3% sevelamer (Fig. 3).

Histological analyses showed that sevelamer increased the trabecular BV, thickness, and number, and decreased the trabecular separation, confirming microCT results. Dynamic histomorphometric bone parameters revealed a 103% higher mineral apposition rate in sevelamer-treated rats compared with untreated OVX rats (Fig. 4). Double-labeled lengths of tetracycline surfaces were 91% longer in sevelamer-treated rats compared with OVX rats. Most importantly, bone formation rate was increased 25% in sevelamer-treated OVX rats compared with OVX rats. Most importantly, bone formation rate was increased 25% in sevelamer-treated OVX rats compared with OVX control animals (Fig. 4).

The three-point bending test was used to determine the mechanical properties of the midshaft femur. Maximal load and stiffness were increased 17 and 15%, respectively, in sevelamer-treated animals compared with OVX rats (Table 1). The indentation test was used to determine the mechanical characteristics of trabeculae of the distal femoral metaphyses. The direct parameters maximal load, stiffness, and energy absorbed were increased about 12, 4, and 12-fold, respectively, in sevelamer-treated rats compared with OVX animals (Table 1). The ultimate strength showed the same trend of a 12-fold increase. Sevelamer treatment improved the trabecular bone parameters compared with OVX rats but did not restore the mechanical properties to those exhibited by sham animals.

Fig. 4. Histology and histomorphometry of distal femora of OVX rats treated with 3% sevelamer. Sevelamer increased double-labeled length (dL.Le), interlabel thickness (IrL.Th), mineral apposition rate (MAR), and bone formation rate over bone surface (BFR/BS), bone formation rate over BV and bone formation rate over trabecular volume (BFR/TV) as evidenced on hemalaun-eosin stained (magnification, ×12) sections. Data are mean ± sem. *, Significantly different from group OVX (ANOVA Dunnett test, P < 0.05) (n = 15 for all groups).
Sevelamer influences activity of osteoblasts and osteoclasts from the bone marrow

Sevelamer treatment of OVX rats stimulated ex vivo the differentiation of BM- mesenchymal stem cells (MSCs) into osteoblast lineage (70%) compared with cells isolated from the bone marrow from OVX rats, as examined by the number of alkaline phosphatase-positive colony forming units, which were comparable to bone marrow cells from sham rats. Furthermore, sevelamer treatment decreased the number of apoptotic osteoblasts by 37% compared with osteoblasts derived from the bone marrow of OVX animals. Further analysis of this set of markers in sevelamer-treated rats resulted in a subset of 20 markers, which expression became similar to sham animals (Fig. 7B). In this subset we identified seven markers that were up-regulated (Fig. 7B, cluster b-1) and 13 markers that were down-regulated after sevelamer intake (Fig. 7B, cluster b-2). This subset of sevelamer-affected markers represents approximately 30% (20 of 69) of markers found to be differentially expressed between sham and OVX animals. These findings support a profound systemic effect after sevelamer treatment on the total serum proteome in OVX animals. Although biomarkers generated with SELDI-TOF technology were anonymous, the technology is very reliable to generate biomarker signatures for estimation of the total treatment effect on the serum proteome. 2-D electrophoresis and HPLC/MS/MS analysis of serum proteins showed that heptoglobin, glutathione peroxidase-3, and retinol binding protein-4 were expressed at a high degree of similarity between spectra from the same experimental group, suggesting good reproducibility of data (Fig. 7A). Clustering analysis using the CiphergenExpress software 3.0 revealed that 69 proteins were differentially expressed in two experimental groups, sham and OVX (with at least 1.5-fold differences in expression). A heat map generated for these markers was shown on the left in Fig. 7B.

Further analysis of this set of markers in sevelamer-treated rats resulted in a subset of 20 markers, which expression became similar to sham animals (Fig. 7B). In this subset we identified seven markers that were up-regulated (Fig. 7B, cluster b-1) and 13 markers that were down-regulated after sevelamer intake (Fig. 7B, cluster b-2). This subset of sevelamer-affected markers represents approximately 30% (20 of 69) of markers found to be differentially expressed between sham and OVX animals. These findings support a profound systemic effect after sevelamer treatment on the total serum proteome in OVX animals. Although biomarkers generated with SELDI-TOF technology were anonymous, the technology is very reliable to generate biomarker signatures for estimation of the total treatment effect on the serum proteome. 2-D electrophoresis and HPLC/MS/MS analysis of serum proteins showed that heptoglobin, glutathione peroxidase-3, and retinol binding protein-4 were expressed at a lower level in OVX rats, and sevelamer treatment increased/ stabilized the levels (data not shown).

Sevelamer increases bone mass in sham rats

To assess the effect of sevelamer on bone in age-matched healthy rats, sevelamer was given with the food for a period of 25 wk to sham animals. By 12 wk after the initiation of sevelamer treatment, in vivo BMD measurements of the hind limbs and lumbar spine showed no significant differences compared with sham rats. However, at 24 wk after the sevelamer intake, the BMD of hind limbs was increased by 5% and of the lumbar spine by 6% compared with untreated sham animals (Fig. 8).

Discussion

We have shown in a rat model of osteoporosis that OVX animals receiving sevelamer in food have increased BV, improved microarchitecture, and bone strength compared with control OVX rats. The use of sevelamer in dialysis patients has been associated with reduction in coronary artery and aortic calcification, as well as with a survival benefit (28). Moreover, it has also been found that sevelamer increases the trabecular BMD in chronic kidney disease (CKD) (stage 5) patients (8). When used to correct hyperphosphatemia, calcium salts lead to a reduction in thoracic trabecular and
cortical bone density, whereas sevelamer-treated patients had a higher BMD, increased serum bone-specific alkaline phosphatase, osteocalcin, and PTH. In patients with low intact PTH levels, sevelamer decreased collagen C telopeptide and increased serum osteocalcin levels (29), suggesting that it elicits bone remodeling response systemically, although sevelamer was not absorbed. The observation that sevelamer therapy decreases collagen C telopeptide and increases osteocalcin levels in OVX rats further corroborates this notion. In a randomized controlled trial performed to test the effects of sevelamer and calcium carbonate (CaCO₃) on bone mineralization and turnover using iliac crest bone biopsies in 119 chronic hemodialysis patients, Ferreira et al. (30) showed that sevelamer increased the bone formation and bone microarchitecture, and maintained the mineralization compared with CaCO₃. Furthermore, a positive effect of sevelamer on bone remodeling was demonstrated in a rat model of CKD-induced vascular calcification and adynamic bone disease; sevelamer therapy reduced established vascular calcification, normalized the serum phosphorus, and reversed the CKD-induced trabecular osteopenia by increasing osteoblast and osteoid surfaces, and more importantly the cortical bone density, whereas sevelamer-treated patients had a higher BMD, increased serum bone-specific alkaline phosphatase, osteocalcin, and PTH. In patients with low intact PTH levels, sevelamer decreased collagen C telopeptide and increased serum osteocalcin levels (29), suggesting that it elicits bone remodeling response systemically, although sevelamer was not absorbed. The observation that sevelamer therapy decreases collagen C telopeptide and increases osteocalcin levels in OVX rats further corroborates this notion. In a randomized controlled trial performed to test the effects of sevelamer and calcium carbonate (CaCO₃) on bone mineralization and turnover using iliac crest bone biopsies in 119 chronic hemodialysis patients, Ferreira et al. (30) showed that sevelamer increased the bone formation and bone microarchitecture, and maintained the mineralization compared with CaCO₃. Furthermore, a positive effect of sevelamer on bone remodeling was demonstrated in a rat model of CKD-induced vascular calcification and adynamic bone disease; sevelamer therapy reduced established vascular calcification, normalized the serum phosphorus, and reversed the CKD-induced trabecular osteopenia by increasing osteoblast and osteoid surfaces, and more importantly the

table 2. Biochemical parameters of serum from sevelamer-treated aged OVX rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>PO₄ (mmol/liter)</th>
<th>Ca (mmol/liter)</th>
<th>Mg (mmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>1.47 ± 0.35⁸</td>
<td>3.04 ± 0.11</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>OVX</td>
<td>1.83 ± 0.24</td>
<td>2.74 ± 0.08</td>
<td>0.92 ± 0.06</td>
</tr>
<tr>
<td>OVX + Sevelamer 3%</td>
<td>1.53 ± 0.26</td>
<td>2.83 ± 0.10</td>
<td>0.80 ± 0.11</td>
</tr>
</tbody>
</table>

Animals were treated for 12 wk starting at 4 wk following OVX; (n = 12 in all groups). Data are mean ± SEM. Significant differences are indicated with respect to OVX group. PO₄, Phosphates.

⁸ P < 0.05 by ANOVA Dunnett test.
FIG. 7. SELDI-TOF profiling of serum samples. A, Gel representation of individual serum samples from three experimental groups (m/z within range of 2–6.5 kDa; CM10 surface): sham, OVX and OVX plus sevelamer 3%. B, Clustered heat map (left) provides an overview of the relative expression levels of 69 differential markers (at least \( \times 1.5 \) fold difference of expression in sham vs. OVX). The expression levels of markers are represented in color (red and green correspond to up-regulated and down-regulated, respectively; black indicates no change in expression). Heat map represents intensities digitally averaged from individual samples. Clustering analysis identified several clusters of markers that shifted their expression back to the sham level after treatment with sevelamer (right).
bone formation rate in the metaphyseal trabeculae of rat tibia and femur (29, 31).

To explore the role of sevelamer intake in bone remodeling, we examined its effects in an aged OVX osteoporosis rat model with high bone turnover due to estrogen deficiency with an intact kidney function. In aged OVX rats, sevelamer reduces serum phosphorus level without an increase in free serum calcium. This transient decrease in serum phosphorus levels may have increased the pulsate secretion of PTH, thus stimulating increased bone formation. However, PTH and FGF-23 serum levels were not different from OVX rats with intact kidney function, examined at 4 wk sevelamer therapy, suggesting a difficulty in capturing potentially minute changes due to pulsate secretion at one time interval. Sevelamer treatment in hemodialysis or peritoneal dialysis patients increased the serum levels of intact PTH (8). In a subsequent study, sevelamer reduced the serum calcium concentration in hemodialysis or peritoneal dialysis patients increased the serum levels of intact PTH (8). In a subsequent study, sevelamer reduced the serum calcium concentration in hemodialysis or peritoneal dialysis patients increased the serum levels of intact PTH (8). In a subsequent study, sevelamer reduced the serum calcium concentration in hemodialysis or peritoneal dialysis patients. Although sevelamer is not absorbed systemically, it reduces serum nonhigh-density lipoprotein cholesterol levels and improves the lipid profiles of hemodialysis patients (31). Furthermore, sevelamer therapy has reduced high-sensitivity C-reactive protein and increased fetuin levels, suggesting an antiinflammatory effect (45). In the present study, we show that sevelamer therapy was able to reverse expression of 30% of proteins that were differentially expressed (up-regulated or down-regulated) in the serum of OVX rats compared with sham animals, suggesting that sevelamer has a profound systemic influence on total proteome in OVX rats. Identification of these sevelamer-responsive protein biomarkers would provide a better understanding of sevelamer mechanism of action in bone remodeling. The observation that sevelamer increased serum levels of haptoglobin, glutathione peroxidase-3, and retinol binding protein-4 in OVX rats (data not shown) suggests a positive role against oxidative stress, hypoxia, and systemic fibrosis (38, 46–49). The effects of sevelamer against oxida-
tive stress-induced systemic inflammation remain to be examined. Further evaluation of sevelamer in healthy age-matched sham rats showed a significant improvement after a longer treatment (25 wk) at hind limbs and the lumbar spine compared with untreated sham animals, suggesting that sevelamer effects are more rapidly pronounced in O VX rats due to a higher bone turnover and a negative bone remodeling. Collectively, these results show that sevelamer improves the BV, microarchitecture, and bone strength in O VX animals.

Acknowledgments

We thank Dijurdjica Car and Mirjana Palcic, University of Zagreb, for their expert technical help in performing animal studies, Tri-Hung Nguyen for cytokine profiles, and Ave Taylor for technical help in performing two-dimensional electrophoresis. We also thank Yves Sabbage for PTH and fibroblast growth factor-23 analyses, and interpretation of the data, and Snivas Shankara for helpful suggestions in interpreting cytokine profiles. We thank Brian DelGiudice for help in the preparation of figures.

Received February 12, 2008. Accepted July 29, 2008.

Address all correspondence and requests for reprints to: T. Kuber Sampath, Ph.D., Genzyme Corporation, One Mountain Road, Framingham, Massachusetts 01701. E-mail: kuber.sampath@genzyme.com; or Sampath, Ph.D., Genzyme Corporation, One Mountain Road, Framingham, Massachusetts 01701. E-mail: sampath.m.e@genzyme.com.

This work was supported by Genzyme Corporation through a Sponsored Research Agreement with University of Zagreb Medical School. Present address for S.K.B.: Protein Therapeutics, Inc., 200 West Street, Walhalls, Massachusetts 02451.

Disclosure Statement: T.K.S., S.M., N.B., and A.B are employed by Genzyme Corporation. S.K.B. was previously employed by Genzyme Corporation. S.K.B. has nothing to declare.

References


