Connecticut Health Center, Farmington, CT, USA, 2Groupe Servier, France. In MSC cultures, continuous treatment with strontium ranelate (3 mM) for 7 days increased ALP activity in this system (preliminary data). To summarize, strontium ranelate stimulates bone formation and increases BMD in OVX rats. These results suggest that OSB may be efficacious as bone anabolic drugs for the treatment of osteoporosis.

Disclosures: S. Choudhary, Osteopharm Inc 1, C. Amantea*1, K. Nguyen*2, D. Squires*1. Osteopharm Inc, Courbevoie Cedex, France 2.


It was previously reported that a fragment of the human CXC chemokine neutrophil activating peptide-2 (NAP-2, CXCL7) stimulated bone mineral apposition in rats (Tam 2004, US Pat.No. 6,693,081). The purpose of this study was to determine whether the modified 8 amino acid peptide Ac-TSGHHPK-amide derived from CXCL7 (OSB) would increase bone formation in ovariectomized (OVX) rats. Virgin SD rats were OVX or sham operated at 23 weeks of age and placed into groups (N=12) on the basis of DEXA measurements of bone and body composition. Eight weeks after surgery the rats were killed, serum samples were collected for biomarker analysis and bones were collected for terminal analysis by pQCT or fixed for histology. Rats treated with OSB had significantly increased total body BMC and BMD compared with OVX control (p=0.03). Terminal analysis of the lumbar spine (L3) by pQCT demonstrated that OSB significantly increased total BMC and BMD (p=0.02) and cortical area and BMC (p=0.006) relative to the OVX control. For tibia and femur, the OSB treated group had significantly higher trabecular BMC and BMD (p=0.03) at metaphyseal sites and higher cortical area and BMC (p=0.04) at diaphyseal sites. The OSB group had significantly higher total BMC and BMD (p=0.03) at metaphyseal sites compared with the OVX control. Analysis of blood samples for markers of collagen degradation showed no differences between OVX and OSB treated animals. Samples analyzed for osteocalcin showed an increase with OSB treatment that was greater in males compared with females (p=0.09). Based on these results we conclude that OSB stimulates bone formation and increases BMD in OVX rats. These results suggest that OSB may be efficacious as bone anabolic drugs for the treatment of osteoporosis.


The novel peptide OSB was derived from the sequence of a cDNA clone in a fetal human liver library. A series of structurally related 10 amino acid peptides were developed based on the active region of the original peptide (Tam 2004, US Pat.No. 6,693,081). The aim of the present study was to test the efficacy of these novel peptides in bone formation. Neonatal rat calvaria (3-5 days old) were cultured with 1 of 3 OSB peptides (10^-5 M), or IGF-1 (50 ng/ml) or left untreated. Mineralized matrix and synthesis were studied based on incorporation of 3H-proline. Calvaria treated with OSB had a significantly higher incorporation of 3H-proline than the untreated control (p<0.05), similar to the IGF-1-treated calvaria. Virgin SD rats were OVX or sham operated at 23 weeks of age and placed into groups (N=12) on the basis of DEXA measurements of bone and body composition. Eight weeks after surgery the rats were killed, serum samples were collected for biomarker analysis and bones were collected for terminal analysis by pQCT or fixed for histology. OSB treated rats had significantly higher incorporation of 3H-proline compared with the untreated control (p<0.05). Terminal pQCT analysis indicated that rats treated with one OSB peptide analog (155M) had significantly higher trabecular BMD at lumbar vertebrae (p<0.05) and at metaphyseal sites in the tibia (p=0.007) and femur (p=0.03) than the OVX rats treated with vehicle. Total BMD of the femoral neck was significantly higher and body weight in the group treated with OSB 155M than in the OVX control. Serum samples tested for markers of bone resorption (RatLaps) or formation (osteocalcin) showed no significant differences between the groups, possibly due to the large variations observed within each group. These results demonstrate that OSB peptides stimulate bone formation in vivo and in vitro. Further these peptides may be useful in the treatment of osteoporosis.

Disclosures: G. O. Ramirez-Yañez, Osteopharm Inc 3.

Characterization of Osteogenic Oxytostin and their Molecular Mechanism(s) of Action. J. A. Richardson*, L. M. Amantea*, K. Neveu*, M. E. Jung*, T. J. Hult* E. Pachani, Modex Corp, UCLA, Los Angeles, CA, USA, Chemistry, UCLA, Los Angeles, CA, USA.

Identification of anabolic agents that enhance bone formation is critical for the better management of bone fractures and osteoporosis. We previously reported that specific oxytocin compounds, products of cholecystokinin, used in combination have potent osteogenic properties when administered to osteoporotic mice in vitro and to neonatal mouse calvarial organ cultures ex vivo. The oxytocin combinations with osteogenic

LOW DOSE STRONTIUM INCREASED THE FORMATION OF NEW LAMELLAR BONE AT DIAPHYSEAL SITES. The novel peptide OSA was derived from the sequence of a cDNA clone in a fetal human liver library. A series of structurally related 10 amino acid peptides were developed based on the active region of the original peptide (Tam 2004, US Pat.No. 6,693,081). The aim of the present study was to test the efficacy of these novel peptides in bone formation. Neonatal rat calvaria (3-5 days old) were cultured with 1 of 3 OSB peptides (10^-5 M), or IGF-1 (50 ng/ml) or left untreated. Mineralized matrix and synthesis were studied based on incorporation of 3H-proline. Calvaria treated with OSB had a significantly higher incorporation of 3H-proline than the untreated control (p<0.05), similar to the IGF-1-treated calvaria. Virgin SD rats were OVX or sham operated at 23 weeks of age and placed into groups (N=12) on the basis of DEXA measurements of bone and body composition. Eight weeks after surgery the rats were killed, serum samples were collected for biomarker analysis and bones were collected for terminal analysis by pQCT or fixed for histology. OSB treated rats had significantly higher incorporation of 3H-proline compared with the untreated control (p<0.05). Terminal pQCT analysis indicated that rats treated with one OSB peptide analog (155M) had significantly higher trabecular BMD at lumbar vertebrae (p<0.05) and at metaphyseal sites in the tibia (p=0.007) and femur (p=0.03) than the OVX rats treated with vehicle. Total BMD of the femoral neck was significantly higher and body weight in the group treated with OSB 155M than in the OVX control. Serum samples tested for markers of bone resorption (RatLaps) or formation (osteocalcin) showed no significant differences between the groups, possibly due to the large variations observed within each group. These results demonstrate that OSB peptides stimulate bone formation in vivo and in vitro. Further these peptides may be useful in the treatment of osteoporosis.

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Characterization of Osteogenic Oxytostin and their Molecular Mechanism(s) of Action. J. A. Richardson*, L. M. Amantea*, K. Neveu*, M. E. Jung*, T. J. Hult*, E. Pachani, Modex Corp, UCLA, Los Angeles, CA, USA, Chemistry, UCLA, Los Angeles, CA, USA.
activity consisted of 22R(-) or 22S(S)-hydroxycholesterol with 20(S)-hydroxycholesterol (RS and SS, respectively). Recently we found that the oxysterol 20S, when used alone at doses of 5-15 µM in cultures of marrow stromal cells, M3-M1094 (M2), induced the formation of mature osteoclasts that were demonstrated by the induction of alkaline phosphatase (ALP) activity, Runx2 DNA binding and protein expression, osteocalcin (OCN) mRNA expression, and mineralization. Treatment or co-treatment of M2 cells with 22R or 22S osteoxysterol greatly enhanced the osteogenic effects of 20S suggesting that 22S and 22R prime the osteoprogenitor cells for better responsiveness to 20S. We have identified other oxysterols with osteogenic properties when used alone or in combination with 22R or 22S. These newly identified oxysterols are 5-cholen-3, 20a-diol 3-acetate, 20a,25-epoxycholesterol, 24a,25-epoxycholesterol (also known as cebrebrosterol), and 26-hydroxycholesterol of which the major markers of osteogenic differentiation in M2 cells. In contrast, 4(S)-hydroxycholesterol and 7α-hydroxycholesterol did not have any osteogenic properties suggesting that the carbon side chain of the sterols and the position of the hydroxyl groups are important characteristics of the osteogenic sterols. Structurally similar molecules to oxysterols, estrogen, estrone, and -estradiol, which do not have the carbon side chain and have in position and number of their double bonds, did not have any osteogenic properties. Pretreatment of M2 cells with the hedgehog signaling inhibitor, cyclopamine (10-10 M), significantly inhibited SS-induced ALP activity, Runx2 protein and OCN mRNA expression, and mineralization. In addition, pretreatment of cells with the inhibitor of Wnt signaling, DKK-1 (1 µg/ml), significantly inhibited induced SS-induced ALP activity, OCN expression and mineralization but not Runx2 protein expression. These results suggest that the osteoclast-induced osteogenic differentiation of cells is mediated through hedgehog- and Wnt-dependent mechanisms. Osteoclasts form a new class of osteoinductive cells that may be useful in the enhancement of local and systemic bone formation.

Disclosures: F. Parnami. None.

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Systemic Administration of Thyroid Stimulating Hormone (TSH) Prevents and Restores Bone Loss in Rats Following Ovariectomy
E. Simic*, R. Sendak, N. Drage*, S. Schavi*, J. McPherson*, S. Vukicevic*
Genzyme Corporation, Framingham, MA, USA; Laboratory for Mineralized Tissues, Zagreb Medical School, Zagreb, Croatia.

Thyroid stimulating hormone (TSH) affects bone remodeling as demonstrated by reduced bone mass in TSH receptor knockout mice (Abe E, et al, Cell 115: 130-140, 2003) and in post-menopausal euthyroid patients following single administration of human TSH (Maxxart G, et al, JBMR 22: S90, 2005). In the present study, we examined whether the systemic administration of TSH could prevent and restore bone loss in an osteoporosis animal model. Female SD rats were ovariectomized (OVX) at 6 months and the TSH therapy was initiated immediately following OVX (day 0). At 4, 8 and 12 months (restoration mode). Animals were divided into six groups (12 rats/group): (1) Sham, (2) Ovariectomized (OVX), (3) OVX + TSH (low dose), (4) OVX + TSH (medium dose), (5) OVX + TSH (high dose) and (6) OVX + TSH (recombinant human TSH) (Thyrogen®) (0.7, 7.0 and 70 µg/rat). Animals were killed at 3 and 15 months of age. pQCT analyses. Serum biochemical analyses suggest that TSH suppresses the ovariectomy-induced bone turnover by decreasing osteocalcin and C-telopeptide levels to sham values. Importantly, low TSH doses had no effect on serum T3 and T4 values, suggesting TSH at these levels may have a direct effect on bone without affecting the thyroid axis. In vitro studies demonstrated two basic cellular responses in rat calvaria cultures. These data corresponded well with observations in vivo of a rapidly proliferating periosteum and its subsequent mineralization. Bioactivity was sequence dependent and resided in two novel and complimentary sequence motifs in the BCSP-1 molecule. The synthesis of these motifs by cell phase peptide chemistry and their subsequent testing in a rat tibia model of bone formation validated the collagen origin of the original BCSS bovine extracted material. The actions of BCSP peptides on cell interactions in osteogenesis demonstrated specificity toward bone cells present in the periosteum as noted by the absence of heterotopic or ectopic bone formation. The rapid onset of the stimulatory effect and the absence of ectopic bone formation make the BCSP peptides a potent site-specific candidate for the treatment of bone fracture and trauma. Osteoinductive BCSP is currently in development for the treatment of local bone repair and systemic treatment of various skeletal diseases.

Disclosures: D. Sindery, Millenium Biologics, 1, 1.

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Department of Internal Medicine, Dankook University College of Medicine, Cheon-An, Republic of Korea, 2Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea.

Postmenopausal osteoporosis is characterized by increased bone resorption due to estrogen deficiency. RANK-Fc, a fusion protein that specifically blocks RANK binding to RANK, has been known to be efficient and well-tolerated in animal models of osteoporosis. Here we show that cell-based gene therapy with RANK-Fc effectively prevented bone loss in ovariectomized (OVX) mice. Twenty-four young adult female C57B1 mice were used and repeated intraperitoneal injection of mesenchymal stem cells(MSCs) transduced with retrovirus was performed as follows: (1) Sham-operated mice (SHAM, n=6) (2) OVX mice treated with PBS (OVX-P, n=6) (3) OVX mice injected with MSCs cells transduced with control retrovirus (OVX-GRP, n=6) (4) OVX mice injected with MSCs transduced with RANK-Fc (OVX-RANK-Fc, n=6). Cellular expression of RANK-Fc was confirmed by Western blot analysis of cell lysates and conditioned medium, and also by ELISA for the mice sera. Microcomputed tomography (micro-CT) and quantitative microcomputed absorptiometry (QPixus) revealed that OVX-RANK-Fc group showed significantly higher BMD (p=0.05) than either the OVX-P group or OVX-GRP group after 8 weeks. The expression of GFP, which is co-expressed with RANK-Fc was observed in the liver, spleen, and intra-abdominal fat of mice but not in femur or freshly isolated bone marrow. Our results suggest that expression of RANK-Fc by genetically modified MSCs may be a feasible option for ameliorating the OVX-induced bone loss.

Disclosures: D. Kim, None.

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Lumbar Spinal Mobility and Back Extensor Strength Are Important Factors for Quality of Life in Patients with Osteoporosis. N. Miyakoshi, M. Hongo, S. Maekawa, Y. Ishikawa*, Y. Shimada*, E. Itoi*.
Orthopedic Surgery, Akita University School of Medicine, Akita, Japan.

We have recently demonstrated that quality of life (QOL) in patients with osteoporosis is affected by the total spinal mobility and that the back extensor strength is the most significant contributor to the total spinal mobility. However, how much thoracic and lumbar spinal mobilities affect QOL has not been clarified. In this study, we evaluated the relation between QOL and thoracic and lumbar spinal mobilities and their related factors in patients with osteoporosis. A total of 174 postmenopausal women with osteoporosis aged over 50 years (mean, 66 years) were included in this study. Their QOL was evaluated using the Japanese Osteoporosis QOL Questionnaire (JOQOL) proposed by the Japanese Society for Bone and Mineral Research. JOQOL contains six domains with higher scores indicating higher levels of QOL. Bone mineral density (BMD) of the lumbar spine, proximal femur, and whole body were measured with dual-energy X-ray absorptiometry. The kyphosis angle and range of motion (ROM) of thoracic and lumbar spine were measured in the upright position and at maximum flexion/extension with a computer-assisted device (SpinalMousetm). The number of vertebral fractures was evaluated with lateral radiographs of the spine. Bilateral grip strengths and isometric back extensor strength were evaluated with dynamometers. JOQOL showed significant correlation (p=0.05) with age (r=0.303), back extensor strength (r=0.455), grip strengths of dominant and non-dominant hands (r=0.275 and r=0.255, respectively), number of vertebral fractures (r=0.282), BMDs of proximal femur and whole body (r=0.200 and r=0.157, respectively), lumbar kyphosis angle (r=0.296), and lumbar spinal ROM (r=0.345). Among these factors, the multiple regression analysis revealed that the back extensor strength and lumbar spinal ROM were the significant contributors to the JOQOL. We conclude that back extensor strength and lumbar spinal mobility (but not thoracic mobility) are the important factors for QOL in patients with postmenopausal osteoporosis.

Disclosures: N. Miyakoshi, None.