Different prostate cancer bone metastasis models respond differently to treadmill exercise (Abstract only)

ARREDONDO, Hector, SPRULES, Alexandria, EATON, Colby and WANG, Ning

delayed bone regeneration, and increased risk of fractures. The use of electroacupunture (EA) for postmenopausal osteoporosis treatment is recent and has been positive for bone quality improvement.

Objective: To verify the effects of EA on the regeneration process in the bone defect in the tibias of ovariectomized rats.

Methods: 48 female Sprague-Dawley rats (aged six weeks) were subdivided into four groups (n=12): OVXDEA: ovariectomy (OVX) + bone defect. + EA; OVXD: OVX + bone defect, without EA; SDEA: SHAM surgery + bone defect + EA; SD: SHAM surgery + bone defect, without EA. OVX surgery was performed. After 90 days, the tibial bone defect was performed bilaterally. EA protocol started after 24h of the bone defect, and used the Zusanli (ST36) Sanyinjiao (SP6) acupoints. Therapy occurred once a day for 20 minutes, for three cycles of 10 days, with one day intervals between them. After euthanasia, bone microarchitecture evaluation by computed bone microtomography (Micro-CT) was performed. Statistical significance between groups were tested using analysis of variance (ANOVA); ($P< 0.05$ was considered statistically significant). The OVXD group had lower values for micro-CT, being statistically significant.

Conclusions: It can be concluded that EA may present the improvement of boné microarchitecture in the bone defect model in osteopenic tibias. Further studies in this area are suggested.

doi:10.1016/j.bonr.2020.100306

ND-P04

A novel mouse model to study fracture healing at the proximal femur

Melanie Haffner-Luntzer[a], Birte Weber[b], Charles Lam[c], Verena Fischer[a], Miriam Kalbitz[b], Anita Ignatius[a], Ralph S. Marcucio[c], Theodore Miclau[c]
[a]*Institute of Orthopedic Research and Biomechanics, University Medical Center Ulm, Ulm, Germany*
[b]*Department of Traumatology, Hand-, Plastic-, and Reconstructive Surgery, University Medical Center Ulm, Ulm, Germany*
[c]*Orthopedic Trauma Institute, Department of Orthopedic Surgery, University of California, San Francisco, United States*

The majority of fractures especially in elderly and osteoporotic patients occurs in metaphyseal bone due to the susceptibility of trabecular bone to microstructural damage. While these injuries are important from a clinical standpoint, adequate small animal models to study them are lacking. Therefore, the aim of the current study was to develop a novel mouse model to study metaphyseal fracture healing at the proximal femur. 12 weeks old female C57BL/6J mice were used for the study (n=6 per group; $p< 0.05$). We successfully combined an open osteotomy approach to the proximal femur with a closed approach for intramedullary stabilization. No animals were lost due to surgical issues or anesthesia. All animals displayed normal limb loading and a physiological gait pattern within the first three days after fracture. µCT analysis revealed successful implementation of the osteotomy between the lesser and the third trochanter in all animals. Bony bridging score increased significantly between d14 and d21 (0.2 vs. 3.5). Bone volume ratio also increased significantly between d14 and d21. Total callus volume decreased significantly between d14 and d21. Histomorphometric analysis of Safranin O-stained sections revealed that all fractured healed via endochondral ossification, whereas relative amount of cartilage decreased and relative amount of bone increased between d14 and d21. All fracture calluses at d21 displayed less than 10% of cartilage tissue, indicating successful cartilage-to-bone transition between d14 and d21. TRAP staining showed high osteoclast abundance and activity at the rims of the fracture callus at d14 and throughout the whole fracture callus at d21 after fracture, indicating that fracture callus remodelling has already started at d21 after fracture. Our novel model provides a fast, reliable and inexpensive way to study metaphyseal fracture healing in mice. Future studies using osteoporotic mice might help to unravel molecular mechanisms of delayed osteoporotic fracture healing.

doi:10.1016/j.bonr.2020.100307

ND-P05

Effects of the escitalopram oxalate on densitometric parameters at the intact and bone callus in growing and young adult rats

Roberta Shimano[a], Ariane Zamarioli[a], Ana Paula Macedo[b], José Batista Volpon[a]
[a]*Department of Orthopedics and Anesthesiology, University of Sao Paulo, Ribeirão Preto, Brazil*
[b]*Department of Dental Materials and Prosthesis, University of Sao Paulo, Ribeirão Preto, Brazil*

Objective: to assess the effect of the escitalopram oxalate intake on densitometric analysis at the intact femur and at the fracture callus in growing and young adult rats.

Methods: Four-week-old and 8-week-old Hannover rats (n=28) were distributed into four groups: GP: growing and placebo; AP: adult and placebo; GE: growing and escitalopram; and AE: adult and escitalopram. Daily administration of 2.0 mg/kg of escitalopram (or saline solution) were orally administered for 35 days. Additionally, a fracture at the right femur was produced in all animals on day 21. Densitometric analysis (BMD and BMC) was performed at the distal metaphysis and at the neck of the intact femur, and in the whole bone callus. Analysis of variance with Bonferroni adjustment was made for comparisons ($p\leq0.05$).

Results: both the BMD at the distal femur ($p=0.039$) and BMC femoral neck ($p=0.043$) were higher in adult than growing animals. The drug-treated growing and young adult animals showed significantly lower BMC ($p=0.042$) and BMD ($p=0.027$) at the distal femur, which infer a negative effect of the drug on bone mass. This decrease in bone density did not differ among immature and mature animals ($p=0.207$). Conversely, the escitalopram oxalate intake did not affect the callus density in either group ($p=0.184$).

Conclusion: the escitalopram oxalate administration equally impaired bone density at the intact femur both in immature and mature animals. However, bone callus density remained unchanged with the pharmacological agent.

doi:10.1016/j.bonr.2020.100308

ND-P06

Different prostate cancer bone metastasis models respond differently to treadmill exercise

Hector Arredondo[a], Alexandria Sprules[b], Colby Eaton[a], Ning Wang[a]
[a]*Oncology and Metabolism, University of Sheffield, Sheffield, United Kingdom*
[b]*Sheffield Hallam University, Sheffield, United Kingdom*

Background: Prostate cancer (PCa) is a leading cause of death in men with a predilection to metastasize into bone, when the disease is considered to be uncurable. Exercise has been suggested to improve the health of patients with PCa but no current studies on its effects on PCa bone metastasis.

Hypothesis: Treadmill exercise can prevent the progression of PCa bone metastasis.

Methods: Human xenograft PCa cell line PC3 and murine syngeneic RM1-BM cells were intracardiacally injected (~1x10 cells/injection) into BALB/c nude (n=8) and C57BL/6J mice (n=12),

respectively. The following day, the mice were subjected to treadmill exercise (12 meters/minute, 5° inclination, 30 minutes/day, 5 days/week) for 3 weeks. Bioluminescence assay was used to track skeletal tumour growth weekly and micro-CT was used to analyse bone morphometrics ex vivo. Naïve mice (n>6) were subject to the same treadmill protocol and used to assess the osteogenic response. Animal procedures were ethically approved by The University of Sheffield, UK.

Results: In the xenograft model, the treadmill exercised mice developed significantly higher tumour burden ($p< 0.05$, Mann-Whitney test) in their hindlimbs compared to sedentary controls. The bone structure was not improved by treadmill exercise according to micro-CT analysis. In contrast, the syngeneic model showed significantly lower tumour burden in exercised mice compared to controls ($p< 0.05$, Mann-Whitney test) and a tendency to significantly improved survival curve ($p=0.07$, Gehan-Breslow-Wilcoxon test). The trabecular thickness (Tb.Th) was found significantly higher compared to controls ($p< 0.001$, unpaired t-test). In the naïve baseline study, the trabecular BV/TV had a 7.5% increase in C57BL/6J but 8.5% reduction in BALB/c nude mice, compared between exercised to sedentary controls.

Conclusion: Treadmill exercise alleviates PCa growth in bones of syngeneic RM1-BM/C57BL/6J but not the xenograft PC3/BALB/c nude model, a possible consequence of different osteogenic response to treadmill by the two mouse strains.

doi:10.1016/j.bonr.2020.100309

ND-P07

Estrogen-mediated downregulation of HIF-1a signaling in B lymphocytes influences postmenopausal bone loss

Xianyi Meng[a], Zhen Lin[a], Iga Janowska[b], Shan Cao[a], Koshiro Sonomoto[a], Darja Andreev[a], Karl Xaver Knaup[c], Michael Sean Wiesener[c], Marta Rizzi[b], Georg Schett[a], Aline Bozec[a]

[a]*Department of Internal Medicine 3, Friedrich-Alexander-University Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany*

[b]*Department of Rheumatology and Clinical Immunology, Medical Center-University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany*

[c]*Department of Internal Medicine 4, Friedrich-Alexander-University Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany*

In the bone marrow, B cells and bone resorbing osteoclasts co-localize and form a specific microenvironment. How B cells functionally influence osteoclasts and bone architecture is poorly understood.

We demonstrate that hypoxia-inducible factors-1a (HIF-1 a) signaling in bone marrow B cells regulates postmenopausal osteoporosis through RANKL-mediated osteoclast formation. Deletion of HIF-1 a in B cells prevents estrogen deficiency-induced bone loss in mice, whereas B cell-specific *Vhl* knockout mice with prolonged HIF-1 a signaling in B cells showed enhanced RANKL production and osteoclast formation. Using high-throughput analyses, we show that estrogen controls HIF-1 a protein stabilization and its downstream *Rankl* transcription through HSP70 induction. Moreover, administration of the HSP70 inducer, Geranylgeranylacetone (GGA), conferred a remarkable protection again ovariectomy-induced bone loss. Interestingly, positive correlation of *RANKL*, and *HIF1A* gene expression in human bone marrow B cells and a reduction of *HSP70* gene expression in circulating B cells from postmenopausal patients, suggesting the HSP70/HIF-1 a axis might serve as new therapeutic targets against osteoporosis.

Hence, these data describe a previously unrecognized role of HIF-1 a signaling for RANKL production by bone marrow B cells, which controls bone homeostasis and osteoclastogenesis.

doi:10.1016/j.bonr.2020.100310

ND-P08

Type 2 diabetes impairs mesenchymal stem cells functions and differentiation

Jonathan Ribot[a], Rebecca Landon[a], Cyprien Denoeud[a], Guilhem Frescaline[a], Morad Bensidhoum[a], Graciela Pavon-Djavid[b], Hervé Petite[a], Fani Anagnostou[a,c]

[a]*Laboratory of Bioingénierie et Biomécanique Ostéo-articulaires - UMR CNRS 7052, Paris 7-Denis Diderot University, Paris, France*

[b]*Laboratory for Vascular Translational Science, Cardiovascular Bioengineering - INSERM U1148, Paris 13 Sorbonne University, Paris, France*

[c]*Department of Periodontology, Service of Odontology,-Pitié Salpetrière Hospital, et Hôtel-Dieu Hospital AP-HP, Paris 7-Denis Diderot University, U.F.R. of Odontology, Paris, France*

Objective: Bone marrow-mesenchymal stem cells (BMMSCs) have the capacity to proliferate and to differentiate into multilineage. They play a key role in osteogenesis and angiogenesis. Type 2 Diabetes Mellitus (T2DM) changes the bone marrow microenvironment and is associated with bone fragility and impaired bone healing. The present study focused to characterize the T2DM-microenvironment impact on BMMSCs select functions pertinent to bone repair.

Materials and methods: BMMSCs were harvested from Zucker Diabetic fatty (ZDF) rats (13-weeks-old; early diabetes) and their LEAN littermates (ZL) as controls. Formation of fibroblastic-like colonies, proliferation, apoptosis and migration were analyzed using established methods. Adipogenic differentiation of BM-MSCs was determined by oil red O staining and adipogenic genes expression. Osteogenic differentiation by alkaline phosphatase activity, calcium content and marker genes expression. Angiogenic potential was evaluated by angiogenic markers expression using matrigel plug *in vitro* et *in vivo*.

Results: The results obtained showed that the ZDF-BMMSCs were fewer, with limited clonogenicity (by 45%; $p< 0.05$), proliferation (by 50%; $p< 0.001$), migration capability (by 25%; $p< 0.05$) and increased apoptosis rate (by 60%; $p< 0.001$), than their ZL counterparts. Compared to ZL-BMMSCs, the ZDF-BMMSCs cultured in adipogenic medium, exhibited enhanced adipogenic differentiation (upregulation of PPARG, adiponectine and FABP4) while in osteogenic medium, their potential to differentiate into the osteoblast phenotype was less impacted. Moreover, ZDF-BMMSCs expressed differentially angiogenic genes, specifically 10 genes were upregulated and 11 genes were downregulated, and exhibited impaired vascular formation *in vivo*.

Conclusion: The results of the present study provide evidence that BMMSCs harvested from a T2DM microenvironment were dysfunctional. They may partially explain the altered bone tissue homeostasis and compromised repair in diabetic patients and set the basis for a rationally designed BMSC-based therapy.

doi:10.1016/j.bonr.2020.100311

ND-P09

SLIT2/ROBO1-axis intensifies inflammation, M1 macrophage polarization, and alveolar bone loss in periodontitis, possibly via the activation of MAPK pathway

Liping Wang[a], Zheng Jing[a], Janak Lal Pathak[a], Lijing Wang[b], Linhu Ge[a]

[a]*Key Laboratory of Oral Medicine, Guangzhou Institute of Oral Disease, Affiliated Stomatology Hospital of Guangzhou Medical University, Guangzhou, China*