Single joint bleeding episode in factor VIII deficient mice causes acute osteoarthritic degradation two-weeks post injury, which is prevented with aggressive factor replacement

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Introduction

The most significant morbidity in patients with severe hemophilia (blood clotting factor VIII or IX deficiency) is recurrent joint bleeding (hemarthrosis), which eventually leads to destruction of joints. In addition to joint damage, osteoporosis has been observed in adults and children with hemophilia. While indirect evidence suggests that primary prophylaxis might help preserve bone mineral density, there is little information available on acute and subacute bone degradation following joint bleeding. The goal of this study is to characterize joint damage response the acute bone loss and from an induced joint bleed in hemophilic mice and determine the efficacy of aggressive factor replacement treatment.

Methods

Study Design

Factor VIII knock-out mice generated by gene targeting (E16 FVIII B6;129S4-F8tm1kaz) were originally supplied by Dr. H. H. Kazazian Jr. (University of Pennsylvania, PA, USA) and bred in house. Twenty-two week old (skeletally mature) FVIII-/- male mice were subjected to knee joint hemorrhage induced by puncture of the knee joint capsule with a 30.5 gauge needle, followed by instillation of 5 microliters of saline into the left knee joint space. The contralateral right knee joint served as the uninjured control.

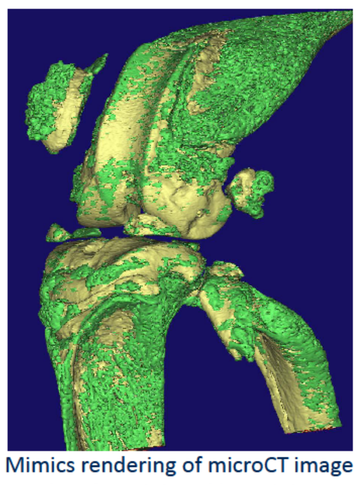
One group of injured mice received intravenous FVIII treatment (n=9), and the placebo group of injured mice was injected with saline (n=9). Thirty minutes prior to the joint hemorrhage mice received an intravenous dose of human recombinant factor VIII 250 IU kg-1 (AdvateTM; Baxter Bioscience, Deerfield, IL) intravenously by tail vein injection. At hours 4, 24, 48, 72 and 96 post bleeding induction, 250 IU kg-1 doses of FVIII were repeated.

Two weeks post-injury, the mice were euthanized and hind limbs were collected for further analysis. Tissues were fixed in formalin for 48 hours and then stored in 70% ethanol.

Analysis

The entire knee joint was scanned using a Scanco µCT80 scanner at 10 µm resolution. MicroCT analysis was performed on 100 slices of the proximal tibia trabecular bone located inferior of the tibial growth plate. Scanco's bone morphometry software was used to calculate trabecular bone volume fraction (BV/TV), Trabecular Connectivity Density, Trabecular Number, Trabecular Thickness, Bone Tissue Mineral Density, and volumetric bone mineral density (vBMD).

Image processing software was used to investigate the morphological changes in the joint resulting from induced joint bleeding. Each joint was rendered in OsiriX™ medical image viewer to look for changes in joint morphology as a result of injury. Joints that showed interesting morphology were further investigated using Mimics in order to isolate and identify regions of mineralization (Right).



Mimics rendering of microCT image data from injured knee joint showing calcification of the patella, patellar tendon, menisci, ligaments, and cartilage rendered in green.

Joints exhibiting large amounts of hemorrhage and swelling were dissected for additional visual inspection (Below).

Upper Left: Uninjured knee joint
Upper Right: Injured knee joint with swelling of the joint capsule and pooling of blood in the lower limb
Lower Left: Dissected Injured knee showing blood pooling in between the muscles

Results

Proximal Tibia Trabecular Bone

Joint bleeding in Factor VIII deficient mice resulted in an acute reduction in the trabecular bone of the proximal tibia measured by BV/TV, Trabecular Connectivity Density and Thickness, bone tissue mineral density, and volumetric bone mineral density (vBMD).

	BV/TV (%)	Trabecular Connectivity Density (per mm^3)	Trabecular Number (per mm^3)	Trabecular Thickness (µm)	Bone Tissue Mineral Density (mgHA/cm^3)	vBMD
Placebo - Control	13.0 ± 2.2	118 ± 22	4.38 ± 0.31	46.3 ± 2.0	815 ± 17	115 ± 18
Placebo - Injury	9.8 ± 2.1*	95 ± 27*	4.19 ± 0.37	40.2 ± 3.0 *	778 ± 32*	84 ± 18*
FVIII - Control	15.7 ± 3.3	98 ± 22	4.25 ± 0.30	57.0 ± 3.0	848 ± 21	138 ± 26
FVIII - Injury	15.2 ± 3.6	97 ± 28	4.26 ± 0.34	54.4 ± 5.0	837± 31	134 ± 32

*Morphometric Parameters from microCT.
Significant changes resulting from joint injury denoted by *(p<0.05)

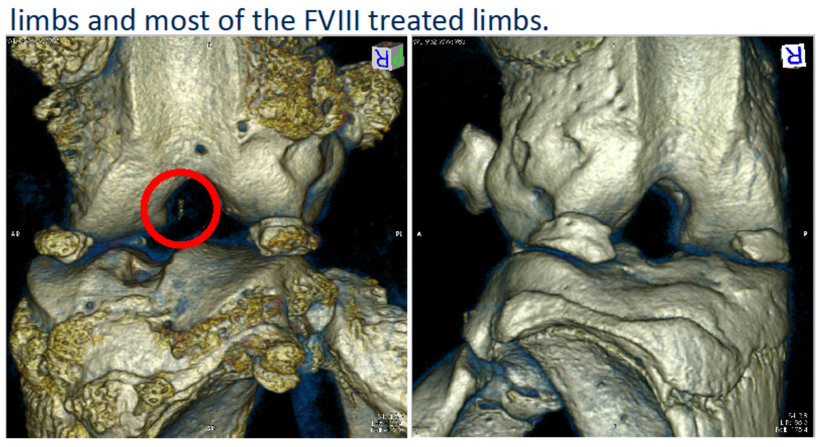
Using the contra-lateral limb as an internal control, joint injury resulted in a 25% decrease in trabecular BV/TV, Trabecular Thickness, Bone Tissue Mineral Density, and volumetric Bone Mineral Density (p<0.05).

	BV/TV (%)	Trabecular Connectivity Density (per mm^3)	Trabecular Number (per mm^3)	Trabecular Thickness (µm)	Bone Tissue Mineral Density (mgHA/cm^3)	vBMD
Placebo: Control vs Injured Limb-% Change	-25.4 %	-20.3 %	-4.3 %	-13.0 %	-4.6 %	-26.9 %
FVIII: Control vs Injured Limb-% Change	-3.2 %*	-2.6%	0.3%	-4.7%*	-1.3 %*	-2.7 %*

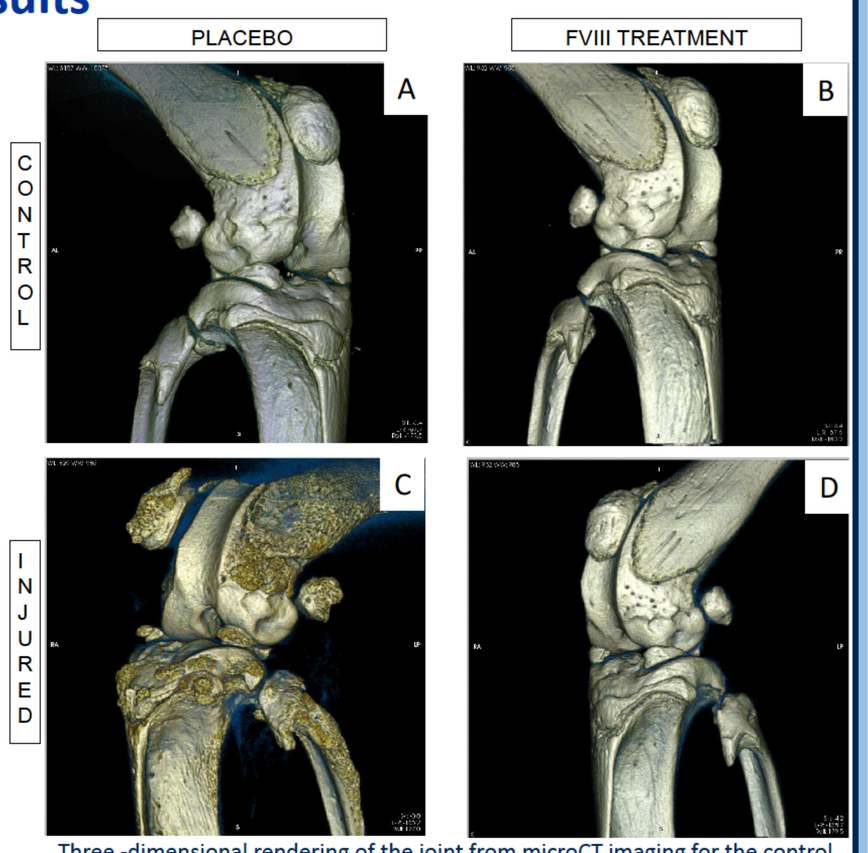
*Percent Change using contra-lateral limb as internal control.
Significant changes resulting from joint injury denoted by *(p<0.05)

Mineralization of Joint Soft Tissues

Joint injury resulted in extensive gross mineralization, which was observed on the femur, tibia, and fibula tendon insertion points as well as the patella, patellar tendon, menisci, ligaments and cartilage (Above Right and Below). FVIIII Treatment prevented this gross joint damage after induced joint bleeding. The gross level of acute mineralization was not observed in any of the un-injured limbs and most of the FVIII treated limbs.



Left: Anterior-Posterior view of Injured Left Knee of untreated mouse. Note the calcification present in the cruciate ligament (red circle)
Right: Anterior-Posterior view of uninjured right knee of untreated mouse has nominal morphology



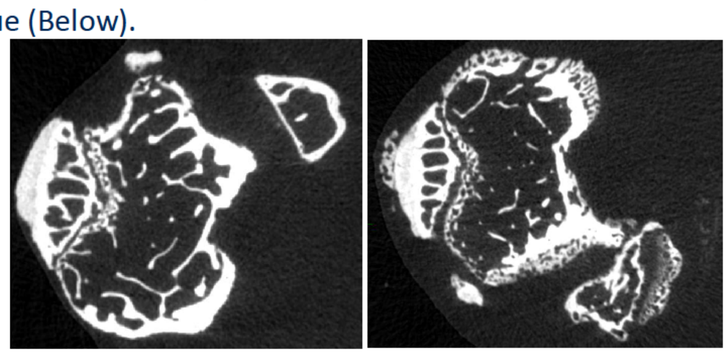
Three -dimensional rendering of the joint from microCT imaging for the control (A & B) and injured (C & D) knee from the placebo (A & C) and FVIII treated (B & D) mice. Injured limb receiving placebo(C) treatment exhibited gross mineralization of the joint soft tissues. Uninjured limbs and limb from FVIII treated mouse look normal (A, B, &D).

Mineralization Density

Some of the injured limbs that received FVIII exhibited very small traces of mineralization (~200mgHA/cc) of the joint soft tissues. Two of the FVIII treated mice exhibited gross levels of mineralization, which also corresponded to FVIII treated mice which lost significant trabecular bone volume (~25% loss of BV/TV). When observed, the quantitative density of the mineralization varied from 500-1000 mgHA/cc, which was less than the observed bone mineral density (+1200mgHA/cc).

Erosion vs Deposition of Mineralized Tissue

The degradation of the joint following bleeding was typically thought of as erosion of the bone surface. However, upon further inspection of the microCT image data, this damage was identified as the deposition rather than erosion of mineralized tissue (Below).



MicroCT image of injured (Left) vs non-injured (Right) limb showing gross mineralization on the exterior wall of the tibia and fibula.

Discussion

The prevalence of low bone mineral density in men and boys with hemophilia is likely multifactorial and has been attributed to the chronic effect of decreased physical activity and disuse atrophy following the development of hemarthropathy, as well as to the chronic effects of HIV and hepatitis infection and their therapies. This study shows that induced joint hemorrhage in hemophilic mice results in an acute loss of trabecular bone in the injured joint as early as 2 weeks after injury. Aggressive replacement of factor VIII prevented the deterioration of these morphologic parameters of proximal tibia trabecular bone after induced injury. The rapid onset of osteoporosis has implications as it is related to the onset of osteoarthritis.

Bleeding and associated inflammation could have long term consequences. Calcification in the joint poses a risk for arthritic joint degradation and has been associated with osteoarthritis in both the hip and the knee. Calcification alters the material behavior of these tissues, leading to altered joint biomechanics, which could further degrade the joint and joint tissues. Future work could use this mouse joint hemorrhage model to investigate the progression of joint degradation and the onset of osteoarthritis associated with these gross soft tissue calcifications following injury.

The current study uses a small needle injury in hemophilic mice to initiate joint bleeding and inflammation and examines acute pathologic changes. The inflammatory response from joint bleeding in hemophilia is expected to be similar to the response following traumatic joint injury in a normal population. However, future work is needed to confirm whether the pathologies exhibited in this hemophilic mouse model are similar to those of a person sustaining traumatic joint injury.

Conclusion

The presented data support the role of acute hemarthrosis in hemophilic bone loss. Whether joint injury is the primary cause of bone loss or a contributing factor deserves further study. Treatment with FVIII prevented both bone loss and joint mineralization caused by joint bleeding. Additionally, the rapid mineralization of the joint soft tissues warrants further study to investigate how these calcifications affect arthritic joint health and the progression to osteoarthritis. Overall, this study contributes to an understanding of the underlying cause of osteopenia and osteoporosis in the setting of FVIII deficiency and identifies calcification in the joint that could be an initiator of osteoarthritic degenerative joint disease.

Acknowledgements

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