

PREVENTION OF GLUCOCORTICOID INDUCED OSTEONECROSIS WITH EITHER PARATHYROID HORMONE OR LLP2A-ALE



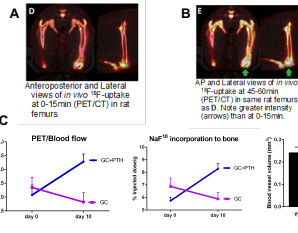
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Introduction

Traumatic osteonecrosis (ON) results from reduced bone vascularity. Glucocorticoids (GC) are a major risk factor for ON. GCs reduce vascular endothelial growth factor, vascular density, and bone mass in mice. LLP2A-Ale is a bone targeted therapy that directs mesenchymal stem cells to bone surfaces. LLP2A-Ale and PTH reduced GC-induced bone changes in mice [Mohan et al. 2017]. The aim of this study was to determine if PTH or LLP2A-Ale co-treatment could prevent GC-induced ON and GC induced changes in bone blood flow.

Methods

- 8-week-old male BALB/c mice were randomized into groups receiving Placebo (PL), GC (4 mg/L dexamethasone in drinking water), GC+250 µg/kg LLP2A-Ale, GC+500 µg/kg LLP2A-Ale (SC, 1X/2 wks), or GC+40 µg/kg PTH (iP/TH (1-34), 5x/wk, SC) (n=8 for PL and 16 for all GC groups). Mice were sacrificed on day 45.
- Mice were MicroCT/PET scanned for 60 mins after IV administration of 10µBq of ¹⁸F at days 0 and 45. MicroFil was given just before necropsy and MicroCT was used to determine femoral bone volume (BV/TV) and vascular density (FVV) (Hawkins et al., J Nuc Med.1992). Serum angiogenic factors were measured at necropsy.
- Both distal femurs (DF) were decalcified, sectioned frontally, and stained with H&E. ON was identified in the DF epiphysis (DFE) using modified criteria [Yang et al., JOR, 2009] (empty osteocyte lacunae, nuclear pyknosis, ghost osteocytes in trabeculae, bone marrow/stromal necrosis in DFE).
- ON was diagnosed when ≥3 of the above features were seen by three independent, blinded observers. Immunohistochemical staining for blood vessels with CD31 and Endomucin antibodies was performed on the DFE.
- References:
Yang L, Boyd K, Kaste SC, et al. A mouse model for GC-induced osteonecrosis. J Orth Res 2009;27:169-172.
Plant M, Zitter TT, Machulle HJ, Becker GA, Jahn M, et al. Blood flow measurements with [¹⁵O]H₂O and [¹⁸F] fluoride ion PET in porcine vertebrae. J Bone Miner Res. 1998;13(8):1328-36.



Figures 1A-E show the ability to acquire/interpret *in vivo* ¹⁸F-fluoride PET/CT scan data from rat femurs. ¹⁸F-PET/CT scanning was done serially at days 0 (baseline) and 10 in rats treated with Methylprednisolone (GC:100 mg/kg, sc, 5x/week) with or concurrent PTH (40 µg/kg, sc, 5x/week) for 10 days. At necropsy, all rats were injected with MicroFil for quantitative identification of vasculature. A: 4x-15 min scan depicts blood flow. B: 45-60 min scan depicts bone localization of ¹⁸F. C: GC caused 20% decline in ¹⁸F-uptake while co-treatment with PTH+GC caused 40% increase ¹⁸F-uptake to bone. PTH caused 78% increase in blood vessel volume. These data suggest that GC reduces blood flow, while PTH prevents/reverses that decline. These data suggest that PTH improves blood vessel volume in GC-treated rodents.

Results

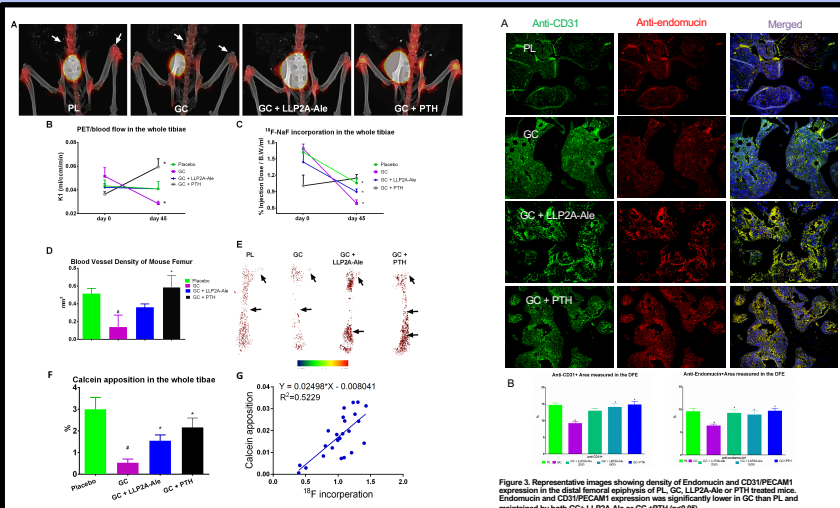


Figure 2. Concurrent treatment of both LLP2A-Ale and PTH prevented GC-induced reduction on blood flow and ¹⁸F-NaF incorporation in bone. (A) Representative PET overlaid to CT images showing blood flow (white arrows) and bone mass (grey) in PL, GC, LLP2A-Ale, and GC + PTH (1-34) treated mice. Note effects on ¹⁸F-NaF distribution in bone, especially in joint areas and vertebrae (white arrows). (B) Quantitative measurement of blood flow in bone. (C) Quantitative measurement of ¹⁸F-NaF incorporation in whole tibiae. (D) Quantitative measurement of bone vessel density in the whole femurs following MicroFil perfusion. (E) Representative blood vessel density micro-CT images in PL, GC, LLP2A-Ale, and GC + PTH (1-34) treated mice. Note blood vessel density in the proximal, middle and distal femoral area (black arrows). (F) Quantitative measurement of calcein apposition in the whole tibiae and (G) correlation to ¹⁸F-NaF incorporation in whole tibiae. *Compared to placebo (p<0.05). #Compared to GC (p<0.05).

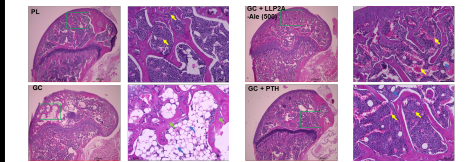


Figure 4. Histologic changes following GC or GC concurrent treatment with LLP2A-Ale or PTH. Representative histopathological sections of the distal femoral epiphyses in PL, GC, GC + LLP2A-Ale (500) or PTH at day 45. In PL, no ON lesions were ever found, with a few fat cells and sinusoids (yellow arrows) being observed in bone marrow. In GC-treated mice, subchondral bone volume was lower. Empty lacunae (green arrows), fewer sinusoids, and bone marrow filled by necrotic fat debris were observed (blue arrows). H&E staining; original magnification 4 and 20x, scale bar, 100 µm.

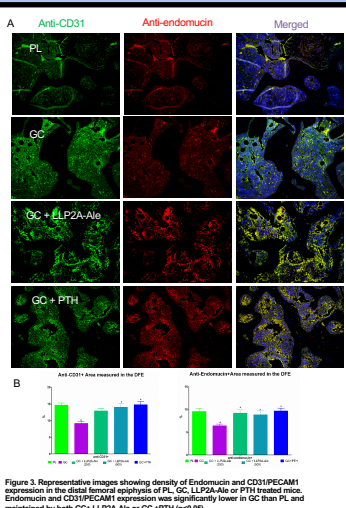


Figure 3. Representative images showing density of Endomucin and CD31/PECAM1 expression in the distal femoral epiphysis of PL, GC, LLP2A-Ale or PTH treated mice. Endomucin and CD31/PECAM1 expression was significantly lower in GC than PL and maintained by both GC+LLP2A-Ale or GC+PTH (p<0.05).

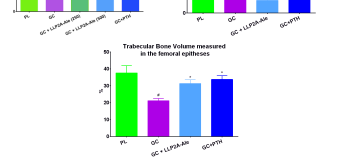


Table 1. Qualitative Assessment for Histopathological Features of Osteonecrosis (Percent of Mice with Feature)

Group	N	Empty osteocyte lacunae (%)	Pyknotic osteocyte nuclei (%)	Marrow necrosis and stromal elements (%)	Fibrin thrombus in blood vessels (%)	Total ON Incident (%)
PL	8	0	0	0	0	0*
GC-only	15	100	100	80	60	60
GC + LLP2A-Ale (500)	14	21	21	29	14	40*
GC + PTH	10	30	30	10	10	10*

*Significantly different from GC-only (p<0.05) by Chi-square analysis.

Summary and Conclusion

PTH or LLP2A-Ale maintained vascularity in the whole femur, and prevented the development of GC induced ON of mice 45 days after treatment. Additional studies are needed to investigate whether treatment with LLP2A-Ale or PTH can reverse GC induced ON.

Acknowledgement

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