Furosemide, a loop diuretic, impairs bone healing in a femoral fracture model in mice

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INTRODUCTION: Loop diuretics (LD), such as furosemide, are widely used in cardiovascular diseases and disorders with fluid accumulation. Treatment with furosemide is associated with increased renal calcium excretion, increased levels of Parathyroid Hormone and bone-specific alkaline phosphatase indicating accelerated bone turnover. Consequently, furosemide treatment has been linked to decreased bone mineral density and an increased risk of hip fractures. However, there is little information on whether LD also affect the process of fracture healing.

METHODS:

The effect of furosemide on bone healing was studied in a stable closed fracture model in mice using intramedullary screw fixation. Animals were treated daily (with intraperitoneal injections of furosemide (15 mg/kg, n=19) or vehicle (dimethyl sulfoxide) (control, n=20). Bone fracture healing was investigated by X-ray, biomechanics, micro-computed tomography (μCT), histology, immunohistochemistry and Western blotting (WB) at 2 and 5 weeks after fracture.

RESULTS: Biomechanics revealed a significantly reduced bending stiffness in furosemide- treated animals at 2 weeks after fracture when compared to controls. This was associated with a significantly lower amount of bone tissue and a higher amount of fibrous tissue within the callus of furosemide-treated animals, indicating impaired fracture healing. Histological analyses revealed a lower number of Tartrate-Resistant Acid Phosphatase (TRAP)-positive osteoclasts, neutrophilic granulocytes and CD68-positive macrophages at 2 weeks after fracture within the callus tissue of furosemide-treated mice. In addition, WB analyses revealed a significantly lower expression of NF-κB ligand (RANKL), cysteine-rich angiogenic inducer 61 (Cyr61) and collagen type X alpha 1 (COL10) within the callus of furosemide-treated animals.

DISCUSSION AND CONCLUSION: Taken together, these results demonstrate that furosemide treatment impairs fracture healing by reducing the activity and number of osteoclasts and affecting the inflammatory response at an early healing time point.