Intravenous Iron Regulates Bone Healing in a Mouse Model of Fracture Induced Iron Deficiency Anemia

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INTRODUCTION: Lower extremity orthopaedic trauma and subsequent surgical repair result in substantial blood loss and lead to injury-associated anemia (defined by hemoglobin [Hb]<12 g/dL) in 95% of patients[1, 2]. Further, studies have shown 96% of these anemic trauma patients exhibit derangements in body iron stores, suggesting there is a high incidence of iron deficiency anemia (IDA) following operative orthopaedic trauma[1]. In orthopaedic patients, anemia is associated with increased fatigue, days in hospital, and rates of infectious complications[2-4]. Blood transfusions are the current standard of care for acute blood loss during trauma. However, transfusions inadequately address IDA after trauma. Transfusions are restricted to severe anemia (Hb<7 g/dL) due to associated increases in infection and morbidity[5]. Moreover, in patients with lower extremity orthopaedic trauma, less than 1% of transfusion recipients normalize Hb within 4 weeks. Thus, roughly 95% of orthopaedic trauma patients with IDA remain insufficiently treated. Intravenous iron therapy (IVIT) is used to restore Hb in cases of severe IDA by replenishing depleted iron levels needed for Hb formation. However, IVIT is not established for use in the orthopaedic trauma setting. To evaluate how IDA impacts bone healing and establish the therapeutic efficacy of IVIT in the orthopaedic trauma setting, we tested if a single dose of IVIT can treat IDA and improve fracture recovery after orthopaedic trauma using a novel mouse model of fracture induced IDA. We hypothesized that IDA would dysregulate bone healing, and IVIT would treat anemia and iron deficiency, alleviate fatigue, and regulate bone healing after orthopaedic trauma.

METHODS: Procedures received IACUC approval and followed NIH guidelines for the ethical treatment of animals. Female, C57BL/6 mice aged 14 weeks during injury were assigned to either control, untreated IDA, or IDA treated with IVIT groups (n=7-8/group). IDA mice were pre-conditioned on an iron-deficient diet (iron: 4 parts per million (ppm)) for 7 weeks. Control mice consumed a standard diet (iron: 67 ppm). During injury, 20% of the total blood volume was drawn from IDA groups to simulate acute blood loss. All mice underwent a 1-mm transverse osteotomy at the mid-femur diaphysis fixed with a stainless-steel intramedullary pin. One day post-injury, IDA mice treated with IVIT received a tail vein injection of low molecular weight iron dextran (Infed, 15 mg/kg). Control and untreated IDA mice received equal volume saline placebo. Longitudinal blood samples were collected weekly via the retro-orbital sinus. Hb was quantified with a complete blood count. The proportion of voluntary time animals spent mobile and recovering limb function were evaluated with weekly dynamic weight bearing (DWB) testing to approximate fatigue. Animals were euthanized 4 weeks post-injury and bone volume of the regenerating callus was measured *ex-vivo* via microCT (Bruker Skyscan 1276). To measure iron deficiency, tissue iron content was quantified in the liver, kidney, and spleen using a colorimetric assay read on a plate reader. 2-way mixed effects analysis tested for significance between groups across multiple timepoints, and T-Tests analyzed significance between groups at the singular endpoint ($p < 0.05$). Multiple tests were accounted for using with Tukey post-hoc correction.

RESULTS: One week post-injury, mice in both saline and IVIT treated IDA groups were anemic with a Hb of 9.8±2.2 and 9.4±1.3 g/dL, respectively. Both IDA groups had significantly lower Hb than the control group 1 week post-injury with 14.2 ± 2.3 g/dL **(Fig 1A**, p<0.0001). At 4 weeks post-injury, Hb of IDA mice treated with IVIT increased to 11.3±0.7 g/dL which was significantly higher than untreated IDA mice with Hb of 7.6±1.6 g/dL (p=0.0009, **Fig 1A**). Compared to controls at 4 weeks post-injury, untreated IDA mice had significantly lower tissue iron content in the kidney, spleen, and liver (p=0.0004, p<0.0001, p=0.0083, respectively; **Fig 1B**), confirming their iron deficient status. Treatment with IVIT significantly increased iron content in the kidney and spleen compared to untreated IDA mice (p=0.0262, p=0.0003; **Fig 1B**). At 4 weeks post-injury, mice with untreated IDA had significantly different mineralized bone volume of 9.1±2.6 mm^3 compared to both the control (6.0±1.8 mm^3) and IDA mice treated with IVIT (5.1±0.9 mm^3) (p=0.0125 & p=0.0022, respectively; **Fig 2A-B**). The proportion of time animals spent mobile during DWB testing was used as a metric indicative of voluntary activity level which is relevant to fatigue. Before surgery and IVIT treatment, both IDA groups spent significantly less time mobile than control mice (p<0.05; **Fig 2C**). 2 weeks post-injury and IVIT treatment, IVIT treated IDA mice spent more testing time mobile (54±4%), which was significantly higher compared to untreated IDA mice (44±8%, p=0.0458). However, IVIT treated IDA animals displayed a gradual decline in mobility between 3–4 weeks post-injury, reaching levels comparable to those of untreated IDA mice (**Fig 2C**). Weightbearing on the injured limb markedly decreased after injury in both groups (**Fig 2D**). Untreated IDA mice were slower to bear weight on the injured limb than IVIT treated IDA mice (week 2: p=0.0073; week 3: p=0.0016) **(Fig 2D)**.

DISCUSSION: In this study, we leveraged a mouse model orthopaedic trauma induced IDA to study the impact of IVIT on IDA, fatigue, and bone healing. We found persistent IDA dysregulates bone healing as untreated IDA mice exhibited significantly elevated callus bone volume 4 weeks after injury compared to controls. A single IVIT dose corrected anemia and iron deficiency, evidenced by increased Hb and tissue iron, while also reducing fatigue and normalizing callus bone volume. We hypothesize IDA could delay healing and increase endochondral ossification pathways during fracture healing by decreasing iron and oxygen availability within the fracture callus, however further studies are needed to test this hypothesis.

SIGNIFICANCE & CLINCAL RELEVANCE: Roughly 95% of lower extremity orthopaedic trauma patients have IDA, however, there are no established treatment options. These preliminary results suggest untreated IDA may dysregulate bone healing and support IVIT as a readily translatable therapeutic approach in orthopaedic trauma to correct IDA, alleviate fatigue, and regulate bone repair.

REFERENCES: (listed as PMID) 40488532[1], 36579920[2], 30214332[3], 11395847[4], 38093439[5]

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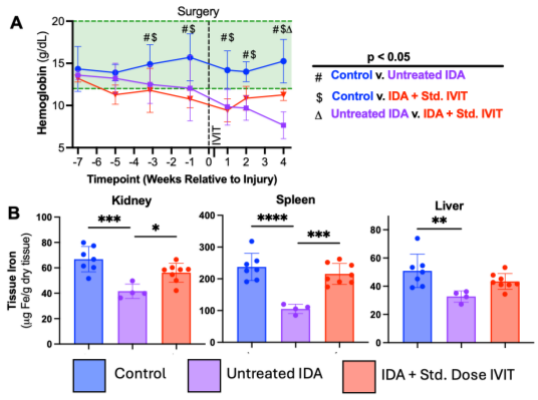

Fig 1: Intravenous iron therapy improves anemia and iron deficiency measures after orthopaedic trauma. (A) Longitudinal hemoglobin; green indicates standard reference range. **(B)** Normalized tissue iron content in the kidney (left), spleen (center), and liver (right) 4 weeks post-injury.

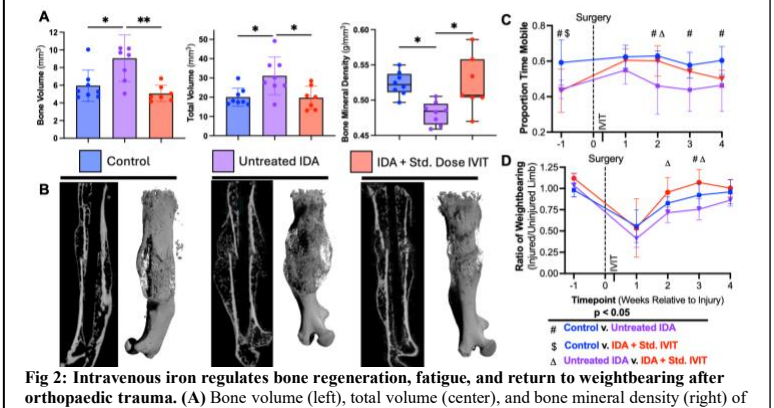

Fig 2: Intravenous iron regulates bone regeneration, fatigue, and return to weightbearing after orthopaedic trauma. (A) Bone volume (left), total volume (center), and bone mineral density (right) of the fracture callus 4 weeks post-injury. **(B)** Representative X-rays and 3D renderings of microCT scans of control (left), untreated IDA (center), and IDA + standard dose IVIT (right) femurs 4 weeks post-injury. **(C)** Longitudinal measurements of the proportion of time mice spent mobile during DWB testing. **(D)** Longitudinal weightbearing ratio of the injured to uninjured limb.