Role of Oxygen in Fracture Repair

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Introduction: Severe trauma to the extremities can result in damage to many tissues including bone and vasculature. Vascular damage can be so severe that an ischemic environment is created whereby skeletal regeneration is compromised. Delayed- and non-union rates are as high as 46% in patients with concomitant vascular injuries [1]. However, the molecular and cellular mechanisms associated with the delay in skeletal healing are unknown. Adequate tissue oxygenation is a prerequisite for satisfactory healing, and the limited supply of this key nutrient could contribute to impaired healing in patients with vascular damage. To begin examining the effect of tissue oxygen on fracture healing, we first examined tibial regeneration in mice that were housed in hypoxic (13%), normoxic (21%), and hyperoxic (50%) environments. In a second experiment, we assessed the ability of hyperoxia to stimulate fracture healing in ischemic limbs.

Materials and Methods: All surgical procedures were approved by the UCSF IACUC. Animals (10–14 week old, male, 129/B6 mice) were anesthetized by intraperitoneal injection of ketamine/medetomidine cocktail. Three-point bending was used to create a transverse fracture at the mid-shaft of tibia. To create ischemic fractures, the femoral artery was ligated and removed before the generation of tibia fractures [2]. All fractures were left un-stabilized and animals were allowed to ambulate ad libitum. Analgesics were provided for the first 24–72 hours. Immediately after recovery from anesthesia, animals were transferred into semi-sealed chambers with controlled atmospheric oxygen levels at 13% (hypoxic), 21% (normoxic), or 50% (hyperoxic). The carbon dioxide concentration and humidity were maintained at <0.5% and between 65–75% respectively.

To assess the effect of atmospheric oxygen levels on fracture healing, non-ischemic fractures were collected at day 5 (13%, 21%, and 50% oxygen, n=5/each group), day 21 (21% and 50% oxygen, n=5/each group), and day 28 (13% and 21% oxygen, n=5, 50% oxygen, n=4). Tissues were fixed, decalcified, embedded, and sectioned (10µm). Sections were stained with the Hall, Bryant Quadruple stain to visualize bone and cartilage, and immunohistochemistry using an anti-PECAM antibody was used to visualize new blood vessels. The volumes of cartilage and bone of day 5 fractures were quantified using histomorphometry.

One way ANOVA and post-hoc test were used to assess how inspired oxygen levels affected the volume of bone (Vbone) and cartilage (Vcartilage) in non-ischemic fractures at 5 days after fracture.

Results: The effect of tissue oxygen on fracture repair in animals with intact blood supply: At 5 days post-injury, bone and cartilage had formed in animals housed in each oxygen environment. Fracture healing in normoxic and hypoxic environments exhibited similar amounts of new bone, but hypoxic fractures had significantly more bone (Fig. 1). Interestingly, both hypoxic and hyperoxic fracture calluses trended towards having more cartilage than normoxic fractures (Fig. 1). At late stages (day 21 or day 28), hyperoxia and hypoxia didn’t have significant effects on fracture healing compared to normoxic controls, while all animals exhibited signs of complete bony bridging.. Visual inspection of angiogenesis using immunohistochemical detection of PECAM suggested that at day 5 post-fracture, there were more blood vessels in hypoxic and hyperoxic fractures compared to normoxic controls (Fig. 2).

Hyperoxia stimulates healing of ischemic fractures: To assess the ability of hyperoxia to stimulate repair in ischemic fractures, we assessed healing in ischemic fractures at 28 days post-injury. The majority of animals (n=4/5) that received an ischemic insult, but no hyperoxia, did not have evidence of bony bridging across the fractured bone ends. In contrast, analysis of histological sections from ischemic animals that received hyper-oxygenation revealed that all animals (5/5) had healed by day 28.

Discussion: Our results indicate that environmental oxygen levels can affect fracture healing. Importantly, hyperoxia was able to stimulate repair of ischemic fractures. However, in addition to this dramatic effect, we also determined that the level of oxygenation could affect differentiation of stem cells during fracture healing. Hyperoxia stimulated bone, and possibly cartilage, formation. Hypoxia, at the level used in this study (13%), was not severe enough to significantly impede fracture healing, but our data suggest that this level of hypoxia may actually enhance chondrogenesis. Finally, both hypoxia and hyperoxia appeared to stimulate new blood vessel formation. Hyperoxic conditions in a wound are likely to enhance angiogenesis by up-regulating the expression of VEGF [3]. Hypoxic conditions may directly stimulate the formation of cartilage, a hypoxic tissue, at the expense of bone. Distinguishing these possibilities is the important next step in our research. Overall, our data provide evidence that increasing the levels of oxygen could provide a simple clinical intervention that may reduce the chance of developing delayed- or non-union.

References:

Acknowledgements: This work is supported by OTA (a research grant to C.L) and NIH (R01 to T.M.).