Is Impaired Fracture Healing in Cigarette Smokers Related to Carbon Monoxide Exposure?

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Purpose: Smoking cigarettes delays fracture healing. Recent evidence has demonstrated that fracture repair can be enhanced by modifying hypoxia signaling during early stages of fracture repair. Additionally, carbon monoxide (CO) exposure in vitro has been shown to block hypoxic signaling through the hypoxic inducible factor (HIF) pathway. This led us to hypothesize that the deleterious effect of cigarettes on fracture healing could be due to CO exposure causing inhibition of physiologic hypoxia signaling via the HIF pathway.

Methods: A sealed environmental chamber was fitted with a CO delivery system so that low dose CO could be delivered cyclically, consistent with exposure seen in heavy smokers. Mice were initially treated with 200 ppm CO for 6 hours, alternating with 6 hours of room air, resulting in peak carboxyhemoglobin levels in the 14% to 20% range. After 2 weeks of accommodation, 240 animals underwent closed femoral fracture in an IACUC (Institutional Animal Care and Use Committee)–approved study. Animals were sacrificed at 7, 10, 14, and 21 days after treatment with cyclic CO or room air and fractures explanted for analysis with micro-CT, histology/immunohistochemistry, and qtPCR (quantitative polymerase chain reaction) for analysis of chondrogenesis, osteogenesis, and angiogenesis.

Results: Significant changes in fracture repair after cyclic CO exposure were readily apparent and occurred primarily at day 10 post-fracture. Micro-CT data demonstrated significant decreases in BV/TV (bone volume/trabecular volume) parameters ($P = 0.0012$) at day 10 after CO exposure, suggesting significant delays in healing. Furthermore, by day 14 the callus size in CO exposed animals was significantly larger than controls ($P = 0.0017$), suggesting delay in transition from chondrogenesis to osteogenesis. qtPCR data were consistent with findings of overall delay in healing by day 10. Expression profiles of angiogenesis genes (Hmox1, Jmjd6, Mmp9, Vegfa, Adora2b) and metabolism genes (Erol, Gys1, Hk2, Ldha, Pfkfb3, Pfkl) all showed more than twofold change from control with CO exposure. Markers of chondrogenesis (Sox9, Col2a1, Acan, Col10) were consistently below controls at all time points. Interestingly, osteogenic markers showed variable effects in Runx2, Col1, and Alk phos mRNA expression throughout the time course.

Conclusion: These data have particular relevance when considering pharmacologic treatments that may help overcome the inhibitory effect of cigarette smoke on healing fractures. Clear and consistent decreases in chondrogenesis and delays in fracture repair were seen with cyclic CO exposure at 200 ppm delivered at 6-hour intervals. This implicates CO as a negative regulator of fracture repair at concentrations consistent with that seen in human cigarette smokers. Altered mRNA expression of genes involved in angiogenesis, and decreases in HIF2a expression at early time points, further implicates HIF signaling in this delayed healing.

See pages 99 - 147 for financial disclosure information.