Heme Metabolism Protects Chondrocyte Mitochondria During Injury

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INTRODUCTION: Post-traumatic osteoarthritis (PTOA) is a leading cause of disability with no disease modifying treatment prior to total joint replacement. Traumatic injury causes acute, pathogenic mitochondrial dysfunction and oxidation of intracellular thiols, including glutathione (GSH) (1). We hypothesized that this same injury pathway might be active in a variety of settings. Considering the likely low intraarticular oxygen content (perhaps 5% O_2 (2)), atmospheric, 21% O_2 exposures play a well-recognized role stressing articular chondrocytes. Several studies have shown low osmolarity saline like that used during arthroscopic procedures is damaging to chondrocytes. Free heme, such as present during hemarthrosis, can damage mitochondria membrane and disrupt proton gradients (3). These stresses interconnect at mitochondria and are present during intraarticular surgical procedures. Activating metabolism of heme *via* carbon monoxide (CO) has been reported to be beneficial in a wide variety of settings including arthritis, but specific intraarticular mechanisms of action have not been described. We have developed a gas entrapping foam that allows delivery of CO in a controlled fashion, and produces antioxidant and anti-inflammatory effects in rodent models of bowel injury (4). We hypothesize CO can protect chondrocyte mitochondria during the variety of stresses associated with trauma by initiating heme metabolism, reprograming mitochondria and improving redox status.
METHODS: We utilized our previously described bovine osteochondral explant model, maintained at 5% O_2 and 5% CO_2. Room air (RAF) or CO (COF) foam were made with PBS and xantham gum as previously described (4). Primary chondrocytes were retrieved from bovine osteochondral explants and 20,000 primary bovine chondrocytes per well were plated on XF96 Extracellular Flux Analyzer (Seahorse Bioscience, Agilent) plates for O_2 consumption as previously described (1). To evaluate the effect of both RAF and COF on intraarticular cartilage *in situ*, RAF or COF was injected intraarticularly with fluoroscopic guidance into intact lapine stifle joints. Thirty minutes later, articular tissue was harvested and placed into 5% O_2 gassed media, cultured for 24 h, and prepared for monochlorobimane (MCB) staining for reduced GSH (5) and Western blotting. RAF or COF was also applied for 60 min prior to an energy-controlled, 2 J/cm^2 impact to osteochondral explants. 24 h after injury, the explants were cross-sectioned and stained with MCB. Explants were visualized at cross-sections of the impact center *via* confocal microscopy and MCB intensities were analyzed and compared among groups *via* t-test with p = 0.05. The opposing face of the specimen was fixed, processed, and embedded for safranin-O staining and immunohistochemistry.
RESULTS: COF treated chondrocytes show higher basal oxygen consumption rate (OCR) and ATP-linked OCR than those treated with RAF, **Fig 1**. COF increased *ex vivo* lapine articular cartilage MCB staining, **Fig 2**. Western blot data indicates that hemeoxygenase-1, the rate-limiting enzyme for heme metabolism, is activated within 1 h after COF treatment (not shown). There are increases in mitochondrial dynamics-related proteins like OPA-1, while total mitochondrial content indicated by TOMM20 staining remains unchanged in COF (not shown). When air, saline, and impact were combined in the bovine explant model, COF was able to protect articular tissue, n=6, **Fig 3**.
DISCUSSION: We have shown that 24 h after CO treatment, articular cartilage demonstrates improved mitochondrial metabolism with stronger OCRs and improved mitochondrial dynamics, similar to other tissues. As little as 30 minutes *in situ* was sufficient to improve cartilage redox status and combinations of hyperoxia, saline exposure, and mechanical injury lead to cartilage damage and oxidation prevented with COF. We note that exposures to room air and saline incur similar oxidation and damage to impact injury alone. CO can protect under each of these conditions, suggesting that heme metabolism is beneficial to cartilage under many stresses. We propose that disabling oxygen metabolism throughout the cell with CO and stimulating heme metabolism thereafter through hemeoxygenase-1 coordinates healthy restoration of mitochondria after injury that might be beneficial in clinical settings.
SIGNIFICANCE/CLINICAL RELEVANCE: This study provides evidence that CO improves intraarticular cartilage health in injury conditions. It also contributes new understanding of how CO prevents multiple stresses at the mitochondrial level within articular cartilage.
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IMAGES AND TABLES:

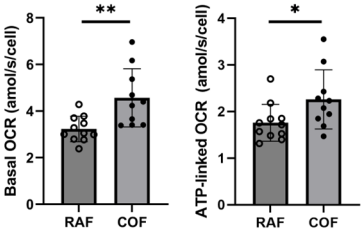

Fig 1. COF Improves Chondrocyte Mitochondrial Metabolism.

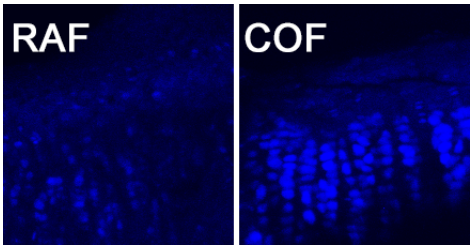

Fig 2. Saline and RAF exposure oxidize GSH, and this is prevented with COF treatment *in situ*.

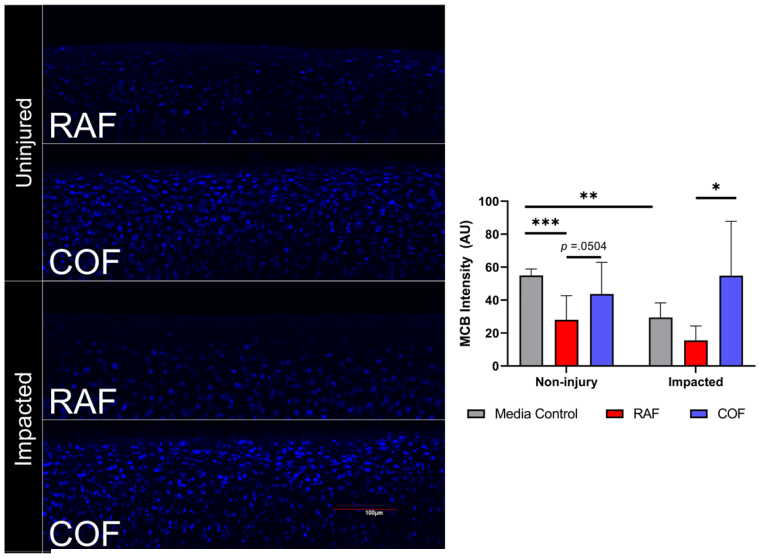

Fig 3. COF Prevents Combined Injuries of Saline, Hyperoxia, and Impact.