

Metformin Treatment Prevents Impact-Induced Degradation of Cartilage Moduli

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INTRODUCTION: Mechanical cartilage overload from a single traumatic impact causes a chondrocyte injury response characterized by mitochondrial dysfunction, cell death, and subsequently catabolic tissue breakdown that culminates in post-traumatic osteoarthritis (PTOA).¹⁻³ Because cartilage has limited intrinsic repair capabilities, there is an unmet clinical need for new methods to treat cartilage injury and inhibit progression of PTOA. The objective of this study was to investigate metformin as a treatment for impact-induced chondrocyte injury. Metformin is one of the oldest oral antidiabetic treatments and is still considered the first line treatment for type II diabetes mellitus.⁴ Oral administration of metformin also prevents or delays the onset of osteoarthritis (OA) in a variety of animal models,⁵⁻⁸ but these models do not include a traumatic cartilage overload. Therefore, the effective dose and timing of metformin treatments for impact-induced chondrocyte injury in cartilage explants were evaluated. First, the metformin concentration that preserved mitochondrial function and chondrocyte viability following a mechanical overload was determined. Next, the effect of metformin treatment regimes designed to mimic oral administration and intraarticular injection on material properties and biochemical composition of impacted cartilage at 28 days post-impact were assessed.

METHODS: Osteochondral cores were aseptically harvested from fresh bovine metacarpophalangeal joints and cultured in defined medium.⁹ Specimens were mechanically impacted with a drop tower instrumented with a load cell and accelerometer and were treated in media supplemented with 0, 10 or 50 mM metformin. Mitochondrial dysfunction (TMRM and MitoTracker Green, both Molecular Probes) and cell viability (LIVE/DEAD viability kit, ThermoFisher Scientific) were determined via confocal microscopy 24 h post-impact. Next, impacted cartilage explants were untreated as controls or were treated with either 1 mM metformin for 28 d or with 50 mM metformin for 24 h post-impact. These treatments were designed to mimic daily oral administration and a single intraarticular injection, respectively. Stress-relaxation indentation was performed before impact and at 1, 7 and 28 d post-impact and was analyzed with a Standard Linear Solid model. Instantaneous (E_0) and equilibrium (E_{eq}) moduli, and time constant (τ) were reported. At 28 d post-impact, GAG content of the tissue was determined with the DMMB assay. Similarly, hydroxyproline (HYP) was quantified with the Chloramine-T assay as a determination of tissue collagen content. Finally, pyridinoline crosslinks (PYD) were quantified in tissue hydrolysates (Quidel MicroVue PYD ELISA). In addition, medium for each specimen was assayed for GAG and HYP to assess matrix degradation during culture. Significance was set at $p < 0.05$ in one-way ANOVAs.

RESULTS: Cartilage was impacted with peak stress of 374 ± 33 MPa, impact duration of 0.86 ± 0.08 s and impact energy density of 22.2 ± 7.9 J/mm³. Mitochondrial polarity was significantly decreased in cartilage explants 24 h post-impact and was rescued with 50 mM metformin but not the 10 mM dose (Fig. 1). Similarly, treatment with 50 mM metformin preserved cell viability at 24 h post-impact significantly better than 10 mM metformin. With the mechanical overload, both E_0 and E_{eq} of impacted cartilage were maintained at days 1 and 7 (data not shown) but were lower than non-impacted tissue by day 28 (Fig. 2A&B). The time constant τ was not altered at any time post-impact (d 28 in Fig. 2C). Viscoelastic material properties of impacted cartilage did not significantly improve when 1 mM metformin was applied for the entire 28 d culture period but were improved by a single day (24 h) of 50 mM metformin (Fig. 2A&B). Neither GAG nor HYP content of the tissue were significantly changed by impact or metformin treatments (Fig. 3A&B). Similarly, no differences were detected in the GAG and HYP released to the media (data not shown). However, PYD crosslink density was lower in the impacted tissue, was not significantly changed by exposure to 1 mM metformin for 28 d, and was rescued by 50 mM metformin treatment for 24 h (Fig. 3C).

DISCUSSION: Findings indicate that 50 mM metformin was necessary to rescue mitochondrial dysfunction and cell viability in impacted chondrocytes 24 h post-impact. This concentration is orders of magnitude higher than expected in the joint with oral delivery, suggesting that intraarticular injection of metformin may be necessary to effectively treat cartilage injury. To test this, we compared the viscoelastic properties and biochemical composition of impacted cartilage treated with a lower 1 mM dose of metformin maintained for the entire 28 d culture period, representing daily oral administration of the drug, and a higher 50 mM concentration for a shorter 24 h duration, representing a single intraarticular injection of metformin. Only the higher concentration maintained viscoelastic material properties of cartilage 28 d post-impact, even though the treatment was limited to the 24 h period following the mechanical overload. These results suggest that rescuing chondrocyte viability and metabolic function in the acute injury phase is required for longer-term preservation of cartilage mechanics. Biochemical analyses did not reveal significant changes in GAG or collagen content of the tissue with mechanical impact or metformin treatments, nor did it indicate significant tissue degradation during culture. However, PYD density was lower in impacted tissue, was maintained with the 50 mM metformin treatment, and was the only biochemical measure that correlated with the changes in moduli. These data highlight the sensitivity of cartilage material properties to collagen crosslinking and identify a new mechanism by which a mechanical overload can modulate the material behavior of cartilage. As increased PYD density is associated with improved cartilage wear resistance,¹⁰ these data also indicate that sufficient levels of metformin may improve functional properties of cartilage post-impact injury.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrated that 50 mM metformin prevents or delays impact-induced markers of chondrocyte injury and degeneration of cartilage mechanics, and may be an effective treatment for cartilage overload injury. Extension of the current findings to an animal model of cartilage trauma may advance intraarticular metformin as a treatment for cartilage injury and the prevention of PTOA.

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