

Evaluation of Soluble Epoxide Hydrolase Inhibitor in an In Vitro Osteoarthritis Model with Equine Synovial Fluid Derived Mesenchymal Stem Cells

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INTRODUCTION: Osteoarthritis (OA) or degenerative changes within the joints is a painful and debilitating condition in humans and animals. Current OA therapies generally provide symptomatic relief and are capable of slowing, but not stopping or reversing, the damage from progression of OA. Recently, the use of synovial-derived mesenchymal stem cells (sMSCs) have been evaluated for the treatment of OA with promising results as disease-modifying treatment [1]. Priming MSCs in vitro with pro-inflammatory mediators has previously been evaluated to enhance efficacy in vivo. However, pro-inflammatory mediators reduce MSC viability [2]. Soluble epoxide hydrolase (sEH) is a novel target for inhibition to reduce pain and resolve inflammation [3]. Sirish et al. (2020) recently demonstrated that concurrent use of an sEH inhibitor with human induced pluripotent stem cell-derived cardiomyocytes improved tissue healing of myocardial infarction. Our objectives were to utilize an established in vitro model of osteoarthritis to evaluate the effect of the soluble epoxide hydrolase inhibitor, t-TUCB (EicOsis), as a primer of and concurrent treatment with sMSCs under inflammatory conditions. We hypothesize that the sEH inhibitor will reduce inflammation and improve sMSC viability.

METHODS: Equine sMSCs, collected under a protocol approved and overseen by the University of Tennessee Knoxville Institutional Animal Care and Use Committee, were cultured (n=3). At 80-100% confluency, cells were treated with one of five primers for 72 hours: phosphate buffered saline (PBS 0.1%), dimethyl sulfoxide (DMSO 0.1%), or t-TUCB at 200 ng/ml, 1 µg/ml, or 4 µg/ml. Primed cells were evaluated for the ability to differentiate into osteocytes and chondrocytes with alizarin red and alcian blue staining, respectively. Primed cells were further subjected to an in vitro inflammatory environment with the addition of interleukin (IL) 1β (10 ng/ml) in PBS for 24 hours. Non-inflammatory conditions were established using PBS (0.01%). One hour post induction, cells were treated with one of six treatments: PBS (0.1%), DMSO (0.1%), phenylbutazone (2 µg/ml), or t-TUCB at 200 ng/ml, 1 µg/ml, or 4 µg/ml for 24 hours. Phosphate buffered saline was used as a negative-control, DMSO served as a vehicle-control for t-TUCB and phenylbutazone, and phenylbutazone was used as a positive-control. At 24 hours post induction, cells were exposed to the standard growth media with respective treatments and maintained for a total of 96-hours post-induction. Cells were evaluated for viability using a Cell Proliferation Assay (CellTiter 96™ Aqueous Nonradioactive Cell Proliferation Assay Kit, Promega) at days 5, 7, and 9. Growth rate, as a measure of cell viability, was calculated from the change in absorbance readings. Media collected pre-induction and 24- and 96-hours post-induction was assayed for the inflammatory mediator prostaglandin E₂ (PGE₂) via a commercially available ELISA (PGE₂ Monoclonal, Cayman Chemical). A mixed model analysis was performed with multiple comparisons. Significance was set at 0.05.

RESULTS SECTION: Primed cells underwent osteogenesis and chondrogenesis. Inflammation reduced sMSC viability and increased PGE₂ levels at 24- and 96-hours (p<0.0001). Under inflammatory conditions, viability of sMSCs was greater in cells primed with t-TUCB at 200 ng/ml, 1 µg/ml, or 4 µg/ml and treated with phenylbutazone or t-TUCB at 1 µg/ml or 4 µg/ml compared to PBS and DMSO (p<0.01). Viability of sMSCs did not differ between primers or treatments under non-inflammatory conditions. Prior to induction (time 0), PGE₂ levels were significantly increased with cells primed with t-TUCB at 4 µg/ml compared to PBS and DMSO (p<0.001). The change in PGE₂ levels did not differ between primers at 24- or 96-hours post induction of inflammatory or non-inflammatory conditions. Under inflammatory conditions at 24- and 96-hours, phenylbutazone reduced the change in PGE₂ compared to PBS, DMSO, and t-TUCB at 200 ng/ml and 1 µg/ml, and t-TUCB at 4 µg/ml reduced the change in PGE₂ levels compared to DMSO (p<0.02). Under non-inflammatory conditions, treatment with phenylbutazone decreased PGE₂ at 24- and 96-hours compared to PBS and DMSO controls (p<0.05).

DISCUSSION: Priming with t-TUCB had no adverse effects on sMSC differentiation into osteocytes and chondrocytes. The increase in PGE₂ observed following priming with t-TUCB may be indicative of increased activation of the PGE₂-dependent proliferation of sMSCs [5]. Priming with t-TUCB at all concentrations improved sMSC viability under inflammatory conditions, suggesting that pre-treating sMSCs with t-TUCB prior to injection into an injured, inflamed tissue may improve sMSC survivability and activity. Treatment with t-TUCB and phenylbutazone both improved sMSC viability. However, the cyclooxygenase (COX) inhibitor reduced PGE₂ under both inflammatory and non-inflammatory conditions as expected, while treatment with the sEH inhibitor resulted in dose-dependent reduction in the change in PGE₂ under inflammatory conditions only. This is consistent with sEH inhibitors indirectly inhibiting the formation of PGE₂ through an alternate mechanism from COX-inhibitors in response to inflammatory stimulants [6]. Additionally, concurrent use of NSAIDs with sMSCs is contraindicated due to the critical role of PGE₂ in sMSC proliferation and negative effects of direct PGE₂ inhibition on bone remodeling and repair [5,6,7]. Treatment with 1 µg/ml of t-TUCB, the concentration measured in vivo in inflamed joints, significantly improve MSC viability within an inflamed environment without directly inhibiting PGE₂ required for proliferation and differentiation [8]. Additional in vivo studies are warranted to further evaluate the combination of sEH inhibitor treatment with sMSCs as a multi-modal approach to osteoarthritis treatment.

SIGNIFICANCE/CLINICAL RELEVANCE: Pre-treatment of sMSCs in-vitro with an sEH inhibitor prior to injection in vivo may improve sMSC viability under inflammatory conditions. In contrast to traditional anti-inflammatory treatments (NSAIDs, corticosteroids), treatment with sEH inhibitors may be able to be used concurrently with sMSCs to reduce pain and inflammation, improve sMSC viability under inflammatory conditions and consequently improve the effectiveness of sMSCs in the treatment of osteoarthritis.

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