Discovery of a Small Molecule Inhibitor of the Wnt Pathway (SM04755) as a Potential Topical Treatment for Tendinopathy

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ABSTRACT INTRODUCTION: Tendinopathy is an inflammatory and degenerative disorder caused by injuries or overuse. Often tendinopathy progresses to a chronic condition with the appearance of fibrosis and micro tears in the tendon that can eventually lead to rupture. Current therapeutic options focus on alleviating the symptoms and pain rather than treatment of the underlying causes. Stem cell and growth factor based treatments are under investigation, but have not definitively established either safety or efficacy. The Wnt pathway is upregulated in chronic tendinopathy and plays an important role in inflammation, tenocyte differentiation and fibrosis. SM04755, a novel, topical, small molecule Wnt pathway inhibitor, was evaluated in preclinical studies to determine its potential to inhibit inflammation, reduce fibrosis and increase tenocyte differentiation, thereby promoting tendon healing.

METHODS: Wnt pathway inhibition was measured via cell-based reporter assay and qPCR. Anti-inflammatory activity was evaluated by measuring TNFα and IL6 secretion using ELISA in lipopolysaccharides (LPS) stimulated THP1 monocytes and antiCD3/antiCD28 stimulated peripheral blood mononuclear cells (PBMCs). Histological expression of scleraxis A (SCXA), tenomodulin, and tenascin C were measured using high-content imaging to evaluate differentiation of human mesenchymal stem cells (hMSCs) to tenocytes. Pharmacokinetics were evaluated by topical application in rats, dogs and mini-pigs, followed by analysis of compound concentrations in tendon and plasma. *In vivo* efficacy of topical SM04755 was evaluated in an intra-tendon collagenase-induced rodent tendinopathy model in both single collagenase injection and multiple collagenase injection conditions, by scoring (range 5-20) several histological indicators of tendon health. Inflammation in the rodent model was measured by chemokine ligand 1 (CXCL1) levels in plasma by ELISA and other pro-inflammatory markers (IL6, TNFa, IL1b, IFNg, IL8) in the tendon by qPCR. Tendon regeneration was evaluated by qPCR based gene expression of tenocyte differentiation markers SCXA and tenascin C. Tendon healing was evaluated by measuring the ratio of Type I to Type III collagen by qPCR as well as polarized light microscopy using Sirus Red staining.

RESULTS SECTION: SM04755 demonstrated potent (EC50 ~152nM) and selective inhibition of Wnt signaling. SM04755 inhibited both LPS and antiCD3/antiCD28 induced TNFα and IL6 secretion (EC50= 500nM) in THP1 cells and PBMCs. SM04755 induced differentiation of hMSCs into SCXA, tenomodulin, and tenascin C expressing tenocytes (EC50 ~ 200nM). A single topical application of SM04755 resulted in tendon concentrations >EC50 for up to 24hrs, with minimal systemic drug exposure or toxicity. In both the single and multiple injection collagenase-induced models, SM04755 treatment improved tendon morphology (Figure A), significantly increased the mean tendon health score (Figure B; n=6, $p<0.01$), decreased the plasma levels of CXCL1 (Figure C; $p<0.05$), reduced gene expression of pro-inflammatory marker qPCR panel ($p<0.05$), and increased expression of SCXA and tenascin C in tendon compared to vehicle ($p<0.05$). Additionally SM04755 treatment increased the gene expression of Type I collagen ($p<0.01$) as well as Sirus Red stained collagen fibers.

DISCUSSION: Wnt signaling has been implicated in tendon inflammation and fibrosis and is upregulated in chronic tendinopathy. Topically applied small-molecule Wnt inhibitor, SM04755 reduced tendon inflammation and an inflammatory marker in plasma, showed evidence of tendon regeneration, and increased tendon health scores compared to vehicle in a rodent tendinopathy model. Plasma exposure was minimal, with no detectable local or systemic toxicity. SM04755 demonstrated potential to promote tendon healing in tendinopathy.

SIGNIFICANCE: Chronic tendinopathy currently has limited therapeutic options, most of which alleviate pain without affecting tendon healing. Most significantly, no single agent actively induces tendon healing and prevents fibrosis. SM04755 demonstrated reduced inflammation, tendon regeneration and reduced fibrosis in a rodent model for acute and chronic tendinopathy. These data suggested that topically applied SM04755 has potential as a safe and effective therapeutic intervention for tendinopathy. Clinical studies are planned.

IMAGES AND TABLES:

Figure. SM04755 inhibited inflammation and promoted tendon healing in a rat collagenase-induced tendinopathy model