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Brief Report

Oral and topical boswellic acid attenuates mouse osteoarthritis

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SUMMARY

Objective: Boswellic acid is a plant-derived molecule with putative anti-inflammatory effects. This study was performed to determine whether oral or topical administration of boswellic acid can attenuate joint damage in a mouse model of osteoarthritis (OA).
Methods: Levels of boswellic acid were measured in the blood and synovium of mice treated with oral or topical boswellic acid. OA was generated by surgical destabilization of the medial meniscus (DMM). Therapy with oral or topical boswellic acid was initiated one day after surgery and continued for 12 weeks, when knees were harvested and scored histologically for degree of cartilage loss, osteophyte formation, and synovitis. Microdissected OA synovium was stimulated with IL-1β or lipopolysaccharide (LPS) in the presence or absence of boswellic acid and cytokine production by quantitative polymerase chain reaction (PCR) or multiplex enzyme linked immunoabsorbant assay (ELISA).
Results: Topical treatment resulted in synovial concentrations of boswellic acid 2–6-fold higher than that measured in plasma. Cartilage loss was significantly reduced in mice treated with oral or topical boswellic acid compared with vehicle control ($P < 0.01$ for both oral and topical therapies). Likewise, treatment with either oral boswellic acid or boswellic acid ointment reduced of synovitis ($P = 0.006$ and 0.025, respectively) and osteophyte formation ($P = 0.009$ and 0.030, respectively). *In vitro*, boswellic acid was able to inhibit IL-1β and TLR4 mediated induction of several inflammatory mediators from OA synovial explant tissue.
Conclusions: Significant synovial concentration and therapeutic efficacy can be achieved with topical boswellic acid treatment. These findings suggest that boswellic acid has potential as a disease-modifying agent in OA.

Introduction

80% of the population has radiographic evidence of osteoarthritis (OA) by age 65, and over 60% of those have symptoms of OA[1]. Current treatments are limited to behavioral interventions, symptomatic medical management, and ultimately, joint replacement surgery. Therapies with potential to slow or halt disease progression are urgently needed. Given that OA is a chronic, nonfatal disease that occurs in an older population with medical and physical comorbidities, such disease-modifying therapies must display a stringent safety profile.

One approach to improving a drug's safety profile is to limit exposure by topical application at or near sites of pathology as has been demonstrated successfully to limit systemic corticosteroid exposure using transdermal or inhaled administration. In the treatment of OA, several agents have been used topically, including analgesics (e.g., salicylates, capsaicin), and indomethacin[2,3].

Boswellia resin, usually derived from the plant *Boswellia serrata*, has been used since biblical times as a natural anti-inflammatory therapeutic in traditional Indian Ayurvedic medicine and traditional Chinese medicine[4]. Findings from small clinical trials suggest that oral Boswellia is efficacious in the treatment of both OA[5,6] as well as rheumatoid arthritis (RA) several other inflammatory conditions (Reviewed in Ref. [4]).

Boswellic acids, especially acetyl-11-keto-β-boswellic acid are potent inhibitors of 5-lipoxygenase (5-LO), an enzyme that catalyzes the generation of leukotrienes including LTB4[7]; a molecule

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strongly implicated in OA-associated inflammation[8]. Additionally, boswellic acid can inhibit toll-like receptor (TLR)-mediated activation of monocytes, suppressing LPS-induced production of nitric oxide, IL-1β, and TNFα[9,10]. Finally, derivatives of boswellic acid have been demonstrated to suppress IL-β induced apoptosis of chondrocytes as well as TNFα induced production of MMP3 by synovial fibroblasts[11] thus demonstrating clear therapeutic potential for the treatment of OA.

To date, there have been few studies of boswellic acid in animal models of OA and, to our knowledge no study has assessed the efficacy of topically therapy. In this study, we used a well-established mouse model of OA to evaluate and compare the therapeutic efficacy of topical and oral boswellic acid preparations in treating post-traumatic OA.

Methods

Animals

20-week-old male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and treated according to the Guidelines for Animal Care of the US National Institutes of Health and Stanford University. All animal experiments were performed under protocols approved by the Stanford Committee of Animal Research.

Surgical mouse model of OA

Mouse OA was generated according to the destabilization of the medial meniscus (DMM) model, which results in articular cartilage loss and synovitis similar to that observed in human OA[12,13]. In the DMM model, the anterior cruciate ligament (ACL) and medial meniscotibial ligament (MML) of the mouse are severed under microscopy, and the mice are sacrificed 12 weeks after surgery. We utilized four groups of eight mice (oral boswellic acid, topical boswellic acid ointment or cream, or vehicle control ointment). This experiment was replicated once with 14 mice per group providing eight mice for histology and allowing an addition six mice for harvesting of synovial tissue to allow quantitation of boswellic acid ($n = 3$) as well as inflammatory cytokines ($n = 3$) in each treatment group. All animals were housed with other mice in their treatment groups however, with the exception of orally dosed, mice, handing was identical between topical treatment and control groups.

Treatment of mouse OA

Starting one day after surgery, we mice were administered either oral (10 mg/kg) or topical boswellic acid cream or ointment twice daily for 12 weeks. Control mice received topical treatment with the formulation ointment base without boswellic acid. For topical application of boswellic acid, we shaved the right stifle joint mice and applied approximately 25 μl of cream or ointment to the joint. Boswellic acid cream and ointment were compounded as described in Supplemental materials.

Evaluation of tissue and plasma levels of boswellic acid

Plasma was obtained by tail-vein bleeding, and synovial tissue was microdissected from the stifle joint. Plasma or tissue samples were precipitated with acetonitrile, and level of beta-boswellic acid were evaluated by liquid chromatography/mass spectrometry (LC/MS) at Climax Laboratories, Inc. (San Jose, CA). The LC/MS analysis was conducted by using Shimazu 10 A HPLC system (Shimadzu Scientific Instruments, Inc.) with ACE C18, 50 × 2.1 HPLC column and ABSciex API-4000 Mass Spectrometer (ABSciex Corp) with Electrospray Ionization (ESI) and negative Multiple Reaction Monitoring (MRM) Scan. A gradient elution was used in separating the test compound with a mobile phase A (0.1% formic acid in 5 mM of NH4AC) and B (0.1% formic acid in acetonitrile).

Scoring of cartilage degeneration, osteophyte formation, and synovitis in mouse OA

Limbs were removed and knees decalcified, paraffin-embedded, and cut into 4-μm-thick sections and stained with Safranin-O. Cartilage degeneration, osteophyte formation and synovitis scores were calculated as previously described[13]. Scores for synovitis were recorded for the femoral-medial and the tibial-medial condyles on the operated side of the joint, and the scores for the two regions were summed.

Stimulation of murine OA synovium

3 months after DMM induction, synovium was microdissected from an additional group of untreated mice and roughly equal pieces (matched by weight) were stimulated in triplicate with LPS in the presence or absence of 140 ug/ml boswellic acid. Tissue was lysed and levels of inflammatory cytokines were measured in supernatants using a multiplex bead-based cytokine assay on the Luminex platform (Millipore) or after extracting RNA for quantitative PCR with results normalized to expression of *gapdh* expression.

Statistical analysis

Cartilage degeneration, osteophyte formation, and synovitis scores as well *in vitro* stimulation assays were analyzed with a two-tailed unpaired *t* test to compare means of treatment groups. For *in vitro* assays, minimal skew of triplicate results was confirmed by eye and for murine studies a Gaussian distribution was confirmed using the D'Agastino-Pearson normality test (GraphPad Prism.) Data are presented as mean ±95% confidence intervals (CI).

Results

Levels of boswellic acid in plasma and synovium after oral or topical administration

Table I demonstrates that topical administration resulted in tissue levels of boswellic acid 2–6 times higher than plasma levels, suggesting local absorption of boswellic acid rather than systemic absorption with local recirculation and deposition. As expected, topical administration resulted in lower plasma levels of boswellic acid than did oral administration and the lipid-based topical formulation (boswellic acid ointment) achieved significantly higher synovial levels than the aqueous topical formulation (boswellic

Table I
Boswellic acid levels in synovial tissue and plasma after oral or topical administration

Boswellic acid	1 h after treatment		
	Oral ($n = 3$ mice)	Ointment ($n = 3$ mice)	Cream ($n = 3$ mice)
Synovial tissue level* (ng/g)	854 ± 479	6,462 ± 1,452	1,052 ± 269
Plasma level (ng/mL)	7,350 ± 3,531	973 ± 320	563 ± 164

* Synovium was microdissected from three mice and independently assayed for levels of boswellic acid as normalized by weight. Results represent average concentration ±95% CI.