Epigallocatechin Gallate Reduces Knee Contracture Formation in a Mouse Model of Arthrofibrosis

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INTRODUCTION: The treatment and prevention of joint stiffness, regardless of origin, remains an unsolved clinical issue with impact across many orthopaedic subspecialties. Although the true incidence of stiffness is difficult to estimate because patients with mild-to-moderate symptoms do not undergo surgical interventions and may not present for clinical evaluation at all, joint stiffness and fibrosis is common and can affect any joint. Loss of 5-to-15 degrees of terminal extension is expected after an elbow dislocation event, and up to 64% of phalangeal fractures experience stiffness even with operative fixation and early mobilization. Stiffness is particularly challenging when present in the knee, as it leads to increased energy expenditure, difficulty performing activities of daily living, and debilitating pain. Although arthrofibrosis of the knee is probably best studied after total knee arthroplasty, an estimated 1% of ACL ruptures that are treated without surgery1 and up to 57% of patients suffering a knee dislocation event2 go onto require a procedural intervention for stiffness. Although there has been increasing interest in recent years in non-surgical methods for the prevention of arthrofibrosis, especially after total knee arthroplasty, we continue to lack effective therapies to both prevent stiffness from developing or accelerate recovery once contracture is established. Recent studies have uncovered the therapeutic potential of epigallocatechin gallate (EGCG) in both preclinical and clinical studies on fibrous conditions.3 EGCG is a polyphenol derived from green tea that is a widely available over the counter, dietary supplement. In this work we explore the impact of EGCG on contracture formation in our mouse model of knee arthrofibrosis.

METHODS: Female C57BL/6J mice aged 14 weeks underwent single hindlimb immobilization using a custom, 3D-printed clamshell cast for 3 weeks prior to release. Mice were treated daily with intra-peritoneal injections of EGCG (Sigma Aldrich, 50mg/kg, n=4) or placebo (saline, n=4) for the study duration. Casts were changed and knee motion was assessed weekly using a custom 3D-printed motion measurement system. Consistent with previous studies, mice were sacrificed 4 days after cast release and tissues were harvested for histology, immunohistochemistry and RNA isolation for gene expression analyses. Mouse knee joints were fixed in 4% formaldehyde, decalcified in EDTA and embedded in paraffin for sectioning. Serial 7µm sections were stained with hematoxylin and eosin (H&E), Masson’s Trichrome, Picosirisus Red or Safranin O/Fast Green for histological analyses. Sections were additionally analyzed using immunohistochemistry to identify markers of myofibroblasts (α-smooth muscle actin, αSMA). Histological quantification was done using QuPath, and statistics were completed in Prism (GraphPad by Domaties).

RESULTS SECTION: After 3 weeks of immobilization, mice treated with EGCG demonstrated improved motion compared to placebo-treated controls. Maximal motion loss among EGCG mice was 50.0±7.2° compared to 68.2±9.9° in control mice (n=4, ANOVA p<0.001) at 4 days after release from immobilization. This was associated with a subjective improvement in leg swelling in EGCG-treated mice. Consistent with the functional range-of-motion analyses, histological evaluation of the placebo- and EGCG-treated mice demonstrated reduced cellularity and attenuated extracellular matrix deposition in the EGCG-treated group.

DISCUSSION: The biological mechanisms leading to arthrofibrosis involve a complex interplay between pro-fibrotic and pro-inflammatory signal cascades. Transforming growth factor-beta (TGFβ) is central among these pathways and has been implicated in the development of arthrofibrosis after ACL rupture and reconstruction,4,5 stiffness after total knee arthroplasty,6 and the development of fibrosis in osteoarthritis.7 Although the TGFβ pathway remains an attractive therapeutic target for many fibrotic conditions, modulation has been challenging due to its pleiotropic actions and multitude off-target effects.8 Recent work has shown that EGCG treatment is a promising and safe non-surgical therapy to treat fibrosis,9 with anti-fibrotic activity associated with the irreversible inhibition of lysyl oxidase-like 2 (LOXL2) and the TGFβ1 and 2 receptors (TGFβR1/2).10 This has translated into very promising results from a recent clinical trial utilizing EGCG to inhibit TGFβ signaling in patients with pulmonary fibrosis, which resulted in improvements in local tissue fibrosis as well as circulating markers of disease activity.11 Here we demonstrate that treatment with EGCG improves knee motion in our mouse single hindlimb immobilization model of knee contracture formation, which is associated with increased collagen accumulation and changes in the TGFβ pathway.12 Further work is necessary to determine the mechanisms underlying the reduced knee arthrofibrosis in mice treated with EGCG.

SIGNIFICANCE/CLINICAL RELEVANCE: In this work we have identified a novel strategy for the prevention of contracture formation associated with knee arthrofibrosis. This work is particularly promising as EGCG has been studied extensively and is a safe and widely commercially available dietary supplement that could be applicable for the prevention of stiffness and fibrosis in a variety of orthopaedic conditions.

REFERENCES:

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