RESULTS: In vivo, Fmod−/− mouse wounds representing a lost-of-function model presented markedly increased myofibroblasts after wound closure compared with wild-type (WT) controls. Intradermal injection of FMOD representing gain-of-function models led to significantly decreased myofibroblast accumulation in the wound areas of multiple animal species, including WT mice and Fmod−/− mice, rats and pigs with high-mechanical loading wounds. Moreover, FMOD injection significantly stimulated IL1β expression, which likely contributed to the diminished number of myofibroblasts. In vitro, FMOD alone stimulated myofibroblast (but not fibroblast) apoptosis as effectively as IL1β. Remarkably, even in the presence of TGFβ1 that completely blocked the effect of IL1β, FMOD promoted myofibroblast apoptosis. Meanwhile, IL1 receptor antagonist (IL1RA) fully rescued myofibroblasts from FMOD-induced apoptosis and blocked FMOD-stimulated myofibroblast IL1β expression. Thus, FMOD selectively promoted apoptosis of myofibroblasts but not fibroblasts via an IL1β-dependent pathway.

CONCLUSION: FMOD accelerates myofibroblast clearance during cutaneous wound healing that significantly reduces scarring, especially hypertrophic scarring, through an IL1β-dependent pathway. Since among mammalian skin porcine skin most closely approximates human skin in anatomic structure, mechanical properties and wound healing and pig models are required by the U.S. Food and Drug Administration (FDA) for human skin product testing, our current study presents a remarkable translational potential of FMOD for human scar management.

PURPOSE: Corneal sensation is a component of reflexes that protect the eye from injury and corneal nerves contain neuromediators that are necessary for corneal epithelial maintenance and healing. Patients with absent corneal innervation develop neurotrophic keratopathy, which is characterized by persistent corneal ulcerations, scarring and vision loss. Corneal neurotisation improves corneal sensation, but it remains unknown whether neurotisation improves ocular surface health. Other than functional sensory data, there remains a paucity of evidence documenting corneal reinnervation after neurotisation. Here we describe our experience with corneal neurotisation, including data documenting ocular surface health, magnetoencephalography and histology of corneal explants after corneal transplantation.

METHODS: Fifteen patients receiving corneal neurotisation were followed prospectively, documenting corneal sensation, ocular surface health, and visual acuity. Corneal sensation was determined using Cochet-Bonnet esthesiometry and visual acuity assessed using best spectacle corrected visual acuity (BSCVA). Magnetoencephalography (MEG) recordings were conducted in an adult patient prior to corneal neurotisation and 8 months afterward. Tactile stimuli were applied to the left and right forehead using an inflating plastic membrane tapped to the skin surface (approx. 200 stimuli at 0.8 pulses/second). The left and right corneas were also stimulated using air-puffs adjusted to the patient’s blink reflex threshold (0.6 pulses/second for 6 minutes). Source localisation was carried out on the stimulus-locked averaged evoked responses (1 to 30 Hz) at 50 millisecond latency using an event-related beam-forming algorithm and superimposed on the patient’s structural (T1) MRI. Histological and immunohistochemical analysis was performed on corneal specimens from three patients undergoing corneal transplantation after neurotisation. In one patient, additional comparison was made to corneal tissue explanted from a corneal transplant prior to neurotisation.

RESULTS: Median central corneal sensation improved from 0 mm pre-operatively (range, 0 to 10) to 60 mm (range, 0 to 60) post-operatively (p < .001). After neurotisation, patients experienced fewer episodes of persistent epithelial defects and there was no further vision loss in any patient undergoing corneal neurotisation with a median follow-up of 16.4 months (range, 1.5 - 43 months). Corneal transplantation to correct pre-existing corneal scarring was uncomplicated in three patients at a median of 30 months (range, 24 - 33), with
improvement in visual acuity six months post-operatively. Histological examination of explanted corneal tissue confirmed the presence of neurofilament+ axon profiles in the cornea after neurotisation. Analysis of MEG data identified a clear absence of evoked response in the anaesthetic (right) cornea prior to neurotisation. Following neurotisation, an evoked response was detected in same (right) cornea, which was localized to the ipsilateral motor cortex, suggesting that the origin of the (right) corneal sensation after neurotisation was derived from the contralateral (left) forehead.

CONCLUSION: Corneal neurotisation reinnervates the cornea using axons from the contralateral face, resulting in improved ocular surface health in addition to sensation. Corneal neurotisation permits successful corneal transplantation, giving visually impaired patients a new opportunity to regain their sight.

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Does NMJ Degradation Initiate the Fibrotic Phenotypic Switch In Skeletal Muscles Post-denervation via a TGFβ-1 Dependent Mechanism?

Jennifer Uong, BS, C’á¬“ndˉ.1, Justin P. Chan, BA1, Henry Hoang, BS, C’á¬“ndˉ.1, Winnie A. På´li˛s¸pi˛s¸, MD2, Ranjan Gupta, MD2

1University of California, Irvine, Irvine, CA, 2University of California, Irvine, Orange, CA

PURPOSE: Traumatic peripheral nerve injuries often result in the permanent loss of muscle function. One reason for the failure of recovery is the end-stage process of muscle fibrosis whereby an increase in extracellular matrix (ECM) interferes with the functional properties of muscles. It has been widely observed that denervation results in the abnormal accumulation of ECM. However, little is known about the cellular and molecular mechanisms of this fibrosis in denervated skeletal muscles in comparison to other tissues. One particular cellular signaling cascade of interest is the transforming growth factor beta 1 (TGFβ-1) as TGFβ-1 and its downstream effectors are widely accepted as being critically important to the development of fibrosis. In addition to TGFβ-1’s role in the fibrotic process, it is also known to play a critical role in synaptogenesis. As neuromuscular junction (NMJ) degeneration and fibrosis both occur following denervation, we sought to establish if there is a temporal correlation between these two phenomena. With TGFβ-1’s ubiquitous involvement in both synaptogenesis and fibrosis, it is our hypothesis that NMJ degradation initiates the phenotypic change of skeletal muscle tissue to fibrotic tissue via a TGFβ-1 dependent mechanism.

METHODS: A denervation model was created in 6 week old C57BL/6 mice by excising 10mm segments from the right sciatic nerve and suturing the proximal nerve to the gluteal muscle with 9-0 suture so as to prevent regeneration. Denervated gastrocnemius muscles were then harvested at multiple time points for analysis by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot for levels of TGFβ-1 and possible downstream targets. Passive muscle stiffness measurements were also performed by subjecting isolated denervated muscles to ramp stretches and then measuring resistance to stretch. Muscle collagen content was measured using a hydroxyproline assay and visualized with Masson’s trichrome staining.

RESULTS: qRT-PCR was used to confirm the transcriptional changes associated with the switch towards a fibrotic phenotype. One week after injury, expression of TGFβ-1 in denervated gastrocnemius muscles was significantly increased compared to muscle from the contralateral side. Connective tissue remodeling proteins including matrix metalloproteinases (mmp-3) and scleraxis (scx) were also significantly upregulated. Genes involved in inflammation (TNFα) and collagen deposition (Col1A1 and Col1A2) were not significantly changed at this time point. Gene expression levels in muscle samples one month after denervation showed a similar trend.

CONCLUSIONS: Our preliminary data suggests that TGFβ-1 signaling plays a key role in the early stages of the phenotypic switch towards muscle fibrosis after nerve injury. There is a temporal correlation between NMJ degradation and upregulation of the pro-fibrotic genes post-denervation. This is an important first step in establishing this correlation prior to determining causality and supports further investigation in this critically important area.

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Growth Hormone Enhanced Recovery After Chronic Denervation Injury: Augmenting Axonal Regeneration, Promoting Motor Reinnervation, and Improving Muscle Function