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A hypothesis for the anti-inflammatory and mechanotransduction molecular mechanisms underlying acupuncture tendon healing

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ABSTRACT

A previous study demonstrated that acupuncture increases the synthesis and reorganisation of collagen molecules in rat tendons after injury. Clinical studies have shown that acupuncture improves pain and functional activity in patients with tendinopathy. However, the molecular mechanisms underlying these effects are unknown. Recent studies have shown that acupuncture can modulate both anti-inflammatory (AI) and mechanotransduction (MT) molecular pathways. Moreover, the modulation of these pathways can increase type I collagen synthesis, which is the main factor that influences tendon biomechanical properties. Our hypothesis is that acupuncture increases synthesis and subsequent reorganisation of type I collagen during tendon healing by concomitant modulation of the Toll-like receptor-nuclear factor-κB AI pathway, the mitogen-activated protein kinases pathway and the Rho/Rac-F-actin MT pathway. Increased collagen synthesis and reorganisation requires that at least one acupoint is anatomically connected with the site of the injury because of the local tenoblast MT mechanism. Confirmation of this hypothesis will increase the knowledge of acupuncture modulation of the previously mentioned molecular pathways, and such confirmation may also help to establish the relationships between the different types of acupuncture needle stimulation and the influence of acupuncture stimuli on pathway activity levels. In addition, the downstream therapeutic effects of acupuncture therapy may be established. This hypothesis can be verified in a rat tendon healing model, and subsequent clinical protocols for tendon healing can be developed and evaluated as standalone therapies or as a component of a combination therapy.

INTRODUCTION

In China, acupuncture has been used to treat several diseases for at least 2000 years.[1] Acupuncture is an ancient healing art that has survived and evolved in China and is currently flourishing in the USA and Europe as both a primary and an adjunctive therapy for a variety of chronic conditions.[2]

According to the ancient theory of oriental medicine, acupuncture is defined as the insertion and manipulation of thin needles into the skin and subjacent tissues at specific sites, known as acupuncture points, for preventive and curative proposes. The needles can be stimulated manually or with a low-voltage electrical current (EA) and heating them with mugwort incense (traditional practice) or with a heat lamp (modern practice). According to this ancient practice, the mechanistic system of therapeutic needling is adjustment of the Qi (vital energy) flow that is believed to circulate in a network of 12 primary channels, also called meridians, which connect 360 principal acupuncture points.[3] Stimulation of the needles is believed to elicit profound psychophysical responses by harmonising or balancing the Qi energy, as well as blood flow throughout the body.[4]

The clinical practice of acupuncture is growing in popularity worldwide and is the most popular complementary and alternative treatment in use today.[5] The WHO has recommended acupuncture as a treatment for over 40 diseases.[5] In the USA, acupuncture therapeutic intervention is widely practised, as shown by an increase in usage from 4.2% to 6.3% of the population, representing 8.19 million and 14.01 million users in 2002 and 2007, respectively.[6] The USA National Institutes of Health (NIH) has listed several diseases that can be treated with acupuncture, including adult postoperative

and chemotherapy-related nausea/vomiting, post-operative dental pain, addiction, stroke rehabilitation, headache, menstrual cramps, tennis elbow, fibromyalgia, myofascial pain, osteoarthritis, low back pain, carpal tunnel syndrome and asthma.[7]

Some clinical studies have demonstrated therapeutic effects of acupuncture on tendinopathy, but well-controlled randomised studies are necessary for confirmation of a causal relationship. In general, these preliminary studies have shown that acupuncture improves both pain and functional activity in study patients.[8–11] One study conducted in our laboratory showed that EA at the ST36 (*Zusanli*) and BL57 (*Chengshan*) acupuncture points increased the concentration and organisation of collagen during the proliferative phase of Achilles tendon healing in rats.[12] This study is unique because it shows that acupuncture has the potential to improve biochemical and morphological characteristics of tendons during healing. However, the molecular pathways underlying these effects are unknown. Accumulating evidence indicates that EA or acupuncture at the ST36 point stimulates anti-inflammatory (AI) properties.[13–16] Several studies have shown that pro-inflammatory molecules are involved in reduction of collagen synthesis.[17–20] Previous studies observed that the insertion and manipulation of acupuncture needles activates cytoskeletal remodelling in subcutaneous connective tissue fibroblasts.[21 22] Downstream effects of cytoskeletal remodelling may include secretion and modification of extracellular matrix components.[23] Recent studies have shown that the application of mechanical stimulation increases production of type I collagen by fibroblasts.[24 25] Based on these and other studies, we discuss in this report the potential AI and mechanotransduction (MT) molecular mechanisms of acupuncture on collagen synthesis during the tendon healing process.

HYPOTHESIS

Our hypotheses state that acupuncture increases type I collagen synthesis and subsequent reorganisation during tendon healing through co-stimulation of acupuncture points with AI effects and acupuncture points located in anatomical sites connected with the injury site (acupuncture points with MT effect). Use of these acupuncture points potentially activates AI mechanisms in inflammatory cells and MT mechanisms in tenoblasts (fibroblasts from tendons) (figure 1). Confirmation of this hypothesis will increase the knowledge of acupuncture modulation of the previously mentioned molecular pathways, and such confirmation may also help to establish the relationships between the different types of acupuncture needle stimulation and the influence of acupuncture stimuli on pathway activity level. Therefore, the confirmation of this hypothesis may lead to novel therapeutic protocols to treat tendon injuries.

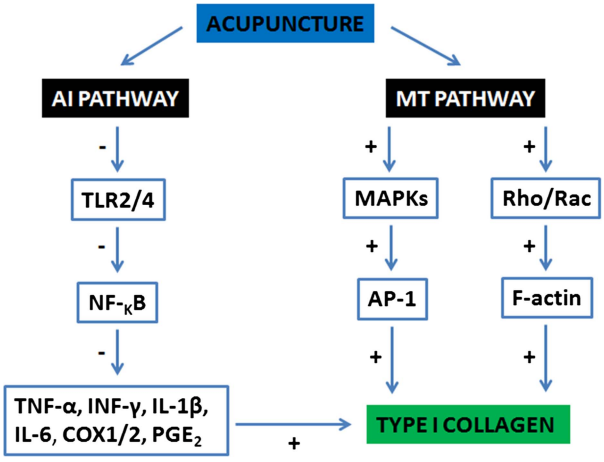

Figure 1 The hypothesis: acupuncture increases type I collagen in the tendon during healing through inhibition (–) of the anti-inflammatory pathway in the inflammatory cells and activation (+) of mechanotransduction pathways in the tenoblasts. AP-1, activator protein-1; COX1/2, cyclo-oxygenase 1 and 2; IFNγ, interferon γ; IL-1β, interleukin 1β; IL-6, interleukin 6; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor-κB; PGE_2, prostaglandin E_2; TLR2/4, toll-like receptor 2 and 4; TNFα, tumour necrosis factor α.

FOUNDATION OF HYPOTHESIS

Inflammatory molecules during tendon healing

After tendon injury, acute inflammation lasts for 3–7 days. The inflammatory process starts with haematoma formation and platelet activation followed by erythrocyte and inflammatory cell (particularly neutrophils) infiltration of the injury site. In the first 24 h, monocytes and macrophages are the predominant cell types at the injury site and serve to phagocytose necrotic material and attract other inflammatory cells from surrounding tissue by releasing vasoactive and chemotactic factors such as vasodilators and pro-inflammatory molecules.[26 27]

During the inflammatory phase the cytokines tumour necrosis factor α (TNFα) and interleukins IL-1β, IL-6 and IL-8 are known to have pro-inflammatory properties.[28] TNFα causes tenocytes to reduce type I collagen deposition and induces production of IL-1β, IL-6, IL-8, IL-10 and prostaglandin E_2 (PGE_2).[19 29] IL-1β is an important pro-inflammatory mediator that promotes prostaglandin synthesis. In damaged tissue, PGE_2 functions to promote vasodilation and pain hypersensitivity.[30] PGE_2, like TNFα and interferon γ (IFNγ), decreases collagen synthesis.[17 18 20]

Acupuncture induces AI properties

As described above, during the inflammatory phase, production of pro-inflammatory molecules reduces type I collagen deposition. Healthy tendon tissue is primarily composed of type I collagen (approximately 95% of the total collagen), which provides strength and elasticity.[31] Therefore, reduction of pro-inflammatory molecule synthesis could increase

synthesis of type I collagen and improve the biomechanical properties of the tendon (figure 1).

ST36 is a key point on the Stomach channel with is commonly used for the treatment of gastric symptoms such as nausea and vomiting.[32] In addition to this action of ST36, several studies have shown that acupuncture at the ST36 exerts AI effects through inhibition of TNFα, IL-1β, IL-6, IFNγ and PGE_2 synthesis.[13–16] The cytokine IL-10 has also been implicated in acupuncture AI effects when the SP6 (*Sanyinjiao*) acupoint is used in a mouse model of peritonitis.[33] Inhibition of Toll-like receptors (TLRs) is one potential molecular mechanism responsible for the observed acupuncture AI effects. TLRs, which are evolutionarily conserved proteins that recognise microbial molecules, initiate the innate immune response and modulate the adaptive immune system. Recent evidence suggests that, in addition to TLR function as sensors of exogenous or foreign pathogen-associated molecular patterns (PAMPs), TLRs can recognise and mediate responses to endogenous stimuli.[34] Heat shock protein 60, a protein released by cells undergoing necrotic cell death, may activate innate immune cells through a TLR4-dependent mechanism.[35] Moreover, necrotic cells were recently shown to activate the nuclear factor-κB pathway (NF-κB) and inflammatory gene production in a TLR2-dependent manner.[36] In the setting of trauma, TLRs detect the release of endogenous ligands, contributing to the pro-inflammatory response to injury.[37 38] One recent study has shown that TLR2, TLR4 and TLR9 play differing and selective roles in both the initial pro-inflammatory response and the adaptive immune response after trauma.[39]

As the downstream effectors of TLR signalling, pro-inflammatory cytokines are known to be elevated during a variety of stress responses. Specifically, the cytokines IL-1β, TNFα and IL-6 were previously proposed to be proximal mediators during the early stages of inflammation.[13] Inhibition of the TLR-NF-κB pathway and downstream effectors may therefore have an AI effect. Recent studies have shown that acupuncture inhibits the TLR2/4-NF-κB pathway and production of the downstream cytokines TNFα, IL-1β and IL-6.[13 14] This is probably one of the molecular mechanisms responsible for the acupuncture AI effect and consequent increase in synthesis of type I collagen during the healing process.

Acupuncture and MT

MT can be defined as the ability of cells to transform mechanical stimuli into biochemical changes.[40] Previous studies have shown that insertion and manipulation of an acupuncture needle results in a mechanical connection of the needle to connective tissue, winding of tissue around the needle, generation of a mechanical signal by pulling of collagen fibres during needle manipulation and MT of the signal into cells.[22]

The acupuncture mechanical signal is delivered into cells through extracellular matrix tensioning, which results in cytoskeletal remodelling and increased cell body cross-sectional area.[21 22] Cytoskeletal remodelling occurs through Rho and Rac signalling as well as actomyosin interactions.[22] Rho and Rac regulate the assembly and organisation of filamentous actin (F-actin) in response to extracellular cues.[41] F-actin composes part of the cytoskeleton, which has emerged as a key structural element allowing transmission of externally applied mechanical forces to the cell and conversion of these forces into biochemical responses.[42] Type I collagen secretion is one potential downstream response to the mechanical signal (figure 1).

Some studies have shown that mechanical stimuli can stimulate the synthesis of type I collagen through the MT pathway composed of mitogen-activated protein kinases (MAPKs), which are the most prominent kinases activated by mechanical stimuli.[43 44] The MAPKs are involved in mechanical force transduction comprising three different pathways: extracellular signal regulated-kinase 1/2 (ERK1/2), c-Jun N-terminal kinase and p38 kinase. These pathways regulate gene expression through activation of transcription factors such as activator protein-1 (AP-1).[45] Nowadays, the MT effects on tissue repair are best characterised in the field of physiotherapy through the application of exercises (mechanotherapy).[46] Recent studies have shown that application of mechanical stimulation increases the production of type I collagen through activation of the ERK-AP-1 pathway in fibroblasts.[24 25] Therefore, these MT data suggest that acupuncture mechanical stimuli have the potential to increase type I collagen synthesis through activation of MAPKs-AP-1 as well as Rho/Rac-F-actin in tenoblasts close to the needle stimulation site (figure 1).

A previous study in our laboratory showed that EA increases the concentration and reorganisation of collagen during the tendon healing proliferative phase in rats.[12] In this previous study we used the ST36 and BL57 acupuncture points. Our hypothesis was that ST36 inhibited inflammation and BL57, which is located at the transition of the triceps surae and the Achilles tendon (an anatomical site connected with the site of the injury), activated the MT pathway in the tenoblasts. According to traditional theory, BL57 is used as a local acupuncture point to relax the muscles and tendons of the lower leg.[47] In this model, both acupuncture points work together to increase collagen concentration and reorganisation.

DISCUSSION

As mentioned previously, the extracellular matrix of healthy tendon tissue is primarily composed of type I collagen (approximately 95% of the total collagen), which provides strength and elasticity.[31] After injury,

type III collagen increases (20–30% of total collagen) compared with uninjured tendon (1–3% of total collagen). Type III collagen tends to produce smaller, less organised fibrils resulting in an increased risk of tendon re-rupture.[48]

Despite the process of remodelling after injury, there is consensus in the literature that the composition, structure and biomechanical properties of the scar tissue of injured tendons never return to the quality of uninjured tendons.[31 48] Therefore, the main goal of regenerative therapies is to improve the quality of scar tissue, and the understanding of mechanisms underlying the effects of these therapies is fundamental to developing them.

The purpose of this report is to present the possible molecular mechanisms underlying the tendon healing process. Our hypothesis is that acupuncture modulates systemic AI and local MT molecular pathways leading to the synthesis of type I collagen. For activation of the MT pathway, the selection of acupuncture points must follow the criterion that at least one acupoint is located in anatomical connection with the site of injury. Supporting this criterion for selecting the acupuncture points, one recent study has shown that the treatment of tendinopathy with dry needling into the pathological tissue provide beneficial effects on pain which did not return with increased physical activity.[49] Another effect of local needling is the increase in blood flow. It has been suggested that the facilitation of tendon blood flow by acupuncture may have a role in the treatment of tendinopathy.[50]

If this hypothesis is confirmed, protocols for other body site injuries including skin, muscle, ligament, joint capsule and nerve could potentially be developed based on the principles of systemic AI and local MT effects of acupuncture. Collagen synthesis during the healing process leads to recovery of the biomechanical and functional properties of these structures. In the future, these protocols may be used separately or combined, depending on the needs of individual patients. For example, in some patients who are intolerant to certain drug classes (eg, AI drugs) due to gastrointestinal, liver or kidney issues, treatment with acupuncture may prove to be a viable alternative. In addition to a collagen synthesis effect, ST36 has an analgesic effect which decreases the necessity of analgesic drugs during the inflammatory phase of the tissue healing.[51] This hypothesis is therefore important to stimulate research in the fields of tissue regeneration and acupuncture mechanism of action.

PERSPECTIVES AND CONCLUSIONS

The hypothesis presented here suggests that acupuncture treatment has the potential to modulate the AI and also the MT molecular pathways. The importance of this concept for tendon healing is the potential for increased synthesis and reorganisation of type I collagen, which is the major tendon structural component. Future studies using tendon healing models to assess the participation of molecular pathways in collagen synthesis and reorganisation will enable us to understand the influence of acupuncture therapy on tendon and other tissue healing. This line of study will potentially lead to a novel therapeutic alternative for treating soft tissue injuries.

Contributors Each author contributed equally according to ICMJE guidelines for authorship.

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