Acupuncture in a neuroimmunological perspective

Peripheral effects
Bacterial challenge from inflammation to pain

Bacterial infection induces inflammation through immune cell recruitment.

Inflammatory pain during infection is triggered by the action of
• immune-derived proteins (e.g. cytokines and growth factors),
• lipids (e.g. prostaglandins)
• amines
• potassium
• protons
on receptors expressed by nociceptors
S. aureus

S. aureus is a major cause of wound and surgical infections, leading to painful abscesses, cellulitis, necrotizing fascitis.

S. aureus releases toxins including hemolysins, Panton-Valentine Leukocidin (PVL), and phenol soluble modulins, which play roles in bacterial dissemination and tissue damage.
Challenging the concept

Woolf and collaborators (Nature 2014)
Pain correlates with bacterial load
*S. aureus* infection induces pain hypersensitivity paralleling bacterial load but not immune activation.
Innate and adaptive host defenses not necessary for *S. aureus* pain
Innate immunity through TLR2/MyD88 and neutrophils/monocytes is not necessary for pain during *S. aureus* infection.
Formyl peptides and α-hemolysin activate nociceptors
Bacterial heat-stable components including N-formylated peptides activate nociceptors
Heat-sensitive *S. aureus* Hla activates nociceptors and contributes to infection-induced hyperalgesia.
Nociceptor neuropeptides regulate inflammation
Nociceptor ablation leads to increased local inflammation and lymphadenopathy following *S. aureus* infection
Woolf and collaborators (Nature 2014) reported that key immune activation pathways were not necessary for hyperalgesia during acute infection.

Bacteria directly activated nociceptors through N-formyl peptides and the pore-forming toxin alpha-hemolysin (Hla).

Nociceptors release neuropeptides that modulate innate immune activation during infection.
Nociceptor derived neuropeptides regulate innate immune activation
Peptides released in the periphery during acupuncture

CGRP: Calcitonin Gene-Related Peptide

VIP: Vasoactive Intestinal Polypeptide

Clinical relevance during needling?
Peripheral and spinal effects of sensory stimulation

Vibration
TENS
Massage
Acu
Acup
EA
Laser
Massage
Moxa

periphery
primary afferent neurons
dorsal root ganglion
spinal cord
to higher centers
dorsal horn
Sensory integration
Autonomic reflexes
Motor reflexes

non-noxious mechanical stimulus
+ hard mechanical stimulus

Aβ fiber

noxious mechanical stimulus

Aδ fiber

noxious heat and chemical stimuli
+ light mechanical stimulus

C fiber

dorsal root projection neurons

gray matter
white matter
Peripheral effects of sensory stimulation (EA and TENS) on rat skin

Transcutaneous electrical nerve stimulation (TENS)
Electro-acupuncture (EA)

FIGURE 3 – Random skin flap raised.

Random skin flaps


Effects of electrical nerve stimulation on vasoactive mediators in rat skin

Electrical stimulation of rat skin

Increased levels of CGRP, VIP, Acetylcholine, ATP, Adenosine, NO

Electrical stimulation induced smooth muscle relaxation

Smooth muscle relaxation – vasodilation – increased nutritive blood flow

Acetylcholine

ATP/Adenosine

CGRP

Peripheral effects of sensory stimulation (EA and TENS) on rat skin

Small diameter myelinated afferents produce vasodilation but not plasma extravasation in rat skin

Jänig et al. Small diameter myelinated afferents produce vasodilation but not plasma extravasation in rat skin
Peripheral effects of sensory stimulation (EA and TENS) on rat skin

Small diameter myelinated afferents produce vasodilation but not plasma extravasation in rat skin.

Conclusion

Acupuncture and Electro-acupuncture induce a local vasodilatory response in the skin.

Can this effect be used in clinical practice in the treatment of acute ischemia/ischemic inflammation in the skin?
Clinical effects of sensory stimulation (ENS) on human skin flaps

Blood circulation was measured by laser doppler flowmetry in fasciocutaneous flaps of 24 patients who underwent reconstructive surgery for mammary carcinoma.

19 of the 24 patients had clinical signs of deficient circulation in the flaps. 14 patients were treated with electrical nerve stimulation (ENS) and 10 with placebo-ENS. Varying degrees of necrosis developed in 8 of the 10 patients who received placebo stimulation but in none of those treated with ENS.

In the 5 patients with good capillary refilling and no signs of stasis or oedema before treatment, only minor increases in blood flow occurred after ENS.

Clinical effects of sensory stimulation (ENS) on human skin flaps

Blood flow was measured in the skin flaps of 20 patients who had undergone reconstructive surgery. All flaps were showing clinical signs of deficient circulation. Local blood flow in skin flaps was significantly increased by electrical nerve stimulation (ENS) (p < 0.001), but not by placebo ENS. Repeated ENS treatment reduced stasis and oedema significantly (p < 0.001), and the capillary refill was also significantly improved (p < 0.001).

The frequency and number of stimuli had any effect turned out to be the case on both counts and the Fig. 3B and C. They show that the greatest skin changes induced with antidromic activation of Aδ-fibres occur when stimuli are delivered at 5–10 Hz. Under these optimal conditions, responses ranged from 14 to 24 s (mean = 24 s) in four animals.

**Effect on inflammation?**
Intravital microscopy

In vivo fluorescence micrographs of the hamster cheek pouch

Figure 2 - A. Hamster cheek pouch (HCP) model. B. The inflatable cuff mounted around the neck of the prepared cheek pouch (HCP).
Calcitonin gene-related peptide (CGRP), but not substance P (SP), was found to inhibit edema-promoting actions of inflammatory mediators (histamine, leukotrine B4, 5-hydroxytryptamine) in vivo in the hamster cheek pouch, human skin, and rat paw.

The effect of CGRP was present in the low nanomolar dose range, and it was mimicked by activation of sensory nerves with capsaicin which caused release of endogenous CGRP-like immunoreactivity (IR).

Suggested mechanisms of action

Acupuncture and Electro-Acupuncture activates afferent A-delta fibers in the skin. Thereby inducing the release of CGRP that results in vasodilation and an anti-inflammatory effect.
Suggested mechanisms of action

Acupuncture and Electro-Acupuncture activates afferent A-delta fibers in the skin thereby inducing the release of CGRP that results in angiogenesis – healing.
CGRP promotes angiogenesis

Neuronal system-dependent facilitation of tumor angiogenesis and tumor growth by calcitonin gene-related peptide

Masaya Toda et al. PNAS 2008 vol. 105 no. 36, 13550–13555
Neuron-induced thickening of the epidermis depends on calcitonin gene–related peptide (CGRP)

*Journal of Investigative Dermatology* (2013) **133**, 1620–1628
Clinical effects of electrical nerve stimulation

A controlled study of the effects of electrical nerve stimulation (ENS) was performed in conjunction with a standard treatment for healing chronic diabetic ulcers on 64 patients divided randomly into two groups.

All patients received standard treatment (paste-impregnated bandage and a self-adhesive elastic bandage) plus placebo ENS or ENS (alternating constant current; frequency, 80 Hz; pulse width, 1 msec; intensity-evoking strong paresthesias) for 20 minutes twice daily for 12 weeks.

Comparison of percentages of healed ulcer area and the number of healed ulcers was made after 2, 4, 6, 8, and 12 weeks. There were significant differences (p < 0.05) in both ulcer area and healed ulcers in the ENS group compared with the placebo group after 12 weeks of treatment.

Clinical effects of CGRP + VIP on human ulcers

A study on the effects of iontophoretic administration of calcitonin gene-related peptide and vasoactive intestinal polypeptide on the healing of venous stasis ulcers of the extremities was carried out on 66 patients.

Two randomized groups of patients were compared, one receiving standard treatment plus iontophoresis of calcitonin gene-related peptide and vasoactive intestinal polypeptide, and the other receiving standard treatment plus placebo iontophoresis. Calcitonin gene-related peptide and vasoactive intestinal polypeptide were administered locally by iontophoresis for 20 min three times weekly for 12 weeks.

To determine the effects of the two treatments, the percentage surface area of ulcer healed and the number of healed ulcers were compared after 2, 4, 6, 8, and 12 weeks of treatment.

The results demonstrate that there were significant improvements of the healing process in the group treated with calcitonin gene-related peptide and vasoactive intestinal polypeptide when compared with placebo iontophoresis.

Electro-Acupuncture, Exercise and Myokines
Swedish father of acupuncture

Early in his career (70s) he became convinced that acupuncture is an effective method of pain control based on the activation of the body’s own systems and that needle stimulation (acupuncture) could represent the artificial activation of such systems.

Sven Andersson (1927-2007).
Professor Andersson emphasised the empirical basis of Traditional Chinese Medicine and when trying to explain TCM he would tell the story of how the Vikings tried to explain lightning and thunder.

According to the Vikings, Thor the god of thunder, son of Odin and a member of the Aesir, smashed giants’ heads with his mighty hammer, thereby causing lightning and thunder: in other words, the lightning and thunder is for real but the rationale given is not.
Professor Andersson was convinced that acupuncture could be integrated into mainstream medicine, and that a prerequisite for this was that the mechanisms of acupuncture could be explained in terms of endogenous systems.

He tried to elucidate what kind of sensory stimulus was most similar to acupuncture, and he suggested that acupuncture excites receptors or nerve fibres in the stimulated tissue, which are also physiologically activated by strong muscle contractions, and the effects on certain organ functions are similar to those obtained by protracted exercise.
Acupuncture: From empiricism to science

Professor Andersson also reported that beta-endorphin levels, important in pain control as well as in the regulation of blood pressure and body temperature, have been observed to rise in the brain tissue of animals after both acupuncture and strong exercise.

He was also inspired by the fact that experimental and clinical evidence suggest that acupuncture may affect the sympathetic system via mechanisms at the hypothalamic and brainstem levels, and that the hypothalamic beta-endorphinergic system has inhibitory effects on the vasomotor centres.

He also demonstrated that there was a post-stimulatory sympathetic inhibition that reached a maximum effect a few hours after acupuncture and which could be sustained for more than 12 hours. This powerful inhibition of sympathetic tone is probably one of the most important effects of acupuncture in the treatment of diseases.
Acupuncture: From empiricism to science

"I don't need that exercise stuff — I cross the pain threshold just getting out of bed in the morning."

Physical pain

Empowerment
Peripheral and spinal effects of exercise/acupuncture stimulation

Ergoreceptors: hard mechanical pressure/contraction
Effects of exercise/acupuncture/electro-acupuncture stimulation

Manual vs 2 Hz burst EA

Muscle Spindle

Muscle Receptors

Golgi Tendon Organ

Microdialysis Push and Pull + Serum, Saliva, Urine, Brain

Muscle contraction
A-alpha efferents

Ergoreceptors: hard mechanical pressure/contraction
Type III–afferents/ A delta–afferents
Effects of exercise/acupuncture/ electro-acupuncture stimulation in rat and human muscles

Increased levels of Calcitonin Gene Related Peptide–like immunoreactivity (CGRP-LI) following exercise (++) and electro-acupuncture (++) but not manual acupuncture. No significant increase in Substance–(SP-LI) or Neurokinin A-like immunoreactivity (NKA-LI) following either modality.

Increased levels of ATP-Adenosine following manual acupuncture (+), electro-acupuncture (+++) and exercise (++++)

Increased levels of Interleukin-6, interleukin-10 following exercise (++) and electro-acupuncture (++) but not manual acupuncture.

Increased levels of Acetylcholine following manual acupuncture (+), electro-acupuncture (+++) and exercise (++++)

Unpublished observations
Effects of exercise/acupuncture/electro-acupuncture stimulation in rat and human muscles
Myokines

The term **myokine** refers to cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert either autocrine, paracrine or endocrine effects.

Of particular interest is the fact that contractile activity plays a role in regulating the expression of these cytokines in skeletal fibers.

**Myostatin** was the first myokine to be identified in 1997.

Both aerobic exercise and strength training in humans and animals attenuate myostatin expression and myostatin inactivation.

Myostatin inactivation potentiate the beneficial effects of endurance exercise on metabolism.


Interleukin-6 (IL-6) (a gp 130 receptor cytokine) was the first myokine that was found to be secreted into the blood stream in response to muscle contraction.

And following 2 Hz burst Electro-acupuncture but not Acupuncture

Unpublished observations
Interleukin-6

IL-6 is secreted by T cells and macrophages to stimulate immune response, i.e. during infection and after trauma especially burns or other tissue damage leading to inflammation.

**Interleukin-6: Pro- or anti-inflammatory?**

IL-6 acts as both a pro-inflammatory cytokine and anti-inflammatory myokine

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<th>Cytokine</th>
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Performance of the various types of fever:
- a) Fever continues
- b) Fever continues to abrupt onset and remission
- c) Fever remittent
- d) Intermittent fever
- e) Undulant fever
- f) Relapsing fever

Mediator of fever - and of the acute phase response

Mobilize extracellular substrates and/or augment substrate delivery

IL-6 acts as both a pro-inflammatory cytokine and anti-inflammatory myokine

Macrophages/Monocytes versus Myocytes

IL-6 signalling

The NFκB signalling pathway

TNF response

Pro-inflammatory - Cytokine Trauma

Anti-inflammatory – Myokine Exercise

Overview

Macrophage

LPS

CD14

TLR4

MyD88

IRAKs

TRAF-6

IKK-α

IKK-β

IKK-γ

NFκB

IL-6

TNF-α

Skeletal Muscle

Calcineurin

p38 MAPK

CREBP

p300

CBP

NFAT

AP-1

IL-6

Ca^{2+}
Interleukin-6: Exercise and Epinephrine

The overall log10-log10 linear relation (straight solid line) between exercise duration and increase in plasma IL-6 (fold change from pre-exercise level) indicates that 51% of the variation in plasma IL-6 increase can be explained by the duration of exercise.

Interleukin-6 and Exercise and Acupuncture

Manual acupuncture = Insertion of microdialysis probe

2Hz burst train Electro-Acupuncture = Exercise

Lundeberg et al. Increases in interleukin-6 and calcitonin gene-related peptide of human skeletal muscle following low force exercise and electro-acupuncture – unpublished observations
IL-6 secreted into the role blood stream in response to muscle contraction:

1. Electro-acupuncture
2. Exercise

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Myokines under investigation showing altered levels during microdialysis during and after Exercise and Electro-acupuncture

- Interleukin 15
- Brain-derived neurotrophic factor (BDNF)
- Myonectin
- Decorin
- Irisin
- Secreted protein acidic and rich in cysteine (SPARC)

Lundeberg et al., in course of publication
The immunomodulatory role of acupuncture may be dependent on more than direct peripheral modulation of nociceptors and high threshold receptors.

1. Spinal reflexes – increased sympathetic tone resulting in the release of immune cells from lymph glands

2. Vagal reflexes

3. Activation of the HPA-axis

4. Deactivation of limbic structures

5. Restoration of the default mode (allostasis-homeostasis)
FIGURE 1. The cholinergic anti-inflammatory pathway.
FIGURE 2. Diffusible versus neural anti-inflammatory pathways.
FIGURE 3. Wiring of the inflammatory reflex.
FIGURE 4. Targeting therapies to the cholinergic anti-inflammatory pathway.
Tack så mycket