The Effect of Acupuncture on the Vagus Nerve to Influence Systemic Inflammation

By
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Abstract

This literature synthesis examines the role the vagus nerve plays in the mechanism of acupuncture to influence inflammatory markers by activating the cholinergic anti-inflammatory pathway. It also compared acupuncture to the use of vagus nerve stimulation (VNS) to modulate these pro-inflammatory cytokines. The synthesis included studies where vagotomy was performed to discern the important role of the vagus nerve and its anti-inflammatory neuro-immune effects. The results showed acupuncture can significantly modulate pro-inflammatory cytokines, dampening the inflammation response and these effects occur via the cholinergic anti-inflammatory pathway. Acupuncture works similarly to VNS to influence these inflammation markers.

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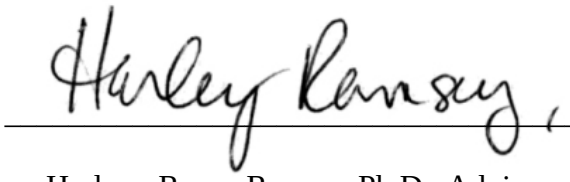

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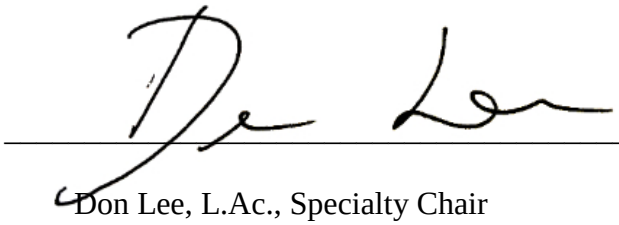

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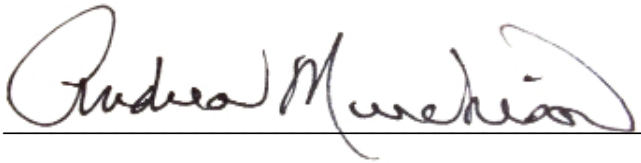

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Table of Contents

Chapter 1: Introduction

Immune System

The immune system plays a vital role in survival. There are two main categories of the immune system: innate and adaptive. The adaptive immune system is the antigen specific response. The innate immune system is the nonspecific defenses that are activated during a pathogen invasion or injury.

Inflammation is an example of an innate protective response to injury in the host's body. It is a complex response that involves several layers of activation: a systemic level, a cellular level, and subcellular level. In healthy individuals, inflammation is a temporary body response, and after an event is resolved homeostasis or balance is restored in the body. It is mostly a beneficial response that will resolve and restore both tissue structure and function (Fernandez et al., 2014). Problems arise when the innate system is not properly regulated. Without proper regulation it could cause excessive and/or continual pro-inflammatory cytokines in the body. Cytokines are low molecular weight signaling proteins that are secreted by the immune system to aid cell-to-cell communication. They bind to specific receptors to affect immune cell differentiation, proliferation, and activity. They direct the inflammatory response to sites of infection and injury and enhance wound healing. Some of the common pro-inflammatory agents are tumor necrosis factor alpha (TNF-α), Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Interleukin-8 (IL-8). Anti-inflammatory cytokines include Interleukin-1 receptor antagonist, Interleukin-10 (IL-10), Interleukin 13 (IL-13), and tumor necrosis factor-binding proteins 1 & 2.

Excessive pro-inflammatory cytokines can lead to both acute reactions such as sepsis in the case of severe or chronic inflammation, which underlies a number of organ

diseases (Pavlov & Tracey, 2012). Additionally "inflammation plays a major role in many chronic and autoimmune diseases involving a complex reaction between pro-inflammatory cytokines, chemokines, neuro-mediators, and other signaling molecules initiating and perpetuating the inflammatory reaction" (Bonaz, Picq, Sinniger, Mayol, & Clarencon, 2013). Chronic inflammation adds to tissue damage, oxidative stress, and increased morbidity and mortality.

The vagus nerve—the main nerve of parasympathetic nervous system—has recently become increasingly important in the study of the innate immune response. The vagus nerve, in Latin the "wandering nerve," is the longest of the cranial nerves. This nerve originates in the brainstem and travels to innervating organs from the neck, thorax, and abdomen, reaching to the colon (Groves & Brown, 2005). The heart, lung, stomach, pancreas, small intestines, half of the large intestines, and liver are all innervated by the vagus nerve. Studies have shown that the vagus nerve plays a critical role in regulating the innate immune response and inflammation in the inflammatory reflex (Pavlov & Tracey, 2012). The inflammatory reflex is a neural circuit that maintains the balance of the innate immune response by regulating the afferent and efferent response of the vagus nerve. When these responses are not balanced there can be either a deficiency in immune response or excessive immune response. The vagus nerve maintains homeostasis through its afferents and through its efferents.

Vagus Nerve Afferent and Efferent Arcs

The afferent arc of the vagus nerve serves to relay signals of inflammation, infection, or injury to the brain, notifying the Central Nervous System of the invasion. It detects these changes in the body from pathogenic molecules, cytokines or other

inflammatory mediators from peripheral tissues and communicates that sensory information to the brainstem, where the afferent vagus neurons terminate (Grundy, 2004) (Pavlov & Tracey, 2012). Tumor necrosis factor alpha (TNF-α) is one the main inflammation mediators sensed. It is synthesized mainly by monocytes/macrophages and T-cells. It also induces other pro-inflammatory cytokines such as IL-6 (Johnston & Webster, 2009). These signals communicated to the brainstem via the vagus nerve afferents induce symptoms such as fever and anorexia (Tracey, 2009).

The other important job of the afferent arc is to stimulate the efferent action of the vagus nerve. The incoming information from the afferents is relayed to the efferent action potentials, which in turn relay the information back to the periphery. The vagal efferent arc is known as the cholinergic anti-inflammatory pathway (CAP). The CAP signaling is initiated in the brainstem nuclei and continues to the celiac and other peripheral ganglia (Levine et al., 2014).

This pathway culminates in the spleen. The spleen is a major source of systemic TNF-α. Parasympathetic innervation of spleen by vagus nerve is controversial: some studies show direct innervation of spleen by vagus nerve, whereas others show no connection. Splenectomy in animal studies has shown to inactivate the CAP, suggesting a link between the spleen and CAP via vagus nerve potentially through splenic sympathetic nerve (Bonaz et al., 2013). The vagal outflow arrives at celiac ganglion, and then releases acetylcholine (ACh) through cholinergic fibers from the splenic nerve, reducing spleen cytokine production (Fernandez et al., 2014). Acetylcholine (ACh)—the main neurotransmitter of the vagus nerve—inhibits the release of TNF and prevents further cytokines by binding to a7 nicotinic acetylcholine receptor (a7nAChR) expressed on

macrophages (Andersson & Tracey, 2012). The suppression of cytokine production inhibits further inflammation. Together the afferent arc (by sensing the inflammation) and the efferent arc (by regulating inflammation) protect against an excessive innate response and cytokine toxicity (Pavlov & Tracey, 2012).

Excessive cytokine production occurs with persistent activation of macrophages and neutrophils. Cytokines can also flow outside of the local area and circulate systemically, which results in an even more widespread inflammation response. Studies show that increased levels of pro-inflammatory cytokines correlate with severity of illness and outcome (Johnston & Webster, 2009).

There is a careful balance the body maintains between pro-inflammatory and anti-inflammatory cytokines. If there is a deficiency in pro-inflammatory cytokines there can be a host of problems that occur with immunosuppression such as secondary infections, whereas an excessive or unregulated cytokine response on the other hand can end up being more detrimental to the body than the original insult (Johnston & Webster, 2009).

Insufficient efferent vagus nerve cholinergic output might have a causative role in the dysfunction of immune regulation (Tracey, 2010). Vagus nerve activity has shown to be decreased in chronic inflammation conditions. Chronic conditions often cause excessive release of host defenses which help with an immediate threat but in the longer term can be more damaging than the initial threat (Libby, 2007). Several autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus, ankylosing spondylitis, and chronic inflammatory bowel disease have a lower vagal tone, measured by a decreased heart rate variable (HRV) and variation in time interval between heart beats, compared to age-matched controls.

The vagus nerve has an important role in detection and inhibition of the inflammatory response. Increasing the vagus nerve output has potential to have an enormous effect on treating inflammation both chronic or acute by down regulating the pro-inflammatory cytokine response and dampening inflammation. Studies have shown that vagus nerve stimulation suppresses local and serum pro-inflammatory cytokine levels. Low-frequency (5 Hz) stimulation activates CAP to induce the anti-inflammatory effect (Bonaz et al., 2013). In experimental models of RA, stimulation of the vagus nerve had an anti-inflammatory effect. The serum cytokine levels were reduced and protection against joint destruction was observed (Koopman, Schuurman, Vervoordeldonk, & Tak, 2014). By targeting the efferent pathway of the inflammation, reflex vagus nerve stimulation can restore balance. Another consideration is the speed of neural conductance. The reflex can provide fast input that will modulate inflammation. The nervous system can also adapt to any changing output because the CAP is fully integrated with the body (Matteoli & Boeckxstaens, 2013).

Vagus Nerve Stimulation

Vagus nerve stimulation is currently a treatment option for people suffering from epilepsy or depression. In 1997 the Food and Drug Administration (FDA) first approved vagus nerve stimulation (VNS) to treat certain types of epilepsy. In 2005 the FDA expanded the use of VNS to treat depression that has been unresponsive to pharmacological interventions. During an outpatient procedure a device is surgically implanted in the chest with a lead to the vagus nerve on the left side. An impulse targeting the vagus nerve is created and the signals are relayed to the brainstem (Gale, 2014). The VNS is programmed to use from 20 to 30 Hz frequencies with 30-second

pulses every five minutes throughout the day. This is adjusted accordingly to accommodate individuals (Bonaz et al., 2013).

In 1998 neurosurgeon Kevin J. Tracey conducted a study on rats that linked the stimulation of the vagus nerve to changes in the immune system, specifically inflammation. He placed a rat under anesthesia and with a hand-held nerve stimulator delivered a one-second electrical impulse to the rat's vagus nerve. Tracey then stitched the rat up and administered a bacterial toxin to the rat in order to promote tumor necrosis factor (TNF) that would trigger inflammation. After an hour blood tests were taken and instead of the rampant inflammation that was expected the TNF had been blocked by 75%. This was a breakthrough for Tracey, and in 2011 he began clinical trials on patients suffering from rheumatoid arthritis. He created SetPoint Medical to create a more efficient VNS device with the goal to use patterns of electrical impulses to treat inflammatory diseases instead of drugs (Behar, 2014).

Acupuncture has been shown in studies both on rats and humans to influence the vagus nerve (XY et al., 2008; Marca et al., 2010). Studies performed on animal models have shown that electrical stimulation of vagus nerve inhibits TNF synthesis and evokes measurable increases in vagus nerve activity (Andersson & Tracey, 2012). Other specific parasympathetic results include increase in gastric tone and gastric acid secretion, lower blood pressure, lower heart rate and reduction of lower esophageal sphincter tone (da Silva & Dorsher, 2014). Acupuncture points in the sub-occipital and neck region as well as *cymba concha*, all located in the vagus nerve distribution area, may have similar effects to VNS (da Silva & Dorsher, 2014). To date there are few studies that show the

same inflammatory potential in humans but there are several ongoing clinical trials (Matteoli & Boeckxstaens, 2013).

With new studies linking the vagus nerve to the immune system's inflammatory reflex the researcher would like to examine the research on the stimulation of the vagus nerve using of acupuncture and its effect on the immune system. Acupuncture may be a valid alternative or adjunct to current inventions in treating inflammation locally and systemically. Acupuncture offers the benefits shown in vagus nerve stimulation without the risk of surgery or some of the adverse effects that have occurred with VNS. Obstructive sleep apnea, chronic laryngeal and pharyngeal muscles in constant contraction have shown to cause reducing airway flow, voice alteration, and cough and throat pain (Gale, 2014).

Research Question & Objective

This study will explore how acupuncture can be used to strengthen the innate immune system and mediate inflammation through its effects on the vagus nerve. The objective is to show acupuncture can increase vagus nerve activity, reduce inflammatory markers and be a beneficial therapy for inflammatory conditions with few or no side effects. The research will also try to bridge research on VNS with inflammatory markers measured compared along with acupuncture.

This research synthesis will be of value to both the Chinese Medicine community and the Western medicine world. It will show the changes in biomarkers that occur with acupuncture and subsequently its effect on inflammation and the damages incurred with chronic inflammation. This research also serves to expand acceptable uses of acupuncture in the United States. Currently insurance coverage for acupuncture has been

limited to pain or nausea. It can lay the foundation to integrate the use of acupuncture as an option of treatment for chronic inflammation.

Abbreviations

HRV: Heart rate variability; variation in time interval between heartbeats

IL-1β: Interleukin-1; pro-inflammatory cytokine

IL-6: Interleukin-6; pro-inflammatory cytokine

IL-10: Interleukin-10; anti-inflammatory cytokine

IL-13: Interleukin 13; anti-inflammatory cytokine

INF-γ: Interferon gamma; pro-inflammatory cytokine

Interleukin-1 receptor antagonist: anti-inflammatory cytokine

NF kappa B p65: master transcription factor controlling the expression of a wide range of pro-inflammatory genes. (Zhao et al., 2012)

TNF-α: Tumor neurosis factor alpha; pro-inflammatory cytokine

Tumor Necrosis Factor-binding proteins 1 & 2: anti-inflammatory cytokine

Chapter 2: Literature Review

Overview

This chapter compiles the data collected from studies that examined the influence of acupuncture on inflammatory markers and studies that examined the influence of VNS on inflammatory markers. These studies were chosen to effectively address the research objective and serve as the foundation of the of the literature synthesis.

Acupuncture and Inflammatory Markers

The researcher examined 7 articles investigating the effect of acupuncture on different inflammatory markers. The 7 articles were in-vivo animal studies. The researcher focused on studies that measured inflammatory markers that are associated with the vagus nerve and cholinergic anti-inflammatory pathway with the purpose of discovering if acupuncture made significant changes in these inflammatory markers as well as if the effect was blocked with a vagotomy.

Du et al. (2013) evaluated the effects of electro-acupuncture (EA) performed at Stomach (ST) 36 on blood pressure, TNF-α and, IL-6 in both plasma and intestine homogenates after hemorrhage and delayed fluid replacement (DFR). Male Sprague Dawley rats that were 8-10 weeks and 240-260g were divided into 6 groups: electro-acupuncture (EA) at ST 36 or electro-acupuncture on non-acupoint (EAN) (.5mm lateral and distal to ST 36) was performed on 3 different groups. The first group experienced hemorrhage (45% blood loss) and then was divided to receive either EA or EAN performed followed by DFR. The second group underwent a vagotomy of the dorsal and ventral vagus nerve on the distal esophagus before blood loss and then EA or EAN was performed followed by DFR. The third group was given α-BGT prior to blood loss and

that was followed by EA or EAN and then again DFR. Each of these groups was then further divided into subgroups. The first subgroup (n=12) survival rate and mean arterial pressure (MAP) was evaluated. The next subgroup (n=18) was used to measure the cytokine levels, organ parameters, gut injury score, ZO1 detection and intestinal permeability.

The research showed EA at ST 36 reduced TNF-α and IL-6 in plasma and intestine ($p < 0.05$). It did not have a significant effect of IL-10 in plasma or intestine. Vagotomy and α-BGT blocked the anti-inflammatory effects of EA on ST 36. In addition the study showed EA at ST 36 reduced damage to intestinal mucosa. The blood pressure was improved after blood loss with those rats that received EA and significant improved survival rate ($p <0.05$). EAN did not have a significant effect on TNF-α, IL-6 and, IL-10. The significance of these results shows that EA at ST 36 attenuates systemic inflammation by reducing TNF-α and IL-6 compared to EAN. It is also able to increase survival rate and improve blood pressure in hemorrhages. Both vagotomy and α-BGT blocked the function of EA on ST 36. It can be deduced that EA at ST 36 increases the efferent activity of vagus nerve through the a7 nicotinic acetylcholine receptor (Du et al., 2013).

The purpose of a study conducted by Torres-Rosas and colleagues (2014) was to investigate the effect of electro-acupuncture (EA) to mediate the vagal modulation of the immune system via dopamine. Wild-type mice, α7nAChr-knockout and, β2AdrR-knockout mice 6-8 weeks old and approximately 25g were randomly assigned. Sepsis was induced by endotoxin, LPS via cecal ligation and puncture procedure. A control group underwent a sham surgery following the same procedures but without electrical

stimulation. EA was performed bilateral on ST 36 for 15 minutes with electric current 40 mA, pulse width of 50 μs and frequency of 10 Hz. Another group was administered sham electro-acupuncture with a wooden toothpick and an additional group received electro-acupuncture on a non-acupoint 3 cm distal to ST 36. Other groups had cervical or sub-diaphragmatic vagotomy, adrenalectomy or splenectomy. The researched evaluated the serum concentrations of cytokines TNF-α, IL-6 and, interferon-γ (INF-γ) from the different groups.

The results showed that EA on ST 36 significantly inhibited LPS induced in serum levels and cytokine pro-inflammatory markers TNF-α, IL-6 and INF-y ($p < 0.01$). They also found the electro-acupuncture is voltage-dependent. Neither the sham electro-acupuncture with wooden toothpick or on a non-acupoint inhibited the cytokine levels. Serum levels of TNF-α, IL-6 and, INF-γ were not affected by EA in the α7nAChr-knockout and β2AdrR-knockout mice. Also cervical vagotomy, subdiapragmatic vagotomy or adrenalectomy, eliminated the anti-inflammatory effect of EA ($p < 0.01$). The mice that underwent splenectomy did not have the effect blocked (Torres-Rosas et al., 2014).

This study was an important contribution for several reasons. It demonstrated that EA at ST 36 is a valuable strategy for vagal stimulation. Also the researchers were able to map another immune regulating neural circuit reflex to the vagus nerve. They found that activating the sciatic nerve in the leg with EA inhibits cytokine release. This circuit was inhibited with resectioning of the vagus nerve. They found that signals arising in the sciatic nerve culminate on vagus nerve efferent signals; these efferent vagus nerve signals terminate on the release of dopamine in the adrenal medulla. This newly discovered

sciatic to vagus nerve circuit plays an important role in the innate immune system (Chavan & Tracey, 2014).

Choi et al. (2010) conducted a study on male Sprague Dawley rats 250-300g to determine the effect of acupuncture on inflammatory markers and functional recovery after spinal cord injury. A laminectomy at T-9-T10 was performed on the rats and T8 and T11 were than clamped together to stabilize the rat. Acupuncture with stimulation was performed for 30 minutes once a day for 2 weeks. One control group did not receive treatment and another control received sham acupuncture with a toothpick on acupoints. A third group received acupuncture 2mm away from the location on DU 26 and Gallbladder (GB) 34. To discover which points offered the best results the researchers had 8 groups including control and needled either DU 26, GB 34, ST 36, Bladder (BL) 60, BL 40, GB 39 or Spleen (SP) 6. They found that DU 26 and GB 34 together offered better results than the others due to less ventral motor neuron loss ($p < 0.05$). Those two points were used together in the study.

Inflammatory markers TNF-α, IL-6 and, IL-1β were measured to find the effectiveness of acupuncture. The above markers were significantly reduced compared to both the control group and sham group ($p < 0.05$). Acupuncture offered neuro-protection in spinal cord injuries with DU 26 and GB 34 being the most potent of the points tested.

Geng, Liu, Song and colleagues (2013) studied the effects of electro-acupuncture performed on ST 36 in smoke-induced chronic obstruction pulmonary disease (COPD) in male Sprague Dawley rats weighing 180-200g. There were four groups-control, sham, COPD and COPD plus electro-acupuncture (COPD-EA). The COPD groups were exposed to cigarette smoke for 1 hour twice a day for 12 weeks. It was set up to mimic

approximately 10 years smoking. EA was performed on ST 36 for 30 minutes a day in both the COPD-EA group and sham group. The EA elicited a small muscle contraction in the hind leg of the rat at alternating 60 Hz for 1.05 seconds and 2 Hz for 2.85 seconds with intensity $\leq$ 1.5 mA. Bronchoalveolar lavage fluid (BALF) was measured to determine the change in TNF-α and IL-1β.

The results showed TNF-α and IL-1β in BALF were increased in COPD rats compared to the control group as expected ($p < 0.01$). The COPD-EA group had decreased levels of both inflammatory markers compared to the COPD group ($p < 0.05$). The COPD-EA group still had higher levels of TNF-α and IL-1β than the control group ($p < 0.05$). There were not significant differences between sham and control group (Geng et al., 2013).

J. Song et al. (2013) conducted a study on male Sprague Dawley rats, 200-300g to evaluate electro-acupuncture's effect on burn induced impairment via the vagal mechanisms. There were 81 total rats divided into 10 groups. There was one sham-burn group left untreated and one burn group left untreated. The burn was induced by immersing the shaved ventral and dorsal area of the rats into 95° C water. The rat was then resuscitated with an injection of 24 mL of Ringer lactate solution. The sham burn rats were shaved and injected with the solution but were not burned. Two other groups were burned and then had EA performed 6 hours following the burn and the other 24 hours following the burn. Two groups were burned and then received sham EA 6 hours after the burn and the other 24 hours after. Sham acupuncture consisted of 2 needles inserted in non-acupoints on the hypochondria and the electric stimulator was off. There were 2 groups of rats that underwent a vagotomy at the sub-diaphragmatic ventral and

dorsal vagal trunk. One of those were also burned and received EA 6 hours following the burns and the other was burned and received sham acupuncture 6 hours following. The last two groups underwent a sham vagotomy where the vagus nerves were only exposed. Both sham vagotomy groups were burned and received either EA or sham EA 6 hours following the burn. Blood was taken on the fourth day before the burn occurred before the rats fasted overnight and also after the burn.

The results showed plasma IL-6 was increased in all groups with the burn. EA significantly lowered IL-6 levels at both 6 hours ($p=0.03$) and 24 hours ($p= 0.003$) following burn compared to sham EA. The same inflammatory modulating effect was blocked on the rats that underwent vagotomy. EA also significantly increased vagal activity measured via ECG. High frequency was measured and significantly higher in EA ($p=0.004$) versus the sham at 6 hours and at 24 hours ($p=0.03$). This study showed EA at ST 36 was able to significantly increase vagal activity and decrease plasma IL-6 via the vagus nerve (J. Song et al., 2013).

The effect of EA at ST 36 was investigated by Song et al. (2014) to discover if it attenuates pro-inflammatory cytokine release via the cholinergic anti-inflammatory pathway in rats with endotoxin challenge. Seventy Wistar rats ranging from 200-240g were divided into 7 groups. Two groups received EA bilateral on ST 36 or sham acupuncture with LPS injection. Two of the groups received bilateral cervical vagotomy and then one group received EA and the other sham. The sham acupuncture was performed on bilateral non-acupoints on the outside of the fibula approximately 1 cm from condyle. Another two groups received α-BGT injection and EA on ST 36 or sham

acupuncture. There was one control group did not receive injection or treatment. Plasma levels of TNF-α and IL-6 were measured.

The researchers found serum levels of TNF-α and IL-6 were significantly higher in the rats that received LPS injection versus the control. TNF-α levels were significantly decreased ($p < 0.05$) with EA and significantly more than the sham EA. Neither EA nor sham EA had any effect of IL-10 levels. EA also had no effect on TNF-α levels when rats were injected with α-BGT or when vagotomy was performed. The researchers concluded EA was able to reduce pro-inflammatory factors (TNF-α) but not able to significantly effect anti-inflammatory factor (IL-10). This effect occurred by activation of the cholinergic anti-inflammatory pathway (Q. Song et al., 2014).

Zhang et al. (2014) observed the effect of EA at ST 36 treatment with postoperative abdominal adhesions. Inflammatory markers were used as the primary measurement of EA's effect. The researchers used male Wistar rats weighing 200-240g as subjects. The rats were divided into 8 groups: the sham control group, abdominal adhesions model, abdominal adhesions plus EA, sham acupoint group, abdominal adhesions with vagotomy, abdominal adhesions with EA after vagotomy, abdominal adhesions with α-BGT and lastly abdominal adhesions with EA after α-BGT. The sham group was not given abdominal adhesions and only had the cecum flipped and then abdomen was sealed. EA was performed 40 minutes after adhesion surgery in those groups that were designated to receive it. Bilateral ST 36 was the point stimulated for 1 hour continuously at 2 mA and 2-100 Hz. One group received EA at a non-acupoint located 5mm below and outside of ST 36. The non-acupoint was stimulated exactly the same as EA groups. The vagotomy groups had bilateral abdominal vagus nerve

transected before abdominal adhesions. The α-BGT was injected into the two groups following the adhesion surgery.

On the third day following surgery, tissue TNF-α levels were significantly increased in all groups compared to sham control. The abdominal adhesions in the groups that received EA group had significantly lowered tissue levels of TNF-α than all groups outside of the sham control ($p < 0.05$). These cytokine levels still remained higher than the control group. EA at ST 36 in vagotomy and α-BGT injection showed no significant differences. The anti-inflammatory protection of EA was diminished with the vagotomy and α-BGT injection (Zhang et al., 2014).

This collection of studies showed the significant role acupuncture played in regulating the immune system by attenuating inflammatory markers. The studies showed acupuncture influences these markers by the cholinergic anti-inflammatory pathway that is blocked when the vagus nerve is not intact.

VNS and Inflammatory Markers

This section consists of 9 rat studies that used vagus nerve stimulation as treatment to discover its effects on inflammatory markers.

Zhao et al. (2012) researched the effect of transcutaneous auricular vagus nerve stimulation (ta-VNS), vagus nerve stimulation (VNS) and transcutaneous acupoint stimulation's (TEAS) effect on pro-inflammatory cytokines: TNF-α, IL-1β and, IL-6 from blood serum collected and NF-kappa B p65 (NF-κB p65) expressions from lung tissue. There were several parts in the study. In the first part using 12-week-old male Sprague Dawley rats ranging from 275-350g were divided into 5 groups. Four groups

were injected with lipopolysaccharide (LPS); an endotoxin to induce inflammation and the fifth group was injected with a saline solution. The rats induced with LPS were treated with either ta-VNS on the auricular concha, TEAS on ST 36, VNS or not treated at all. The study also observed the effects of these inflammatory markers after a vagotomy. Rats in this part of the study had a vagotomy performed and then were treated with saline solution, or injected with LPS and then treated with ta-VNS on auricular concha, TEAS on ST 36 or VNS. Another component involved in the study was to look at the effect after nicotinic acetylcholine receptors (a7nAChr) antagonist, α-bungarotoxin (α-BGT) injected. Rats in this part were divided in 4 groups as well: saline treated, endotoxemia model (injected LPS), rats injected with a7nAChr antagonist and then LPS were either treated with TEAS on ST 36 or ta-VNS on the auricular concha. The VNS group underwent stimulation and hour and half following LPS set at 1 mA, 10 Hz for 10 minutes. Both ta-VNS and TEAS on ST 36 were electrically stimulated an hour and a half after LPS and intensity was set to elicit a muscle twitch at a maximum of 1 mA.

The results showed that LPS increased the cytokine expressions of TNF-α, IL-1β and IL-6. VNS and ta-VNS inhibited cytokine concentrations of TNF-α, IL-1β and IL-6 strongly ($p < 0.01$ & $p < 0.05$ respectively). TEAS on ST 36 only lowered TNF-α significantly ($p < 0.05$) and had no significant effect on IL-β and IL-6 in serum. The suppression of cytokines with ta-VNS and TEAS were blocked in both the models that were injected with the a7nAChR agonist and the models that underwent vagotomy. NF-κB p65 immunohistochemistry staining was performed on lung tissues. NF-κB p65 was strongly inhibited in the VNS and ta-VNS groups induced with LPS ($p < 0.01$) but were

not significantly affected by TEAS. Again these effects were blunted when vagotomy was performed.

This study shows ta-VNS on the auricular concha can be used to suppress pro-inflammatory cytokines TNF-α, IL-1β and IL-6 via the a7nAChR mediated cholinergic anti-inflammatory pathway and regulate NF-κB activities. It also demonstrates serum TNF-α can be reduced by TEAS on ST 36 (Zhao et al., 2012).

Schulte et al. (2014) investigated how loss of vagal tone aggravates inflammation in endotoxemic rats. Lewis rats weighing 275-300g were the subjects in this study. There were 2 sham groups, one that was not injected with LPS and one injected with LPS. Three other groups received right cervical vagotomy and injections of LPS. They then received either no VNS; VNS for 2 minutes or VNS for 20 minutes which all began 5 minutes after LPS was injected. The stimulation was performed on the vagal trunk and set at 5v, 2 ms and 1 Hz.

The results showed TNF-α and IL-1β from both serum and tissues from the ventricle were slightly increased compared to the control. The levels in rats that underwent vagotomy were significantly increased ($p < 0.05$). VNS on the vagal trunk was able to attenuate the inflammatory markers although it remained higher than the control ($p < 0.05$). The significance of the study shows that vagotomy caused an increase in the inflammatory response potentially because it is not able to balance it by activating the efferent response (Schulte, Lichtenstern, Henrich, Weigand, & Uhle, 2014).

The aim of the study conducted by Zhou et al. (2014) was to illuminate the neuro-protective mechanism of the vagus nerve on traumatic brain injury induced on rabbits. Twenty-eight New Zealand rabbits were randomly grouped into 4 separate groups:

control group-which did not receive any treatment; sham surgery group-had their skull opened but no injury or VNS; explosive injury; VNS group. The brain injury was inflicted on the rabbits in the explosive injury and VNS group by first performing a craniotomy with exposure of right parietal cortex, then the midpoint of a lightning firecracker was placed .5 cm from brain tissues and 5 pieces of metal were placed in the cortex the firecrackers were lit outdoors to create the explosive injury model. The right cervical vagus nerve was stimulated at 10 V, 5 ms, and 5 Hz for 20 minutes. Blood was collected from the rabbits 6 hours following the injury.

Zhou and colleagues found TNF-α and IL-1β levels in the serum and brain tissue were significantly higher in the VNS group ($p < 0.01$) compared both the sham and control group. They were also significantly lower than the explosive injury group ($p < 0.01$). Serum and tissue IL-10 levels were significantly higher in the VNS group compared to explosive injury group ($p < 0.01$). The significance of these results was that VNS was able to decrease pro-inflammatory cytokines, TNF-α and IL-1β and increase anti-inflammatory cytokine IL-10 (Zhou et al., 2014).

The purpose of the study by Kyzyzaniak et al. (2011) was to evaluate VNS on lung tissue after an abdominal vagotomy. Male balb/c mice were divided into groups. In the sham group mice were shaved in dorsal area and had right cervical incision with the exposure of the vagus nerve but no stimulation. Another group of mice were steam burned for 7 seconds to create about 30% total body surface area burn. The third group had an abdominal vagotomy prior to burn and VNS. Both burn groups received VNS with 2 mA at 1 second intervals for 10 minutes prior to burn.

The NF kB pathway was analyzed and the researchers found NF-κB p65 in the lung tissues were significant higher in the burn group that did not receive VNS versus both the sham group and VNS + burn group ($p \leq 0.001$). The vagotomy group of mice did not receive the protection from VNS seen in the group with vagus nerve intact. Their levels were similar to the untreated burn group. The importance of this study was to show VNS can attenuate the NF kB pathway but this effect is blocked or diminished with abdominal vagotomy (Krzyzaniak et al., 2011).

Kox et al. examined the effects of vagotomy and VNS on systemic inflammation. The researchers chose to measure the cytokines TNF-α and IL-10 and, IL-6. Male Sprague Dawley rats weighing between 300-450g were divided into 3 groups, sham operation group, vagotomy group and VNS group. All three groups were injected with LPS. A cervical incision was made to expose the left vagus nerve. The sham group only had the nerve exposed and then covered with gauze. The VNS group received 3 minutes of stimulation at 5 V, 5 Hz and 2 ms. The vagotomy group had both the left and right vagus nerves transected. Blood serum and pulmonary levels of pro-inflammatory cytokines were examined.

Vagotomy resulted in significantly increase in pulmonary levels of TNF-α when compared to sham or VNS rats ($p < 0.05$) and had no effect on IL-10. Plasma cytokines levels measuring systemic inflammation were not affected by vagotomy. VNS had no effect on plasma or pulmonary levels of cytokines. The researchers found that while vagotomy increased the inflammatory response in pulmonary inflammation it did not affect systemic inflammation. Also VNS did not have any effect on pro-inflammatory or

anti-inflammatory cytokines. They researchers questioned the ability of VNS to attenuate cytokines as seen in many other studies on inflammation (Kox et al., 2012).

Sun et al. evaluated how chronic VNS can affect rats against 2, 4, 6-trinitrobenzsnsulfonic acid (TNBS) used to induce colitis. Both male and female Sprague Dawley rats weighing 180-220g were split into 4 groups: sham VNS and saline injection, VNS and saline injection, sham VNS and TNBS and, VNS and TNBS. Surgery was performed on all rats to expose the left cervical vagus nerve. Electrodes were placed with leads and stimulator. The sham VNS groups underwent the same procedure but did not receive stimulation. Seven days after the surgery TNBS was administered and the VNS stimulation continued from day 1 to day 6 at .25 mA, 20 Hz, 500 ms with 30 seconds on and 5 minutes off for 3 hours each day. The researchers measured HRV along with TNF-α and IL-6 in colonic tissues. The expression of NF-κB p65 was also monitored.

The results showed TNF-α and IL-6 were elevated with the TNBS group but the group receiving VNS was able to significantly inhibit the pro-inflammatory cytokines ($p < 0.05$). TNBS also activated the NF-κB p65 expression in the tissue collected from the colon and VNS was able to reverse this effect ($p < 0.05$). The balance of sympathetic-vagal balance also showed the TNBS decreased HF. VNS was able to balance and significantly increase HF and decrease LF ($p < 0.05$) (Sun et al., 2013).

Jiang et al. (2014) investigated how vagus nerve stimulation attenuates acute cerebral ischemia via the cholinergic pathway in rats. Male Sprague Dawley rats weighing between 250-250g were divided into 3 groups, sham w/ VNS, focal cerebral ischemia and reperfusion (I/R) and, I/R with VNS. I/R was established in the rats using the intraluminal occlusion technique to cause middle cerebral artery occlusion. The

subjects had the right cervical vagus nerve exposed and the stimulating electrode attached. The sham group received the same treatment minus the occlusion. Thirty minutes following the occlusion VNS was administered for 30 s every 5 minutes for a total of 60 minutes. The stimulation was set at .5 mA, 20 Hz, and .5 ms. The researchers measured concentrations of TNF-α, IL-1β and, IL-6 obtained from the ischemic cortex tissues.

The pro-inflammatory cytokines measured in the study were significantly decreased in the I/R with VNS compared to I/R group ($p < 0.05$). VNS was able to reduce the expression of TNF-α, IL-1β and IL-6 (Jiang et al., 2014).

In a similar study Mihaylova, Schweighöfer, Hackstein and Rosengarten designed a study to measure the effect of vagus nerve stimulation on LPS induced rats on blood and spleen lymphocyte subsets. The study also looked at how cervical vagotomy would influence the vagus nerve stimulation. Male Sprague Dawley rats weighing 290-320g were divided into 6 groups. Three groups received LPS injection and another 3 were sham control groups given saline solution. In each of the sham and LPS groups there was a group that received no treatment and acted as control, bilateral cervical vagotomy (VGX) or bilateral cervical vagotomy and VNS on distal trunk of left vagus nerve. The stimulation began directly after LPS was administered. The stimulation occurred for 10 minutes at 2 mA, 2 Hz and .3 ms and then off for 35 minutes. The process repeated for 4.5 hours, which was the length of the experiment. Cytokine concentrations of IL-10, TNF-α and IFNγ were analyzed.

The levels of IL-10, TNF-α and IFNγ were significantly increased in all LPS groups compared to sham. The group that underwent vagotomy and VNS while trended

to lower values of the pro-inflammatory cytokines did not reach statistical significance. While this study did not show a statistical decrease in the cytokines listed above with VNS this may be attributed to the vagotomy (S. Mihaylova, Schweighofer, Hackstein, & Rosengarten, 2014).

Mihaylova and colleagues had another very similar study as the one previously mentioned that look at the effect of VNS on the cerebral microcirculation in endotoxinemic rats. The exact same study design was used on the Sprague Dawley rats and measurements of plasma TNF-α, IL-10, IL-6 and, IFNγ were evaluated in the VNS and vagotomy rats. The levels of cytokines in the VNS group that also underwent vagotomy were statistically insignificant from the other groups. The trend showed VNS did attenuate the inflammatory response while the vagotomy group without VNS had an increased inflammatory response (Stanka Mihaylova et al., 2012).

The studies researched the effects direct stimulation of the vagus nerve by surgical insertion of a stimulator on the vagus nerve had on inflammatory markers. These studies showed the importance of the vagus nerve in the neuro-endocrine signaling to influence the immune system and regulate inflammation.

Conclusion

The studies investigating the effect of acupuncture and VNS on inflammatory markers both show that these therapies can increase vagus nerve activity. Acupuncture and VNS have the ability to modulate the inflammatory markers. The studies also showed the vagus nerve was a critical component of this mechanism by also measuring this effect when a vagotomy was performed.

The researcher's objective was to bridge the research on both therapies to demonstrate acupuncture increases vagal activity as VNS does making it an important consideration for patients with decreased vagal tone.

Chapter 3: Method

Statement of Methodology

The researcher performed a retrospective review of studies in order to evaluate the effect of acupuncture on the vagus nerve to influence inflammation and the immune system. A literature synthesis was performed as an effective way to measure the outcomes that relate to vagus nerve activity on inflammatory markers. This evaluative method to review the research is effective in presenting data of both acupuncture and VNS to identify the potential connections and integrate the research of the two therapies(Cooper, 2009).

Procedures

Databases used in the search for studies were EBSCOhost, Mary Ann Liebert, Google Scholar, PubMed and, Research Gate. The search was compiled using terms: acupuncture, electro-acupuncture, vagus nerve stimulation AND vagus nerve, inflammation, immunity, cytokine, immune system, vagotomy.

Inclusion/Exclusion Criteria

This initial search brought up over 100 results. Any articles that were not entirely available in English, had only an abstract available or were published earlier than 2000 were initially excluded. Only peer-reviewed articles were chosen during the research. This left 92 articles, 28 Western background articles and 64 pertaining to acupuncture or vagus nerve stimulation. Eighteen of the 28 Western articles were used as background information in Chapter 1. The ten were excluded because they had only very basic information of the vagus nerve or immune system that was greatly expanded on in the other 18 articles. Of the 64 acupuncture articles 16 met the inclusion criteria. They measured cytokines related to the vagus nerve. Articles conducted in any country were included. All articles defined statistical significance as $p < 0.05$. Articles with less than 10 subjects were removed as well as those with poor methodology quality. Poor methodology quality was determined when the studies did not give sufficient information in their design to be able to replicate the study. The 16 remaining articles were divided into 2 groups. There were 7 that measured cytokine concentrations with acupuncture and 9 that measured cytokine concentrations with vagus nerve stimulation. After careful consideration only animal studies were used and the human studies were eliminated.

This was done to focus the studies on the effect acupuncture or VNS had specifically via the vagus nerve and not just any general effect on cytokines.

Chapter 4: Results

This chapter looks at the connections and common patterns that emerged from the articles presented in Chapter 2. Statistical significance for the articles included in the research were $p < 0.05$. Only a few of the articles reported the actual p values in their studies. The significant data was compiled and analyzed to answer the research question: How does acupuncture affect the vagus nerve to influence inflammation and the immune system?

Acupuncture Studies

There were several key elements in the acupuncture studies that were analyzed. The effect of acupuncture on the cytokines measured in the studies was the foundational data used to address the research objective. The effect of a vagotomy prior to acupuncture was analyzed. Another important research component was the effect acetylcholine receptors (a7nAChr) antagonist, α-bungarotoxin (α-BGT), had on subjects. Lastly the data that measured the effectiveness of sham acupuncture versus acupuncture was compiled.

Table 1 illustrates the different cytokines measured in the studies when acupuncture was performed on the subjects. The number of studies that measured each different cytokine and the subsequent effect on them when acupuncture was performed is shown in the table.

Table 1: Acupuncture & Cytokines

Pro-Inflammatory Cytokines	**Anti-Inflammatory Cytokines**
TNF-α:	IL-10:
serum: ↓ (5/5 studies)	serum: NSC (2/2 studies)
tissues:↓ (1/1 studies)	
IL-1β:	
serum: ↓ (2/2 studies)	
IL-6:	
serum: ↓ (4/4 studies)	
INFγ:	
serum: ↓ (1/1 studies)	

(↓ represents significant decrease in levels $p < 0.05$) (NSC: no significant change $p > 0.05$)

The acupuncture articles measuring cytokines all showed there were significant decreases in pro-inflammatory cytokines as shown in Table 1. Every pro-inflammatory cytokines measured changed significantly with the administration of acupuncture.

Within these 7 articles, 6 of them used EA at ST 36 and 1 used manual acupuncture on DU 26 and GB 34. Two studies looked at anti-inflammatory cytokine, IL-10 and showed there was no significant change in levels.

The effect of acupuncture on the cytokines when a vagotomy is performed is displayed in Table 2. This data is significant because it demonstrates the critical role the vagus nerve plays in the mechanism to decrease cytokines with acupuncture.

Table 2: Vagotomy with Acupuncture & effect on cytokines

TNF-α:
serum: Blocked effect (3/3 studies)
tissues: Blocked effect (1/1 studies)
IL-6:
serum: Blocked effect (3/3 studies)
INFγ:
serum: Blocked effect (1/1 studies)

(Blocked effect of acupuncture)

Five of the acupuncture studies researched the effect a vagotomy would play in the mechanism of acupuncture to modulate cytokines. All five studies showed that vagotomy blocked the ability of acupuncture to significantly decrease cytokines that otherwise were significantly decreased in the subjects when vagus nerve was intact. (Table 2)

The studies that evaluated a7 nicotinic acetylcholine receptors (a7nAChr) antagonist, α-bungarotoxin (α-BGT) are shown in Table 3. Acetylcholine, the main neurotransmitter of the vagus nerve inhibits the release of TNF and prevents further cytokines by binding to a7nAChR expressed on macrophages (Andersson & Tracey,

2012). With the injection of α-BGT, researchers were confirming the role a7nAChr via the vagus nerve.

Table 3: a7AChR agonist with Acupuncture & effect of cytokines

TNF-α:
serum: Blocked effect (2/2 studies)
tissues: Blocked effect (1/1 studies)
IL-6:
serum: Blocked effect (1/1 studies)

(Blocked effect of Acupuncture)

There were three acupuncture studies that researched the α-BGT injected in subjects. The injection of α-BGT blocked the effects of acupuncture on the cytokines in every study that otherwise were decreased in the subjects that did not undergo injection. (Table 3)

Table 4 looks at the two different forms of sham acupuncture used in studies. This data was compiled to show the effectiveness of sham acupuncture versus acupuncture. This analysis is important to verify acupuncture is not a placebo.

Table 4: Acupuncture versus Sham

Sham on Non-acupoint	**Sham with Wooden Toothpick**
TNF-α:	TNF-α:
serum: NSC (3/4 studies); ↓ (1/4 studies)	serum: NSC (2/2 studies)
tissues: NSC (1/1 studies)	IL-1β:
IL-1β:	serum: NSC (1/1 studies)
serum: NSC (1/1 studies)	IL-6:
IL-6:	serum: NSC (2/2 studies)
serum: NSC (2/2 studies)	INFγ:
	serum: ↓ (1/1 studies)

(NSC: no significant change p >0.05)

The majority of the studies showed sham acupuncture either on a non-acupoint or with a wooden toothpick on the acupoint did not significantly reduce cytokines. These cytokines were significantly reduced with acupuncture. There was one article that showed a significant decrease in cytokine, TNF-α, when a non-acupoint was used for treatment. Overall, sham acupuncture is not as efficacious as acupuncture. (Table 4)

Vagus Nerve Stimulation Studies

VNS studies were analyzed to determine their effect on inflammatory markers. Along with the individual inflammatory markers analyzed the effect of vagotomy with VNS was also evaluated. These components were identified by the research as important to compare with acupuncture studies.

Presented in Table 5 are the different inflammatory cytokines evaluated in the studies when VNS was performed on the animals. The number of studies that measured each different cytokine and the effect on them when VNS was performed is shown in the table. Both significant and non-significant changes are noted.

Table 5: VNS & Cytokines

Pro-Inflammatory Cytokines	**Anti-Inflammatory Cytokines**	**Other Markers**
TNF-α: serum: ↓ (3/4 studies); NSC (1/4 studies) tissues: ↓ (2/3 studies); IL-1β: serum: ↓ (3/3 studies) tissues: ↓ (1/1 studies) IL-6: serum: ↓ (1/1 studies) tissues:↓ (2/2 studies)	IL-10: serum: ↑ (1/1 studies); NSC (1/1 studies)	NF κB p65: tissues: ↓ (3/3 studies)

(↓ represents significant decrease in levels $p<0.05$)(↑ significant increase in levels $p<0.05$)(NSC: no significant change $p>0.05$)

Six of the seven of articles measuring inflammatory markers with VNS showed VNS significantly attenuated pro-inflammatory cytokines or NF κB p65. Only one out of two studies that looked at anti-inflammatory cytokine, IL-10 showed it increased with VNS. (Table 5)

The consequence of vagotomy with VNS is demonstrated in Table 6. This data is comparable to the data of the acupuncture studies. In addition it shows the importance of an intact vagus nerve in modulating inflammatory markers.

Table 6: Vagotomy with VNS & effects on Inflammatory Markers

TNF-α:
serum: ↓ (1/3 studies); Blocked effect (1/3 studies); NSC (1/3 studies)
tissues: ↑ (1/1 studies);
IL-1β:
serum: ↓ (1/2 studies); Blocked effect (1/2 studies)
IL-6:
serum: Blocked effect (1/2 studies)
NF κB p65:
tissues: Blocked effect (2/2 studies)

(Blocked effect of acupuncture)

Six out of nine articles examined the effect of vagotomy with VNS. Of those, two articles not included in Table 6, but of importance, looked exclusively at vagotomy with VNS. They did not have subjects that only received vagus nerve. Both articles showed the VNS had no effect on cytokines TNF-α, IL-6, IL-10 and, INF-γ. Two out of four articles that looked at both vagotomy with VNS compared to just VNS showed that the vagotomy blocked the anti-inflammatory effects seen in subjects with the vagus nerve intact. (Table 6)

Conclusion

The studies measuring acupuncture and VNS both showed the therapies were able to significantly modify inflammatory markers. They also both showed the importance of the vagus nerve to effect these markers. Acupuncture and VNS increased vagal activity proved by the diminished cytokines. When vagotomy is performed the ability of either acupuncture of VNS was considerably diminished or blocked.

Chapter 5: Discussion

This literature synthesis examined the effect of both acupuncture and VNS on inflammatory markers in animal subjects. It also observed the role a vagotomy played in the mechanism. This researcher's intention was to discover if acupuncture could significantly affect inflammation comparably to VNS via the vagus nerve by activating the cholinergic anti-inflammatory pathway. Another objective was to use the analysis of the studies to discover the inferences that emerged to bridge the relationship of the acupuncture and VNS.

Summary of Findings

The collection of articles supports the hypothesis that acupuncture significantly impacts pro-inflammatory cytokine levels in animal models. These studies also showed acupuncture is significantly more effective than sham acupuncture in modulating these cytokines. These same studies showed that the effects of acupuncture were blocked or greatly diminished if a vagotomy was performed prior to stimulation. This supports the idea that acupuncture influences inflammatory markers via the vagus nerve and cholinergic anti-inflammatory pathway. In addition, 3 of the acupuncture studies researched the a7 nicotinic acetylcholine receptors (a7nAChr) antagonist, α-bungarotoxin (α-BGT) injected into subjects. The injection of α-BGT also blocked the effects acupuncture had on reducing pro-inflammatory cytokines. This analysis shows that acupuncture acts on the vagus nerve, which releases acetylcholine and inhibits the release of TNF by binding to a7nAChR (Andersson & Tracey, 2012). This demonstrates the importance of the vagus nerve in the mechanism of acupuncture to attenuate cytokines.

Vagus nerve stimulation studies were also analyzed to discover their effects on inflammatory markers and to compare those result with acupuncture results. These studies also performed on animals showed VNS significantly decreased pro-inflammatory markers. The vagotomy performed prior to VNS showed that in 2/3 of the studies that the vagotomy blocked the benefits of the VNS.

Implications for Theory

Inflammation is a key immune defense in the body. When there is a disruption in the balance of the immune system one consequence is unnecessary excessive inflammation. This is seen in chronic inflammatory diseases, where there is an excessive amount of pro-inflammatory cytokines (Pavlov & Tracey, 2012). As shown in the studies presented in this research acupuncture has ability to modulate the parasympathetic nervous system, in effect reducing inflammatory markers by increasing vagal activity. Acupuncture is a great tool in addressing both chronic and acute inflammation, which can be detrimental to many organ systems.

Implications for Practice

The research supports the theory that acupuncture is a valid alternative or adjunct to current interventions in treating systemic inflammation. It should be a serious consideration for those using pharmacological interventions to treat chronic inflammatory diseases that show a decrease in vagal activity and increase in pro-inflammatory cytokines (Johnston & Webster, 2009). Long-term uses of immunosuppressive drugs are associated with many side effects that reduce longevity (Koopman et al., 2014). Acupuncture has little or no side effects compared to the current pharmacological

interventions. It also offers hope to individuals that do not respond to the drug interventions.

Another objective of the research was to bridge the research on the stimulation of the vagus nerve using bioelectronics devices with the use of acupuncture and its effect on the immune system. More research is being focused on the vagus nerve and its role in immunity. There are several pilot studies just beginning or recently completed examining the safety and efficacy of VNS with chronic inflammatory diseases such as rheumatoid arthritis, postoperative ileus or Crohn's disease (Levine et al., 2014). While VNS may be a great therapy to combat inflammation as seen in the research, acupuncture offers the benefits shown in vagus nerve stimulation. Acupuncture does not involve the risk of surgery or some of the adverse effects that have occurred with VNS such as obstructive sleep apnea, chronic laryngeal and pharyngeal muscles in constant contraction have shown to cause reducing airway flow, voice alteration, cough and throat pain (Gale, 2014). Acupuncture can be a safe alternative with little to no side effects to treat immune system disorders by using the same mechanism of action. Both acupuncture and VNS increase vagal activity and activate the cholinergic pathway to regulate the immune system. The analysis of research shows acupuncture is a valid therapy to treat systemic inflammation and should be tried before more drastic measures are considered.

This research can be of value in the Chinese Medicine communities. The data compiled by the researcher illustrates the strength acupuncture has in modulating inflammatory markers and increasing vagal activity. In practice, this has a wide range of benefits to many sufferers of chronic illnesses such as rheumatoid arthritis, systemic

lupus, erythematosus, ankylosing spondylitis, chronic inflammatory bowel disease or other autoimmune disease.

In the Western community this research can be of value for the prescription of acupuncture as a treatment modality for diseases as mentioned above. Currently acupuncture is covered by health insurance for a limited number of diagnoses. Objective data that illustrates the effect of acupuncture such as that presented by researcher has the potential widen the range of health issues covered by insurance.

Limitations of the Study

There were several limitations identified by the researcher. The first was the small number of studies available with quality methodology to measure acupuncture and biomarkers with vagotomy. Another limitation was the source of measurement for the biomarkers. The evaluation techniques were different in some studies. They measured different biomarkers in their study so a broader range of biomarkers from both serum and tissues were considered for analysis. The researcher used animal models to better prove the effect of acupuncture via the vagus nerve by using studies that included subjects with a vagotomy. Therefore human studies were excluded. The researcher assumed the same outcomes in animals translated to human models. Another reason animal studies were used was due to the lack of available data for the VNS performed on humans. VNS is currently being explored in several studies that are either beginning, current or just completed and have not published any findings as of now. Another limitation was the small variety of acupuncture points used in the studies (ST 36, DU 26, GB 34). In the VNS articles the vagotomy was performed at different locations, cervical or abdominal,

which could have affected the outcome in the studies and differences when analyzing the articles together is this research.

Recommendations for Future Studies

Future studies have great potential to better illuminate acupuncture's role on cytokines and inflammatory markers via the vagus nerve. Studies performed on human subjects in a disease model can compare both acupuncture and VNS together. There were not any longitudinal studies available. Human trials over a longer period of time would add more weight to the findings by showing if acupuncture has a cumulative effect or not and make it more comparative to VNS.

The use of different acupuncture points in studies especially the inclusion of ear points while measuring cytokines can also prove to be helpful. Ear acupuncture stimulates the vagus nerve via the cymba concha and has the potential be even more comparable even more to VNS. One article brought up an interesting point about stimulation of vagus nerve possibly leading to immune-suppression, and dampening the immune response can be damaging in situations such as the case of infectious disease (Matteoli & Boeckxstaens, 2013). While acupuncture may have more of a regulatory effect instead of just down regulation of the immune system this is an important point for researchers to consider and possibly measure when developing future studies.

Conclusion

The intention of this research synthesis was to determine the effect of acupuncture on the vagus nerve to affect the immune system by measuring inflammatory cytokines. Those results were then compared to the influence of VNS on the vagus nerve. The researcher sought to prove that acupuncture could be a first line of treatment for systemic inflammation due to its ability to act similarly to VNS on the inflammatory markers with minimal side effects if any at all.

Acupuncture was shown to significantly attenuate inflammatory markers, reducing the damaging effects of excessive and/or unwarranted inflammation. The neuro-immune system is immeasurably complex with new pathways constantly being explored and discovered. Information in this field is rapidly and continually growing as researchers learn more about the immune system. These discoveries regarding the vagus nerve offer many benefits for patients with low vagal activity, autoimmune diseases, or acute inflammation. Acupuncture can have a great impact on these individuals by supporting those in the disease state. There could be more control over inflammation with fewer side effects than either pharmacological effects or VNS. It should be explored and considered as valuable method of treatment before more extreme measures of surgical implantation of vagus nerve stimulator or long-term immunosuppressive drugs are implemented. Acupuncture has the ability to promote longevity by diminishing the impacts of acute and chronic inflammation that diminishes quality of life. These benefits come without having to endure from side effects as a trade-off.

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Appendix A: IRB Approval Letter

October 8th, 2014

Zaria Valentine
1533 Jackson St., Apt 315
Oakland, CA 94612

Dear Zaria,

Your research proposal has been approved, with no additional recommendations effective through March 31, 2016.
Should there be any significant changes that need to be made which would alter the research procedures that you have explained in your proposal, please consult with the IRB coordinator prior to making those changes.

Sincerely,

For/Penny Weinraub, L.Ac.
IRB Coordinator

Appendix B: Data Abstraction Form

Article Abstraction Form **Date:**

Title

Health or Disease Condition:_____

Animal: Randomization: Y/N

Intervention: VNS___Acupuncture___ Electro-acupuncture ___ Other___

Control Groups:

Data Measured:

Data analysis:

Results:

Significance:

Study Limitations:

Appendix C: Initial Data Extraction

Article #	Year	Animal/Human	# Participants	Healthy/Disease	Type of Treatment	Points	# Sessions	Randomization	Blinded	Control	Sham	Data Measured	Significance
1	2012	Animal-Male Sprague Dawley rats		LPS induced inflammation	VNS TEASta-VNS saline treated	ST 36 (TEAS) auricular concha (cavum and cymba) (ta-VNS)	1	N/A		saline treated	none	NF-κB p65 (Lung tissue) TNF-α (serum) IL-1β IL-6 w/ vagotomy w/ a7AChR agonist	VNS: TNF-α ↓, 1β □, IL-6 □ NF-κB p65 □ ta-VNS: TNF-α ↓, 1β □, IL-6 □ NF-κB p65 □ TEAS: TNF-α □,IL-1β (NSC), IL-6 (NSC), NF-κB p65 (NSC) *All effect blunted /blocked vagotomy or a7AChR agonist
21	2014	Male Lewis rats		LPS	VNS	vagal trunk	1			yes	yes	TNF-a IL-1B	VX: increase TNF-a & IL-B response (p <0.001) VNS: □ TNF-a & IL-1B (serum) (p<0.05)
23	2014	New Zealand Rabbits		Traumatic Brain Injury	VNS	R cervical VN	1			yes	yes	TNF-a IL-1B IL-10	VNS: TNF □ IL-1B □ (p<0.01) vs brain injury IL-10 □(<0.01) vs brain injury

24	2011	balb/c mice		Burn	VNS	R cervical VN	1			yes	yes	NF-κB p65 (Lung tissue)	VNS: ↓NF-κB p65 vs burn Abd VX: blocked effect
25	2012	Sprague Dawley Rats		LPS	VNS VX	L cervical VN	1			no	yes (operation)	IL-10 TNF-a	VNS: NSC TNF-a (plasma or pulmonary) VX (no VNS): NSC plasma; ↓TNF-a (pulmon)
26	2013	Sprague Dawley Rat		TNBS	VNS	L cerival VN	6			yes	yes	IL-6(colon) TNF-a (colon) NF kB p65 (colon)	VNS: TNF-a ↓ 6 ↓, NF kB p65 ↓ < 0.05)
27	2014	Sprague Dawley Rats		acute cerebral ischemia (I/R)	VNS	R cervical VN	1				yes	TNF-a IL-1B IL-6 (coret ex tissue)	I/R + VNS: TNF-a ↓ IL-1B ↓ IL-6 ↓ compared I/R (p<0.05),
29	2014	Sprague Dawley rats		LPS		L distal trunk	1			yes	yes		VNS/VX (LPS): TNF-a:NSC IL-10:NSC INFy: NSC although lower than VGX/LPS

36	2012	Sprague Dawley rats		LPS		L distal trunk				yes	yes		VNS/VX (LPS): TNF-a:NSC IL-10:NSC INFy: NSC although lower than VGX/LPS
5	2013	Animal-Sprague Dawley rats		45% blood loss, DFR	EA	ST 36 (EA)	1	n/a		none	EAN: acup .5mm lateral & distal to ST 36	MAP TNF-α (plasma & intestinal) IL-6 (plasma & intestinal) IL-10 survival rate	EA: TNF-α □, 6 □, IL-10 (NSC) (plasma & intestinal) EAN: not sig reduced EA w/vagotomy: block EAN w/ vagotomy: block EA w/α-BGT: block EAN w/α-BGT: blocked
6	2014	Animal: Wild type rats, α7nAChr-knockout, β2AdrR-knockout in vitro		LPS induced sepsis	EA	ST 36 (EA)	1a	yes		sham surgery	wooden toothpick instead of electrodes-3cm distal ST 36	TNF-α IL-6 INF-γ	EA: TNF-α: □ 6: □ F-γ: □ (p <0.01) EA w/ wood: no sig effect EA on non-acup: no sig □ VNS: TNF-α: □ 6: □ NF-γ: □ (p < ?) cervical, subdiaphragmatic, adrenelectomy: EA

													effects blocked
7	2010	Animal-Male Sprague Dawley rats		SCI (laminectomy T9-T10)	Acupuncture	DU 26* GB 34* ST 36 BL 60 BL 40 GB 39 SP 6	30 min/daily for 2 weeks			yes (no acup)	wooden toothpick used at points	TNF-α IL-6 IL-1β	Acup (w/ Stim): TNF-α: ↓ 6: ↓ 1β: □ (p <0.01) vs control Sham: no sig
20	2013	Animal-Male Sprague Dawley rats	28 in 4 groups (n=7)	COPD	EA	ST 36	30 min day for 14 days		pathologist blinded	yes	sham (no COPD) EA at ST 36	TNF-α IL-1β	COPD EA vs COPD: TNF-α ↓ 1β □ (p<0.05) control vs sham: no difference
28	2013	Male sprague dawley rats	81 in 10 groups	Burn induced	EA	ST 36	1			yes	sham EA (hypo) sham burn shm VX	IL-6 (plasma) HRV	EA:IL-6 □(mpared to sham 6hrs (p=.03; 24hr (p=.003) Sham-EA: not sig VX w/ EA: blocked HRV: HF □ / EA)
33	2014	Wistart rats	70	LPS injection	EA	ST 36	1.5 hour			yes	yes on non acup point	TNF-a IL-10	EA: TNF □(p <0.05) and versus sham, IL-10 NSC Sham-: reduced TNF, IL-10 NSC VX: blocked effect a-BGT:

													blocked effect
35	2014	Wistar rats		abdominal adhesions	EA	ST 36	1			yes	yes (5 mm below and outside ST 36)	TNF-a	sham-no sig

Appendix D: Inflammatory Markers Data

Article #	TNF-α (Serum)	TNF-α (other)	NF κB p65 (LU)	IL-1β (serum)	IL-1β (culture)	IL-6 (serum)	IL-6 (other)	IL-10 (Serum)	INF-γ	IL-4	IL-2	Vagotomy	a7AChR agonist
1	☐		☐(ta-VNS & VNS only)	☐ (ta-VNS & VNSonly)		☐ (ta-VNS only)						blocked effect	blocked effect
21	☐			☐								☐ inflamm response VNS reduced, VNS still decreased	
22	☐			☐				☐					
24			☐									Abd VX-blocked effect	
25	VNS: NSC VX: NSC	(pulmo)VNS: NSC VX: ☐						NSC				VX (no VNS): NSC plasma; éTNF-a (pulmon	
26		(colon)VNS: ☐	(colon)VNS: ☐				(colon)VNS: ☐						
27		(cortex)☐			(cortex) ☐		(cortex)☐						
29		**NSC (VX+ VNS)**						**NSC (VX+ VNS)**	**NSC (VX +VNS)**				
36		**NSC (VX+ VNS)**				**NSC (VX+ VNS)**		**NSC (VX+ VNS)**	**NSC (VX +VNS)**				

5*	☐					☐		NSC				**blocked effect**	**blocked effect**
6**	☐					☐			☐			**blocked effect**	
7**	☐			☐		☐							
20	☐			☐									
28***						☐						**blocked effect**	
33	☐							NSC				**blocked effect**	**blocked effect**
35		(tissue) ☐										**blocked effect**	**blocked effect**
		p <0.05 unless otherwise noted											
		* intestinal markers same											
		**(p <0.01)											

Appendix E: Article # Coding

Article #	Article Name
1	Transcutaneous Auricular VN Simulation Protects Endotoxemic Rat from Lipopolusaccharide-Induced Inflammation
21	Loss of Vagal tone aggravates systemic inflammation and cardiac impairment in endotoxemic rats
23	Neuroprotective effects of vagus nerve stimulation on traumatic brain injury
24	Efferent Vagal Nerve Stimulation Attenuates Acute Lung Injury Following burn: the important of gut-lung axis
25	Effects of Vagus Nerve Stimulation and Vagotomy on Systemic and Pulmonary Inflammation in a Two-Hit Model in Rats
26	Involvement of MAP/NF-κB Signaling in the Activation of the Cholinergic Anti-Inflammatory Pathway in Experimental Colitis by Chronic Vagus Nerver Stimulation
27	Vagus Nerve Stimulation Attenuates Cerebral Ischemia and Reperfusion Injury via Endogenous Cholinergic Pathway in Rat
29	Effects of anti-inflammatory vagus nerve stimulation in endotoxemic rats on blood and lymphocyte subsets
36	Effects of anti-inflammatory vagus nerve stimulation on the cerebral microcirculation in endotoxinemic rats
5	Electroacupuncture Improves Gut Barrier Dysfunction in Prolonged Hemorraghic Shock Rats through Vagus Inflammation Mechanism
6	Dopamine Mediates the Vagal Modulation of the Immune System by Electroacupuncture
7	Acupucture-mediated inhibition of inflammation facilitates significant funtion recovery after spinal cord injury
20	Effects of Electroacupuncture at Zusanli (ST 36) on inflammatory cytokines in a rat model of smoke-induced COPD
28	Electroacupuncture improves burn-induced impairment in gastric motility mediated via the vagal mechisnism in rats
33	Electroactupuncture at Zusanli point attenuates pro-inflammatory cytokine release and organ dysfunction by activating cholinergic anti-inflammatory pathway in rat with endotoxin challenge
35	Inhibiting Effect of Electroacupuncture at Zusanli on Early Inflammatory Factor Levels Formed by Postoperative Abdominal Adhesions