VAGUS NERVE STIMULATION REDUCES INFLAMMATION OF THE SMALL INTESTINAL MUCOSA IN THE INDOMETHACIN-INDUCED ENTEROPATHY MODEL

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Introduction

Homeostasis through the cholinergic anti-inflammatory pathway (CAP)

The brain integrates inflammation signals and utilizes the vagus nerve to reflexively reduce inflammatory responses through delivery of acetylcholine to immune cells (Figure 1).

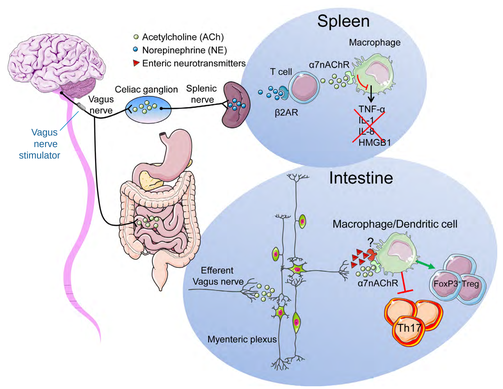


***Figure 1** (adapted from Matteoli, Gut, 2012) :*
Neurostimulation of the cholinergic anti-inflammatory pathway (CAP)

- **The CAP signal in the vagus nerve targets:**
  - **splenic macrophages and circulating leukocytes through interfacing with the splenic nerve** *(Rosas-Ballina, 2008)* **and a specialized subset of acetylcholine producing T-cells** *(Rosas-Ballina, 2011)*.
  - **intestinal macrophages and dendritic cells through interfacing with nerves of the enteric nervous system** *(Olofsson, 2012)*.

The vagus nerve provides tonic inhibition of inflammation in the gut through integrity of the CAP.

- **Vagus nerve function has been shown to be impaired in patients with ulcerative colitis** *(Lindgren, 1993)*.
- **Colitis in mice is exacerbated by vagotomy as well as by nicotinic receptor antagonists and is attenuated by nicotine** *(Ghia, 2006)*.
- **Susceptibility to colitis in mice is enhanced in mouse models of depression with associated reduction in intestinal acetylcholine and is reversed with antidepressants. This treatment normalizes intestinal acetylcholine levels and is dependent on an intact vagus nerve** *(Ghia, 2008)*.

Augmenting CAP activity by brief, intermittent electrical neurostimulation of the vagus (VNS) is effective in the treatment of clinical rheumatoid arthritis *(Koopman, 2012)* **and in a variety of animal models of acute and subacute inflammation.**

- **VNS reduces inflammatory infiltrates in the small intestinal muscularis in a post-surgical ileus model** *(De Jonge, 2005)*.
- **VNS reduces multivariate index of colitis in TNBS challenged rats** *(Meregnani, 2011)*.
- **VNS reduces gut injury due to trauma-hemorrhagic shock** *(Levy, 2012)*.
- **CAP effects have also been observed in models of sepsis, pancreatitis, peripheral inflammation in subcutaneous tissue, ischemia-reperfusion injury, myocardial infarction, and colitis** *(reviewed in Tracey, 2009)*.

Activation of the CAP by VNS using implantable stimulation devices holds promise as a potential therapeutic approach for chronic human inflammatory diseases including Crohn's disease and ulcerative colitis (Figure 1). To date, there is no experimental evidence linking vagus nerve stimulation to disease therapy in the small intestinal mucosa. To provide evidence for the feasibility of this non-pharmacological clinical approach, we examined the effect of VNS in the rat indomethacin-induced enteropathy model.

Methods

Rat Indomethacin-induced Enteropathy Protocol

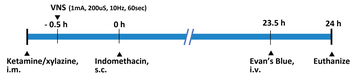


Figure 2 :
Experimental protocol; rat indomethacin-induced enteropathy

Experimental Groups
- **Disease induced / Sham (-) VNS**
- **Disease induced / (+) VNS**

Assessments
- **Gross pathology**
  - **Formalin-fixed intestine**
- **Tissue specific markers**
  - **Snap frozen intestine, liver, serum**

The experimental design is presented in Figure 2. A bipolar cuff electrode (Figure 3) was implanted around the left cervical vagus nerve of male Sprague Dawley rats. VNS or Sham VNS was delivered for 60 seconds. The characteristics of the electrical waveform utilized in stimulation are summarized in Figure 4.

VNS was followed with subcutaneous injection of 10mg/kg indomethacin to induce intestinal lesions. 24 hours thereafter, animals were injected i.v. with Evans blue to stain mucosal ulcerations, and euthanized (Figure 2).

Figure 3 :
Electrode implantation

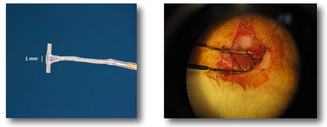
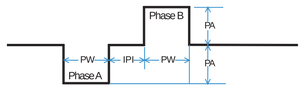


Figure 4 :
Electrical stimulation waveform



- **Charge-balanced biphasic pulse, frequency =10Hz**
- **Pulse Width (PW): 200uS**
- **Pulse Amplitude (PA): ±1mA**
- **Inter-Pulse-Interval between phase A & B (IPI): 50uS**

The small intestine was excised, cleaned, formalin-fixed, flat-mounted, photographed, and the total lesion area quantitated using digital morphometry, performed by a blinded scorer. In some animals the proximal ilium, distal jejunum, and liver were snap-frozen in liquid nitrogen and homogenized.

IL-23 and HMGB1 were measured by immunoblot in snap-frozen intestine and serum, respectively. TNF was measured by ELISA in snap-frozen liver.

Intestine IL-23 levels were normalized to β-actin loading control and TNF levels were normalized to sample protein content.

Results

Figure 5a :
VNS reduces intestinal lesion area

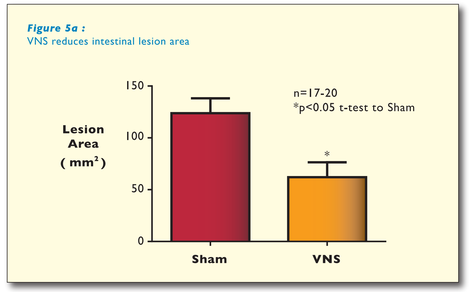


Figure 6 :
VNS reduces IL-23 in the intestine

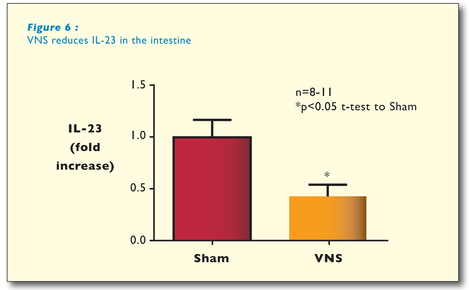


Figure 7 :
VNS reduces serum HMGB1

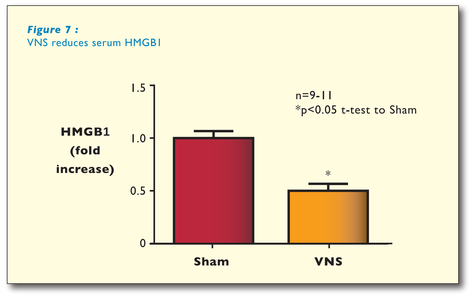


Figure 8 :
VNS reduces hepatic TNF

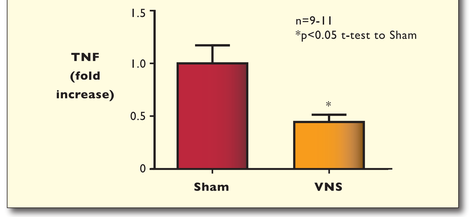
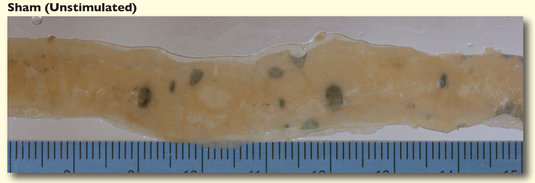
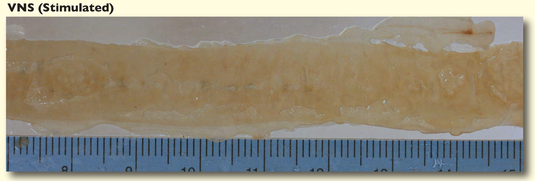


Figure 5b :
VNS improves gross pathology of intestine

Sham (Unstimulated)



VNS (Stimulated)



As expected, the majority of the lesions were localized to the distal jejunum and the proximal ileum. VNS significantly inhibited disease severity in the small intestinal mucosa as determined by gross pathology assessment as well as tissue-specific and systemic markers of inflammation and damage:

- **The total lesion area in the intestine was reduced by 50% (Figure 5a). An example of VNS-induced improvement in gross pathology is shown in Figure 5b.**
- **Intestinal IL-23, an important mediator of inflammation in both Crohn's disease and ulcerative colitis, was reduced by 57% (Figure 6).**
- **Serum levels of HMGB1, a marker and promoter of intestinal tissue damage in this model** *(Nadatani, 2012)* **was reduced by 50% (Figure 7).**
- **Hepatic inflammation coincident with enteropathy** *(Elson, 1995)* **was reduced (Figure 8), as indicated by TNF levels.**

Summary & Conclusions

Activation of the Cholinergic Anti-inflammatory Pathway by electrical stimulation of the vagus nerve in rats alleviates structural damage and reduces production of tissue specific and systemic inflammatory mediators in a model of intestinal injury induced by systemic indomethacin administration.

Implications

These observations represent the first demonstration of electrical vagus nerve stimulation ameliorating enteropathy observed in the small intestinal mucosa. These findings provide supportive preclinical evidence for clinical study on the efficacy of implantable neurostimulation devices in the treatment of Crohn's disease, ulcerative colitis, and other diseases of inflammation.

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DISCLOSURE OF INTEREST

Y.L., A.C., M.F., A.A. and R.Z. are employees of SetPoint Medical Corporation.

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