Non-invasive Interactive Neurostimulation (InterX®) elicits significantly greater physiological response than TENS: *Lymphocyte Metabolism and Cytokine production*

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Presented as a poster at the 13th World Congress on Pain, Montreal. Aug 29th - Sept. 2nd, 2010. PH 255

Introduction:

Non-invasive interactive neurostimulation (InterX) has shown to be clinically effective for a range of acute and chronic conditions [1,2,3] and in experimental pain research [4]. InterX also demonstrated reduced inflammation in patients following ankle surgery [2] . While mechanisms for pain relief for this type of therapy are well understood [5,6], it has previously been demonstrated that trancutaneous electrical stimulation does not affect the inflammatory response [7].

In a previous study, the effects of InterX pre- and post-treatment were examined. It was demonstrated that lymphocytes post treatment may be better able to respond to stimuli when necessary, which could result in enhanced healing ability post injury. The genomic profile seen after just 20 minutes is indicative of an acute response that would infer improved ability of these cells to respond to injury types of stimuli. We extended this study to include volunteers treated with a TENS device to compare physiological response following a different form of electrical stimulation.

Hypothesis:

In this study it is hypothesized that significantly greater physiological response will occur following InterX treatment than following TENS. This is suggested in view of the higher amplitude (approximately 3 - 5 times higher) and current density (approximately 90 times higher) output of the InterX device.

Method:

Treatment protocol: A multidisciplinary approach was used to examine the effect of transdermal interactive neurostimulation using the InterX 5002 device compared to the Biomed 2000XL TENS device on lymphocyte metabolic function and cytokine production. Blood was drawn from 8 healthy adults (4M/4F) prior to and 20 minutes following a treatment session. Treatment applications consisted of 10 minutes of treatment on the lateral elbow of the arm from which blood was drawn and 10 minutes over the corresponding spine root. The TENS device had two channels so that the direct area around the elbow, as well as the corresponding spine root could be treated simultaneously to follow the InterX protocol (Fig 1). The manufacturer recommendations to increase the intensity to a level just below that which causes muscle contraction were followed. 100Hz frequency was used with a pulse width of 150 microseconds. These parameters matched the InterX protocol as closely as possible. Using a higher frequency (250Hz) caused greater muscle contraction which thus meant using a lower amplitude so in the interests of comparison, frequency was sacrificed in favor of amplitude. The InterX device has small, closely spaced electrodes which allow for the use of high amplitude stimulation without causing muscle contraction. As TENS electrodes need to be larger and are spaced further apart, muscle contraction occurs at much lower amplitude.

Fig 1. InterX and TENS treatment areas

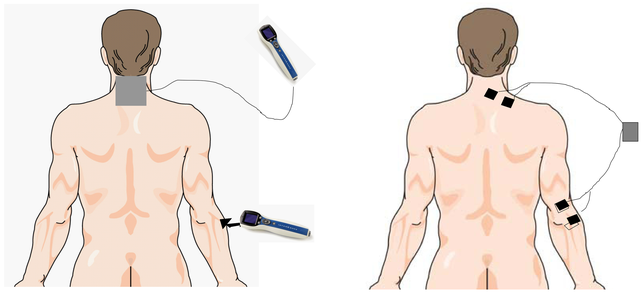
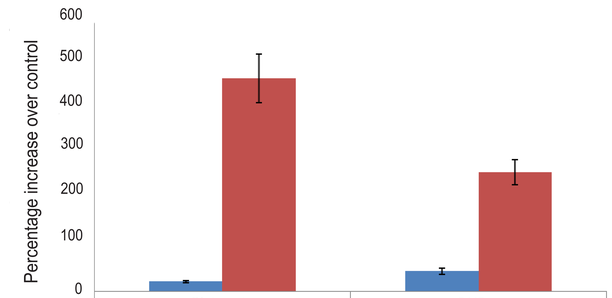


Fig 2. InterX vs TENS. Percentage increase in lymphocyte respiration following activation



	Glutamate	DNP
TENS, n=4	21.7%	44.5%
InterX, n=4	476.2%	266.9%

Blood samples were obtained from consented adult volunteers. Non red blood cells (mitochondrial containing cells) were isolated and placed in the chamber of a high resolution respirometry machine (Oroboros, Innsbruck, Austria). All experiments were performed in duplicate. Respiration of the cells in the presence of Glutamate (a favored substrate of lymphocytes) and 2, 4-Dinitrophenol (mitochondrial uncoupling for maximum respiration) was recorded. In order to account for differing numbers of the cell in the chamber, a BCA protein assay was performed to determine total protein to which the respiration data was normalized.

Results:

The TENS treatment when compared to pre-treatment values, slightly but not significantly increased the maximal respiration in the uncoupled state and there was no discernible difference due to Glutamate over control (p>.05) (Fig. 2)

The InterX treatment however elicited significantly (p<0.05 by ANOVA) greater respiration both in response to Glutamate and DNP compared with pre-treatment control and compared with TENS.

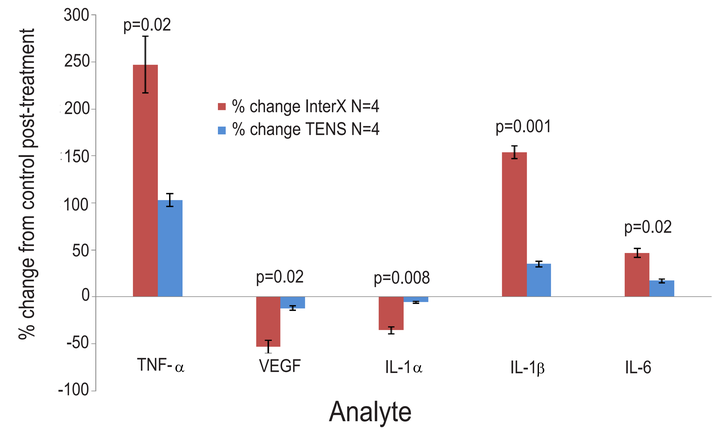
FIG.3 shows the percentage change in lymphocyte cytokine content, expressed as percent change from pre-treatment to 20 minutes post-treatment. N=4 for InterX, N=4 for TENS. Two lymphocyte extractions were performed on each subject's blood sample and the assays were performed in triplicate.

IL-1β has been shown to increase mitochondrial oxidative metabolism in pancreatic islet cells in vitro, concomitant to the release of isulin and increased glucose oxidation [8] . IL-6 and TNF-α have both been implicated in liver hypermetabolism, eliciting significantly increased mitochondrial oxidative metabolism [9] . Increased IL-1β levels, both in arterial wall and systematic circulation, accelerated vascular neointimal formation after injury, and surpressed matrix-metalloproteinase-2 expression (and therefore further vessel wall damage) 6 days following injury[10] . In mice lacking the gene for IL-6, bone fracture callus formation, mineralization and remodelling are signifcantly delayed, although by 6 weeks, healing had progressed to the same level as in wildtype mice. This indicates a role for IL-6 in early fracture healing[11] . IL-6 has also been shown to play a pivitol role in the healing of CNS following trauma. The effect of IL-6 appear to be most evident in revascularization and prevention of blood-brain barrier breakdown [12] .

IL-1α has been found to be elevated in chronic wounds. Following treatment with a hydroactive dressing (Cutinova cavity), accelerated wound healing was associated with reduced IL-1α levels. This mechanism is thought to be an IL-1α mediated upregulation of matrix metalloproteinase-1, which, when present at elevated levels, prevents wound resolution[13]. There are conflicting reports of the role of VEGF in wound and bone healing literature. A systematic review of vascularity and healing of bone fractures concluded that VEGF was necessary for bone healing[14]. However, wound healing in diabetic mice can be improved by gene transfer of angiopoetin-1, without increased VEGF expression [15] and blockade of VEGF has been proposed as a potential mechanism for improving cartilage healing in osteoarthritis[16].

Analysis was done to compare the effects of InterX to the effects of TENS and demonstrated a statistically significant greater response in the InterX group, compared to the TENS group (Fig.3).

Fig 3. InterX vs TENS. Percentage change in lymphocyte cytokine content



Conclusion:

This comparative study clearly demonstrates that both TENS and InterX influence similar responses in relation to lymphocyte metabolism and cytokine up and down regulation. However, the magnitude of that influence is significantly different with InterX having a much greater response in all parameters measured. TENS has a relatively small effect in some cases and a negligible effect in others.

The necessary level of lymphocyte activation needed to elicit a meaningful response to physiological stresses such as energy is unknown, though previous literature has demonstrated that TENS does not have an anti-inflammatory effect [7]. InterX has previously been shown to have an anti-inflammatory effect [2]. The significant differences in the response to the two different types of stimulation may be explained by the fact that InterX delivers a much higher intensity and density of current than TENS as well as delivering stimulation specifically to optimal treatment points.

These data provide only a partial explanation of the inflammatory response and studies relating to circulation and lymphatic flow are warranted to further understand the complex interaction of mechanisms that can elicit a reduction of inflammation in patients. However, this study confirms the hypothosis that InterX elicits greater physiological response than TENS.

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Acknowledgements: This work was funded by Neuro Resource Group, Inc. Plano, TX. Author PM is a paid consultant for Neuro Resource Group and cointributed to protocol design and treatment delivery.