



## Grant Information Summary:

# Pre-Exposure Effects of Ultrasound on Spleenocytes Proliferation and Lymphokine Production

### Practical Significance:

**O**ur data supports the hypothesis that 1 MHz ultrasound may be effective on injuries associated with chronic inflammation.

#### Background

A recent review (Johns, JAT, September 2002) outlines and proposes a hypothesis that therapeutic ultrasound effects key regulatory components that direct the immune response and injury repair. The literature shows that significant differences exist between the mounting in vitro evidence clearly demonstrating ultrasound's effects on inflammation and the lack of quality clinical data to support the use of ultrasound. This disparity may suggest that energy thresholds relating to total energy delivered per treatment area may play a critical role in stimulating the non-thermal therapeutic effects of ultrasound.

#### Objective

The specific aim of this study was to determine whether 1 MHz ultrasound significantly effected spleenocyte proliferation or lymphokine production.

#### Design and Setting

Single cell suspensions generated from mice spleen were treated with 1 MHz ultrasound at either 0.1 or 0.5 W/cm<sup>2</sup> for 10 min. One hour later the cells were activated with a T cell activator (ConA) and analyzed for effects of non-thermal ultrasound on cell growth and production of four lymphokines central in modulating an inflammatory response.

## Subjects

Mouse strain, Balb/c.

## Measurements

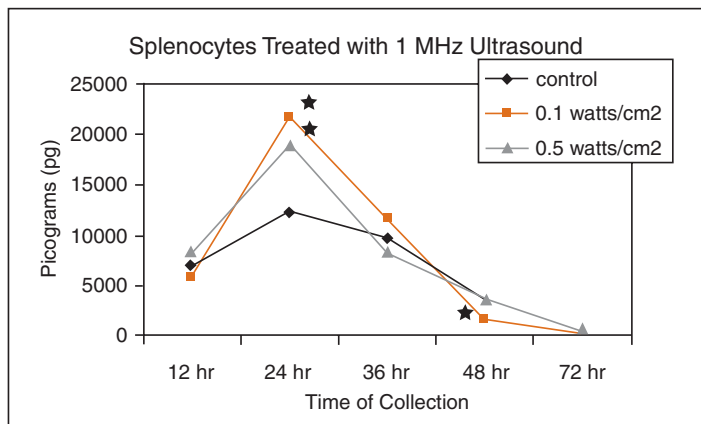
Cell proliferation was measured via MTT assay. Cytokine production was measured by ELISA.

## Results and Conclusions

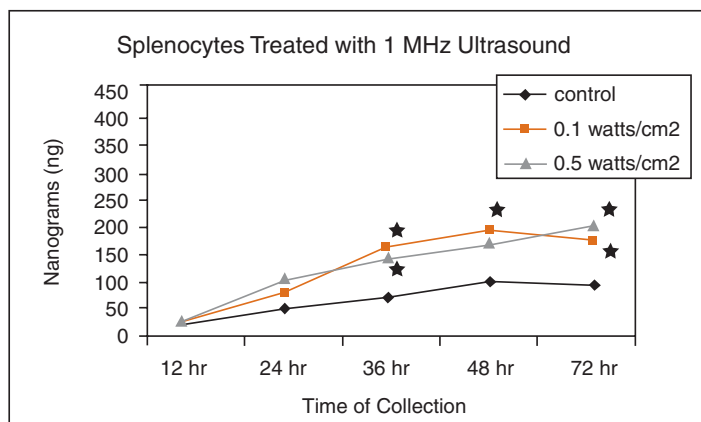
The data show that pre-exposure of T cells to 1 MHz ultrasound at 0.1 and 0.5 W/cm<sup>2</sup> did not significantly affect growth. However, significant increases in T cell growth factors Interleukin-2 (Fig. 1) and Interleukin-4 (Fig. 2) were observed in all experiments. The same sets of experiments found that ultrasound significantly

affected two additional lymphokines that have clear roles in injury and injury repair. First, significant decreases in Tumor Necrosis Factor-alpha were found. Tumor Necrosis Factor-alpha is known to induce tissue injury, increase vascular permeability and fever. Second, significant increases in Interferon-gamma were found (Figure 2). Interferon-gamma is known to stimulate production of collagen in fibroblasts, enhance debridement activity of macrophage and inhibit activity of the T cell subpopulation, Th2. While our data support the contention that 1 MHz ultrasound may be effective on chronic inflammation, the integration of these data into clinical recommendation is presently unclear.

**Figure 1** The Effect of 1 MHz Ultrasound on IL-2 Cytokine Production. Splenocytes were exposed to various treatment levels of ultrasound (1 MHz and either 0.1 watts/cm<sup>2</sup> or 0.5 watts/cm<sup>2</sup>), rested for 1 hour, then stimulated with 5mg/ml of ConA. Supernatants were collected at various time points and analyzed for IL-2 via ELISA. The data point is an average of two replicates. The graph is representative of three separate experiments. The star indicates a p value of  $\leq 0.05$ .



**Figure 2** The Effects of 1 MHz Ultrasound Treatment on IFN-g Production. The splenocytes were exposed to a 1 MHz frequency ultrasound treatment with either a 0.1 watts/cm<sup>2</sup> or a 0.5 watts/cm<sup>2</sup> intensity, rested for 1 hour, then stimulated with 5 mg/ml of ConA. Supernatants were collected at various time intervals and analyzed for IFN-g levels via an ELISA. Each data point is an average of two replicates. The graph is representative of 3 separate experiments. The star indicates a p value of  $\leq 0.05$ .



## Primary Investigator:



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## Publication & Presentation List

Johns, L.D., Cellular and Molecular Effects on Therapeutic Ultrasound: The Frequency Resonance Hypothesis. In Press (2002):

*Journal of Athletic Training.*

Johns, L.D., Colloton, P., Neuenfeldt, J. and Krupo, K. Effect of therapeutic levels of ultrasound on splenocyte proliferation and the production of cytokines.

NATA, June, 1999. (Presentation)

Johns, L.D., Colloton, PA. Effects of Ultrasound on Splenocyte Proliferation and Lymphokine Production, NATA, June, 2002. (Presentation)

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