UNIVERSITY of WISCONSIN GREEN BAY

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*The Effects of Oral Consumption of *Echinacea* on Human Neutrophils

Introduction

Echinacea is a popular, commercially available herbal supplement used to treat infectious diseases of the upper respiratory tract, including the common cold and influenza in humans (Goel et al., 2005). A large number of studies have examined the effects of *Echinacea* on preventing or treating the common cold in humans. However, relatively few studies have examined the mechanism by which *Echinacea* affects the immune system (Barret et al, 2010). Neutrophils are powerful phagocytic white blood cells, constituting 90% of white blood cells found in human blood. Neutrophils are the first cells to infiltrate infected tissues during inflammatory responses and play an important role in infections caused by bacteria, fungi and viruses, including respiratory infections caused by viruses. During the process of phagocytosis, neutrophils internalize pathogens into a phagosome. The phagosome fuses with lysosomes within the neutrophil, allowing the neutrophil to digest the internalized pathogen through the release of lysosomal enzymes and the generation of superoxide anion (O_2^-).

The focus of this study was to examine the effects of *Echinacea* on superoxide anion production by freshly isolated neutrophils from human volunteers taking commercial strength *Echinacea* capsules or placebo capsules for seven days to explore the mechanism by which Echinacea mediates the therapeutic benefits reported in the literature. Superoxide anion production by freshly isolated neutrophils was analyzed in blood samples from pre-treatment and post-treatment blood samples to evaluate *Echinacea*-mediated effects on neutrophil function.

Materials

Source: Planet Natural

- **Plant Material:** Nature's Way Whole Herb *Echinacea* herbal supplement (400mg/capsule) was generously donated by Nature's Way (Green Bay, WI).
- **Blood draws:** All blood draws were performed by registered nurses with phlebotomy training at the Counseling and Health Center at UW-Green Bay.
- **Consent and questionnaire forms:** Consent was obtained for all subjects and health/background questionnaires were administered and completed by study subjects prior to each blood draw.

Methods

Neutrophil Isolation
Neutrophils were isolated from whole blood by a modified double gradient procedure described previously (English and Andersen, 1974). 20mls of whole blood were layered onto a double gradient, containing 9mls of Histopaque 1077 layered onto 11mls of Histopaque 1119. Polypropylene tubes were used to reduce binding of cells to the tube wall. The blood was centrifuged at 700xg for 30 minutes at room temperature (Figure 1). The neutrophil layer was washed by centrifugation in Hank's Balanced Salt Solution (HBSS). The resulting cells were suspended and maintained in HBSS. Cell viability was determined by Trypan Blue analysis and/or by Bio Tek Cytation 1 analysis.

Superoxide Anion Production
Superoxide anion production was measured by a discontinuous assay, the superoxide dismutase(SOD)- inhibitable reduction of cytochrome c (Babior et al. 1973). Neutrophils (1×10^6) were treated with PMA (50ng/ml) for 30 minutes at 37°C. A medium control containing cytochrome c and HBSS alone was used to determine background values. Reduction of cytochrome c was measured by a Bio Tek Cytation 1 multimode plate reader with a reading wavelength of 550nm and a reference wavelength of 570nm. Superoxide anion-mediated reduction of cytochrome c was determined by subtracting the reduction of cytochrome c in the presence of optimal concentrations of superoxide dismutase (SOD) from the reduction of cytochrome c in the absence of SOD (Figure 2). The mean absorbance readings of a minimum of duplicate samples ±SE were recorded. Analysis of variance was performed by a two-tailed Student's *t*-test for unmatched pairs with $p \leq 0.05$ used to establish statistical significance (n=17).

*IRB Study Protocol #Spr-17-13-Ext) approval was extended through the Spring term of 2019.

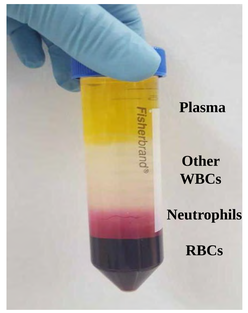


Figure 1: Neutrophil isolation (Step 1)

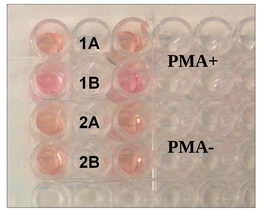
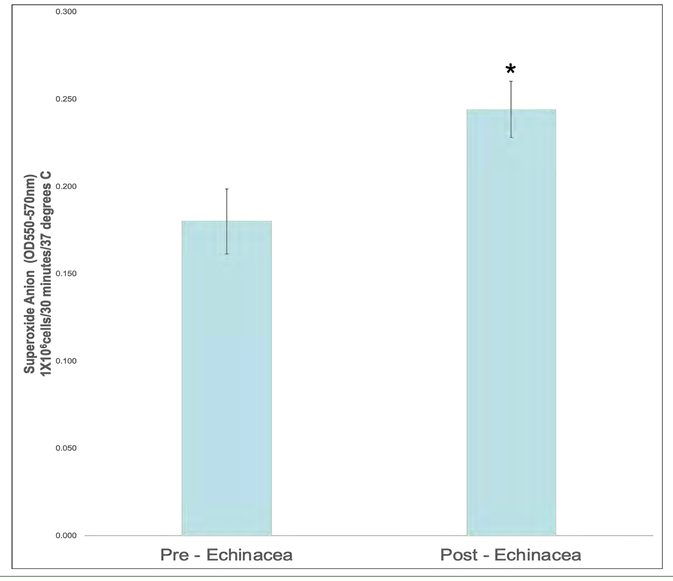


Figure 2: Supernatants from PMA treated neutrophils and background controls.

Results

Figure 3: The Effects of Oral Consumption of Echinacea on Superoxide Anion Production by Human Neutrophils



* $p \leq 0.05$ (n=17)

Conclusions

This study investigated the effects of *Echinacea* on superoxide anion production by freshly isolated human neutrophils. As shown in Figure 3, superoxide anion levels were statistically higher in post-treatment blood samples ($p \leq 0.05$). Ongoing analysis of superoxide anion production in pre-treatment and post-treatment blood samples from placebo controls is required to explore further the significance of these promising data.

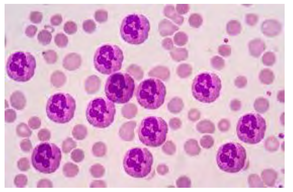


Figure 4: Human Neutrophils viewed under a microscope.
Source: Bel Marra Health

Literature Cited

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