Peripheral Blood Mononuclear Cell Cytokine and Proliferative Response to In Vitro Echinacea Stimulation in Male College Wrestlers and Soccer Players During Preseason Practice

Nisarg B. Shah1, Daniellie M. Doty2, David S. Senchina3

1Pharmacy Program and 2Biology Department, Drake University, Des Moines, IA, 50311

ABSTRACT:
The effects of dietary botanical supplements on the immune response in athletes are unknown, despite a recent increase in herbal supplement use by both college and professional athletes. We conducted 2 separate studies to examine the effects of in vitro Echinacea stimulation on the immune responses of peripheral blood mononuclear cells (PBMCs) taken from athletes during preseason training. College-level male competitive athletes from 2 sports (wrestling and soccer) participated in the studies. PBMCs were isolated from blood sampled either pre- or post-practice, standardized to the same concentration, and then stimulated with extracts from Echinacea pallida, Echinacea simulata, or solvent vehicle control. Cytokine production (TNF, IL-1, and IFN-γ) was measured from supernatants collected 24-72 hrs contingent on the specific cytokine; proliferation was assessed in both studies. Following exercise, PBMC production of TNF, IL-10, and IFN-γ production either decreased or was unaffected. IL-1β levels showed no change in either study. PBMC proliferation increased in the wrestlers as a result of training, but decreased in the soccer players. In conclusion, observed effects were contingent on species chosen, time point within preseason training, and sport (training type).

BACKGROUND & RATIONALE:
- Herbal supplements (such as Echinacea) are increasingly used by athletes to offset exercise-induced immunosuppression (Petrotz & Nachbau 2009).
- Production of cytokines and cytokine tumor necrosis factor (TNF) are known to be influenced by acute exercise and exercise training, though studies report differences in direction and degree specific to each cytokine and Echinacea and also across studies (Suzuki et al. 2002; Pedersen et al. 1998).
- Different species of Echinacea have different immunomodulatory effects in vitro; within the genus, E. pallida and E. simulata have discrepant effects (Senchina et al. 2006).

MATERIALS & METHODS:
Plant Materials: Echinacea purpurea (PE31275) and E. simulata (PE31604A) were harvested from the North Central Regional Plant Introduction Station in Ames, IA and dried for 16 months as intact plants in a climate-controlled facility. Dried roots were used to generate 50% ethanol, 50% water tinctures at a ratio of 1:9 C until use. A solvent vehicle control was generated from the same reagents but contained no plant material.

Human Subjects: All procedures for wrestler portion of the study were approved by the Iowa State Institutional Review Board and all procedures for the soccer portion of the study were approved by the Drake University Institutional Review Board. Eight male wrestlers (HT, WT) from the 2006-2007 Iowa State Wrestling Team and seven male soccer players (HT, WT) from the 2007 Drake University Men’s Soccer Team were recruited to the study.

Exercise Protocol: For the wrestlers, practice on Days 1 and 15 was identical and consisted of 2 h intense aerobic drills (target HR 170-180) bookended by 10-minute transition sessions (target HR 130-160). For the soccer players, the single practice session consisted of 1 h aerobic drills followed by 1 h scrimmage.

Phytochemistry: Blood was sampled at rest or post-exercise. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficol density centrifugation and standardized to 1 x 10^9 cells/mL in AIM-V medium.

Immune Assays: E. pallida extract, E. simulata extract, and control vehicle preparations were further purified by 1.25 g/L in 0.1 M sodium phosphate solution. 4 mL of cells were cultured with 50 μL of the three preparations. ELSA was used to assess TNF production at 24, 48, 72, or 96 h or IFN-γ production at 48 h, and IL-10 at 72 h. For proliferation, 100 μL of cells were cultured with 5 μL of either extract for 72 hours. Each condition was replicated in triplicate and proliferation quantified using a formazine salt assay (Calfilter).

Statistica: Each subject served as their own control. All statistical procedures were conducted in SPSS. Main effects of time, treatment, and treatment x time interactions were determined by ANOVA. When significant effects were discovered, follow-up post-hoc tests (LSD) were performed.

DISCUSSION:
- In the wrestling portion of the study, E. simulata consistently stimulated greater levels of PBMC cytokine production (Fig. 1 and Table 1) and proliferation (Fig. 2) relative to E. pallida or control. Wrestlers’ immune responses to Echinacea extracts changed during the course of pre-season training; these changes depended on the training as well as the species.
- In the soccer portion of the study, acute exercise decreased IFN-γ (Fig. 3), IL-10 (Fig. 4), and proliferation (Fig. 5). The E. simulata extract was able to consistently enhance cytokine production or PBMC proliferation compared to either solvent vehicle control or E. pallida extract (Fig. 3-5). Acute exercise caused a statistically significant decrease in E. pallida extract for IFN-γ (Fig. 3); this was seen for IL-10 (Fig. 4) and TNF (not shown), but was not significant.
- The difference in results between the soccer and wrestler studies could be due to the different training regimens and demand to each sport.
- Extracts were phytochemically profiled to quantify known bioactive compounds (Table 3). The only consistent positive correlation between phytochemical composition and cytokines was chlorogenic acid which significantly increased cytokine production and proliferation (Table 4). Other phytochemicals were inconsistently related with observed immune effects. Given the modest differences in chlorogenic acid between the two extracts, and that many immunomodulatory effects were observed, data suggest that other compounds not profiled here may be the mechanism behind the observed effects.

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REFERENCES:

Table 1: Winnsting production of TNF and IL-10 in both studies. Asterisks indicate statistically significant differences (p < 0.05). There were significant effects of extract but no extract x time interactions.

Table 2: Soccer study production of TNF and IL-10 at 24-72 hrs culture, respectively, expressed as pg/mL. Standard errors are given for each value. Asterisks indicate statistically significant differences as compared to control and E. pallida (p = 0.05). There were significant effects of extract but no significant effect of time for these cytokines.

Table 3: Phytochemical composition of E. pallida and E. simulata extracts used in this study, expressed as mg/100 g. The following phytochemicals could not be detected in any of the extracts: E. pallida, E. simulata, and Echinacea control. ND = not detected.

Table 4: Correlations between extract phytochemical composition and observed effects. Bivariate correlation Pearson values (r) are given for TNF (E. pallida and E. simulata) and IFN-γ (E. pallida and E. simulata) extracts used in both studies. Asterisks indicate statistically significant differences (p<0.05) and dagger indicate trends (0.05< r< 0.1). Plus and minus symbols indicate correlation direction and strength.

Figure 1: TNF Production in the Wrestling Study. There were time (pre vs. post) treatment (E. pallida vs. control) and time x treatment (p<0.001) interactions. E. simulata induced production was higher than both control and E. pallida induced TNF production at the Pre time point (both p<0.001). There was a significant reduction in TNF production with E. pallida-stimulated cultures from the Pre to Day 1 time points (p<0.001).

Figure 2: Proliferation in the Wrestling Study. There were time (pre vs. post), treatment (E. pallida vs. control) and time x treatment (p<0.001) interactions. E. simulata induced proliferation was higher than both control and E. pallida induced TNF production at the Pre time point (both p<0.001). In conclusion, observed effects were contingent on species chosen, time point within preseason training, and sport (training type).