Echinacea Purpurea and Mucosal Immunity

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- immunoglobulin A
- immune function
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Abstract

This investigation examined the effects of Echinacea purpurea on mucosal immunity and the incidence and duration of upper respiratory tract infection (URTI). 32 subjects completed an exercise protocol known to affect mucosal immunity. Saliva was collected prior to and five minutes after completion of exercise testing. Subjects then took either a placebo (C) or Echinacea supplement (E) for 4 weeks and the testing procedure was repeated. Each time, s-IgA concentrations and saliva flow rate were measured and the secretion rate of s-IgA was calculated. In addition, standard logs indicating symptoms of URTI were completed throughout the study. Both groups demonstrated significant exercise induced reductions in s-IgA (C – 69%; E – 43%) and the secretion rate of s-IgA (C – 79%; E – 53%) at the beginning of the study ($p < 0.05$). Following the 4-week intervention, only the control group experienced the post intervention decrease in s-IgA (C – 45%; E + 7%) and the secretion rate of s-IgA (C – 45%; E – 7%). Further, while there was no significant difference in the number of URTI between groups, the reported duration was significantly different (C 8.6 days vs. E 3.4 days). The results suggest that Echinacea may attenuate the mucosal immune suppression known to occur with intense exercise and reduce the duration of URTI that subjects incur.

Introduction

Extracts of Echinacea have been used therapeutically for centuries by Native Americans and also are increasingly used by the general population for their potential effects in the prevention and treatment of upper respiratory tract infections (URTI) [31]. However, despite their widespread popularity, their efficacy remains controversial. While several clinical studies found that Echinacea extracts decreased the frequency, symptoms and severity of the common cold [3,7,14,15,22], other studies showed Echinacea had no beneficial effect on lessening cold and flu symptoms [2,13,23,30]. Currently, three main species of Echinacea are used for medicinal purposes: those derived from dried roots and underground stems of *Echinacea angustifolia* or *Echinacea pallida* and those derived from the fresh juice of the roots or above ground structures of *Echinacea purpurea* [31]. A 2003 review of the medicinal properties of Echinacea, reported that the species *Echinacea purpurea* is most effective for the treatment of acute upper respiratory tract infection [1].

While a growing body of *in vitro* and animal laboratory research shows the ability of *Echinacea purpurea* to affect cellular immune function, there are no published studies that have examined the effect of *Echinacea purpurea* on immunoglobulin A (s-IgA) which is the immune factor most closely linked with URTI [19]. The mucosal immune system of the upper respiratory tract provides the first barrier to pathogenic microorganisms that can cause URTI. This is accomplished primarily through the secretion of immunoglobulin A. The levels of s-IgA in salivary fluids have been found to correlate more closely with resistance to respiratory infection than do serum antibodies or other immune parameters and changes in secretory s-IgA are now widely used as an important physiological biomarker of the mucosal immune system [19].

Strenuous physical exertion and heavy training have often been shown to be associated with depressed immune function and both athletes and coaches are searching for methods to attenuate this effect [19–21]. Therefore, the purpose of this investigation was to examine whether *Echinacea purpurea* supplementation can serve as a nutri-

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tional countermeasure to attenuate the experimentally exercise-induced decrease in s-IgA and reduce the incidence and duration of URTI. It was hypothesized that subjects who received Echinacea would have fewer URTI than those who received the placebo and of those subjects who acquired an URTI, those taking Echinacea would experience the symptoms for a shorter period of time.

Methods

Subjects

Thirty-two, non-smoking, active adults aged 19 – 46 completed this investigation (**Table 1**). Following a detailed review of their medical history, present health status, and a 12 lead resting ECG, subjects were first cleared for participation by a physician. Only those subjects that were healthy, habitually active, free of respiratory disease, free of signs and symptoms of URTI the preceding week, not taking any medications and/or dietary supplements, and who exhibited no contraindications to strenuous exercise testing were included in the study. All subjects provided a written informed consent following a thorough explanation of the purpose and possible risks and all procedures were approved by the Institutional Human Investigation Committee.

Procedures

The study was conducted using a randomized, double-blind, placebo-controlled parallel group design. Subjects were randomly assigned to a group based on a computer generated table of random numbers. Each subject was laboratory tested at the same time of day [32] before and after a 28 day dietary supplementation period of either *Echinacea purpurea* or a placebo. Subjects were instructed to avoid strenuous physical activity the day before and leading up to the laboratory test and to abstain from food and caffeine for three hours prior to the test. The pre and post-trial laboratory protocols were the same except that during the post-trial session, subjects were asked specific questions related to compliance with the daily intake of capsules, avoidance of other dietary supplements, and the occurrence of any adverse effects.

Prior to experimental testing, subjects completed medical history forms and standardized questionnaires to assess the incidence and duration of signs and symptoms of URTI not related to allergies over the previous two weeks (sore throat, cold, cough, and running nose). The form subjects completed asked them to distinguish allergies from the symptoms of a URTI. If subjects were unable to make the distinction, they were excluded from the study. Inflammation appears to be the central event leading to symptoms of infectious rhinosinusitis (the common cold) and pharyngitis (sore throat), the primary illnesses for which Echinacea is currently used [1].

Saliva collection

Subjects were required to thoroughly rinse out their mouths with water, and unstimulated saliva was collected for exactly four minutes into 15 ml polypropylene tubes prior to exercise and at minute five post exercise to evaluate resting levels of s-IgA. Subjects were instructed to allow the saliva to collect in their mouth while occasionally drooling it into a collecting test tube. The saliva was measured for volume to the nearest 0.1 ml and then stored at – 70 °C until analysis.

Table 1 Descriptive characteristics of subjects (Mean ± SD)

	Echinacea (n = 18)	Placebo (n = 14)
Age (yr)	27.7 ± 7.8	24.9 ± 5.8
Height (cm)	169.1 ± 6.8	169.9 ± 6.7
Weight (kg)	66.2 ± 12.2	68.2 ± 11.2

Determination of secretory Immunoglobulin A

Immunoglobulin concentrations were measured for s-IgA using the enzyme-linked immunosorbent assay (ELISA) described by Mackinnon et al. [21]. Briefly, microplates were precoated with unconjugated goat antihuman IgA (Sigma) diluted 1 : 2000 in a carbonate buffer and left refrigerated overnight at 4 °C. After being brought to room temperature (RT), they were washed with PBS-T (Sigma) and the plates were blocked with 1 % Bovine Serum Albumin (BSA) (Sigma) PBS-T and incubated at room temperature for 30 minutes. Saliva was spun at 10 000 rpm/5 min, diluted 1 : 1500 with 0.1 % BSA PBS-T, added to the plates, and incubated at RT for 60 minutes. The plates were washed with PBS-T after which biotin conjugated antihuman IgA diluted 1 : 2000 with 0.1 % BSA PBS-T was added to each well and allowed to incubate at RT for 60 min. Horseradish Streptavidin Peroxidase (HRP-SA) (Sigma) diluted with 0.1 % BSA PBS-T was added to each well and allowed to incubate at RT for 30 min. The plates were washed, PBS and K Blue Substrate were added, and the reaction was immediately stopped with 3 % HCL. The plates were read on a microplate reader (Spectra Maxplus [ELX 800], Molecular Devices Corp., Sunnydale, CA, USA) at 450 nm. Standards of known concentrations of purified IgA were included on each microplate and absolute concentrations (µg/ml) were calculated from the standard curve. To avoid interassay variability, all samples from each subject were assayed on the same microplate. The coefficient of variation for s-IgA was 10.4 %.

The secretion rate of s-IgA (in µg/min), which represents the total amount of s-IgA appearing on the mucosal surface per unit of time, was calculated by multiplying absolute s-IgA concentration (µg/ml) by saliva flow rate (ml/min). This latter value was calculated by dividing the total volume of saliva obtained in each sample by the time taken to produce each sample (four minutes).

Exercise protocol

Prior to testing, subjects were seated on a previously calibrated, mechanically-braked Monark cycle ergometer (Model 818, Varberg, Sweden) with the height of the seat adjusted for leg length. Each subject underwent a standardized warm-up procedure consisting of pedaling against a light frictional resistance for three minutes. The warm-up was designed to elevate the heart rate of a subject to approximately 60 % of their age-predicted maximum. In addition, two brief cycling sprints (3 – 4 seconds) against an increased resistance were interspersed to provide each subject with an opportunity to experience how the frictional resistance setting used during the actual test would feel. Subjects were then permitted to rest quietly for three to five minutes to eliminate any acute fatigue that may have resulted from the warm-up and ask questions.

The exercise protocol consisted of three consecutive, all-out effort, 30-second Wingate eg cycling tests. The frictional resistance was set at 0.075 kg/kg body mass. Tests were separated by a three minute recovery interval consisting of 90 seconds of active recovery during which the subjects pedaled at a rate of 50 rpm against a light frictional resistance (1 kp) and 90 seconds

of passive recovery during which the subject sat quietly on the cycle ergometer.

Pedal revolutions were counted using an infrared beam optical sensor (SMI OptoSensor 2000™, Sport Medicine Industries, St. Cloud, MN, USA). The sensor cable was fed into an RS232 serial port of an IBM microcomputer to provide an on-line analysis of the power output data using the SMI Power™ software program (Sport Medicine Industries). The following two standard power output performance characteristics were obtained: (a) peak anaerobic power, which is the highest mechanical power output generated during a single five-sec period of each 30 sec test, and (b) mean anaerobic power, which is the arithmetic average of the total power output generated during a 30-second test period ($W \cdot kgBW^{-1}$). Heart rates were obtained at the end of each 30 second Wingate test using 15 second electrocardiographic chart paper recordings derived from a bipolar V_5 configuration and telemetry (Model G-2400T, EatonCare Telemetry, Ann Arbor, MI, USA). Each subject was then asked to provide a post-exercise saliva sample obtained in the same manner as previously described for the pre-exercise resting saliva sample.

Dietary supplementation

At the beginning of the study, subjects were provided either a placebo or standardized *Echinacea purpurea* supplement (Nature's Way®, Springville, UT, USA) to be consumed over a 28 day period. The specific Echinacea type, dosage, and treatment duration were selected based on an extensive literature review, including the recommendations of the German Commission E [4]. A leading American supplement manufacturer (Nature's Way®) provided the study capsules free of charge and without any expectations or other agreements between the researchers and the manufacturer regarding the conduct and dissemination of the study findings. They had no input into the study design or execution. The placebo treatment was prepared in-house and consisted of gelatin capsules containing a sugar mixture (white confectioners powdered sugar consisting of 97% sucrose and 3% corn starch and brown sugar consisting of 90% sucrose combined with molasses) and had the same size, weight, shape, and color as the Echinacea capsules. It was necessary to use the described sugar mixture to color match the Echinacea capsules. The placebo and Echinacea capsules were placed into identical containers prior to distribution and were randomly assigned and coded, by a third party, to assure allocation concealment.

Subjects were given detailed take home instructions regarding the intake and storage of the capsules. Subjects were instructed to consume eight capsules per day, two with each meal and two at bedtime. This same standardized supplement, except in liquid form, (8 ml/day of Echinacin Madaus Liquidum) was used safely for four weeks [24] and eight weeks [13] in previous studies.

During the 28 day intervention period, subjects completed standardized logs documenting the type, frequency, duration, and severity of any symptoms of URTI unrelated to allergies. In addition, study participants were instructed to maintain their usual physical activity level and a normal diet without taking any dietary supplements other than those provided by the researchers.

Statistical analysis

The Wingate test data were analyzed using 2 groups (Echinacea vs. control) × 2 times (pre intervention vs. post intervention) × 3 tests (Wingate test 1, 2, and 3) Analysis of Variance (ANOVA) with repeated measures on the last two factors. Post hoc (Least Significant Difference: LSD) analyses were performed on all significant main effects. Interactions were subjected to simple main effects analysis, followed by post hoc (LSD) analysis. This analysis was used to assess whether participants provided full effort on the Wingate tests (enough to show that performance declined in the direction expected, justifying an examination of s-IgA). The dependent variables of absolute salivary s-IgA concentration, saliva flow rate and the secretion rate of salivary s-IgA, were analyzed separately using a 2 groups (Echinacea vs. control) × 2 times (pre vs. post) × 2 test sessions (before intervention vs. after intervention) ANOVA with repeated measures on the last two factors. Incidence of URTI, and duration of URTI were analyzed using independent samples *t*-test. All statistical decisions were based on $\alpha = .05$ and follow-up analysis on interactions were performed using simple effects tests. Observed power for the dependent variables in this study ranged between .76 with an effect size of .70 (flow rate) and .94 with an effect size of .75 (s-IgA). The statistical package used to run all analysis was SPSSX (Ver. 10.5), Chicago, IL, USA.

Results

Concentration of s-IgA

The final sample consisted of 32 subjects (aged 26 ± 6 years). Subject characteristics by group are shown in **Table 1**. Analysis of the concentration of absolute s-IgA data revealed main effects for Test, $p = 0.031$, and time factors, $p < 0.002$, as well as a significant test × time interaction, $p = 0.05$ and a significant group × time interaction, $p < 0.045$. A simple main effects analysis of the test × time interaction on the time factor revealed that the expected post exercise decrease in s-IgA occurred prior to the treatment in both the Echinacea and control group. A simple main effects analysis of the group × time interaction on the time factor revealed that after the treatment, only the control group experienced the expected post exercise decline. The Echinacea group had no significant decrease in s-IgA (**Fig. 1**).

Secretion rate of s-IgA

Analysis of the secretion rate of s-IgA data revealed main effects for test, $p = 0.004$, and time factors, $p = 0.000$. The test × time interaction approached significance, $p = 0.07$. Post hoc analysis for the main effects, test × time, revealed that the expected post exercise decrease in the secretion rate of s-IgA occurred prior to the treatment in both the Echinacea and control group. However, after the intervention, only the control group experienced the expected post exercise decline while the Echinacea group had a significant increase in the secretion rate of s-IgA (**Fig. 2**).

Saliva flow rate

Analysis of the saliva flow rate (SFR) data revealed only a significant main effect of time, $p = 0.005$. Post hoc analysis revealed that the Echinacea group experienced a post exercise decline in SFR after exercise in the pre-intervention test (0.425 ± 0.005 ml vs. 0.336 ± 0.004 ml).

Wingate data

Analysis of the Wingate mean anaerobic power $p < 0.000$ and mean peak power $p < 0.000$ data revealed a significant main effect for test. Post hoc analysis of the test factor revealed the expected decline in mean and peak anaerobic power values from test 1 to 2 to 3 for both groups (**Table 2**).

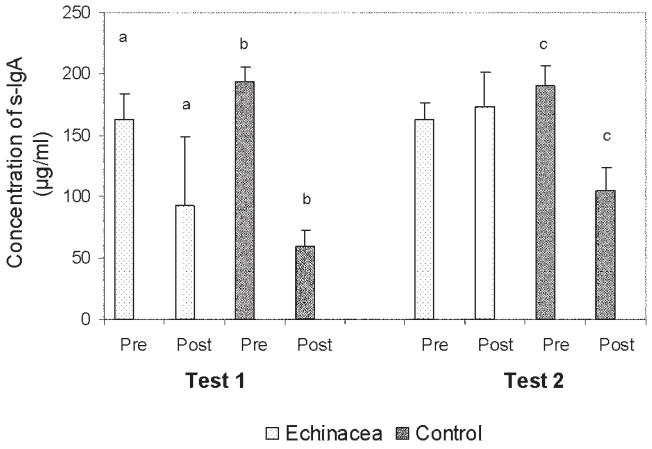

Fig. 1 Concentration of s-IgA (µg/ml) before exercise (Pre) and after exercise (Post) and before intervention (Test 1) and after intervention (Test 2). Like letters (a, b, c) indicate significant time differences ($p < 0.05$). Values are mean ± SE.

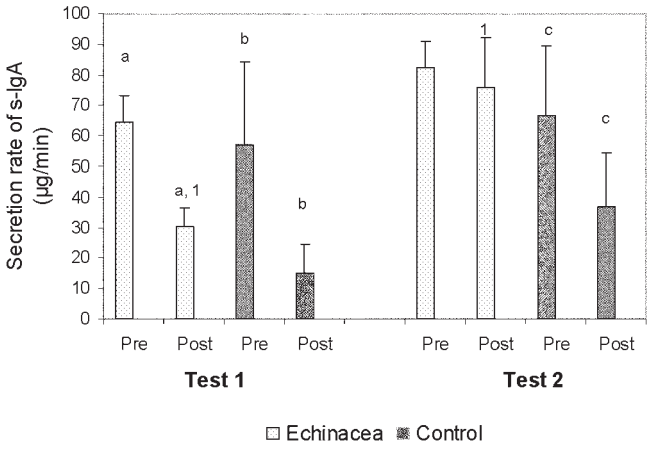

Fig. 2 Secretion rate of s-IgA (µg/min) before exercise (Pre) and after exercise (Post) and before intervention (Test 1) and after intervention (Test 2). Like letters (a, b, c) indicate significant time difference and like numbers (1) indicates significant test difference ($p < 0.05$). Values are mean ± SE.

Table 2 Wingate test: Peak anaerobic power and mean anaerobic power (W·kg BW-1) (mean ± SE). Each subsequent test was significantly different from the one preceding it for both pre and post intervention for both groups ($p < 0.05$)

	Echinacea						Placebo					
	Pre intervention			Post intervention			Pre intervention			Post intervention		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Peak anaerobic power	8.3 ± 0.3	7.3 ± 0.3	6.5 ± 0.3	8.3 ± 0.3	7.3 ± 0.2	6.5 ± 0.3	9.1 ± 0.3	8.0 ± 0.3	7.2 ± 0.3	9.3 ± 0.3	8.5 ± 0.2	7.4 ± 0.2
Mean anaerobic power	6.4 ± 0.2	5.4 ± 0.2	4.8 ± 0.2	6.3 ± 0.2	5.4 ± 0.2	4.8 ± 0.2	6.7 ± 0.2	5.7 ± 0.2	5.1 ± 0.2	6.7 ± 0.3	5.7 ± 0.2	5.1 ± 0.2

Incidence and duration of URTI

Subjects were required to turn in a log where they documented signs and symptoms consistent with URTI, but unrelated to allergy for the duration of the intervention. If the infection lasted more than three days and the symptoms were cough, runny nose and nasal congestion, it was assumed to be viral in nature. Analysis of the number of URTI revealed no significant differences in the number of colds $p = 0.735$. However, for those who had colds, the duration of the cold was significantly less for the Echinacea group vs. the control group (3.4 days vs. 8.6 days) $p = 0.000$ (**Fig. 3**).

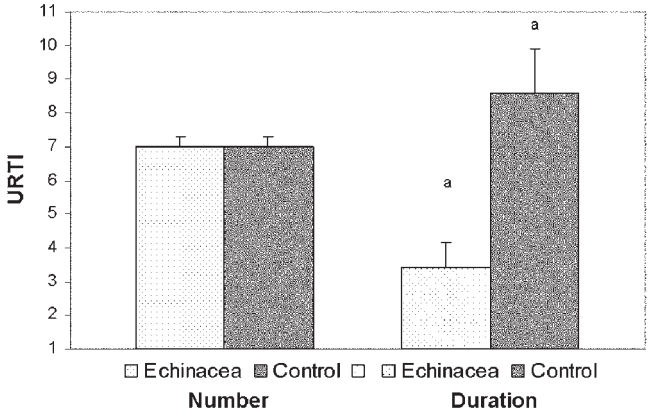

Fig. 3 Number of URTI and duration of URTI (days) for Echinacea and control subjects. Values are mean ± SE.

Discussion

The present study used a laboratory tested protocol that has been previously demonstrated to result in mucosal immunosuppression [9,10]. The most important finding of this study is that *Echinacea purpurea* exhibited a physiological protective effect on the mucosal immune system as evidenced by the lack of expected post-exercise decrease in s-IgA and the secretion rate of s-IgA. Moreover, there was evidence that it effectively reduced the duration of clinical symptoms of URTI. This stands in contrast to findings on another promising "immune boosting" agent, ginseng [9], using the same laboratory testing procedures.

Upper respiratory tract infection is the most common illness prevalent in the United States and the average adult experiences 2 – 4 of them per year. A recent estimate states that over $2 billion annually is spent on over the counter remedies for these infections [11]. Currently, 2 – 8 million Americans use an Echinacea product at least once in a given year [1]. Due to the significant decrease in symptom days among the treatment group, the results from the current investigation suggest that *Echinacea purpurea* may be successful as an early treatment for acute URTI. These observations extend previous work by Hoheisel et al. [14] who used a similar preparation and reported that subjects taking Echinacea had a markedly shorter length of illness than those taking a placebo (mean duration of 4 days vs. 8 days respectively).

Echinacea is widely promoted for its ability to "boost" the immune system, yet data to support this claim are inconclusive. It has been demonstrated in both *in vitro* and animal models that Echinacea has the ability to increase immunologic activity. No single component has been found to be responsible for this immunostimulating activity, but several explanations are plausible. One explanation is that the immunomodulation occurs through increased levels of interferon which may increase phagocytosis and lymphocyte activation [26]. Another possible explanation involves the cytokines. Previous studies report increases in interleukins (IL) 1 and 6 as well as increased tumor necrosis factor (TNF) after supplementation with *Echinacea purpurea* [8,16]. Although the function of cytokines is still under investigation, it is commonly accepted that they are important modulators of the immune response. IL-1 is responsible for both T and B cell activation while IL-6 activates B cell growth and differentiation into plasma cells. TNF is active in mediating a defense against an invading virus [17]. Many of the cytokines have similar and overlapping functions and thus it is difficult to isolate their specific actions, however, the studies of Burger et al. and Luettig et al. [8,16] indicate that *Echinacea purpurea* activates the immune system through cytokine pathways.

Other studies have found that extracts of *Echinacea purpurea* enhance cellular immune function, primarily through the proliferation of peripheral blood mononuclear cells [25,27,28]. Most important in the incidence of URTI are the B cells which have been shown to increase proliferation after *in vivo* mixture with *Echinacea purpurea* [29]. The mucosal immune system is complex. B cells that contain the potential for J-chain expression are stimulated in the mucosa-associated lymphoid tissue (MALT), migrate through lymph and blood to the glands and are then differentiated to Ig-producing plasma cells [6]. Mucosal s-IgA is a dimer containing two IgA molecules linked to a polypeptide J chain and a secretory component fragment [18], which serves as a transport mechanism. When the MALT encounters an antigen, it signals the B cells which then proliferate and differentiate into J-chain dimeric IgA for translocation to the lumen as s-IgA [5]. Since s-IgA production is dependent on B cells, it is possible that as *Echinacea purpurea* increases B cell production, it enhances mucosal immunity.

Both coaches and athletes have long been interested in finding a means to counter the immune suppression known to accompany intense exercise [10] or endurance training [12]. More recently, researchers have begun to examine the effectiveness of nutritional agents to counter this immunosuppression [3,9]. Although Echinacea has previously been widely studied, given the lack of consistent available evidence regarding its efficacy, there is a need for additional studies examining its potential. In particular, there is a need to examine the effects of Echinacea supplementation on athletes in training. Berg and colleagues examined the effect of *Echinacea purpurea* supplementation on a group of triathletes before and after a sprint triathlon, comparing cellular measures of immune function and incidence of URTI [3]. The athletes who were supplemented were less likely to experience a URTI than those who were not which the authors attributed to decreased levels of IL-2 and increased levels of IL-6.

In conclusion, in this investigation, *Echinacea purpurea* was able to attenuate the mucosal immune suppression known to occur with intense exercise as well as the duration of URTI that subjects incurred.

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