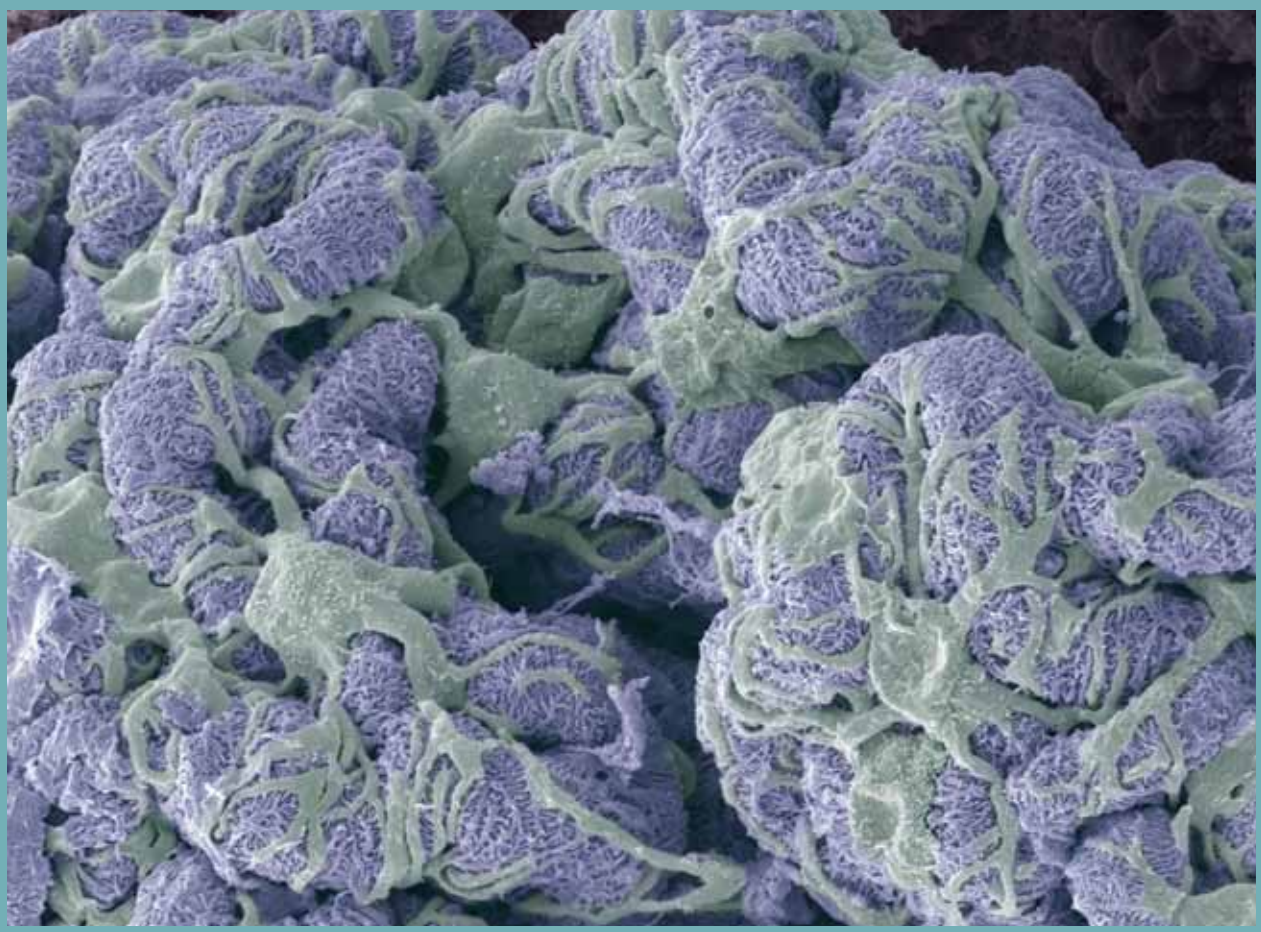
Alternative Medicine Review®

A Journal of Clinical Therapeutics

March 2012

Volume 17, Number 1



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The Official Journal of The American College for Advancement in Medicine

Use of a Standardized Extract from *Echinacea angustifolia* (Polinacea®) for the Prevention of Respiratory Tract Infections

Francesco Di Pierro, Giuliana Rapacioli, Tarcisio Ferrara, Stefano Togni

Abstract

Echinacea preparations are extensively used for the prevention and the management of the common cold. Despite this popularity, the clinical studies on Echinacea have produced mixed results, possibly in part because of the poor characterization of the extracts investigated and the use of different species and/or plant parts for the preparations investigated in the various trials. To address this issue, Polinacea®, a highly standardized extract from a well-defined botanical source (roots of *Echinacea angustifolia)* with a specific phytochemical profile (presence of the complex polysaccharide IDN5405, the phenylethanoid echinacoside, and substantial lack of alkamides) was developed. We have studied whether Polinacea® could enhance the immune response subsequent to the influenza vaccination, and whether the use of this preparation could translate into a decreased morbidity from influenza. The preliminary results were encouraging, and suggest that Polinacea® could be used for improving the immune response to influenza vaccine.

(*Altern Med Rev* 2012;17-1:36-41)

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Introduction

Preparations from Echinacea are used for the prevention and the treatment of the common cold; however, research on efficacy has produced mixed results.[1] In contrast to a large number of positive studies conducted according to a non-controlled design or on a limited population, controlled and wider-ranging studies have frequently yielded negative results, or, at best, have only suggested a positive trend for efficacy.[1] These mixed findings are not surprising, since the term "Echinacea-based preparations" encompasses extracts (1) obtained using varying extraction methods and solvents, (2) from different Echinacea species, and (3) from different parts of these plants (e.g., aerial versus underground parts), with therefore marked differences in terms of constituent profiles.

There are three medicinal species of Echinacea (*Echinacea angustifolia* D.C., *E. purpurea* (L.) Moench, and *E. pallida* Nutt.). All three of these species can be found in at least some Echinacea products in the healthcare market.[2] The roots of these three species are difficult to distinguish from one another, which can lead to difficulty in botanical identification. In part because of confused botanical identification, a number of preclinical and/or clinical studies on Echinacea lack botanical traceability and the exact species of Echinacea used cannot be determined.[1] This is a critical issue, since substantial phytochemical differences exist between the three commercial species of Echinacea, and within each species between the aerial and underground parts. Furthermore, the phytochemical profile of all Echinacea plant parts is characterized by the presence of compounds that span a wide range of polarities, and with often opposite, rather than complementary bioactivity (i.e., some constituents are water-soluble and are extracted better using certain methods or solvents, while others are lipid-soluble and would be poorly extracted using these same methods or solvents).[3] Generally, the polar and water-soluble polysaccharides – which are shared by all three species – have immunostimulating properties, while the lipophilic alkamides (isobutylamides) – typical of *E. angustifolia* and *E. purpurea* – have a powerful anti-inflammatory action, mediated by the activation of the peripheral cannabinoid receptor.[4] Consequently, consumption of isobutylamide-rich Echinacea

Keywords Echinacea, vaccine, influenza, immunoglobulins, T lymphocytes

preparations may contribute to mitigation of the inflammatory response during an infection in progress, but might not exert any immuno-stimulating or preventive action on morbidity.[5] When this information is combined with their chemical instability[5] and the current limited knowledge of their toxicity, it provides a rationale for removing alkamides from Echinacea extracts intended to be used exclusively for immuno-stimulating purposes.

From the standpoint of preventing respiratory tract infections, the most interesting Echinacea constituents appear to be the polysaccharides and echinacoside (a polyphenol conjugate).[1] The polysaccharide fraction of various species of Echinacea is a powerful activator of the alternative route of the complement system – a non-specific defense mechanism – and of cytokines capable of triggering macrophage activation.[1] These effects increase objective immunological parameters, like the number of T cells, the plasma levels of neutrophils, the macrophage phagocytosis index, as well as the activity of natural killer cells.[1] Echinacoside is also a weak antibacterial agent that can inhibit bacterial hyaluronidase,[5,6] a class of enzymes necessary for bacteria to spread through the skin and mucosal membranes.

To address the uncertainties of composition that plague so many commercial Echinacea preparations, a specific botanical preparation (Polinacea®) was developed from the roots of *E. angustifolia*, the most researched Echinacea species in terms of its ethnopharmacological and medicinal uses.[7] Polinacea® is a hydro-alcoholic extract obtained from the roots of this plant. It is triply standardized for echinacoside (>2%), IDN5405 (>5%), and alkamides (< 0.1%).[8] [Note: IDN5405 is a polysaccharide characterized by a highly branched galacturonic structure, well distinct from the endophyte-derived lipopolysaccharides ubiquitous in plant tissues.]

Since this standardized Echinacea preparation is intended for long-term prophylactic use as a means to improve immune system performance, the profile of the product is intentionally kept very low in its contents of alkamides. Part of the rationale for this decision is that, while these compounds have shown powerful immuno-stimulating activity *in vitro*, as exemplified by the stimulation of IFN-γ production by T lymphocytes (not by other types of lymphocytes), they might be inactive *in vivo*.[8] The anti-inflammatory and cytokine-inhibiting properties of alkamides,[4] though undoubtedly useful for the mitigation of symptoms once an infection is in progress,[5] might also potentially be, in fact, either neutral or counter-productive in products aimed at the prevention of infections.[5] The peculiar composition of Polinacea® was established using cell assays, and was then validated at the pre-clinical level using an animal model (*Candida sp*. infection in normal- or cyclosporine-treated immunosuppressed mice). In this model, common endophytic bacterial lipopolysaccharides, usually active on macrophages, proved totally inactive.[8]

There has been a growing interest for natural products capable of modulating the strength and the duration of the immune response, in particular linked to seroconversion subsequent to vaccinations.[9] Most studies in this area have been done using extracts containing saponins or polysaccharides of bacterial/fungal origin, frequently in association with polyphenols.[10] The preliminary results obtained from studies in this area provide a rationale for assessment of other natural products, which might potentially augment the immune response to vaccinations. This research was undertaken to provide preliminary data on whether Polinacea® might augment the immune response to influenza vaccination,[11] and whether its use would have any clinical relevance in terms of influenza morbidity in instances where vaccination was not administered.

Clinical Assessment

Two explorative pilot investigations – one in adults affected by respiratory disorders (e.g., chronic bronchitis, respiratory insufficiency, and asthma), and the other in healthy pediatric age individuals – were conducted. Exclusion criteria were the presence of any disease of cardiological, immunological and/or oncological nature. The first pilot study was aimed at assessing the reduction of respiratory complications associated with ailments diagnosed as common colds (influenza and/or parainfluenza) in persons with an existing respiratory disease who received the influenza vaccine while taking Polinacea®. The second pilot study was carried out in healthy pediatric age individuals, and was conducted to evaluate the number of influenza and/or parainfluenza episodes diagnosed in a fixed period after the use of the product prophylactically.

Preparations Used

The following products were used:

- Monoselect® Echinacea (PharmExtracta, Pontenure, PC), containing 100 mg Polinacea® (Indena, Milan) per tablet;
- Fluad® (Novartis Vaccines and Diagnostics, Italy), polyvalent anti-influenza type A and B vaccine, consisting of surface antigens, haemagglutinins and neuraminidase, obtained from influenza viruses cultured in hen's embryonic eggs;
- Be Total Plus Tablets (Pfizer, Italy), containing B complex vitamins (B1, B2, B6 and B12).

First Pilot Study (Adults with Existing Respiratory Disorders)

Thirty-eight volunteers of either sex, aged between 38 and 79 years, with diagnosed chronic bronchitis (19 individuals), respiratory insufficiency (13 individuals), or intrinsic asthma (6 individuals), were enrolled at local health centers in Salerno and Piacenza. The volunteers were sorted into 3 groups on the basis of the treatment. Group 1 received influenza vaccine only (V group). Group two received Monoselect® Echinacea only (ME group). Group three received the influenza vaccine and Monoselect® Echinacea (V + ME group). The influenza vaccine was administered between 15 October and 15 November 2008. The Monoselect® Echinacea tablets were administered from October to December 2008 according to the following schedule: 2 tablets/day for the first 15 days, with dosage reduced to 1 tablet/day for the next 15 days, and then further reduced to 1 tablet every other day for the next 60 days. Participants were instructed to take the tablets on an empty stomach at the same time of the day.

Assessment was based on symptoms characteristic of influenza and/or parainfluenza episodes, as well as the onset of respiratory complications, and was made by collecting data from all the patients between 1 November 2008, and 28 February 2009. Results are presented in table 1.

Five of the 14 volunteers treated with the vaccine therapy (V group) suffered from parainfluenzal episodes, each lasting for fewer than 5 days, without attacks of fever (or at least with an evening temperature not exceeding 37.5 °C [99.5 °F]). Three of the subjects in the V group also suffered from respiratory complications - tracheitis, retrosternal pain with an irritable, dry cough at onset followed by a deep productive cough. In the ME group, 2 of the 12 individuals suffered from symptoms typical of common cold disorders. One of these might have been affected by influenza, since temperature exceeded 37.5 °C (99.5 °F) and duration of the disease was greater than 7 days. This individual experienced respiratory complications similar to those noted in the V group. In the combined (V + ME) group, only 1 of the 12 individuals exhibited episodes of a parainfluenzal nature. The individual had symptoms consistent with a common cold, including moderate fever, and duration of less than 5 days.

Table 1. Number of individuals in the first pilot study from each treatment group that developed symptoms suggestive of influenza, parainfluenza, and respiratory complications

Group (n)	Influenza-like Symptoms	Parainfluenza-like Symptoms	Respiratory Complications
V Group (14)	0	5	3
ME Group (12)	1	1	1
V + ME Group (12)	0	1	0

Note: Symptoms were more frequent in the vaccination group than in the ME and ME +V groups. Fisher exact test12 indicated that the probability volunteers did not suffer complications by chance is 7-fold higher in the combination group and the ME group than in the vaccination group, suggesting that ME, either alone or in combination with vaccination, might be more efficacious than the vaccination alone.

In an attempt to ascertain whether and which immune parameters might have been affected by treatment with Monoselect® Echinacea, blood analyses that included hematochrome, leukocyte formula, platelet parameters, serum proteins, and antibody production were conducted on the volunteers enrolled in the ME group. Blood was sampled upon enrollment (t = day 0) and 15 days after the last day of supplementation (t = day 105). Results are shown in Table 2. At both time points, most values were within normal limits, or deviated only slightly. Antibody production parameters, although still within the normal range, showed a marked increase of Immunoglobulin G (IgG), a change suggestive of an increased immune response.

Table 2. Measured immune parameters prior to and 15 days following Monoselect® Echinacea supplementation

Test	Day 0	Day 105	Reference Range
White Blood Cell Indices			
Leucocytes[a]	6.7	8.3	4.3-10.8 K/microL
Neutrophils[a]	45%	41%	55-65%
Lymphocytes[a]	44%	48%	23-35%
Monocytes	8%	9%	4-8%
Eosinophils	2%	1%	2-4%
Basophils	1%	1%	0-1%
Red Blood Cell Indices and Platelet Count			
Red Blood Cell Count	4.20	4.35	4.2-5.9 M/microL
Hemoglobin	13.1	13.3	12-18 g/dL
Hematocrit[a]	38.5%	40%	37-52%
Platelet Count[a]	350	325	150-400 K/microL
Serum Proteins			
Albumin	54%	53%	60%
Alpha-1	5%	4%	1.4-2.9%
Alpha-2	10%	11%	7.3-12.6%
Beta Zone	14%	14%	9-14.8%
Gamma Zone	17%	18%	8-18.2%
Immunoglobulins			
IgG[a]	1150	1659	723-1685 mg/dL
IgM[a]	298	169	48-271 mg/dL
IgA	192	183	81-463 mg/dL

a $t < 0.001$; Statistical analysis was done according to the t-test for dependent samples or, alternatively, according to the Wicoxon S-analysis for paired samples.[12]

Second Pilot Study (Healthy Pediatric Age Individuals)

Thirty-four healthy pediatric volunteers of both sexes, aged between 9 and 15 years old, were enrolled at local health centers in Salerno and Piacenza. Volunteers received either Monoselect® Echinacea (ME group) or Be Total Plus (BTP group) tablets. Supplementation took place from October to December 2008 according to the following schedule: 1 tablet/day for the first 30 days, and then reduced to 1 tablet/day every other day for the following 60 days. Participants were instructed to always take tablets on an empty stomach at the same time of the day.

Participants were evaluated for symptoms of influenza and/or parainfluenza, as well as the onset of respiratory complications. Evaluation was carried out between 1 November 2008, and 28 February 2009. Results are presented in Table 3.

Two out of the 14 volunteers in the ME group had onset of symptoms consistent with common cold disorders. One of these cases might have been influenza virus, since temperature exceeded 37.5 °C (99.5 °F) and symptoms persisted for more than 7 days. This individual did not exhibit any respiratory complications. In the BTP group, 6 of the 20 volunteers exhibited episodes of a parainfluenzal nature with symptoms of colds, moderate fever and a duration of symtoms of less than 5 days. Another 2 individuals from the BTP group had symptoms that strongly suggested influenza. None of the individuals in the BTP group suffered from respiratory complications.

Discussion

In order to avoid the issues of (1) poor characterization of constituents used, and (2) ambiguity of the source of the Echinacea being used, we used a standardized Echinacea preparation (triple standardization for IDN5405 >5%, echinacoside >2%, and alkamides <0.1%) that was derived exclusively from underground parts from a single source of known botanical origin (*E. angustifolia*).

This product (Polinacea®) was investigated in two distinct groups of volunteers – adults with existing respiratory disorders and healthy adolescents – to determine whether alone or, in the case of the adults with existing respiratory disorders, in combination with the influenza vaccine, it could influence the occurrence of symptoms suggestive of influenza or parainfluenza infection. Results showed a statistically significant tendency for this triple-standardized Echinacea preparation, either alone or in combination with vaccination, to be more efficacious than the vaccination alone, suggesting that this product has the potential to act synergistically with influenza vaccination. The small number of participants precludes any definitive inferences or conclusions in this area.

In a subset of the first pilot trial – the ME group – lab work was conducted for some parameters of immune function. The results observed suggest that the active treatment might have altered immunoglobulin levels, since IgG increased and IgM decreased. White blood cell indices, including monocyte-macrophage and/or neutrophil-mediated response, were not affected by supplementation.

Table 3. Number of individuals in the second pilot study from each treatment group that developed symptoms suggestive of influenza, parainfluenza, and respiratory complications

Group (n)	Influenza-like Symptoms	Parainfluenza-like Symptoms	Respiratory Complications
ME Group (14)	1	1	0
BTP Group (20)	2	6	0

Fisher exact test[12], although not reaching significance due to the overall relatively low frequency of the events considered, showed, nevertheless, a tendency to a higher proportion of cases free of symptoms of influenza- and parainfluenza in the ME group than in the BTP group (86% vs 60%, respectively).

Limitations of this study include the small size of the sample population, a lack of a comparison with other Echinacea preparations, and the failure to monitor aspects of immune performance in all participants. Because of these limitations, our results should be considered preliminary. There were no complaints of side effects in our two pilot studies, and in the first pilot study, there were fewer respiratory complications when the Echinacea preparation was provided either alone or in combination with the vaccine. This excellent tolerability of the extract used, combined with our preliminary results suggesting a potential clinical benefit, provide a rationale to undertake a systematic study on the effects of standardized Echinacea preparations alone or in combination with influenza vaccination in larger trials in order to determine whether these types of products can reduce influenza episodes in individuals at risk (e.g., adolescents, elderly people).

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