

The Potential Use of *Echinacea* in Acne: Control of *Propionibacterium acnes* Growth and Inflammation

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Acne is a chronic inflammatory disorder of skin follicles caused by the gram-positive bacterium *Propionibacterium acnes*. The possibility was investigated that a standardized preparation of *Echinacea purpurea* (Echinaforce®), with known antiviral, antiinflammatory and antibacterial properties, might provide a useful alternative treatment in the control of the disease. The herbal extract readily killed a standard laboratory strain of the bacterium and several clinical isolates. In cell culture models of human bronchial epithelial cells and skin fibroblasts, *P. acnes* induced the secretion of substantial amounts of several pro-inflammatory cytokines, including IL-6 and IL-8 (CXCL8), as determined by means of cytokine-antibody arrays. However, the *E. purpurea* completely reversed this effect and brought the cytokine levels back to normal. Thus Echinaforce® could provide a safe two-fold benefit to acne individuals by inhibiting proliferation of the organism and reversing the bacterial-induced inflammation. Copyright © 2010 John Wiley & Sons, Ltd.

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INTRODUCTION

Acne vulgaris is a multifactorial chronic disorder of the skin pilo-sebaceous follicles. Its pathogenesis is not completely understood, although *Propionibacterium acnes*, the dominant microbe in the sebaceous glandular regions, and inflammation, possibly initiated by this bacterium, appear to be the two main instigators and drivers of the disease. In addition, other important contributing factors include several hormones and host nutritional status (Kurokawa *et al.*, 2009; Lyte *et al.*, 2009). One explanation for the chronic nature of the disease is that *P. acnes* induces the production of pro-inflammatory cytokines and chemokines, as well as other inflammatory mediators, which attract leukocytes to the site of infection and thereby set up a cascade of inflammatory responses, which also involve the production of reactive oxygen species and other radicals, all of which in combination lead to the development of the acne lesion (Docherty *et al.*, 2007; Kurokawa *et al.*, 2009).

Conventional therapy has targeted the development of the lesions, by means of retinoic acid analogues and other compounds, and antibiotics directed against the bacterium (Docherty *et al.*, 2007). Needless to say, the continued application of antibiotics entails the risk of resistant bacteria emerging. As an alternative approach, several recent reports have indicated the possibility of using plant extracts to counter the growth of the bacteria and/or the inflammatory response, although these have yet to be evaluated *in vivo* (Chomnawang *et al.*, 2007; Joo *et al.*, 2008; Kim *et al.*, 2008).

Another prospective safe alternative is the use of standardized preparations of *Echinacea* species, which are currently very popular herbal remedies for 'colds and flu', although in traditional medicine in North America *Echinacea* preparations were also advocated for the treatment of skin lesions, especially for wound healing (Barrett, 2003; Barnes *et al.*, 2005). More recent monographs have suggested the topical use of *Echinacea purpurea* in semi-solid or liquid form for cutaneous use and the treatment of small superficial wounds (HMPC Monograph, 2008). This suggests possible value as an antibacterial and antiinflammatory agent. However, there is considerable variation in the bioactivities of preparations derived from different species and parts of the plant (Hudson *et al.*, 2005; Vohra *et al.*, 2009). It was reported recently that a commercial standardized preparation of *Echinacea purpurea* (Echinaforce®) possesses efficient and selective antiviral and antibacterial properties, in addition to antiinflammatory properties, with no cytotoxic effects (Sharma *et al.*, 2009a). Therefore it was decided to evaluate Echinaforce® for its ability to control or inactivate *P. acnes*, and to inhibit bacterial induction of pro-inflammatory cytokines, as determined by cytokine antibody arrays. The advantage of array technology is that multiple cytokines and chemokines can be analysed simultaneously, and this formed the basis of our recent study on virus-induced pro-inflammatory responses and their reversal by Echinaforce® (Sharma *et al.*, 2009a).

MATERIALS AND METHODS

Echinacea. The herbal preparation was Echinaforce® (A. Vogel Bioforce AG, Switzerland, batch number 018451), a 65% ethanol extract of freshly harvested

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aerial parts of *Echinacea purpurea* (L.) (drug extract ratio 1:12) Moench supplemented with 5% *E. purpurea* roots (drug extract ratio 1:11). HPLC analysis documented the presence of caftaric acid, 264.4 µg/mL; chlorogenic acid, 40.2 µg/mL; cichoric acid, 313.8 µg/mL; echinacoside, 6.9 µg/mL; PID 8/9 (alkylamide), 36.3 µg/mL; but no caffeic acid, cynarin or polysaccharides (Sharma *et al.*, 2009a). The recommended oral dose is approximately 1.0 mL Echinaforce in 10.0 mL water, corresponding to a final concentration of 1.6 mg/mL dry mass per volume and 6.5% ethanol v/v. No endotoxin was detected (<0.1 unit per mL), according to the Lonza endotoxin test kit (Lonza, Walkersville, MD).

Antibacterial assays. The standard ATCC (American Type Culture Collection) strain of *Propionibacterium acnes* was obtained from PML Microbiologicals (Wilsonville Oregon). Seven clinical isolates of *P. acnes*, identified in routine blood cultures, were obtained from Dr Diane Roscoe (Microbiology, Vancouver General Hospital). They were all propagated and assayed on sheep blood agar plates, in anaerobic chambers at 35°C. All culture plates and related reagents were obtained from PML Microbiologicals (Oregon, USA).

The standard assay method was as follows. Several isolated colonies of each strain were removed and dispensed into PBS (phosphate buffered saline) by vortex mixing, to give a homogeneous suspension of approximately 1×10^8 cfu (colony forming units) per mL. Aliquots of suspension were mixed 1:1 with the diluted extract to give final concentrations of Echinaforce® 1:100 (in PBS), equivalent to 160 µg/mL dry mass per vol plus 0.65% ethanol, or PBS alone, or 0.65% ethanol in PBS, in transparent sterile plastic tubes in ambient light or covered in aluminium foil (for dark exposure), for a period of 60 min with incubation at room temperature (20°C) on a rocker platform. The rationale for comparing light and dark exposure was based on evidence indicating the existence of photoactive ingredients in *Echinacea* extracts (Hudson *et al.*, 2005).

Following the treatment, each mixture was serially diluted ($10 \times$ dilutions) in PBS, and replicate 2.5 µL aliquots were spotted onto blood agar plates, divided into sectors for each dilution, and spread uniformly with sterile plastic loops, allowed to dry, and the plates incubated under the conditions described above. After incubation of the plates for 72 h, colonies were counted manually and compared with untreated samples.

Cytokine analysis. BEAS-2B cells, a tracheo-bronchial epithelial cell line, and A-549 cells, a lung epithelial cell line, were obtained from ATCC. Human skin fibroblasts (courtesy Dr Aziz Ghahary, Prostate Research Centre, Vancouver) were used in their sixth passage. They were all propagated in Dulbecco MEM (Invitrogen, Ontario), supplemented by 5–10% fetal bovine serum (Invitrogen), without antibiotics or antimycotic agents. For experimental purposes, they were grown in complete medium, in 6-well trays, to produce freshly confluent monolayers. Bacteria were added to the cells at 70 cfu/cell for 1 h, followed by aspiration and replacement by a 1:100 dilution of *Echinacea* in DMEM without serum, corresponding to a final concentration of 160 µg/mL (dry mass/vol). Cell-free culture supernatants were harvested at 48 h after infection (Sharma *et al.*, 2008a) for ELISA tests and Quantibody cytokine

array analysis. In additional experiments it was shown that medium alone, with or without an equivalent volume of ethanol, and cell-free supernatant derived from control uninfected BEAS-2B cells, did not induce the secretion of cytokines.

ELISA assays. These were carried out according to the instructions supplied by the companies (R&D Systems, Minneapolis, for IL-8 and TNFα, or e-Bioscience, San Diego, CA, for IL-6).

Cytokine antibody arrays. The Raybiotech fluorescent antibody array system was used. The array format (QAH-CYT-1 from Raybiotech Inc. Norcross GA) consisted of quadruplicate antibody spots for 20 cytokines and inflammation-related mediators. The array slides were incubated with cell-free supernatants, and processed according to the manufacturer's instructions. Data acquisition was performed via a Perkin Elmer ScanArray Express laser microarray scanner (courtesy the Vancouver Prostate Centre Microarray Facility) and subsequent quantification using ImaGene 8.0 software from BioDiscovery. Signal intensity medians were background corrected and the means and standard deviations of the replicates calculated. Some of the slide wells were treated with pure antigens (as part of the Raybiotech fluorescent antibody array system kit) in order to calculate a standard curve. The standard curve was used to convert the calculated mean intensities to concentrations (pg/mL).

RESULTS

The *Echinacea* extract (Echinaforce®) showed potent antibacterial activity against the ATCC and clinical strains of *Propionibacterium acnes*, consistently reducing the cfu titre by more than 4 log₁₀. Figure 1 shows the

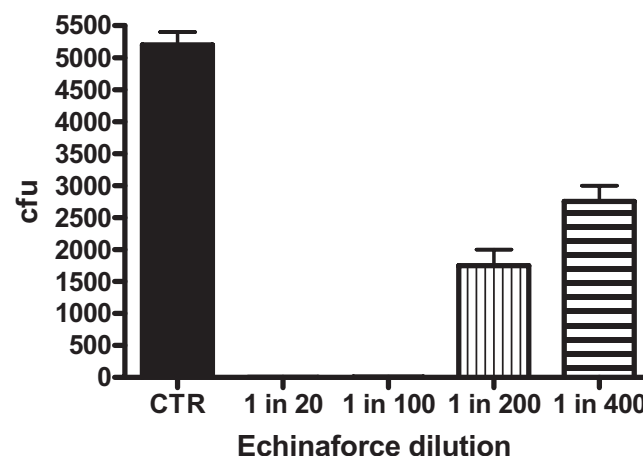


Figure 1. Inactivation of *P. acnes* by different concentrations of Echinaforce®. Aliquots of *Propionibacterium acnes* (ATCC strain, $\sim 6 \times 10^8$ cfu/mL) were mixed with various dilutions of *Echinacea*, in PBS, and incubated, in ambient temperature and light, for 30 min. Following incubation, serial ten-fold dilutions were made in PBS and enumerated for surviving cfu on blood agar plates. Control bacteria (CTR) were incubated in PBS. No colonies were observed at the 1:20 and 1:100 dilutions. The normal recommended dose for oral consumption is a 1:10 dilution (corresponding to 1.6 mg/mL dry mass per volume).

Table 1. IL-6 & IL-8 secretion in BEAS-2B cells

Supernatant	IL-6 pg/mL	IL-8 pg/mL
Control	10.8 ± 2.26	209.5 ± 4.95
Control+ E (Echinaforce®)	31.8 ± 25.17	299.0 ± 1.41
<i>P. acnes</i> ATCC	302.6 ± 35.36	1652 ± 4.95
<i>P. acnes</i> ATCC + E	16.4 ± 0	41.0 ± 0
<i>P. acnes</i> clinical 1	172.4 ± 14.14	1671 ± 32.53
<i>P. acnes</i> clinical 1 + E	18.4 ± 2.83	42.5 ± 2.12
<i>P. acnes</i> clinical 2	283.6 ± 6.22	1688 ± 47.83
<i>P. acnes</i> clinical 2 + E	53.20 ± 3.39	42.5 ± 2.12

dose response effect up to an *Echinacea* dilution of 1:400, equivalent to 40 µg dry mass per mL, or 1/40th of the recommended dose for oral consumption. Dilutions of 1:10 and 1:100 showed complete elimination of the test bacteria. In contrast the common skin bacterium *Staphylococcus aureus* showed no more than a 1 log₁₀ decrease under the same conditions. Incubations were carried out in light and dark conditions, in case the antibacterial activity was due to bioactive photosensitizers, which are known to be present in some *Echinacea* species (Hudson *et al.*, 2005). However, light was not a factor in this study.

The pro-inflammatory activity of *P. acnes* was measured in two human epithelial cell lines, the tracheo-bronchial line BEAS-2B and the lung-derived epithelial cell line A549, and in early passage human skin fibroblasts. Cell-free supernatants were analysed by ELISA assays for cytokine IL-6 and chemokine IL-8 (CXCL8). The cell lines normally secrete only low levels of these two cytokines, but incubation with *P. acnes* ATCC strain or either of the two clinical strains tested (clinical 1 and clinical 2 in Table 1) resulted in substantial increases in both IL-6 and IL-8, as shown in Table 1. In all cases treatment with *Echinacea* strongly inhibited the secretion of both cytokines. The *Echinacea* by itself had no significant effect on the uninfected cells. Similar results were obtained for the other two cell lines (data not shown).

In order to assess the generality of this anticytokine effect, similar cell-free supernatants were analysed by quantitative cytokine antibody arrays, which contained 20 different cytokines/inflammatory mediators, in quadruplicate spots, together with known amounts of the individual antigens to permit construction of standard curves. Following incubation with the supernatants, the arrays were analysed by means of a laser scanner, and the results were converted into concentrations of each cytokine in pg/mL. Figure 2 shows a typical set of arrays, with colours representing relative fluorescent intensities of the spots, from red (highest level) through green and blue. The actual cytokine concentrations in pg/mL are shown below the figures for the responders and several non-responding cytokines. Absence of a coloured spot indicates relatively little or no cytokine detected. Arrays are shown only for the *P. acnes* stimulated and *P. acnes* + *Echinacea* samples, since the control and control + *Echinacea* arrays were indistinguishable from the *P. acnes* + *Echinacea* array shown. The bacteria stimulated the secretion of IL-6 and IL-8 (7.0 and 3.0 fold, respectively), in confirmation of the results of the ELISA assays, as well as GROα (approximately 2-fold) and a slight but reproducible increase in TNFα. However,

Echinacea reduced these levels of secretion to control levels. In addition MCP-1 and VEGF, which were usually observed in control cells but were not stimulated by *P. acnes*, were also reduced by *Echinacea*. All of the other cytokines present in these arrays (indicated on the side bars of each array) were unaffected by *P. acnes*. These patterns of bacterial stimulation and its reversal by *Echinacea* were also observed in arrays of supernatants derived from cells infected by either of the two clinical strains shown in Table 1.

DISCUSSION

The objective of the present study was to evaluate a standardized *Echinacea* extract (Echinaforce®) for its ability to inactivate *P. acnes* and to inhibit the possible pro-inflammatory effect of this organism. According to our general understanding of acne pathogenesis these are the two main factors that initiate and perpetuate the disease, although other hormonal and nutritional factors play important roles (Kurokawa *et al.*, 2009). The organism itself was readily inactivated even by dilutions of extract well below the normal recommended dose for topical treatment or for oral consumption in the control of colds and flu symptoms.

Several respiratory and skin bacteria were reported previously to be vulnerable to *Echinacea* extracts (Sharma *et al.*, 2008b), although this property was not common to all types of extract tested, and not all bacteria were equally susceptible. However, Echinaforce® is a standardized preparation that is consistently active against *P. acnes*. Furthermore, the bacterial induction of pro-inflammatory cytokines, evident in all three cell lines examined, was also inhibited by Echinaforce®, which suggests that this extract could offer dual benefits to acne patients. The bacterial-induced cytokines included IL-6 and IL-8 (CXCL8), and to a lesser extent TNF-α, which are hallmarks of inflammatory responses, and would be expected to lead to an influx of various inflammatory leukocytes. In addition, the secretion of GROα normally results in the attraction of monocytes. Such a combination of cytokines could well explain the production of inflammation at the site of the infection; consequently an agent capable of safely reversing this effect should be beneficial to the patient.

Other plant extracts have recently been reported with anti-*Propioni* bacteria properties, e.g. *Garcinia mangostana* (Chomnawang *et al.*, 2007), *Selaginella involvens* (Joo *et al.*, 2008) and several Korean extracts (Kim *et al.*, 2008), in addition to the isolated phytochemical resveratrol (Docherty *et al.*, 2007). The advantage of Echinaforce® is that it is a standardized and chemically characterized commercial preparation that has been licensed for oral administration, and therefore should be safe for skin applications (Sharma *et al.*, 2009b).

Several other useful properties have been attributed to various *Echinacea* preparations, including antiviral activities, immune modulating actions, antioxidant activities (Barrett, 2003; Barnes *et al.*, 2005; Sharma *et al.*, 2009b), which could help to control the free radicals associated with acne, and wound healing and for which *Echinacea* has been described as useful (Rousseau *et al.*, 2006). The latter properties could be impor-

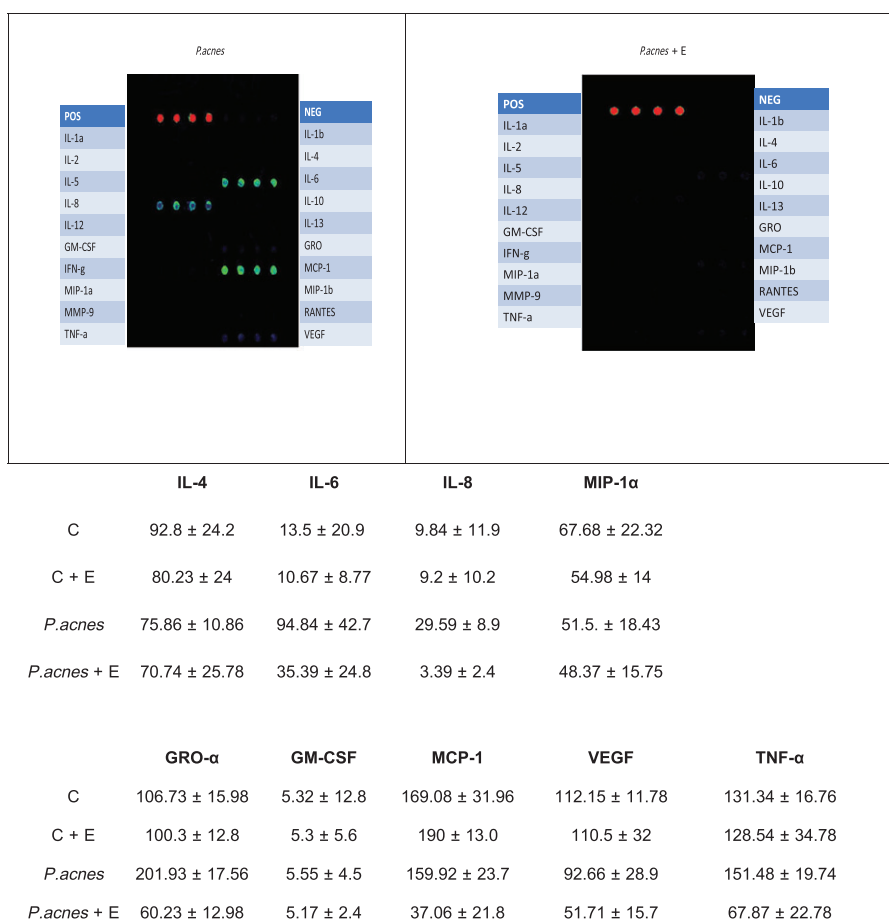


Figure 2. Cytokine antibody arrays of supernatants from cells infected with *P. acnes* \pm Echinaforce®. Replicate BEAS-2B monolayer cultures were infected with *Propionibacterium acnes* (ATCC strain, 70 cfu/cell) for 1 h, followed by Echinaforce® (E) at 1:100 dilution. Control uninfected cells were also treated, or not, with Echinaforce®. At 48 h after infection, cell-free supernatants were harvested for analysis by cytokine antibody arrays (as described in Materials and Methods). The fluorescent quadruplicate spots of each cytokine are shown. Pos and Neg spots represent internal positive and negative controls. The relative intensities of the reactions are indicated by colour, with red being the highest reaction and blue the least. Intensity values were converted to cytokine concentrations, in pg/mL, by means of built-in standard curves (not shown) and these values are displayed below the slides for the most significant responders. The arrays for control uninfected cells, \pm E, were indistinguishable from *P. acnes* + E. The significant responders were IL-6, IL-8 (chemokine CXCL8) and GRO α , which were all inhibited by Echinacea. In addition the normal unstimulated levels of MCP-1, TNF α and VEGF were decreased by Echinacea.

tant in skin applications of *Echinacea*, although they have not yet been described for Echinaforce® per se, which was designed primarily for oral consumption. Cytotoxicity is not a factor in Echinaforce applications (Sharma *et al.*, 2008a, 2009a); and clinical trials have not revealed any side effects (Schoop *et al.*, 2006); consequently safety is not a concern in its use. Therefore we believe that trials of Echinaforce® for the treatment of acne would be worthwhile.

The active ingredients responsible for these activities have not been identified. Polysaccharides, caffeic acid derivatives and alkylamides, common but not universal constituents of *Echinacea* extracts, have been incrimi-

nated in various biological effects, but evidence for any of them individually as the 'active ingredient' is inadequate (Vimalanathan *et al.*, 2009). In addition, polyynes, which have known antibacterial properties (Deng *et al.*, 2008), are often found in *Echinacea* extracts. It is conceivable therefore that a combination of bio-active compounds, including polyynes, flavonoids or other phenolic compounds, is required.

In conclusion, we believe that the combination of antibacterial and antiinflammatory properties shown by certain Echinacea preparations, especially the standardized Echinaforce, could make this herb a useful adjunct treatment for acne.

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