**Echinacea purpurea** diminishes neovascular reaction induced in mice skin by human cancer cells and stimulates non-specific cellular immunity in humans

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**Abstract**

**Objective:** The first aim of the present study was to evaluate the effect of *Echinacea purpurea*-containing drug (Immunal forte tablets) on neovascular response induced in mice skin by human lung and kidney cancer cells, 3 days after their intracutaneous grafting. The second aim was to evaluate the effect of Immunal forte tablets, administered for seven days to healthy human volunteers, on the proportion of natural killer (NK) cells in their blood and on the activity of their blood granulocytes, tested by chemiluminescence test.

**Results:** *Echinacea* preparation caused inhibition of angiogenesis induced by human lung and kidney cancer cells, as evaluated 3 days after intradermal cells injection. In human volunteers Immunal forte administration resulted in the stimulation of granulocytes metabolic activity and have increased the incidence of CD16⁺ and CD56⁺ NK cells in their blood.

**Key words:** *Echinacea purpurea*, human cancers, angiogenesis, granulocytes, NK cells.

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**Introduction**

*Echinacea purpurea* belongs to the most important herbal remedies with immunostimulatory properties. This plant is widely used for the prevention and treatment of upper respiratory tract infections. *Echinacea* extracts contain many compounds with immunomodulatory and anti-inflammatory activity – alkamides, polysaccharides, polyphenols, glycoproteins, essential oils, tannins, and others. A number of in vitro and animal studies have shown that *Echinacea* increases immunologic activity and may enhance phagocytosis, cellular respiratory activity, and lymphocyte activation through release of various cytokines [1-4]. In in vitro studies *Echinacea* reduced human cancer cells viability [5]. Some studies in cancer patients undergoing chemotherapy showed that polysaccharide fraction isolated from *E. purpurea* may counteract the chemotherapy-induced leukopenia [6].

In our previous studies performed in mice we have found that some medicines of natural origin posses antiangiogenic activity, enhance immunity in mice and stimulate various functions of human blood leukocytes [7-10].

The aim of our present study was to evaluate: firstly, the effect of *E. purpurea* preparation, Immunal forte tablets, on cutaneous neovascular response induced in mice by human cancer cells, and, secondly, the influence of Immunal forte administered to human volunteers on the number of their blood natural killer (NK) cells and on the activity of their blood granulocytes.
Material and Methods

Part 1

Lung cancer tissue (squamous cancer) was obtained from Surgery Department of Institute of Tuberculosis and Lung Diseases, Warsaw. Kidney cancer tissue (carcinoma clarocellualre) was obtained from Department of Urology, Postgraduate Medical Center.

Animals: 6-8 weeks old female inbred Balb/c mice. Mice have been of local laboratory breed, weighing 20 g each. Animals were handled according to the Polish law on protection of animals and NIH standards.

The following material was studied: Immunal forte tablets (LEK) – 3 × 0.64 mg/day, 3 × 0.32 mg/day and 3 × 0.16 mg/day.

Tumor tissue was sliced in a sterile ice-cold PBS and then treated with an enzyme mixture containing collagenase 0.1 mg/ml and DNAse 0.004 mg/ml, at room temperature, and stirred for 45 min. Then the obtained suspension was filtered through a sieve, washed twice in PBS and suspended in Parker culture medium at a concentration of 5 × 10⁶ cells per ml. Viability of tumor cell suspensions as assessed by trypan blue exclusion test was about 80%.

Cutaneous angiogenesis assay was performed according to Sidky and Auerbach [11] with own modifications [12]. Briefly, multiple 0.05 ml samples of lung cancer cells or kidney cancer cells were injected intradermally into partly shaved, anesthetized Balb/c mice (at least 2-4 mice per group). In order to facilitate the localization of cell injection sites, the suspension was colored with 0.1% of trypan blue. Next, mice were fed Echinacea preparation in doses described previously, for 3 consecutive days. After 72 hours mice were sacrificed with lethal dose of Morbital. All newly formed blood vessels were identified and counted in dissection microscope, on the inner skin surface, at magnification of 6 ×, in 1/3 central area of microscopic field. Identification was based on the fact that new blood vessels, directed to the point of cells injection are thin and/or differ from the background vasculature in their tortuosity and divarications.

All experiments were accepted and supervised by the local Ethical Committee.

Part II

Study was performed in 25 healthy male volunteers, 20-40-years old. Blood from cubital vein was obtained twice, before and after 7 days treatment. The following materials were studied:
1) Echinacea purpureae aerial parts succus siccum (Immunal forte – tablets, LEK), 160 mg (2 tablets) daily for 7 days – 14 persons,
2) placebo, 2 tablets daily for 7 days – 11 persons.

The following parameters were studied:

- analysis of NK cells was done by monoclonal antibody staining of specific markers CD16+, CD56+ using DAKO APAP KIT System 40, USA, in Lymphoprep-isolated blood mononuclear cell suspensions, according to producer instruction;
- chemiluminescent activity (CL) of blood granulocytes, according to [10]; briefly 0.05 ml of heparinized blood was diluted 1:4 with PBS supplemented with 0.1% of glucose and 0.1% of BSA; 0.05 ml of such diluted blood was added to 0.2 ml of luminol solution (10⁻⁵ M) in PBS and placed in the scintillation counter Rackbeta 1218, LKB Wallac, in the out of coincidence mode for spontaneous CL measurement; cells were activated by addition of fMLP (Sigma) in final concentration 10⁻⁷ M; CL of stimulated cells was then measured for 15 min; the results were calculated as a maximal CL value (in cpm) for 1000 granulocytes.

All experiments were accepted and supervised by the local Ethical Committee.

Statistical analysis of the results was performed using Student t-test.

Results

The inhibitory effect of Echinacea preparation on neovascular reaction induced in mice by injection of cancer cells was observed. We noticed statistically significant reduction of new blood vessels formation in high doses of Echinacea preparation (Table 1 and Table 2). Such effect

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<th>Table 1. inhibitory effect of Echinacea on neovascular reaction induced in mice by intradermal injection of human pulmonary cancer cells</th>
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<th>Table 2. inhibitory effect of Echinacea on neovascular reaction induced in mice by intradermal injection of human kidney cancer cells</th>
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was observed as well in the case of lung cancer cells as kidney cancer cells. There was no influence of low dose of *Echinacea* preparations on neovascularization.

Table 3 presents the number of mononuclear cells with NK specific markers in blood of persons treated with *Echinacea* preparation. *Echinacea* intake statistically significantly increased NK cells number, particularly CD16+. Placebo had no effect.

Significant stimulation of granulocytes activity was observed in comparison to the values obtained before treatment or after placebo (Table 4).

### Discussion

The results of the present study demonstrated that *E. purpurea* preparation Immunal forte tablets caused inhibition of angiogenesis induced by human cancer cells. Chicca et al described that *E. pallida* extract was able to induce apoptosis of human pancreatic cancer and colon cancer cell lines by increasing significantly caspase 3/7 activity and promoting nuclear DNA fragmentation [5]. It was found that flavonoids, present also in *Echinacea* extract, inhibit activities of metalloproteinases and some serine proteases important for angiogenesis [13]. Other polyphenolic compounds have ability to block VEGF binding to its receptor and inhibit VEGF receptor phosphorylation [14]. It seems that *Echinacea* ingredients exert multifunctional inhibitory effects on tumor angiogenesis.

Experiments performed by Razina et al [15] on mice with transplanted Lewis lung carcinoma showed that the officinal *E. purpurea* preparation did not influence the efficacy of cytostatic therapy, however, a hydrophilic polysaccharide complex isolated from *Echinacea* increased the antitumor and antimetastatic activity of cyclophosphamide.

*Echinacea* is immune system stimulator. In patients with immunodeficiency, e.g. with neoplasia disease undergoing the antitumor therapy, it is important to reduce infections. In our previous study *E. purpurea* succus (Immunal drops) stimulated proliferative activity of blood mononuclear leukocytes, increased blood granulocytes activity and elevated CD4/CD8 ratio [10]. In the present study another *Echinacea* preparation (Immunal forte, tablets) stimulated human blood granulocytes chemiluminescence activity. It is known that the generation of the reactive oxygen species during oxidative burst is the most important process in the killing of microbial pathogens. Roessler et al. reported that the polysaccharides purified from large-scale cell cultures of the plant *E. purpurea* could induce acute phase reactions and activation of phagocytes in humans [16]. Melchart et al. showed that polysaccharides isolated from *E. purpurea* might be effective in reducing chemotherapy-induced leukopenia in patients with advanced gastric cancer [6].

In the present study *Echinacea* preparations augmented the number of NK cells, which is known to play a major role in the rejection of tumors and cells infected by viruses. Curier et al. reported that in aging mice *E. purpurea* increased new NK cells production in bone marrow, leading to an increase of NK cells in the spleen. This increase was paralleled by an increase in NK cells anti-tumor activity [17]. The authors did not notice the difference in number of mature granulocytes and their precursors, as well as lymphocytes T and B, and the red blood cell precursors in both the spleen and the bone marrow [18].

The dietary administration of *E. purpurea* extract to mice bearing leukemia increased number of NK cells and prolonged life span [19].

In summary, *Echinacea* preparation used in our study showed an anti-angiogenic properties and succored human immune system. It seems that *Echinacea* preparations might be effective in cancer patients with impaired immunity as an immunoenhancing and anti-angiogenic herbal medicine.

### References


