

# Angiomodulatory properties of some antibiotics and Tołpa Peat Preparation

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## Abstract

Deterioration of the immune system due to antibiotic therapy can be restored by immunomodulator application. In this paper we estimate the effect of ampicillin, amikacin, doxycycline, rifampicin, rifamycin and immunomodulator Tołpa Peat Preparation (TPP) on neovascular reaction induced in murine skin by human mononuclear cells (MNC) injection. MNC originating from 15 healthy volunteers were injected intradermally to Balb/c mice. Antibiotics (3, 15, or 75 mg/kg of body weight) alone or with TPP (10 mg/kg of body weight) were administered subcutaneously to mice on three consecutive days. The number of newly formed blood vessels was measured in dissection microscope 72 hours after cell injection. Results: TPP stimulated angiogenic activity of MNC at the dose 5 and 10 mg/kg. Rifamycin exerted strong stimulatory action, ampicillin slightly stimulated immune response, while doxycycline and rifampicin downregulated it. Amikacin did not influence the results of angiogenesis tests. Studied antibiotics (15 mg/kg), except rifamycin, inhibit the angiostimulatory effect of the tested immunomodulator. TPP should be applied after antibiotic therapy to maintain its stimulatory effect and restore proper host immune function.

**Key words:** antibiotics, angiogenesis, Tołpa Peat Preparation (TPP), amikacin, ampicillin, doxycycline, rifamycin, rifampicin, immune system.

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## Introduction

Antibiotics are an essential part of modern medicine and are the only cure for numerous infectious diseases. However, this kind of therapy may produce side effects that can cause serious medical disorders. A negative impact on the immune system is one of them [1]. Correction of disturbed defensive system function may be assured by immune system modifiers, including immunomodulators. These substances are able to restore proper function of the immune system, i.e. increase the function that had been downregulated and suppress upregulated processes [2]. Tołpa Peat Preparation (TPP) belongs to this group of remedies.

Peat, a source of natural humic substances, has long been used in physiotherapy, rheumatology, and sports medicine. The study of biologically active substances isolated from peat was started in 1945 and was continued since 1956 by Wrocław Agricultural Academy, resulting in isolation of Tołpa Peat Preparation [3]. In 1988 the

Wrocław Agricultural Academy sold the licence for the production of the peat preparation on an industrial scale to the Polish-American company Torf Corporation based in Wrocław. TPP is known to have immunomodulatory and antioxidant properties, regulating a lot of processes, including immune response. Inglot *et al.* [4] described the stimulatory effect of TPP on cytokine production, such as tumour necrosis factor  $\alpha$  and interferon  $\alpha$  and  $\gamma$  by human peripheral blood leukocytes. The same group published immunoprotective activity of peat preparation, performing studies of oral treatment of athletes with TPP [5]. Diminished angiogenic response of geriatric patients and patients with coronary heart disease was upregulated by TPP application in studies by Skopińska *et al.* [6]. The preparation restored abnormally high angiogenic activity of human leukocytes from rheumatoid arthritis (RA) patients, as well. Furthermore, spontaneous IL-1 production of monocytes from RA patients was inhibited by TPP. Krzemiński *et al.* [7] presented cardioprotective and im-

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munostimulatory effect of this remedy after experimental myocardial infarction *in vivo* (rat model).

Nowadays, TPP is offered in tablets containing 2.5 mg of peat with specific indication for recurrent infections. The preparation is of potential interest to dermatologists and cosmetologists. Because of mild astringent and anti-inflammatory activity, humic substances are useful adjuncts in the topical therapy of inflammatory skin diseases, like atopic dermatitis, cheiropodopompholyx, psoriasis, and mild focal hyperhidrosis [8].

Angiogenesis, the process of new blood vessels formation from pre-existing ones, plays a key role in many physiological processes, including embryogenesis, reproductive function, or wound repair. As neovascularisation is a part of cell immunity, it can be a good model for the examination of immune response [9].

Knowledge concerning the antibiotic effects on the immune function is essential because antibiotic therapy is still prevalent. Therefore, the question of whether immunomodulators should be administered during or after antibiotic therapy is still valid. In this paper we evaluate the effect of five popular antibiotics and TPP (applied alone or together to mice) on the angiogenesis process in order to learn more about this issue.

## Material and methods

The studies were conducted based on mononuclear cells (MNC) obtained from peripheral blood of 15 healthy volunteers from the Institute of Tuberculosis and Lung Diseases, Warsaw, Poland, all of whom signed Informed Consent forms.

The following antibiotics and immunomodulator were analysed:

- ampicillin (Polfa, Poland),
- amikacin sulphate (Amikacin, Polfa, Poland),

- doxycycline hydrochloride (Vibramycin, Polfa, Poland),
- rifampicin (Polfa, Poland),
- rifamycine (Rifamycin SV, Glaxo),

Tolpa Peat Preparation branch OHPG9R (Torf Corporation, Wrocław, Poland).

## Isolation of mononuclear leukocytes

Isolation of mononuclear leukocytes (MNC) was performed according to Boyum method and was based on centrifugation of a blood sample on a gradient of Ficoll/Uropoline [10]. The viability of isolated cells was estimated by trypan blue exclusion and was always higher than 98%.

## Angiogenesis test

Angiogenesis test was conducted according to Sidky and Auerbach [11] with Skopińska-Różewska *et al.* [12] modification. Experiments were performed on inbred Balb/c mice, 6-8 weeks old, from the Polish Academy of Sciences breeding colony. MNC ( $5 \times 10^5$  per inoculum) resuspended in Parker medium were injected intradermally (4-6 injections per mouse) into flanks of pre-anaesthetised Balb/c mice. Antibiotics were administered subcutaneously to the mice in three daily doses: 3, 15, or 75 mg/kg of body weight (calculated from recommended human daily dose), for three consecutive days, beginning from the day of MNC injections. Tolpa Peat Preparation was administered subcutaneously at the following daily doses: 0.025, 0.05, 0.25, 0.5, 1, 2.5, 5, 10, 25, and 50 mg/kg of mouse body weight. To examine the combined effect of antibiotic and TPP, the antibiotic was administered at the dose of 15 mg/kg and TPP 10 mg/kg to studied animals. The groups always consisted of three mice. Each experiment was repeated at least two times. After 72 hours the mice were sacrificed and newly formed blood vessels were counted using dissection microscope (magnification 6x) on the inner skin surface.

The experiments were approved by the Local Ethical Committee.

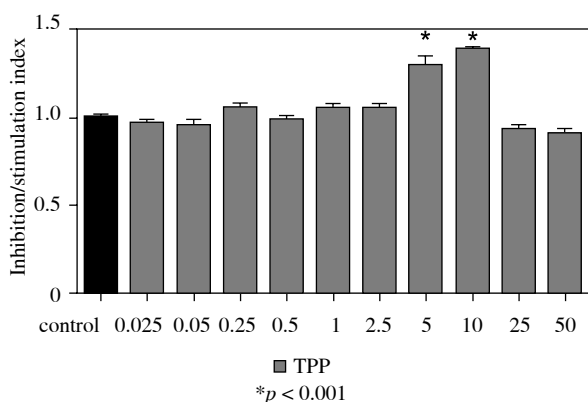
## Statistical analysis

Angiogenic activity was calculated as a number of newly formed blood vessels and presented as quotient of: a) the newly formed blood vessels in tested group, and b) the mean number of newly formed blood vessels in control group, called the inhibition/stimulation index ( $I = a/b$ ). All data are shown as a mean  $\pm$  SEM. The differences between the groups were calculated by two-way ANOVA followed by Bonferroni post-test (GraphPadPrism software). The differences were considered significant at a  $p$ -value  $< 0.05$ .

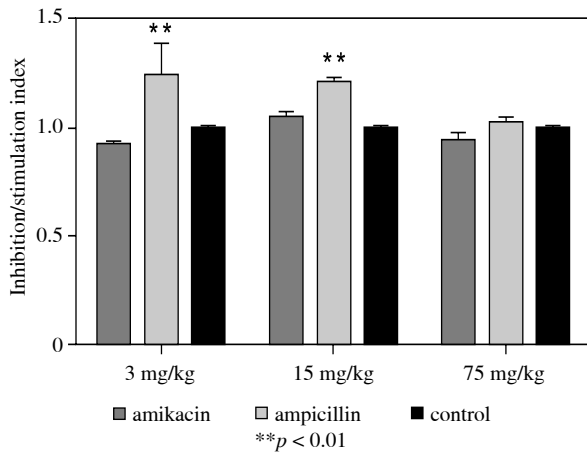
## Results

The results are shown in Figs. 1-5.

Tolpa Peat Preparation exerted a dose-dependent effect in angiogenesis test (Fig. 1).



**Fig. 1.** The influence of various doses of Tolpa Peat Preparation (series: OHP G9R) on angiogenic activity of mononuclear cells



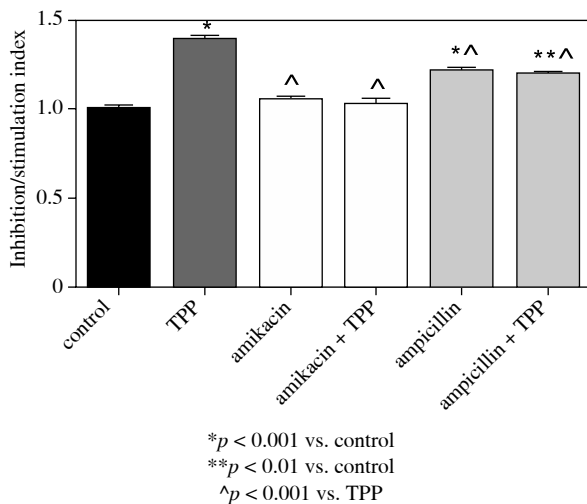
**Fig. 2.** The influence of various doses of ampicillin and amikacin on angiogenic activity of mononuclear cells

Among 10 TPP doses, ranges 0.025-50 mg/kg of body weight, only 5 and 10 mg/kg stimulated ( $p < 0.001$ ) neovascular reaction. The most effective was 10 mg/kg.

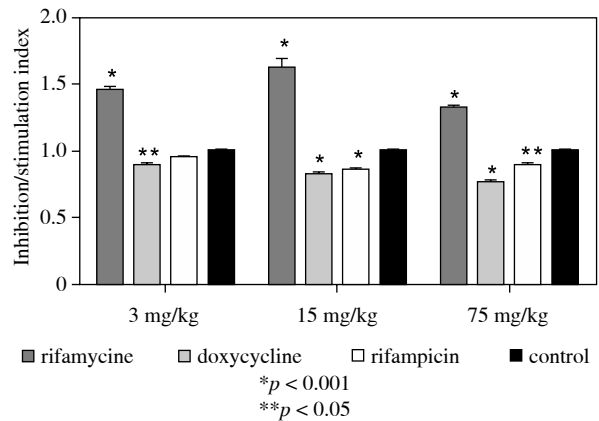
Ampicillin has shown angiostimulatory activity at lower doses (3 and 15 mg/kg), although the effect was not strong ( $p < 0.01$ ). The highest dose did not change MNC activity in angiogenesis test (Fig. 2).

Amikacin did not influence the angiogenic activity of the studied cells (Fig. 2).

Strong stimulation of angiogenesis was obtained for rifamycine administered to mouse at each studied dose. The highest effect was seen at 15 mg/kg (Fig. 3).



**Fig. 4.** Combined effect of antibiotic (ampicillin or amikacin) and Tolpa Peat Preparation (TPP) on angiogenic activity of mononuclear cells. Antibiotic and TPP were administered subcutaneously at the dose 15 mg/kg and 10 mg/kg of body mass, respectively

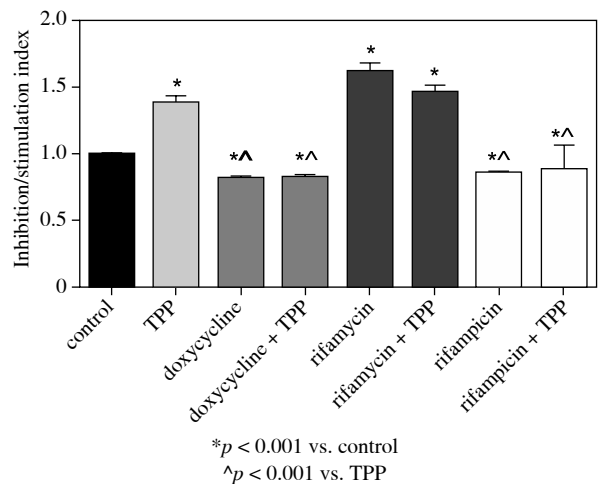


**Fig. 3.** The influence of various doses of doxycycline, rifamycine, and rifampicin on angiogenic activity of mononuclear cells

Rifampicin at the dose of 15 mg/kg ( $p < 0.001$ ) and 75 mg/kg ( $p < 0.05$ ) suppressed angiogenesis, while the smallest dose had no effect in neovascular reaction (Fig. 3).

Doxycycline diminished the results of angiogenesis test in each dose: 3 mg/kg ( $p < 0.05$ ), 15 mg/kg ( $p < 0.001$ ), and 75 mg/kg ( $p < 0.001$ ) (Fig. 3).

Almost all studied antibiotics (15 mg/kg), except rifamycine, abolished the angiostimulatory effect of TPP (10 mg/kg) (Figs. 4 and 5). The results of angiogenesis tests in the groups receiving antibiotic (ampicillin, amikacin, rifampicin, doxycycline) and TPP were significantly ( $p < 0.001$ ) decreased compared to the results from the



**Fig. 5.** Combined effect of antibiotic (doxycycline, rifamycine, or rifampicin) and Tolpa Peat Preparation (TPP) on angiogenic activity of mononuclear cells. Antibiotic and TPP were administered subcutaneously at the dose of 15 mg/kg and 10 mg/kg of body mass, respectively

groups receiving TPP alone. There was no statistical difference between groups of mice receiving antibiotic alone (ampicillin, amikacin, rifampicin, doxycycline) and antibiotic with TPP. The results of angiogenesis tests in the groups injected rifamycine with TPP and rifamycine alone were at the same level (no statistical difference was seen).

## Discussion

The knowledge of modulatory influence of antibiotics on immune function should be propagate and used in clinical practice. Occasionally, even suppression of some immune parameters can be of benefit in particular medical cases. An example is angiogenesis, a part of the cell immune response, which is abnormally increased in many diseases, including cancer, rheumatoid arthritis, and psoriasis. In those cases, during bacterial infection, application of antibiotic diminishing angiogenesis could have a double beneficial effect: antibacterial and restoring disturbed angiogenesis [1]. Antibiotic therapy in patients presenting normal immune function often causes a decrease of some immune function [13]. Our previous reports [9, 14] have shown that antibiotic effect on angiogenesis process depends on the type and dose of antibiotic. Cephalosporins, cephadrine, cefsulodin, and ceftriaxone, in angiogenesis test, showed inhibitory activity, while cefuroxime and cefazidime stimulated angiogenic response. Cefoperazone did not change the neovascular reaction induced by injection of healthy donors' MNC [14]. However, it stimulated angiogenic response of cells derived from bronchoalveolar lavage of sarcoidosis patients [15].

The modulatory effect of ampicillin on various parameters of immunity was reported several times [16-18]. It stimulated GvH reaction and antibody production in mice, while antibody-dependent cell-mediated cytotoxicity (ADCC) and PHA-induced reaction in mice was lowered by its administration [16, 17]. Furthermore, ampicillin modulated human lymphocyte proliferation response to PHA – this effect depended on the dose [16]. The study of Wlodarska *et al.* [18] demonstrated that ampicillin administered to pregnant C3HW mice modulates the immune reactivity of their offspring. The adult progeny of these mice presented lowered cellular immunity and a reduced percentage of T lymphocytes in the lymph nodes, while humoral immunity was enhanced.

The present study shows an increase in human MNC-induced angiogenesis at two studied doses (3 and 15 mg/kg) of ampicillin, meaning that this antibiotic is safe for patients with proper or diminished immune function and can be safely used in bacterial infection.

Artsimowich *et al.* [19] demonstrated that amikacin did not influence humoral and cellular response (GvH) of healthy mice and stimulated these parameters in cyclophosphamide-immunosuppressed animals. *In vitro* study showed [20] that humoral response of human lymphocytes

was not modified by amikacin. Our investigation confirms these results. The angiogenic response of human MNC was not altered by amikacin in the present study. However, Roszkowski *et al.* [21] reported that seven-day amikacin Balb/c mice treatment by 3, 15, and 75 mg/kg of this drug lowered humoral response and LPS-induced lymphocyte activity.

Most investigators have reported inhibitory effects of doxycycline on various immune functions, such as chemotaxis, phagocytosis, intracellular bacteria killing, antibody production, and synthesis of proteins [13]. Delayed hypersensitivity reaction, mitogen-induced blast transformation of lymphocytes, in *in vitro* and *in vivo* studies was also reduced by doxycycline administration [13, 22]. The results of the angiogenesis tests presented in this paper showed an inhibitory effect of doxycycline administered to mice at each studied dose.

Rifamycines are a group of antibiotics that are synthesised either naturally by the bacterium *Amycolatopsis rifamycinica*, or chemically. Rifamycines are particularly effective against mycobacteria, and are therefore used to treat tuberculosis, leprosy, and *Mycobacterium avium* complex (MAC) infections. Rifamycine has been shown to increase many parameters of immune function, e.g. phagocytosis of mouse peritoneal macrophages after one week of administration (at doses 10-30 mg/kg) [23]. Amagay *et al.* [24] showed that rifamycine did not alter antibody production and Th lymphocyte activity and enhanced suppressor T lymphocyte activity. Ten-day therapy with this antibiotic did not influence delayed hypersensitivity reaction in mice [1].

The results of our work show a strong increase in angiogenic activity of human MNC at each dose of rifamycine. These results indicate the possibility to safely use this drug in the therapy of patients with suppressed immune function. Although rifampicin belongs also to rifamycines, it exerts contrary effects, diminishing angiogenic response at the dose of 15 and 75 mg/kg. The smallest dose had no influence. Our previous results demonstrated that rifampicin administered at the dose of 50 mg/kg also inhibited angiogenesis [25]. Moreover, this study showed that rifampicin slightly stimulated the production of anti-SRBC antibodies and suppressed the cellular response *in vivo* in B6AF1 mice. It decreased PHA- and Con A-induced proliferation of lymphocytes (dose 70 µg/ml) in *in vitro* studies. According to other investigations [26], long-term rifampicin treatment inhibits production of anti-SRBC antibodies (20 mg/kg) or exerts no influence [27]. Inhibition of mitogen-stimulated proliferation of lymphocytes and blast transformation was also shown in *in vitro* and *in vivo* study. It suppressed chemotactic activity of neutrophils. Long rifampicin treatment of tuberculosis patients diminished cellular response parameters, which returned to normal levels afterwards [27].

Tolpa Peat Preparation stimulated angiogenic activity of human MNC in average doses (5 and 10 mg/kg of body mass), exerting no influence on angiogenesis at lower and higher doses. Studied antibiotics (15 mg/kg), except rifamycine, abolished the angiostimulatory effect of 10 mg/kg TPP. A similar effect was obtained for erythromycin and cephadrine in our previous investigations [28]. This fact implicates that TPP should be administered after therapy with these antibiotics. In contrast, rifamycine used with TPP did not alter the angiogenic activity of MNC. Clindamycin was demonstrated to have similar activity [28].

To sum up, our studies show a modulatory effect of antibiotic administration on immune function measured by angiogenesis test. Rifamycine exerted strong stimulatory action, ampicillin slightly stimulated immune response, while doxycycline and rifampicin downregulated it. Amikacin did not influence the results of the LIA test. The studied antibiotics (15 mg/kg), except for rifamycine, abolished the angiostimulatory effect of TPP.

## Conclusions

Antibiotics modulate angiogenesis and immune response.

Tolpa Peat Preparation should be applied after antibiotic therapy, in order to maintain its stimulatory effect and restore proper immune function.

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*The authors declare no conflict of interest.*

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