The effect of clindamycin and lincomycin on angiogenic activity of human blood mononuclear cells

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Abstract

Clindamycin and lincomycin have a similar mode of antibacterial action, similar antibacterial spectra and they are used frequently in clinical practice. Irrespective of their anti-microbial properties they are suggested to modulate immune function. The aim of our study was to estimate the effect of these antibiotics on neovascular reaction induced in mice skin by mononuclear cells (MNC) collected from the blood of 8 healthy volunteers. Mononuclear cells were injected intradermally into anaesthetized Balb/c inbred mice. Clindamycin, lincomycin or PBS (control) were administered subcutaneously into the mice over 3 days at doses of 3, 15 and 75 mg/kg of the body mass. The number of newly formed blood vessels was counted in dissection microscope 72 h after cells injection. Lincomycin did not change the results of angiogenesis tests comparing to the control . On the contrary, clindamycin administration resulted in strong stimulation of angiogenic response at all used doses. Monocytes and CD8+ cells inhibited angiostimulatory effect of clindamycin administration.

Key words: *clindamycin, lincomycin, angiogenesis, immune system modifiers, mononuclear cells.*

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Introduction

Antibiotics are used in clinical practice very frequently. One should realize, however, that antibiotic therapy may deeply influence human organism changing its homeostasis. It may concern many important processes including host inflammatory and immune response [1].

Clindamycin and lincomycin belong to lincosamides with chemical structure consisting of amino acid and sugar moieties. Their antimicrobial activity is based on protein synthesis inhibition by binding to the 50S ribosomal subunit [2]. Lincomycin occurs naturally, while clindamycin is a semi-synthetic chlorinated derivative of lincomycin and exhibits improved antibacterial activity. Staphylococci and streptococci as well as *Bacteroides fragilis* and some other anaerobes are sensitive to lincosamides treatment. Clindamycin therapy is effective in dental infection, toxic shock syndrome and directly block the M protein production that leads to the severe inflammatory response. It has high oral absorbtion and significant tissue penetration including bone [3].

Formation of new blood vessels from preexisting ones, defined as angiogenesis, is essential for a variety of physiological processes like embryogenesis, reproductive functions and wound repair. The process of angiogenesis is regulated by various angiogenic and angiostatic/angioinhibitory factors. Increased angiogenesis plays a critical role in pathogenesis of various diseases including cancer, diabetic retinopathy, rheumatoid arthritis and other chronic inflammatory disorders. On the other hand, a decreased new blood vessels formation underlies poor wounds and fractures repair, coronary heart disease and other ischemic conditions [4].

Angiogenesis is regarded as a part of cell immunity and that is why it can be also a good model for immune response examination (immunological angiogenesis). Lowered angiogenic activity of MNCs collected from the blood of elderly people, patients suffering from candidosis, patients with coronary heart disease and patients with scleroderma was reported using leukocyte-

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induced mice cutaneous angiogenesis test, LIA [5-7]. Increased angiogenic activity of MNCs isolated from the blood and synovial fluid of rheumatoid arthritis patients and from the blood of patients with psoriasis or epidermodysplasia verruciformis were reported as well [8-10]. Several natural drugs and herbal extracts behaved as stimulators of immunological angiogenesis induced by MNCs; among them Tołpa peat preparation (TPP), stimulating angiogenic activity of T cells and monocytes, water extracts of *Hypericum perforatum* L and *Melissa officinals* L; *Echinacea purpurea* extracts, ether fraction of poplar leaves water extract and isolated from this fraction phenolic acids: caffeic, gallic, salicylic, ferulic and gentisic. Chlorogenic acid behaved as immunomodulator. It increased angiogenic activity of MNCs collected from healthy donors, and decreased abnormally high angiogenic activity of MNCs collected from the blood of rheumatoid arthritis patients. The same was observed for TPP [8, 11-13].

The results provide evidence that antibiotics may affect angiogenesis. Previous study of our group revealed a different influence of cephalosporin antibiotics using LIA test. The results depend on the kind of cells used for angiogenesis induction as well as on the kind of antibiotic applied and its doses [14, 15]. Some antibiotics (cefradine, ceftriaxone and cefsulodin) suppressed, while cefuroxime and ceftazidime enhanced, neovascularization of healthy people's MNC. Cefoperazone did not exert any effect on angiogenic response of healthy people's MNC, althought it increased angiogenic activity of bronchalveolar lavage cells obtained from sarcoidosis patients. Lung cancer cells' as well as ovarian cancer cells' angiogenic activiy was diminished after cefoperazone and cefuroxime administration, indicating that this kind of antiinfecive therapy could be beneficial for cancer patients.

The information concerning the effect of lincosamides on angiogenic activity of mononuclear leukocytes is scarce. Also, information on the effect of antibiotics from this group on immunity is limited [16-24]. Clindamycin and lincomycin are often used in bone and joint surgery, where correct healing process is extremely important. Knowledge about the effect of selected antibiotic on an important part of reparation process – angiogenesis – should be obligatory. Therefore, the aim of our work was to evaluate the effect of two antibiotics from lincosamides group – clindamycin and lincomycin – on neovascular reaction induced in mice by intradermal grafting of healthy volunteers blood mononuclear cells.

Materials and methods

All experiments were performed with mononuclear cells (MNC) obtained from peripheral blood of 8 healthy volunteers from Institute of Tuberculosis and Lung Diseases which signed Informed Consens.

Following antibiotics were tested:
- Dalacin (Clindamycin phosphate), Upjohn, Belgium,
- Lincomycine HCL, Upjohn, Belgium.

Isolation of mononuclear leukocytes

Mononuclear leukocytes (MNC) were isolated from blood by centrifugation on Ficoll/Uropoline gradient, according to Boyum [25]. The viability of isolated cells was determined by trypan blue exlusion and was always higher than 98%.

Monocyte elimination

Mononuclear cells suspension was mixed with carbonyl iron (15 mg/10^7 cells) and incubated for 1 h, at 37°C in plastic bottles. Non adherent cells were poured into the tubes, and phagocytic and lymphoid cells were separated using a magnet. The lymphoid cells (0-2% peroxidase possitive, < 3% BMA 0310 positive) were washed twice and resuspended in PBS – FCS or Parker medium.

CD8 positive cells elimination

Monocyte – depleted cell suspensions (2×10^7 cell/ml) were incubated for 30 min at 4°C with 5 μg/ml monoclonal antibody (BMA 081). Then the cells were washed twice and resuspended in PBS medium supplemented with 2% inactivated FCS. Cell suspensions were placed on plastic Petri dishes precoated with affinity purified rabbit anti-mouse IgG for 30 min at room temperature and then 30 min in 37°C, and washed in PBS. Cell suspensions enriched with CD4 positive cells contained less then 1% CD8 possitive cells, as estimated by APAAP method.

Angiogenesis test

Angiogenesis test was performed according to Sidky and Auerbach [26] with some modifications [27]. All experiments were performed on inbred 6-8 week old Balb/c mice, from the Polish Academy of Sciences' breeding colony. Various fractions of human MNC were injected intradermally in 4-6 places (5×10^5 per inoculum) into anesthetized mice. Afterwards, antibiotics were administered subcutaneously into the mice (3 mice in one group) for 3 consecutive days at daily dose of 3, 15 and 75 mg/kg of the body mass. Mice from the control group were injected with PBS. Each experiment was repeated at least 2 times. After 72 h mice were sacrificed and newly formed blood vessels were counted in dissection microscope (magnification 6 ×) on the inner skin surface.

Experiments were approved by Local Ethical Committee.

Statistical analysis

Angiogenic activity was calculated as a number of newly formed blood vessels and presented as an index (I) counted from the formula: I = a/b, (a: newly formed blood vessels in tested group; b: mean number of newly formed blood vessels in the control group)

All data are shown as a mean ± SEM. The differences between the groups were evaluated by two-way ANOVA followed by Bonferroni post-test. The differences were considered significant at p-value < 0.05. Evaluation of the results of experiments with cell fractions was done by one-way ANOVA followed by Tukey's post-test (GraphPadPrism software).

Results

Results were shown in Table 1, Table 2, Fig. 1 and Fig. 2.

The effect of lincosamides on neovascular response depended on the type of antibiotic and was dose-independent. Lincomycin (each dose), did not alter neovascular response comparing to the control group of mice. Strong stimulation of angiogenic response was observed for each used dose of clindamycin. In recipients of monocyte-depleted MNC statistically higher stimulatory effect of clindamycin (15 mg/kg) was observed, than in recipients of full suspension of MNC. The highest stimulation of neovascular response was observed in monocyte and CD8+ cells depleted cell suspension.

Discussion

Regardless of the dose used in our experiments, clindamycin produced a strong stimulatory effect on new blood vessels formation, while lincomycin did not exert any effect in cutaneous angiogenesis test. We also observed monocyte- and CD8+ cells- dependent inhibition of angiostimulatory response of the full MNC suspensions after clindamycin administration.

There aren't many publications concerning immunomodulatory activity of lincomycin. This antibiotic in subminimal doses increased the susceptibility of *S. aureus* on cytotoxic activity of human MNC [16]. Lincomycin administered before and 6h after surgical ovariectomy prevented development of an infection [17]. Lincomycin used at the dose of 17 mg/kg/day was found to be effective in protecting mice from endogenous septic shock in β-glucan-indomethacin model, by modulating gut microbial flora [18]. Furthermore, proinflammatory cytokines production (IL-6, TNF-α) was decreased by lincomycin in *in vitro* spleen cell cultures.

Clindamycin was described to have stimulatory effect on immune system.

Ammurio *et al.* [19] have shown stimulation of phagocytic activity of mouse macrophages *in vivo* at the dose of 40 mg/kg and lack of the effect at 15 mg/kg after 1 week of treatment. Stimulation of phagocytic activity by this antibiotic was also reported by other author in *ex vivo* and *in vivo* experiments [20]. Moreover, clindamycin did not exert any effect on protein synthesis nor chemotaxis [21]. Roszkowski *et al.* [22] has found that LPS or Concanavalin A induced lymphocyte proliferation as well as humoral immunity was not influenced by clindamycin.

In the present work we obtained evidence of beneficial stimulatory effect of clindamycin on the ability of human peripheral blood mononuclear leukocytes to release proangiogenic factors. Experiments with cell fractions revealed that stimulatory effect is significantly more pronounced after removing monocytes and CD8+ lymphocytes from the injected cell suspension. It suggests that cell target for clindamycin is CD4+ lymphocyte. The same cell target was observed in experiments with two angiostimulatory herbal remedies- Tołpa Peat Preparation (TPP) and *Rhodiola rosea* hydro-alcoholic extract [8, 28, 29].

We previously reported, that the highest angiogenic activity was expressed by T cells forming rosettes with SRBC and bearing both the receptor for the Fc portion of

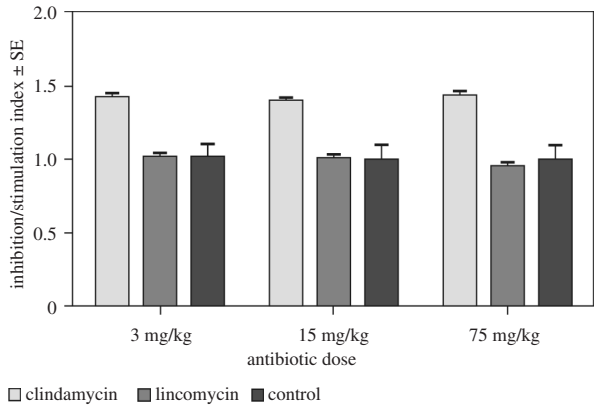

Fig. 1. The effect of clindamycin and lincomycin on angiogenic activity of MNC

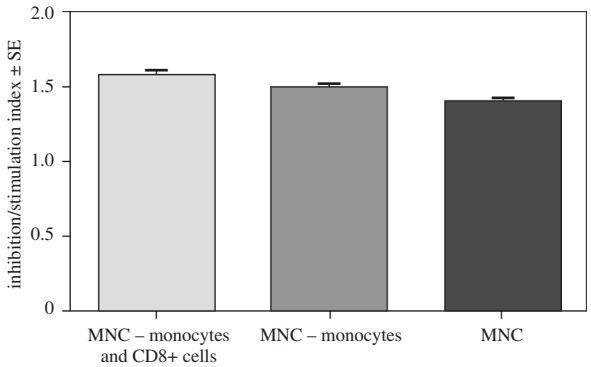

Fig. 2. The effect of clindamycin on various fractions of MNC in angiogenesis test

Table 1. Two-way analysis of variance of experiments with clindamycin and lincomycin (full MNC)

Two-way ANOVA				
Source of Variation	% of total variation	*p*-value		
Interaction	0.27	0.9332		
Drug	37.90	< 0.0001		
Dose	0.03	0.9542		
Source of Variation	*p*-value summary	significant?		
Interaction	NS	No		
Drug	***	Yes		
Dose	NS	No		
Source of Variation	Df	sum-of-squares	mean square	F
Interaction	4	0.05562	0.01390	0.2090
Drug	2	7.673	3.836	57.66
Dose	2	0.006233	0.003116	0.04684
Residual	188	12.51	0.06653	
Number of missing values	64			
Bonferroni posttest				
control vs. clindamycin				
dose	control	clindamycin	difference	95% CI of diff.
3 mg/kg	1.000	1.420	0.4200	0.2068 to 0.6332
15 mg/kg	1.000	1.400	0.4000	0.1825 to 0.6175
75 mg/kg	1.000	1.440	0.4400	0.2297 to 0.6503
dose	difference	t	*p*-value	summary
3 mg/kg	0.4200	5.252	< 0.001	***
15 mg/kg	0.4000	4.904	< 0.001	***
75 mg/kg	0.4400	5.579	< 0.001	***
control vs. lincomycin				
dose	control	lincomycin	difference	95% CI of diff.
3 mg/kg	1,000	1,020	0,02000	–0,1978 to 0,2378
15 mg/kg	1,000	1,010	0,01000	–0,1899 to 0,2099
75 mg/kg	1,000	0,9600	–0,04000	–0,2525 to 0,1725
dose	difference	t	*p*-value	summary
3 mg/kg	0.02000	0.2449	> 0.05	NS
15 mg/kg	0.01000	0.1334	> 0.05	NS
75 mg/kg	–0.04000	0.5019	> 0.05	NS

Table 2. One-way analysis of variance (experiments with cells fractions and clindamycin)

One-way ANOVA				
P-value	< 0.0001			
P-value summary	***			
Are means signif. different? ($p < 0.05$)	Yes			
Number of groups	3			
F	13.7			
R square	0.3246			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	0.8113			
P-value	0.6666			
P-value summary	NS			
Do the variances differ signif. ($p < 0.05$)	No			
Tukey's Multiple Comparison Test	mean difference	q	significant? $p < 0,05$?	summary
MNC vs. MNC – monocytes	–0.1	4.731	Yes	**
MNC vs. MNC – monocytes and CD8+ cells	–0.18	7.268	Yes	***
MNC – monocytes vs. MNC - monocytes and CD8+ cells	–0.08	3.396	No	NS

IgG and a CD4 surface antigen [30, 31]. Later we described two subpopulations of highly angiogenic human T lymphocytes: first, CD4+ FcG+ with CD2 receptors sensitive to theophilline, and second, CD4+ FcG– with CD2 receptors resistant to theophilline [32, 33].

Conclusion

Results of our work indicate that clindamycin may be used during infections of patients with low angiogenesis level (for example ischemic heart disease), for improving new vessels formation during prolonged repair and healing processes in infected patients. It may also be used for antibiotic treatment to prevent infectious complications in joint replacement [34] and for the treatment of infected pseudoarthrosis.

This work was partly performed in the Institute of Tuberculosis and Lung Diseases, Płocka 26, Warsaw, Poland.

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