The effect of selected cephalosporins on angiogenic activity of human blood mononuclear cells

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Abstract

Some antibiotics have been found to modify immune response of the host. Cephalosporins belong to the most frequently used antibiotics. The aim of our study was to evaluate the influence of some cephalosporins on neovascular reaction induced in mice skin by mononuclear cells (MNC) collected from the blood of 12 healthy volunteers. Mononuclear cells or their fractions were injected intradermally into Balb/c mice. Cefradine, cefuroxime, ceftriaxone, cefsulodin, ceftazidime, cefoperazone or PBS (control) were administered subcutaneously into the mice over 3 days at doses of 3, 15 and 75 mg/kg of the body weight. The number of newly formed blood vessels was counted in dissection microscope 72 h after cells injection. Cefradine (each dose), ceftriaxone (75 mg/kg) and cefsulodin (15 and 75 mg/kg) inhibited angiogenic response while cefoperazone did not exert any effect. Cefuroxime (3 and 75 mg/kg) and ceftazidime (15 mg/kg) enhanced neovascular response . Inhibitiory effect of cefradine disappeared after removing monocytes and $CD8^+$ cells from MNC suspension.

Key words: *cephalosporins, angiogenesis, human blood mononuclear cells, mice.*

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Introduction

The word antibiotic comes from the Greek *anti* meaning "against" and *bios* meaning "life". Selective toxicity is the essence of antibiotic mode of action. Antibiotic should destroy or inhibit bacterial (or other microorganism) growth not affecting their host organism. Hovewer, it is known that these drugs may disturb biological homeostasis of organism manifesting adverse or side effects [1]. There is a need to investigate this unwanted influence to manage antibiotic therapy in a proper way. Interestingly, suppression of some immunological parameters caused by antibiotic administration can be beneficial in some specific disorders.

Angiogenesis, a new blood vessels formation from existing one, is a necessary process for proper functioning of the organism. Angiogenesis can be characteristically disturbed in many diseases. In malignancy or arthritis angiogenic activity is too high, while in e.g. coronary heart disease it is diminished [2]. So in the first case during bacterial infection it would seem reasonable to use the drug which decreases angiogenesis, while in the second one the drug enhancing angiogenesis is more desirable. So, the doctor should knows patients immunological status to aplicate him a therapy which compensates disturbed immune defence.

Cephalosporins belong to the most frequently used class of antibiotics. They are highly active against wide spectrum of bacteria, reaches effective concentrations in blood and respiratory secretions, giving infrequent adverse effect (mainly hypersensitivity reaction 1-5% and nonspecific gastrointestinal reaction 5-10%) [3].

Te aim of our study was to evaluate the influence of some cephalosporin antibiotics on neovascular reaction induced in mice by intradermal grafting of healthy volunteers mononuclear cells.

Materials and methods

All experiments were performed with mononuclear cells (MNC) obtained from peripheral blood of 12 healthy volunteers which signed Informed Consent.

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The following antibiotics were tested belonging to cephalosporin group:
- Sefril (Cefradine, Polfa, Poland)
- Biotrakson (Ceftriaxone, Glaxo, USA)
- Pseudomonil (Cefsulodin, Ciba, Denmark)
- Biocefal (Cefuroxime Glaxo, USA)
- Biofort (Ceftazidime, Glaxo, USA)
- Cefobid (Cefoperazone Pfizer, USA)

Daily doses of antibiotics (3, 15, 75 mg/kg mice body mass) corresponded with the doses used in human therapy.

Isolation of MNC

Mononuclear leukocytes (MNC) were isolated from blood by centrifugation on Ficoll/Uropoline gradient, according to Boyum [4]. The viability of isolated cells was determined by trypan blue exlusion and was always higher than 98%.

Monocyte elimination

MNC 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, less then 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 $CD4^+$ cells contained less then 1% $CD8^+$ cells, as estimated by APAAP method.

Angiogenesis test

Angiogenesis test was performed according to Sidky and Auerbach [5] with some modifications [6]. All experiments were performed on inbred 6-8 week old Balb/c mice. Various fractions of human MNC were injected intradermally (5×10^5 per inoculum) into anesthetized mice .Afterwards, antibiotics were administered subcutaneously into the mice (3 mice in one group) for 3 consequitive days at the 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 and Bonferroni post-test to compare replicate means by rows. The differences were considered significant at p-value < 0.05. Evaluation of the results of experiments with cell fractions was done by one-vay ANOVA and Tukey's post-test.

Results

Results were shown in Tables 1, 2, Figures 1, 2.

Cephalosporins exerted different effects on angiogenic activity of MNC, depending on type of antibiotic and its dose (Figure 1, Table 1). Inhibitory action was noted for 3 antibiotics from this group. The strongest effect was obtained for cefradine which diminished angiogenic response in each used dose. The smallest number of new blood vessels was observed for 15 mg/kg ($p < 0.001$). Cefsulodin slighty ($p < 0.05$) decreased angiogenesis at doses 15 and 75 mg/kg, while 3 mg/kg did not differ from the control group. Lowering ($p < 0.05$) of angiogenesis level was observed also for the highest dose (75 mg/kg) of ceftriaxone. There was no effect of cefoperazone on angiogenesis test. Cefuroxime and ceftazidime stimulated angiogenic response. In the case of cefuroxime this effect was observed for doses 3 and 75 mg/kg ($p < 0.01$). Significant ($p < 0.001$) stimulatory effect was produced by 15 mg /kg of ceftazidim dose, while the doses 3 and 75 mg/kg didn't change the level of angiogenic response. Studies concerning mechanism of cefradine inhibitory action were performed using the most inhibitory dose of this drug (15 mg/kg) (Table 2, Figure 2). Depletion of monocytes from MNC suspension caused slight increase of new blood vessels number, while depletion of monocytes and $CD8^+$ cells caused further growth of angiogenesis level. The differences between groups were statistically significant ($p < 0.001$).

Discussion

Cephalosporins belong to the group of antibiotics with heterogeneous influence on immune function. Cephalosporins of the first (like cefradine) and second generation (cefuroxime) were considered by many authors inhibitory or neutral for immune response, while cephalosporins of

Table 1. Two-way analysis of variance experiments with various cephalosporins

Source of Variation		**Significant?**		
Interaction	*	Yes		
Column Factor	***	Yes		
Row Factor	NS	No		
Source of Variation	**Df**	**Sum-of-squares**	**Mean square**	**F**
Interaction	12	0.4018000	0.0334800	2.125000
Column Factor	6	3.1020000	0.5170000	32.810000
Row Factor	2	0.0002167	0.0001084	0.006876
Residual	509	8.0210000	0.0157600	
Number of missing values	331			
Bonferroni posttests				
control vs. cefradine				
row factor	difference	t	*p*-value	summary
3 mg/kg	–0.1500	3.955	< 0.001	***
15 mg/kg	–0.1700	5.851	< 0.001	***
75 mg/kg	–0.1100	3.036	< 0.010	**
control vs. ceftriaxone				
row factor	difference	t	*p*-value	summary
3 mg/kg	–0.04000	1.055	> 0.05	NS
15 mg/kg	–0.07000	2.131	> 0.05	NS
75 mg/kg	–0.09000	2.754	< 0.05	*
control vs. cefsulodin				
row factor	difference	t	*p*-value	summary
3 mg/kg	–0.02000	0.4325	> 0.05	NS
15 mg/kg	–0.08000	2.4350	< 0.05	*
75 mg/kg	–0.08000	2.4480	< 0.05	*
control vs. cefoperazone				
row factor	control	cefoperazone	difference	95% CI of difference
3 mg/kg	1.000	1.000	0.0	–0.10500 to 0.1050
15 mg/kg	1.000	1.070	0.07000	–0.02114 to 0.1611
75 mg/kg	1.000	1.060	0.06000	–0.03607 to 0.1561
row factor	difference	t	*p* value	summary
3 mg/kg	0.00000	0.000	> 0.05	NS
15 mg/kg	0.07000	2.309	> 0.05	NS
75 mg/kg	0.06000	1.877	> 0.05	NS
control vs. ceftazidime				
row factor	control	ceftazidime	difference	95% CI of difference
3 mg/kg	1.000	1.030	0.03000	–0.08216 to 0.1422
15 mg/kg	1.000	1.140	0.14000	0.05614 to 0.2239
5 mg/kg	1.000	1.050	0.05000	–0.05361 to 0.1536

Table 1.

row factor	difference	t	*p*-value	summary
3 mg/kg	0.03000	0.804	> 0.050	NS
15 mg/kg	0.14000	5.018	< 0.001	***
75 mg/kg	0.05000	1.451	> 0.050	NS
control vs. cefuroxime				
row Factor	control	cefuroxime	difference	95% CI of difference
3 mg/kg	1.000	1.120	0.12000	0.01249 to 0.2275
15 mg/kg	1.000	1.060	0.06000	–0.03203 to 0.1520
75 mg/kg	1.000	1.120	0.12000	0.01107 to 0.2289
row factor	difference	t	*p* value	summary
3 mg/kg	0,1200	3,355	< 0,01	**
15 mg/kg	0,06000	1,960	> 0,05	NS
75 mg/kg	0,1200	3,311	< 0,01	**
3 mg/kg vs. 15 mg/kg				
row factor	3 mg/kg	15 mg/kg	difference	95% CI of difference
row factor	difference	t	*p*-value	summary
cefradine	–0.02000	0.5624	> 0.05	NS
ceftriaxone	–0.03000	0.7776	> 0.05	NS
cefsulodin	–0.06000	1.2640	> 0.05	NS
cefoperazone	0.07000	2.0850	> 0.05	NS
ceftazidime	0.11000	3.2330	< 0.01	**
cefuroxime	–0.06000	1.7310	> 0.05	NS
75 mg/kg vs. 15 mg/kg				
row factor	75 mg/kg	15 mg/kg	difference	95% CI of difference
row factor	difference	t	*p*-value	summary
cefradine	–0.06000	1.6870	> 0.05	NS
ceftriaxone	0.02000	0.5262	> 0.05	NS
cefsulodin	0.00000	0.0000	> 0.05	NS
cefoperazone	0.01000	0.3075	> 0.05	NS
ceftazidime	0.09000	2.7360	< 0.05	*
cefuroxime	–0.06000	1.6310	> 0.05	NS

NS - non-significant

third generation were seen as rather stimulating. In our studies cefradine inhibited angiogenic activity of MNC in all used doses. Studies concerning cefradine immunological effect presented by Chaperon *et al*. [7] and Ogawa *et al.* [8] are in line with our results. They revealed that cefradine administration inhibited lymphocyte response to PHA and Con A and decreased production of anti-SRBC antibodies by mouse splenocytes. The inhibition produced by cefradine, in the context of our present results, might have arisen from the stimulation of $CD8^+$cells and monocytes which, in our experiments, synergically diminished angiogenic response of MNC. Similar effects were obtained by Eismary group [9]. They revealed that suppression activity of $CD8^+$cells was associated with the presence of monocytes and $CD4^+$cells. Cefsulodin was shown [10] to decrease humoral response in mouse model. Inhibitory effect of this

Table 2. Statistical analysis of the results (experiments with cell fractions)

One-way analysis of variance				
p-value	< 0.0001			
p-value summary	***			
Are means signif. different? (p < 0.05)	Yes			
Number of groups	3			
F	44.44			
R square	0.5326			
Bartlett's test for equal variances				
Bartlett's statistic (corrected)	1.899			
p-value	0.3868			
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	–0.13	6.655	Yes	***
MNC vs. MNC - monocytes and $CD8^+$ cells	–0.28	13.24	Yes	***
MNC-monocytes vs. MNC - monocytes and $CD8^+$ cells	–0.15	6.685	Yes	***

drug used at therapeutic doses (15 and 75 mg/kg) was obtained also in our work.

Ceftriaxone in our experiments diminished angiogenic response at dose 5 times higher (75 mg/kg) then therapeutic. Subminimal concentration of ceftriaxone, not studied in our paper, according to Raponi *et al*. [11] increased polymorphonuclear leucocytes (PMN) phagocytic activity of *Klebsiella* oxytoza and *Staphylococcus aureus*.

Cefuroxime exerted stimulatory effect on angiogenic response at doses 3 and 75 mg/kg, as demonstrated. Chemotaxis of leukocytes and their spontaneous activation was enhanced by administration of cefuroxime. This drug did not influenced phagocytosis and fungicidal activity. Increased production of IgM antibodies but not IgG by BDF and Balb/c nu/nu mice as well as no influence on PWM induced leucocytes migration were presented for cefoperazone [12]. In our present work this antibiotic was the only drug that didn't influenced neo-vascular reaction.

Our previous studies [13] revealed stimulatory effect of cefoperazone on angiogenic activity of bronchoalveolar

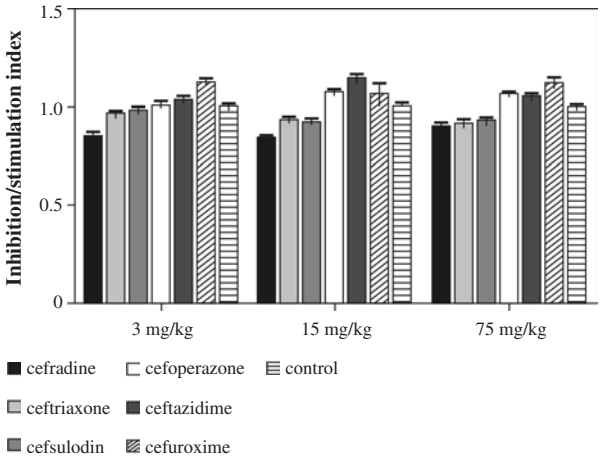

Fig. 1. The effect of cephalosporins on angiogenic activity of MNC

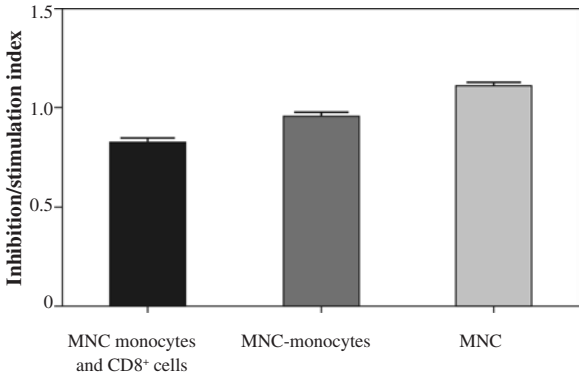

Fig. 2. The effect of cephradine on various fractions of MNC in angiogenesis test

lavage cells obtained from sarcoidosis patients. We have also shown that angiogenic activity of lung cancer cells was diminished after cefoperazone and cefuroxime administration. The same drugs inhibited angiogenesis induced by ovarian cancer cells [14]. Such observations suggested that these cephalosporins can regulate production of angiogenic growth factors. Administration of these drugs could be beneficial during bacterial infections in cancer patients.

There is a lot of studies of ceftazidime effects on immune defences. In our experiments ceftazidime enhanced angiogenesis only at 15 mg/kg – the dose 2 times lower than therapeutic. Stimulation of immune parameters was observed for subminimal concentration of this drug. Cuffini *et al.* [15] demonstrated enhanced phagocytic and bactericidal activity of human and mouse macrophages after administration of subminimal doses of this antibiotic. Elevation of humoral response and bactericidal activity of macrophages was obtained after administration of 2,5-3 mg/kg ceftazidime in Balb/c mice. Other *in vivo* and ex vivo ceftazidime studies indicated stimulation of phagocytosis, production of IL-1 and IFN-γ in immunologically impaired patients and laboratory animals [16]. Single dose of ceftazidime enhanced blood concentration of some pro-inflammatory cytokines (IL-6 and TNF-α) in bloodstream of rats [17]. However supratherapeutic concentrations of ceftazidime inhibited production of TNF-α by human PBMC stimulated with heat-killed *Stenotrophomonas maltophilia* [18].

Analysis of these results suggest that ceftazidime mode of action is dependent on its concentration.

Conclusions

1. Cephalosporins exerted heterogeneous effect on angiogenic MNC response depending on the type of antibiotic and their dose.
2. The mechanism of inhibition probably rely on activation of suppressor/cytotoxic function of CD8 positive cells with monocytes cooperation.
3. Cefradine and cefsulodin are suggested to exert a beneficial effect in therapy of patients with high level of pathological angiogenesis, while cefuroxime and ceftazidime are expected to enhance immunological and angiogenic response of angio- and immuno-compromised patients.

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