Effects of Antibiotics on Reactive Oxygen Species Generation by Neutrophils

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Antibiotic therapy of patients with exacerbation of chronic obstructive bronchitis exposed and not exposed to ozone did not improve oxidative metabolism in neutrophils. Cefazolin, ceftizoxime, and gentamicin normalized functional biocidal reserves of neutrophils, which correlated with pronounced therapeutic effects.

Key Words: *antibiotic therapy; biocidity; neutrophils; ozone*

The system of reactive oxygen species (ROS) generation by blood neutrophils plays an important role in the body and is involved in the pathogenesis of various diseases [1]. The effects of antibiotics widely used for the therapy of infectious diseases are of particular interest in this respect. Antibiotics and ROS generated by blood neutrophils possess antibacterial activity. Antibacterial properties of antibiotics are associated with their bactericidal and bacteriostatic effects, while ROS enhance biocidity of phagocytes [2,4].

The effects of antibiotics on chemiluminescence (CL) of phagocytes are poorly understood [5,6].

Here we studied the effects of antibiotics widely used in clinical practice on ROS generation by neutrophils from patients with chronic obstructive bronchitis (COB) exposed and not exposed to ozone.

MATERIALS AND METHODS

Antibacterial therapy was performed in 45 workers employed in industrial production of plasticizers and exposed to ozone in a concentration of 0.10-0.23 mg/m^3 and 48 city residents not contacting with ozone. All examinees had COB relapse. Benzylpenicillin sodium salt, oxacillin sodium salt, ampicillin sodium salt, cefazolin sodium salt, and ceftizoxime sodium salt, gentamicin sulfate, and lincomycin hydrochloride were administered in doses routinely used for the therapy of chronic bronchitis. The patients also received bronchodilators and mucolytics. The sensitivity of sputum microbial flora to antibiotics was determined. Antibiotic therapy was performed for 8-10 days. ROS generation was studied before the start of therapy and 1 day after antibiotic withdrawal.

The patients were subjected to general clinical examination. Fifty healthy donors served as the control.

ROS generation was studied by recording whole blood CL on a KhL-003 chemiluminometer [3]. Heparin (20 U/ml), luminol (final concentration 10^{-5} M), and Hank's solution were added to blood samples (0.1 ml). CL was induced by adding zymosan in a concentration of 2 mg/ml. The leukocyte activation coefficient (AC) was calculated as the ratio between zymosan-induced and spontaneous CL [3]. The results were analyzed by Student's t test. The differences were significant at $p \leq 0.05$.

RESULTS

In employers with COB relapse ampicillin, cefazolin, and ceftizoxime did not change spontaneous CL. Zymosan-induced CL in employers receiving penicillin, oxacillin, ampicillin, and lincomycin did not differ from that observed before therapy (Table 1). Cefazolin, ceftizoxime, and gentamicin increased leukocyte AC. However, this parameter remained 2.2-2.4-fold below the control. Antibiotics changed only the type of latent ROS insufficiency in employers with COB (Table 1).

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TABLE 1. ROS Generation in Employers and City Residents with COB Relapse before and after Antibiotic Therapy (*M*±*m*)

Antibiotics	Employers		City residents	
	before therapy	after therapy	before therapy	after therapy
Penicillin				
CL, arb. units				
spontaneous (Δ, %)	12.5±2.2*** (44)	19.2±3.2*$^{+++}$ (121/53^{x})	30.0±3.6* (246)	25.5±3.7* (194/-15)
zymosan-induced (Δ, %)	13.3±3.2* (-77)	13.2±1.7*$^{+++}$ (-77/-1)	56.7±4.4 (-3)	63.5±3.3 (9/12)
Leukocyte AC	1.1	0.7	1.9	2.5
ROS insufficiency	LI	LI	R	R
Oxacillin				
CL, arb. units				
spontaneous (Δ, %)	17.2±2.4*** (98)	11.2±1.9^{+++} (29/-35)	33.3±3.5* (285)	27.0±3.6* (212/-19)
zymosan-induced (Δ, %)	16.7±1.8* (-71)	18.7±1.9* (-68/12)	35.8±2.6* (-39)	37.8±2.6* (-35/6)
Leukocyte AC	1.0	1.7	1.1	1.4
ROS insufficiency	LI	LII	LI	LI
Ampicillin				
CL, arb. units				
spontaneous (Δ, %)	11.5±1.7*** (33)	11.7±2.0*** (35/0)	34.2±3.1* (294)	20.8±2.5*$^{+}$ (140/-39)
zymosan-induced (Δ, %)	10.8±2.3* (-81)	11.7±2.6* (-80/8)	57.8±6.9 (-1)	36.8±4.5*$^{++}$ (-37/-36)
Leukocyte AC	0.9	0.0	1.7	1.8
ROS insufficiency	LI	LI	R	LI
Cefazolin				
CL, arb. units				
spontaneous (Δ, %)	14.5±2.8 (29)	6.7±3.0 (-23/-40)	23.8±2.7* (175)	12.8±2.6^{++} (48/-46)
zymosan-induced (Δ, %)	13.7±2.4* (-77)	20.0±2.3*$^{+++}$ (-66/46)	34.7±1.9* (-41)	34.2±3.7* (41/-1)
Leukocyte AC	1.2	3.0	1.5	2.7
ROS insufficiency	LII	LII	LI	LI
Ceftizoxime				
CL, arb. units				
spontaneous (Δ, %)	8.3±1.6	10.8±1.6*** (25/30)	35.0±5.2* (304)	15.8±1.9*$^{+}$ (83/-55)
zymosan-induced (Δ, %)	8.3±1.7* (-86)	32.0±4.6*$^{+}$ (-45/284)	62.2±5.4*** (7)	44.3±9.1*$^{++}$ (-24/-29)
Leukocyte AC	0.0	3.0	1.8	2.8
ROS insufficiency	LII	LI	R	LI
Gentamicin				
CL, arb. units				
spontaneous (Δ, %)	9.7±1.8 (12)	13.3±2.1***$^{+++}$ (54/38)	31.2±4.5* (265)	19.2±4.3*$^{+++}$ (121/-39)
zymosan-induced (Δ, %)	9.3±1.1* (-84)	37.7±5.2*$^{+}$ (-35/304)	35.0±3.4** (-40)	35.0±5.1** (-40/0)
Leukocyte AC	1.0	2.8	1.1	1.8
ROS insufficiency	LII	LI	LI	LI
Lincomycin				
CL, arb. units				
spontaneous (Δ, %)	8.0±1.4 (-8)	11.5±1.7***$^{+++}$ (33/44)	38.0±5.3* (339)	22.5±8.1*$^{+++}$ (16/-41)
zymosan-induced (Δ, %)	12.5±2.2* (-79)	11.3±2.0*$^{+++}$ (-81/-9)	40.3±3.3* (-31)	23.3±3.7*$^{+}$ (-60/-42)
Leukocyte AC		1.5	1.0	1.1 1.0
ROS insufficiency	LII	LI	LI	LI

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to healthy donors (spontaneous and zymosan-induced CL 8.7±0.3 and 58.3±5.8 arb. units, respectively, leukocyte AC 6.7); ^{+}p<0.001, ^{++}p<0.01, and ^{+++}p<0.05 compared to parameters before therapy. ^{x}Numerator: compared to healthy donors; denominator: compared to parameters before therapy. LI and LII: type I and II latent ROS insufficiencies, respectively; R: relative ROS insufficiency.

Thus, therapy with antibiotics inhibited ROS generation by neutrophils in employers with COB relapse.

Penicillin and oxacillin had no effect on spontaneous CL in patients with COB not exposed to ozone (Table 1). Moreover, penicillin, oxacillin, cefazolin, and gentamicin did not change zymosan-induced CL in these patients. Leukocyte AC increased in patients treated with penicillin, cefazolin, and ceftizoxime, but remained 2.4-2.7-fold below the control (Table 1). Relative ROS insufficiency was transformed into type I latent ROS insufficiency. In some patients the type of ROS insufficiency remained unchanged.

Thus, antibiotic therapy inhibited ROS generation in patients with COB relapse not exposed to ozone. This effect in city residents was less pronounced than in employers, which is probably related to the influence of ozone on the bronchopulmonary system.

Clinical examination showed that treatment with cefazolin, ceftizoxime, and gentamicin produced most pronounced therapeutic effects in employers. By contrast, penicillin, ampicillin, cefazolin, ceftizoxime, and gentamicin were especially effective during the therapy of city residents with COB. In both groups of patients these antibiotics increased leukocyte AC, which indicates that their influence on the ROS-generating system correlated with clinical course of COB.

These data show that antibiotic therapy did not normalize ROS generation by blood neutrophils in patients with COB exposed and not exposed to ozone. After antibiotic therapy biocidity of neutrophils providing antimicrobial protection in the body remained low. Our results indicate that antibiotics killing pathogenic microorganisms do not increase biocidal activity of phagocytes and, therefore, can not prevent exacerbations of COB and decrease the severity of this disease (particularly, in patients exposed to ozone).

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