Effects of Different Antibiotics on Free Radical Oxidation *In Vitro* and *In Vivo*

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Effects of naturally occurring and semisynthetic penicillins, cephalosporines, aminoglycosides, tetracyclines, and lincomycin on free-radical oxidation were studied in 4 model systems and in intact Wistar rats. Liver and kidney tissue and blood plasma were examined by chemiluminescence. Antibiotics of all groups in therapeutic concentrations exerted antioxidant effect *in vitro* and *in vivo*.

Key Words: *antibiotics; free-radical oxidation; chemiluminescence*

Antibiotics play the leading role in chemotherapy of bacterial infections [6]. Impaired regulation of free-radical oxidation (FRO) is a nonspecific component in the pathogenesis of many diseases, including diseases of bacterial etiology [3]. Today we know that many drugs possessing pro- and antioxidant properties can influence FRO [2]. From this viewpoint, new approaches to antibiotic therapy can be developed, aimed at altering FRO, i. e., the production of active oxygen forms and lipid peroxidation (LPO). A great variety of antibiotics (more than 6000) and their great significance in the management of inflammatory processes prompt the search for simple test systems for predicting effects on FRO [2,3,8].

We studied of the effects of widely used antibiotics on FRO *in vitro* and *in vivo* using the chemiluminescent (CL) registration [2,3,7]. Our objectives were as follows:

1. Study of antibiotic effects in model systems generating active oxygen forms and in a liposome suspension in which LPO reactions occur.

2. Study of antibiotic effect on LPO *in vivo* in blood plasma and hepatic and renal tissue of intact Wistar rats after an 8-day course of intramuscular injections in therapeutic concentrations.

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MATERIALS AND METHODS

The following antibiotics were used: benzylpenicillin, oxacillin, ampicillin, carbenicillin, cephasoline, cephtisoxime, kanamycin, gentamycin, and lincomycin. Free-radical processes were modeled in the following systems:

- citrate-phosphate-luminol (KH_2PO_4 20 mM, KCl 105 mM, pH 7.45, Na citrate 45 mM, luminol 10^{-5} M) [1];
- heparin-treated (50 U/ml) blood, intact and activated with prodigiosan (1 mM) [5];
- suspension of liposomes obtained by homogenization of egg yolk in phosphate buffer (1:5) [4].

Preparations in concentrations compatible with therapeutic doses were added to model systems, and the maximum amplitude of slow flash, light sum, and latent period between fast and slow CL flashes were recorded. Changes in CL intensity were used as the indicator of oxidative processes activity in the presence of antibiotic [3].

Experiments were carried out on 46 Wistar rats weighing 150-170 g. After an 8-day course of intramuscular injections of antibiotics in therapeutic doses, the parameters of Fe^{2+}-induced CL of blood plasma, hepatic and renal tissues was measured [7].

CL intensity was expressed in relative units of radiation. A CLM-003 device was used for recording

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superweak fluorescence. The results were processed using Statgraphics software. The differences were significant at $p<0.05$.

RESULTS

In the first series of experiments, we examined the effects of antibiotics in different concentrations on FRO in model systems in order to assess their pro- and antioxidant properties.

In the citrate-phosphate-luminol model, CL developed after addition of bivalent iron salt (Haber-Weiss [1] reaction), which was selectively stimulated by luminol. Maximum amplitude of slow CL flash decreased 4.7 times after addition of penicillin (28.5 U/ml), 6.6 times after lincomycin (1.4 mg/ml), 5.8 times after cephasoline (4.7 mg/ml), 2.6 times after gentamycin (0.32 mg/ml), and 1.4 times after kanamycin (0.3 mg/ml). Light sum was inhibited in parallel: 7.6 times by penicillin, 7.1 times by cephasoline, 5.2 times by lincomycin, and 2.3 times by aminoglycosides. Cephasoline and kanamycin prolonged the latent period 1.9 times, penicillin 1.8 times, lincomycin 1.7 times, carbenicillin 1.5 times, and gentamycin 1.3 times. The semisynthetic penicillins ampicillin (3.5 mg/ml), oxacillin (3 mg/ml), and carbenicillin (3.3 mg/ml) slightly decreased CL, while tetraoleane (0.15 mg/ml) and cephtisoxime (4 mg/ml) in fact extinguished it. Tenfold lower concentrations of antibiotics decreased 10-fold the intensity of CL, and 100-fold decreased concentrations did not change CL (control).

Then we examined the effects of antibiotics on CL caused by generation of active oxygen forms in intact and activated whole blood of healthy humans. After incubation of intact blood with antibiotics, maximum amplitude of slow flash and light sum of CL decreased by 20% with penicillin (2.85 U/ml) and oxacillin (0.3 mg/ml), decreased by 90% with tetraoleane (0.015 mg/ml) and cephtisoxime (0.4 mg/ml), decreased by 80% with cephasoline (0.47 mg/ml) and carbenicillin (0.33 mg/ml), decreased by 60% with gentamycin (0.032 mg/ml) and ampicillin (0.35 mg/ml), and decreased by 45% with lincomycin (0.14 mg/ml).

Production of active oxygen forms by prodigiosan-activated blood was suppressed in the presence of antibiotics in the same concentrations. The maximum amplitude of slow flash and light sum of CL were decreased 2-fold by oxacillin and ampicillin, 1.4 times by gentamycin, 2.3 times by lincomycin, 2.4 times by penicillin, 8.3 times by carbenicillin and cephasoline, and 16 times by kanamycin. Cephtisoxime and tetraoleane virtually extinguished CL. Thus, cephalosporines, aminoglycosides, and tetraoleane in

Table 1. Changes in LPO processes in the Liver, Kidneys, and Plasma of Wistar Rats after an 8-Day Course of Intramuscular Injections of Antibiotics in Therapeutic Doses (*M±m*, *n*=9)

Drug	Liver CL			Kidney CL			Plasma CL		
	LP	S	I_{max}	LP	S	I_{max}	LP	S	I_{max}
Control	21±1.65	31±2.87	190±3.22	35±1.26	9±0.37	105±3.36	5±0.33	10±0.32	65±3.34
Penicillin, 100 U/kg	25±1.04 (119)	27±2.2 (87)	155±6.58 (82)	40±3.11 (114)	7±0.64 (78)	90±6.18 (86)	9±0.86 (180)	8±0.32 (80)*	45±3.09 (69)
Oxacillin, 100 mg/ml	24±1.51 (114)	20±3.85 (65)*	147±7.2 (77)*	41±2.08 (117)*	6±0.61 (67)	79±3.31 (75)*	10±0.64 (200)	8±0.5 (80)*	42±2.17 (65)
Ampicillin, 50 mg/kg	32±1.76 (152)*	14±1.74 (45)*	126±9.2 (66)	53±2.53 (151)*	7±0.46 (78)	94±3.02 (90)*	8±0.61 (160)	9±0.39 (90)	46±2.91 (71)
Carbenicillin, 300 mg/kg	30±1.39 (143)*	14±0.96 (45)	139±5.4 (73)*	48±4.93 (137)	6±0.35 (67)	85±3.23 (81)*	11±0.64 (220)*	7±0.3 (70)	42±2.46 (65)
Cephasoline, 66 mg/kg	15±0.74 (71)*	23±2.3 (74)*	120±7.92 (63)	42±2.42 (120)	6±0.64 (67)*	76±3.23 (72)*	4±0.28 (80)*	7±0.53 (70)	41±2.22 (63)*
Cephtisoxime, 50 mg/kg	84±3.57 (400)	6±0.35 (19)	65±4.82 (34)	79±3.0 (226)	8±0.37 (89)	94±8.44 (90)	15±1.63 (300)	7±0.24 (70)	33±1.29 (51)*
Kanamycin, 10 mg/kg	34±2.56 (162)*	12±1.93 (39)*	87±5.75 (46)	68±4.36 (194)	5±0.45 (56)	48±3.43 (46)	5±1.19 (100)	6±0.39 (60)*	26±1.83 (40)
Gentamycin, 4 mg/kg	25±1.3 (119)*	7±0.69 (23)	99±7.37 (52)*	41±2.05 (117)	5±0.66 (56)*	62±2.97 (59)*	10±1.09 (200)	8±1.01 (80)	44±4.62 (68)
Tetraoleane, 25 mg/kg	14±0.53 (67)	24±1.01 (77)*	121±3.41 (64)	23±1.14 (66)*	6±0.59 (67)	76±2.19 (72)*	2±0.04 (40)	8±1.35 (80)	44±1.07 (68)*
Lincomycin, 15 mg/kg	20±0.45 (95)	24±1.21 (77)	98±3.36 (51.5)*	34±1.99 (97)	5±0.66 (56)*	59±0.94 (56)	6±0.35 (120)	7±0.35 (70)*	31±1.47 (48)

Note. Control group consisted of 10 rats. The percentage is given in parentheses. LP: latent period between fast and slow CL flashes; S: CL light sum; I_{max}: maximum amplitude of slow CL flash.

therapeutic concentrations inhibit the production of active oxygen forms by activated blood more intensely than antibiotics of other groups. Analysis of the effects of drugs on whole blood CL, reflecting phagocyte function, helps predict their effect on defense functions of the organism [3,5].

In the second series of experiments we studied antibiotic effect on LPO in a suspension of liposomes. The latent period was the most demonstrative in the record of CL of liposome suspension. Addition of penicillin (28.5 U/ml) prolonged it 3.7 times in comparison with the control, ampicillin (3.5 mg/ml) and oxacillin (3 mg/ml) 3 times, carbenicillin (3.3 mg/ml) and kanamycin (0.3 mg/ml) 2.7 times, gentamycin (0.32 mg/ml) 2.4 times, lincomycin (1.4 mg/ml) 2.2 times, cephasoline (4.7 mg/ml) and cephtisoxime (4 mg/ml) 2 times. Tetraoleane (0.15 mg/ml) decreased CL to a zero level. Drugs possessing antioxidative properties prolong the latent period [2,7].

Some drugs, inert in model systems, modify FRO *in vivo*, and vice versa, many substances decelerating oxidation in simple chemical reactions loose this capacity in a live organism [2]. Changes in the main CL parameters of liver and kidney tissues and of plasma of rats after an 8-day course of intramuscular injections of antibiotics in therapeutic concentrations are summarized in Table 1. Prolongation of latent period was paralleled by a marked decrease in the maximum amplitude of slow CL flash in hepatic tissue after cephtisoxime and aminoglycosides. Kanamycin and lincomycin markedly decreased renal and plasma CL. Lincomycin, cephtisoxime, and aminoglycosides suppressed LPO in different substrates *in vivo*.

Thus, all tested antibiotics were capable of suppressing the intensity of FRO in model systems and in laboratory animals. Complex spatial chemical structure, additional hydroxyl and amino groups, and their different screening enhanced the antioxidative activity of aminoglycosides and cephalosporines *in vitro* and *in vivo* in comparison with other antibiotics. The CL decrease in intact rat liver and kidneys after a course of antibiotics correlated with the drug tropism to this or that organ.

Development of an acute inflammation is associated with impairment of FRO regulation [3,5,7]. Probably, under such conditions antibiotics exert not only antioxidative, but also the prooxidant effect, but this is to be studied in a clinical setting.

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