In Vitro Effects of Gentamicin, Ampicillin, and Cefobid on Energy Supply and Antioxidant Protection Systems of Venous Blood Erythrocytes in Newborns

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We studied the effects of antibiotics of different classes on reserve capacities of the energy supply and antioxidant protection systems in newborns. Erythrocyte catalase activity was high in children with perinatal asphyxia. Penicillin antibiotics, aminoglycosides, and cephalosporins decreased enzyme activity of the glutathione-associated antioxidant system. Ampicillin most significantly inhibited ATP synthesis in erythrocytes under conditions of hypoxia. Gentamicin was least potent in this respect. Impairment of ATP synthesis in erythrocytes was associated with inhibition of antioxidant enzymes catalase and glutathione reductase. Ampicillin increased glutathione peroxidase activity in response to addition of H_2O_2. None of the antibiotics modulated activity of cytosolic superoxide dismutase in blood erythrocytes from healthy newborns and newborns with perinatal asphyxia.

Key Words: *newborns; erythrocytes; antibiotics; energy supply system; antioxidant protection system*

The energy supply system and activation of reactive oxygen species (ROS) generation play an important role in the development and progression of various diseases. Therapy with antibiotics is of considerable importance in this respect. Antibacterial protection is determined by bactericidal and bacteriostatic properties of antibiotics and biocidal phagocyte activity of ROS [1,2,5]. Erythrocytes can inhibit proliferation and cause death of unicellular organisms [7,8]. These effects are related to degradation of H_2O_2 produced by the bacterial cell. The reaction is catalyzed by catalase located in the erythrocyte plasma membrane [7]. Catalase activity decreases at the site of contact between plasma membranes of the erythrocyte and bacterial cell, which results in microexplosion, impairment of membrane integrity, and plasmolysis of the bacterium and erythrocyte [6-8].

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Here we studied the effects of antibiotics widely used in clinical practice on the energy supply system and ROS generation in erythrocytes from conventionally healthy newborns and children with perinatal asphyxia.

MATERIALS AND METHODS

We examined 13 conventionally healthy newborns (average age 45 days) and 15 children with chronic or acute perinatal hypoxia or asphyxia (Apgar score 5-6, no uteroplacental bleeding). The method and protocol of the study were approved by Local Ethics Committee. We performed routine clinical examination, electrocardiography and electroencephalography (when indicated), clinical blood test, and biochemical blood test (glucose and hemoglobin fractions). The children with perinatal asphyxia received therapy with gentamicin and ampicillin (5 days), antioxidant vitamin E, antihypoxant lithium hydroxybutyrate, and riboxin.

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The blood was taken under standard conditions (average age of erythrocytes 23±7 days). Commercial preparations of ampicillin, gentamicin (SP Labo, Heist), and ceftriaxone (Hoffman La Roche) were dissolved in 0.9% NaCl to a final concentration of 10 mg/ml. Erythrocyte suspension was incubated with 10 mg/ml antibiotic for 20 min. After incubation erythrocytes were precipitated and resuspended in Dulbecco's medium with or without 0.1 mM H_2O_2 at 37°C (20- and 60-min incubation). Intracellular ATP concentration was measured using luciferin luciferase at 5-2500 nM (absorption wavelength 259 nm, extinction coefficient 15,400). We also measured activities of glucose-6-phosphate dehydrogenase (G-6-PDG) [4], glutathione peroxidase, glutathione reductase [11], and catalase [9]. The results were analyzed by Student's *t* test (STAT Soft software).

RESULTS

Penicillin antibiotics, aminoglycosides, and cephalosporins *in vitro* had little effect on the concentrations of ATP and ADP (Table 1). ATP and ADP concentrations in the erythrocyte suspension from healthy full-term infants remained practically unchanged after administration of exogenous H_2O_2. However, cephalosporins increased the ATP/ADP ratio. This cephalosporin-produced shift toward the increase in ATP concentration reflects compensatory activation of the synthesis of available energy source in erythrocytes. This conclusion was supported by activitation of a key glycolytic enzyme G-6-PDG under the influence of cephalosporins (by 43% relative to the baseline level). G-6-PDG activity did not increase during H_2O_2-induced oxidative stress (Table 1).

Blood ATP concentration in children with perinatal asphyxia decreased by 43.5%. The concentration of ATP in the blood decreased by 54.5% after administration of H_2O_2. The resistance of erythrocytes to oxidative stress decreased. G-6-PDG activity increased by 34% (Table 1). Blood methemoglobin concentration sharply increased, while oxyhemoglobin concentration decreased over the first hours after birth. These changes reflect the development of intoxication [2,3,7].

ATP concentration *in vitro* remained unchanged after administration of gentamicin, but decreased upon treatment with ampicillin and cephalosporin (by 17 and 19%, respectively, compared to blood erythrocytes from newborns with asphyxia). The content of adenyl nucleotides decreased more significantly during severe oxidative stress produced by H_2O_2. It manifested in a decrease in the ATP/ADP ratio after administration of ampicillin. Inactivation of G-6-PDG reflected the decrease in ATP concentration and inhibition of its synthesis (Table 1). These data indicate that erythrocytes from newborns with moderate perinatal asphyxia are more susceptible to oxidative stress. They are characterized by more pronounced inhibition of the glycolytic pathway of ATP synthesis in response to treatment with penicillin antibiotics, cephalosporins, and to a lesser extent aminoglycosides.

The test antibiotics produce different effects on catalase and glutathione peroxidase activities. It remains unclear which enzyme is of greater significance to the organism [10,11]. After treatment with antibiotics catalase activity remained unchanged, while glutathione peroxidase activity decreased (Table 2). In the presence of antibiotics, H_2O_2 increased catalase activity to a level observed in intact erythrocytes. Glutathione peroxidase activity increased upon treatment with ampicillin, but did not reach the level observed in the absence of this antibiotic. Glutathione reductase activity increased, tended to decrease, and remained unchanged in the presence of gentamicin, ampicillin, and ceftriaxone, respectively. Administration of H_2O_2 had little effect on glutathione reductase activity. Activity of superoxide dismutase (SOD) remained unchanged (Table 2).

Catalase activity was high in erythrocytes from children with perinatal asphyxia. After treatment with antibiotics H_2O_2 produce no compensatory increase in enzyme activity; moreover, erythrocyte catalase activity tended to decrease under these conditions. Activity of H_2O_2-degrading glutathione peroxidase decreased by 29% in erythrocytes from newborns. These changes were more pronounced after administration of exogenous H_2O_2. The decrease in glutathione reductase activity reflects exhaustion of reserve capacities of the glutathione-associated antioxidant system in newborns with moderate perinatal asphyxia. The observed changes became more pronounced after treatment with antibiotics.

Cytosolic Cu/Zn-dependent SOD is a key enzyme that rapidly utilizes superoxide anion radicals. Enzyme activity was low in children born in asphyxia and underwent little change under the influence of H_2O_2 and incubation with penicillin antibiotics, aminoglycosides, and cephalosporins.

Doxycycline, Zinnat, Keiten, metacycline, Panglob, rifampicin, Septrin, tinidazole, metronidazole, Ceprova, cephalexin, and ericyclin inactivate SOD, succinate dehydrogenase, and glutathione reductase. Published data show that doxycycline, Ceprova, and ericyclin can inhibit some enzymes, but increase activity of other enzymes [6-8].

Our results showed that ampicillin most significantly inhibits ATP synthesis and decreases ATP/ADP ratio in erythrocytes under conditions of hypoxia. Gentamicin is least potent in this respect. These changes are associated with inhibition of antioxidant enzymes

TABLE 1. Effects of Antibiotics on Energy Supply System under Normal Conditions and after Perinatal Asphyxia (*M*±*m*)

Parameter	Control/ control+ H_2O_2	Control		Asphyxia/ asphyxia+ H_2O_2	Asphyxia	
		antibiotic	antibiotic+ H_2O_2		antibiotic	antibiotic+ H_2O_2
ATP, μmol/mg protein	8.5±0.9	8.6±0.9	8.2±1.0	4.8±0.6***	4.0±0.5***	3.8±0.4***
	8.8±0.8	8.4±0.6	8.1±0.8	4.6±0.6***	3.9±0.7***	3.2±0.4***+
		9.0±0.5	9.1±0.7		4.2±0.6***	3.7±0.3***+
ADP, μmol/mg protein	2.3±0.4	2.2±0.3	2.1±0.3	1.9±0.3	1.9±0.2	1.8±0.2
	2.3±0.3	2.1±0.2	2.3±0.3	2.1±0.2	2.1±0.2	1.9±0.3
		2.0±0.2	1.9±0.2		1.7±0.2	1.55±0.15+
ATP/ADP	3.7±0.3	3.9±0.3	3.9±0.2	2.5±0.3***	2.1±0.3**	2.1±0.2
	3.8±0.2	4.0±0.2	3.5±0.2	2.2±0.2***	1.86±0.13**	1.68±0.12+
		4.5±0.3*	4.8±0.3+		2.5±0.2	2.4±0.1
G-6-PDG, U/mg protein	2.1±0.5	1.3±0.3*	2.1±0.3	1.1±0.3***	0.7±0.1***	0.7±0.1***
	1.2±0.4	1.1±0.4*	1.0±0.2	0.8±0.2***	0.9±0.2**	0.3±0.2***
		3.0±0.4*	1.3±0.2*+		1.0±0.2**	0.9±0.1***

Note. Here and in Table 2: antibiotics: gentamicin (row 1), ampicillin (row 2), and ceftriaxone (row 3). *p<0.05, **p<0.01, and ***p<0.001 compared to the control. +p<0.05, ++p<0.01, and +++p<0.001 compared to the same group before antibiotic treatment.

TABLE 2. Effects of Antibiotics on Antioxidant Protection System in Erythrocytes under Normal Conditions and after Perinatal Asphyxia (*M*±*m*)

Parameter	Control/ control+ H_2O_2	Control		Asphyxia/ asphyxia+ H_2O_2	Asphyxia	
		antibiotic	antibiotic+ H_2O_2		antibiotic	antibiotic+ H_2O_2
H_2O_2-degrading catalase, ml/min	3.5±0.3	3.9±0.4	8.2±1.0	4.8±0.6*	4.0±0.5	3.8±0.4***
	8.8±0.8	3.4±0.6	8.1±0.8	4.6±0.6*	3.9±0.7	3.2±0.4***+
		3.0±0.5	7.1±0.7		4.2±0.6	3.7±0.3***
Glutathione peroxidase, μmol	0.92±0.06	0.77±0.07*	1.9±0.3	0.54±0.05	0.49±0.03	0.52±0.02
reduced glutathione per min	2.3±0.2	0.69±0.05*	1.7±0.2*	0.60±0.02	0.41±0.02	0.49±0.03
		0.52±0.04**	1.9±0.2		0.57±0.03*	0.55±0.05
Glutathione reductase,	89±10	107±9*	91±9	59±7***	47±9**	41±6***++
μmol NADPH per min	115±14	77±7***	81±11***	65±4***	47±7***	31±4***+++
		88±7***	86±7***		58±7***	36±7***++
SOD, arb. units/mg protein	265±25	249±13	289±21	167±12**	149±11***	152±10***
	278±25	291±22	299±13	160±11***	161±12***	143±9***
		221±23*	255±15		157±11***	155±11***

catalase and glutathione peroxidase. After treatment with ampicillin, H_2O_2 slightly increases glutathione peroxidase activity. The test antibiotics did not modulate activity of cytosolic SOD in erythrocytes from conventionally healthy newborns and newborns with perinatal asphyxia.

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