Mechanisms of Penicillin Antibiotic Interactions with Human Erythrocytes

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Significant effects of penicillin antibiotics, used in pediatrics, on the erythrocyte structural characteristics were detected by recording kinetic curves of osmotic and acid hemolysis. Possible relationships between erythrocyte properties and concentrations, duration of exposure to, and structure of antibiotics were revealed. Recommendations for dose correction for effective use of antibiotics in medicine with consideration for individual features of the patient are proposed.

Key Words: *antibiotics; hemolysis; osmotic and acid resistance; erythrocyte membranes*

Studies of the mechanisms of antibiotic effects on various components of human somatic cells are essential for rational use of antibiotics and prevention of toxic reactions and side effects.

Osmotic resistance of blood cells is an important parameter characterizing erythrocyte membrane permeability, status of lipoprotein structures and their conformation properties, and protein-lipid interactions in the membrane [5,8].

We studied the interactions of penicillin antibiotic (benzylpenicillin, BP; amoxiclav, AC) with eukaryotic cells *in vitro* in an erythrocyte-antibiotic model system.

MATERIALS AND METHODS

Experiments were carried out on suspensions of erythrocytes from male donor blood. Whole blood was diluted in saline (1:10) and centrifuged 3 times at 1500 rpm for 10-15 min, the cells were washed from the stabilizer and plasma with 0.9% NaCl solution between centrifugations.

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Antibiotics were used in a wide concentration range. The drug doses were selected on the basis of our notions on possible intoxication as a result of cumulative effect during the treatment typical of all antibiotics [6]. The doses were calculated on the basis of the therapeutic pediatric daily dose (C2): 2.4×10^{-6} mol/liter for BP and 6.9×10^{-7} mol/liter for AC [1,11]. Intoxication was simulated by applying antibiotic in a dose 10-fold surpassing its therapeutic dose (C1): 2.4×10^{-5} mol/liter for BP and 6.9×10^{-6} mol/liter for AC. Minimum concentration of the drug (C3) was used for detecting minimum sensitivity: 2.4×10^{-7} mol/liter for BP and 6.9×10^{-8} mol/liter for AC.

Automated method for registration of osmotic and acid erythrograms consisted in photometric registration of erythrocyte hemolysis. The kinetic curves (erythrograms) graphically reflect gradual involvement of cells with different stability into hemolysis [2].

The device for erythrogram registration consisted of an optic block (PEC-56M photoelectrocolorimeter including a differential amplifier or KFK-2MP), recorder block (LKD4-003 two-coordinate recorder and a V7-20 type digital voltmeter), and a thermostat block (UTU-6 ultrathermostat).

Erythrocyte hemolysis was carried out in thermostated cuvettes (20×40×10 mm; 4 ml working vo-

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lume). Light transmission was measured at λ=490 nm, because molar extinction coefficient for water solutions of HbO_2 is minimum in this spectrum band [3].

The device recorded the integral curve, reflecting the time course of summary shifts in light diffusion in the studied solution during erythrocyte hemolysis.

The kinetics of acid hemolysis was recorded after adding 100 μl acid (0.1 N HCl) into working cuvette with 5 ml erythrocyte suspension. The choice of HCl is explained by its stability during storage and by the presence of both ions (H^+ and Cl^- in the blood plasma).

The maximum number of latent structural injuries to erythrocytes, accumulated over 30-min incubation in hypo-osmotic medium, was evaluated by the G_{max} value [2].

RESULTS

Analysis of the relationships between osmotic resistance of erythrocytes and BP concentration in the incubation medium showed that the maximum hemolytic effect was induced by this modifier in C2 (Fig. 1, *a*), leading to hemolysis of 8% erythrocyte. Benzylpenicillin in C3 incorporated into the membranes of not only old low resistant, but of also medium- and highly resistant erythrocytes, which was shown by registration of the spherulation phase (1.5%). Intoxication (use of C1) induced degradation of 2% cells, which could be characterized as the membrane structure stabilizing effect of the antibiotic accumulation.

Experiments with AC showed that the intensity of the succession of spherulation processes correlated with drug content in incubation medium. Treatment with AC in C1 led to 15% spherulation (Fig. 1, *b*). Further reduction of AC concentration (to the therapeutic concentration) led to the development of the latent phase of hemolysis, which was recorded during the first seconds of hemolytic process with gradual increase of the spherulation constituent. As a result, spherulation for AC in C2 reached 5%. Treatment with AC in C3 led to 5% hemolysis (G).

A common feature of kinetic curves of hemolysis induced by BP and AC is the presence of peaks or fluctuations (particularly during the first seconds of recording) reflecting sensitivities of erythrocyte populations to antibiotics. Erythrocyte interactions with antibiotics seem to be regulated by a common mechanism, membrane permeability being determined by the age of cells for both drugs [10].

Amoxiclav is characterized by less pronounced (compared to BP) hemolytic effects and lower membrane permeability. On the other hand, interactions of "old" cholesterol-depleted or damaged erythrocytes with AC in C2 and C1 can lead to the development of spherocytosis processes, which can result in edema, increase of blood viscosity, hemodynamic disorders, and development of other side effects associated with this antibiotic [7,9].

Benzylpenicillin exhibited its hemolytic effects in the hypoosmotic medium even without preliminary incubation (Fig. 2, *a*). The maximum (8%) degradation of erythrocytes was observed after addition of BP in C2. The effect of apparent stabilization of membranes in medium- and highly resistant erythrocytes was detected in simulated "intoxication" (the G_{max} values close to the control). The C3 completely leveled the hemolytic process.

Both antibiotics are long acting, exhibiting their significant hemolytic effects after 30-min preincu-

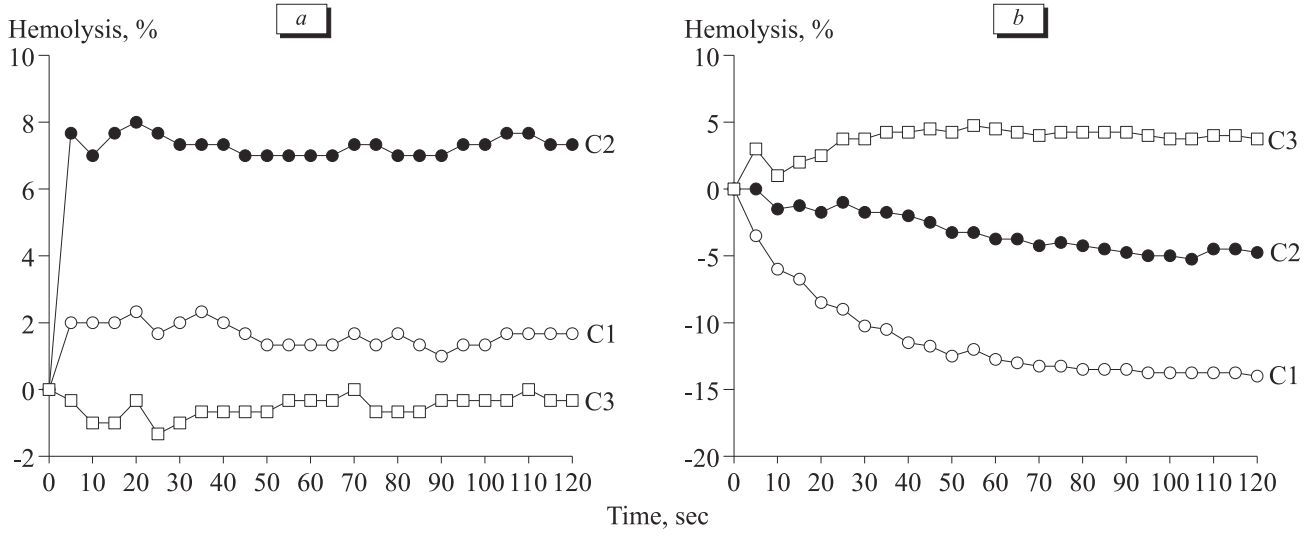

Fig. 1. Kinetics of hypoosmotic hemolysis of erythrocytes modified by BP (*a*) and AC (*b*).

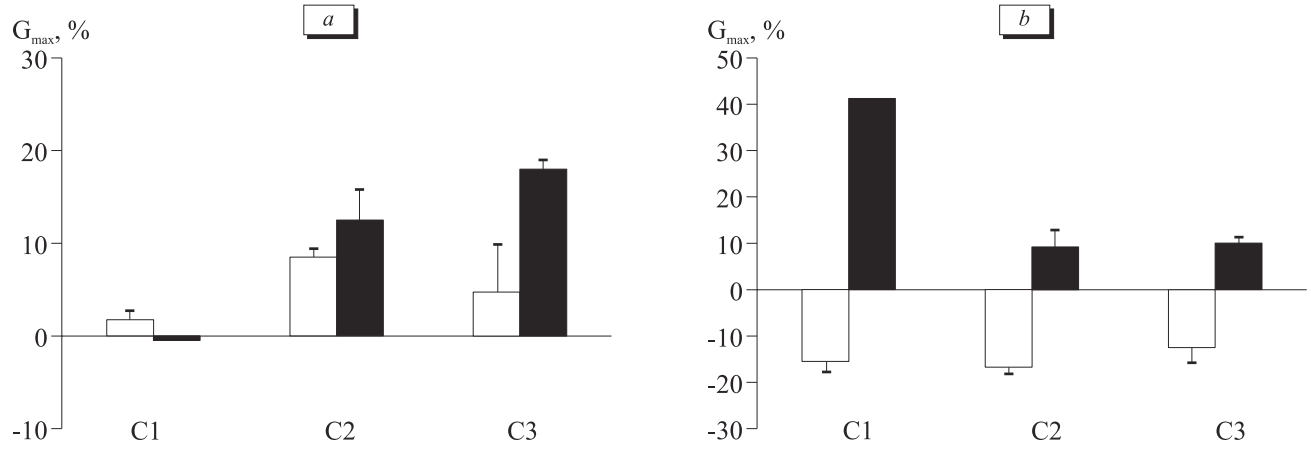

Fig. 2. G_{max} of hypoosmotic hemolysis of erythrocytes induced by BP (*a*) and AC (*b*). Here and in Fig. 3: light bars: no incubation; dark bars: 30-min pre-incubation.

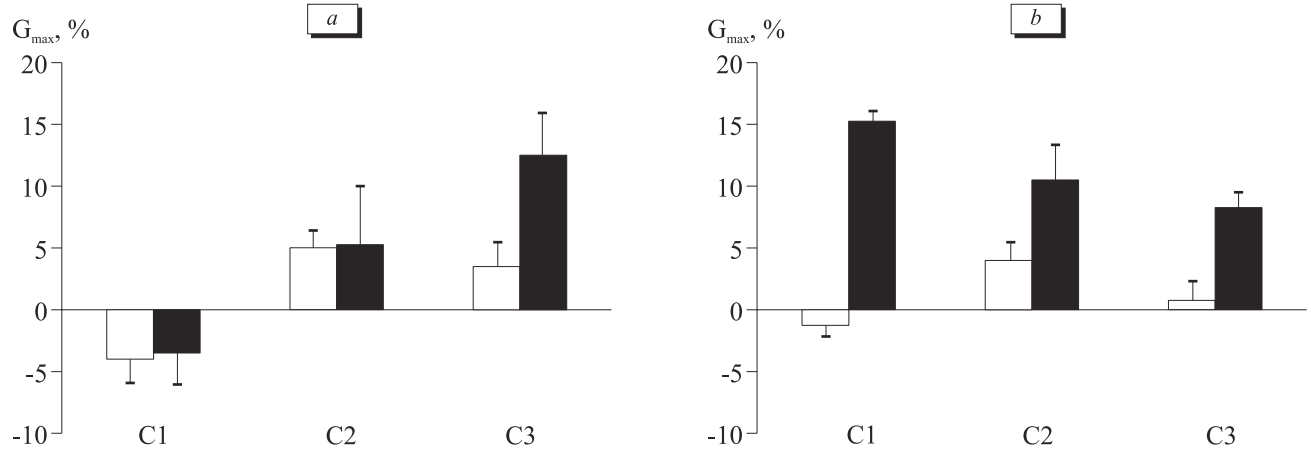

Fig. 3. G_{max} of erythrocyte autohemolysis induced by BP (*a*) and AC (*b*).

bation. An inverse relationship between G_{max} values and BP concentration in the incubation medium was detected (Fig. 2, *a*). Direct relationship between G_{max} and drug concentration was observed for AC. In simulation of intoxication AC induced degradation of 41% cells after preincubation, while the hemolytic effects of its C2 and C3 were about same (Fig. 2, *b*).

AC easily binds to the erythrocyte membrane without preincubation, modifies it, and presumably penetrating inside the cell, inducing spherocytosis phase slightly correlating with the concentration and status of erythrocytes (Fig. 2, *b*).

Treatment with BP and AC in C2 without preincubation led to degradation of 4% erythrocytes in comparison with G_{max} in the control. Antibiotic treatment in C1 led to spherulation and hemolysis, the latter process slightly predominating. The BP C3 caused destruction of 3% cells, while G_{max} after AC C3 was close to the control.

BP in C1 caused a negative increment in G_{max} value, as a result of which spherulation of 4% erythrocytes was observed (Fig. 3, *a*). Reduction of the concentration to C2 and C3 increased the proper hemolysis constituent.

A direct relationship between G_{max} and AC concentration in the medium was detected, but this effect manifested only after preincubation. Amoxiclav in C1 induced autohemolysis of 15% cells, while in C2 and C3 this value decreased to 11 and 8%, respectively (Fig. 3, *b*).

The majority of antibiotics can easily incorporate in cholesterol-depleted ("old") and normal cells and then diffuse through their lipid bilayer into the cytosol space. We hypothesized that several types of molecular mechanisms underlie changes in erythrocyte hemolytic activity: chemical, electrostatic, and hydrophobic reactions of the antibiotic molecules with protein and lipid components of membranes. Possible blockade of ionogenic groups of polypeptide chains and charged lipid sites with antibiotics of different structure can lead to modification of the summary charge of the erythrocyte

TABLE 1. Main Parameters of Acid Hemolysis of Erythrocytes Induced by Penicillin Antibiotics

Parameter	BP			AC		
	C1	C2	C3	C1	C2	C3
K_{max}, rel. units						
no incubation	-0.59	0.02	0.18	-0.33	-0.05	-0.19
after incubation	-0.47	-0.43	-0.16	-0.13	0.03	0.08
Latent period, sec						
no incubation	-87	-53	-40	-22	-33	-17
after incubation	-17	-17	0	-7	-10	-5

membrane, which eventually impairs blood cell resistance to the destructive effects of hemolytic agents (H^+ ions) [4,5]. Polar nature of antibiotics used in our study suggests not only modification of electrostatic forces, but also of hydrophobic interactions between proteins and lipids, serving as the backbone in the cells and determining the strength of permeability barrier for charged ions [2,3]. The contribution of these effects of antibiotics on erythrocyte membrane structure was evaluated indirectly by comparing acid resistance of modified and intact erythrocytes.

Analysis of the main parameters of acid resistance of BP-modified erythrocytes revealed a direct relationship between the duration of latent period of hemolysis and antibiotic content in the incubation medium. Under conditions of strong intoxication with this penicillin, chemical resistance dropped in comparison with the control at all the studied concentrations without preincubation (Table 1). After 30-min incubation of erythrocytes in an isosmotic solution, the latent period characterizing the sensitivity of the membrane lipid bilayer mainly in erythrocytes with low resistance to the destructive agent [2] decreased to 17 sec under the effect of BP in C1 and C2. After treatment with BP in C3, the latent period virtually did not differ from the control. Analysis of the data showed that BP actively incorporated in membranes of erythrocytes with low resistance (which is in line with the data on osmotic resistance), thus reducing significantly their hydrophobic barrier for hydrogen ions.

Comparison of the maximum velocity constants (K_{max}) of BP-induced erythrocyte acid hemolysis also revealed its relationship with the concentration. Without preincubation K_{max} sharply changed from C1 to C3, while in C2 it virtually did not differ from the control. Preincubation led to reduction of the homogeneity of erythrocyte subpopulations, which gradually normalized under the effect of antibiotic C3.

Analysis of acid resistance of blood erythrocytes modified by AC showed a reduction of the hemolysis latent period in comparison with the control at all the studied concentrations. Hence, without preincubation AC leads to reduction of the hydrophobic barrier, prolonged interaction between the antibiotic and erythrocytes leading to normalization of the cell structure. The degree of erythrocyte differentiation in this case is 0.33 rel. units higher than in the control during exposure to AC in C1 and virtually the same as in the control in exposure to AC in C3 or C2. Cell homogeneity in erythrocyte populations virtually did not change after 30-min preincubation, as the maximum velocity constants were close to the zero at all AC concentrations used in our study.

It seems that the studied antibiotics reduce the hydrophobic interactions between proteins and lipids performing the skeleton functions in the cells and determining the extent of permeability barrier for charged ions (H^+) as early as during the first minutes of circulation in the body. Hence, these drugs should be prescribed only for vital indications, with obligatory monitoring of patient's status during therapy.

Drug interactions with isolated human somatic cells can be studied by this method, and therefore, it should be introduced in medical practice for choice of individual drug doses with consideration for individual features of the patient.

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