Effect of UV Irradiation and Magnetic Field on Immunometabolic Effects of Antibiotics Immobilized in Cell Carriers

A. I. Lazarev, G. V. Siplivyi, A. V. Kukureka, and L. E. Siplivaya

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The effects of UV and magnetic radiation on the immunometabolic activity of ampicillin and cephazolin immobilized in erythrocytic and leukocytic carriers were studied in intact Wistar rats and animals infected with staphylococci. Erythrocytic and leukocytic carriers with antibiotics were obtained. Injection of free antibiotics stimulated the immunosuppressive, pro-oxidant, and hepatotoxic effects, associated with staphylococcal infection. Treatment with antibiotics in erythrocytic and leukocytic carriers stimulated (to different degrees) the activity of the immune system and stabilized the parameters of LPO, antioxidant defense, cytolysis, and cholestasis. Ultraviolet irradiation and magnetic field modified (to different measures) the immunometabolic effects of ampicillin and cephazolin, immobilized in erythrocytic and leukocytic carriers, in animals with staphylococcal infection.

Key Words: *immobilized antibiotics; UV and magnetic exposure*

Infusion of autologous blood extracorporeally exposed to UV radiation is now widely used for the treatment of cardiovascular and pyoinflammatory diseases, burns, *etc.* Reinfusion of UV-irradiated blood increased the counts of leukocytes and erythrocytes, their volume and residual resistance, and content of oxygen bound by erythrocytes. UV-irradiated blood is a peculiar "biological preparation", which being infused to patients with pyoinflammatory diseases associated with sharp suppression of nonspecific resistance and immunological reactivity factors increased phagocytic activity of leukocytes, complement activity, and lysozyme concentration, and of immunological defense factors (counts and functional activities of T and B lymphocytes, immunoglobulins) [7].

Kursk State Medical University, Federal Agency for Public Health and Social Development, Russia. ***Address for correspondence:*** farmchim@rambler.ru. L. E. Siplivaya

The leukocyte counts in human blood and phagocytic activity of these cells increase after infusion of blood exposed to magnetic field of certain intensity. High immunomodulating activity of erythrocyte fractions exposed to UV and magnetic field was detected and studied in health and diseases associated with the development of stable immunodeficiency [8].

Directed transport of antibiotics consisting in their immobilization on cell carriers stabilizes or significantly improves the parameters characterizing immunometabolic shifts caused by toxins and bacteria [4]. The study of changes in immunometabolic efficiency of antibiotics immobilized in cell carriers after cell exposure to UV and magnetic field seemed to be an interesting problem.

We studied the immunometabolic activities of ampicillin and cephazolin immobilized in erythrocytic and leukocytic carriers, prepared from erythrocytes and leukocytes exposed to UV and mag-

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netic field in intact animals and in staphylococcal infection.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats (n=369; 180-220 g). Staphylococcal infection was induced by a single intraperitoneal injection of pre-tittered doses of a 24-h agar culture of *Staphylococcus aureus* (Zhaev strain) containing 10^8 bacterial bodies/0.5 ml solution. Ampicillin sodium (0.25 or 0.5 g flasks; Ferein) and cephazolin (0.25 or 0.5 g flasks; Ferein) were used.

The antibiotics were immobilized in erythrocyte carriers by a modified [1] method of hypoosmotic hemolysis. Leukocyte suspension was prepared by mixing heparin-treated (25 U/ml) blood with 3% gelatin (0.1 ml/ml) and incubated for 15-20 min at 37°C. After erythrocyte precipitation, lymphocyte-rich plasma layer was transferred into silicone-treated tubes. The cells were precipitated by 10-min centrifugation at 1500 rpm, washed twice in Hanks' solution, and transferred into medium 199. The cells were counted under a microscope in a Goryaev's chamber. Antibiotics were immobilized in leukocytic carriers by a previously described method [5].

In some cases, allogenic erythrocytes and leukocytes were put into quartz cuvettes and pre-exposed to UV light (λ=250 nm) for 20 min. An NKN-11 quartz lamp served as the source of UV light; the lamp was placed at a distance of 20 cm from the tubes at the same horizontal level. The intensity of UV exposure of cell carriers was expressed in radiation energy Q, calculated by the formula:

$$Q=E_l \times A_{ir} \times t,$$

where E_l is surface density of radiation flow for this source, A_{ir} is the cuvette area open for irradiation (m^2), and t is the duration of exposure (sec). The intensity of UV exposure was 90 J/m^2 (120 J/sec× 0.00006 m^2×1200 sec).

In some experiments erythrocytes and leukocytes were pre-exposed to permanent magnetic field of 500 Oe (39 788.75 A/m) for 20 min. Permanent magnetic field was created by a stationary electric magnet fed from a rectifier transformer at 1.3 A current and 15 V voltage. The devices used in the study provided even exposure of the entire volume of cell carriers to physical factors. Ampicillin and cephazolin were injected intramuscularly, 5 injections at 12-h intervals, in single doses of 120 and 60 mg/kg, respectively. Single doses of ampicillin and cephazolin immobilized in erythrocytic and leukocytic carriers were 60 and 30 mg/kg, respectively. Erythrocytic and leukocytic carriers with incorporated antibiotics were injected intravenously (2 injections at 24 h interval). The concentration of antibiotic incorporated in cell carriers was evaluated in accordance with the recommendations of standard documents for the drugs.

Humoral immune response was induced by a single intraperitoneal injection of sheep erythrocytes (SE; 2×10^8 cells/0.1 kg). The intensity of humoral immune response to SE was evaluated on day 5 after immunization by measuring the number of cells producing antibodies to SE (by direct local hemolysis [6]) and of cells forming rosettes to SE in the spleen [2].

Serum concentrations of bilirubin and activities of ALT, AST, and alkaline phosphatase were measured in experimental animals by universal methods with standard reagents. Activities of AST and ALT served as the indicators of the cytolysis syndrome, activity of alkaline phosphatase and bilirubin concentration indicated the cholestasis syndrome.

The erythrocyte antioxidant potential was evaluated by SOD and glutathione reductase activities. The intensity of LPO in erythrocytes was evaluated by the content of conjugated dienes and MDA.

Normal rats immunized with SE (thymus-dependent antigen) served as controls for evaluation of humoral immune response, intact rats served as controls for measurements of biochemical values.

The results were processed using the Student and Wilcoxon—Mann—Whitney tests. The means and standard errors of the means were calculated.

RESULTS

Intramuscular injection of ampicillin and cephazolin caused a slight reduction of immune response induced by SE in normal animals, which was seen from decreased number of antibody-producing and rosette-forming cells in the spleen. Serum concentrations of bilirubin, activities of ALT, AST, and alkaline phosphatase increased in normal animals after 5 intramuscular injections of ampicillin and cephazolin. Changes in the antioxidant potential of erythrocytes and LPO intensity in healthy animals treated with antibiotics were within the normal range.

Staphylococcal infection caused a drop of immunological reactivity to T-dependent antigen; bilirubin concentration, activities of ALT, AST, and alkaline phosphatase increased. The erythrocyte antioxidant potential decreased and LPO intensity increased. Five injections of antibiotics stimulated the immunosuppressive, hepatotoxic, and prooxidant effects of staphylococcus (Table 1).

TABLE 1. Changes in Humoral Immune Response, Cytolysis and Cholestasis Processes, and LPO after Injections of Antibiotics (n=8-10)

Experiment conditions	APC, thousand/organ	AST, μcat/liter	Bilirubin, μmol/liter	CD, nmol/ml
Control (no antibiotics)	25.2±2.4	0.24±0.03	7.20±0.51	4.2±0.5
Infection	15.4±1.6*	0.73±0.08*	14.1±1.8*	7.5±0.6*
Healthy rats, ampicillin injection	21.4±1.9*	0.45±0.03*	10.5±1.1*	4.4±0.4
Healthy rats, cephazolin injection	20.9±1.8*	0.37±0.04*	10.9±0.9*	4.3±0.5
Infection+ampicillin	12.4±1.2^{+}	0.97±0.07^{+}	18.6±2.2^{+}	10.4±0.8^{+}
Infection+cephazolin	11.9±1.4^{+}	0.89±0.07^{+}	19.1±1.4^{+}	9.1±0.8^{+}

Note. Here and in Tables 2, 3: APC: antibody-producing cells; CD: conjugated dienes. Here and in Table 2: p<0.05 compared to: *control, $^{+}$infection.

TABLE 2. Changes in Humoral Immune Response, Cytolysis and Cholestasis Processes and LPO after Injection of Antibiotics Immobilized in EC and LC (n=8-10)

Experiment conditions	APC, thousand/organ	AST, μcat/liter	Bilirubin, μmol/liter	CD, nmol/ml
Control	24.6±2.4	0.25±0.02	7.4±0.6	4.1±0.4
Infection	14.8±1.5*	0.71±0.60*	14.8±1.2*	7.8±0.6*
Healthy rats, EC with ampicillin	33.8±2.6*	0.24±0.03	7.6±0.5	4.4±0.3
Healthy rats, EC with cephazolin	35.1±2.8*	0.27±0.02	7.3±0.6	4.3±0.4
Healthy rats, LC with ampicillin	24.1±2.6	0.26±0.03	7.5±0.7	4.2±0.3
Healthy rats, LC with cephazolin	23.9±3.1	0.23±0.02	7.2±0.6	4.5±0.4
Infection+EC with ampicillin	25.6±1.4	0.31±0.03	7.6±0.5	4.2±0.4
Infection+EC with cephazolin	25.4±2.4	0.29±0.02	7.1±0.6	4.1±0.5
Infection+LC with ampicillin	18.4±1.4^{*+}	0.41±0.03^{*+}	10.4±0.9^{*+}	6.1±0.5^{*+}
Infection+LC with cephazolin	19.2±1.6^{*+}	0.44±0.04^{*+}	11.1±0.8^{*+}	5.9±0.4^{*+}

Note. EC: erythrocytic carriers; LC: leukocytic carriers.

TABLE 3. Changes in Humoral Immune Response, Cytolysis and Cholestasis Processes, and LPO after Injections of Antibiotics Immobilized in UVEC and MFEC (n=8-10)

Experiment conditions	APC, thousand/organ	AST, μcat/liter	Bilirubin, μmol/liter	CD, nmol/ml
Control	24.6±2.4	0.25±0.02	7.2±0.5	4.0±0.3
Infection	14.8±1.5*	0.71±0.6*	13.1±0.8*	7.3±0.7*
Infection+EC with ampicillin	25.6±1.4*	0.24±0.03	7.4±0.4	4.5±0.5
Infection+EC with cephazolin	25.4±2.4*	0.27±0.02	7.6±0.5	4.4±0.4
Infection+UVEC with ampicillin	35.6±3.4^{*o}	0.24±0.03	7.0±0.3	3.8±0.4
Infection+UVEC with cephazolin	37.1±4.1^{*x}	0.26±0.04	7.4±0.5	4.1±0.4
Infection+MFEC with ampicillin	46.4±4.4$^{*+,++}$	0.23±0.02	7.3±0.4	3.9±0.3
Infection+MFEC with cephazolin	47.1±4.6^{*+xx}	0.27±0.04	7.5±0.5	4.2±0.4

Note. p<0.05 compared to: *control, ^{o}infection+EC with ampicillin, ^{x}infection+EC with cephazolin, $^{+}$infection, $^{++}$infection+UVEC with ampicillin, ^{xx}infection+UVEC with cephazolin. UVEC: erythrocytic carriers prepared from erythrocytes exposed to UV radiation; MFEC: carriers prepared from erythrocytes exposed to magnetic field.

Injection of antibiotics immobilized on erythrocytic carriers increased the humoral immune response, had no negative effects on the cytolysis and cholestasis processes and LPO in normal animals and normalized these parameters in infected rats (Table 2). Injections of ampicillin and cephazolin

immobilized on leukocytic carriers caused no negative shifts in the immunometabolic parameters of normal rats and reduced these values in infected animals (Table 2). Injection of antibiotics immobilized in erythrocytic carriers, prepared from erythrocytes exposed to UV radiation or magnetic field, sharply amplified their influence on the intensity of immune response to SE, normalized cholestasis parameters, LPO intensity, and increased the antioxidant potential of erythrocytes in infected animals. Antibiotics immobilized on erythrocytic carriers obtained from erythrocytes exposed to magnetic field exhibited more pronounced immunomodulating effect (Table 3).

Preliminary exposure of leukocytes in magnetic field did not increase activity of leukocyte carriers prepared from these cells, while UV exposure significantly stimulated their influence on the intensity of the studied processes in infected rats.

The use of erythrocytes and leukocytes of infected animals as containers for antibiotic transport is an interesting problem. Immunostimulating, hepatoprotective, and antioxidant activities of erythrocytic and leukocytic carriers of antibiotics, prepared from infected animal cells, was comparable to that of carriers obtained from normal animal cells, due to which the use of donor cells for obtaining the carriers can be excluded or minimized.

Hence, antibiotics immobilized in erythrocyte and leukocyte carriers and injected to infected animals exhibited hepatoprotective, immunostimulatory, and antioxidant effects.

These effects of immobilized antibiotic cell forms can be explained by changes in their pharmacokinetics, formation of stable erythrocyte-antibiotic or leukocyte-antibiotic complexes, and gradual release of the antibiotic from this complex, stimulation of phagocytosis processes and reactions with immunocompetent cells.

Pre-exposure of erythrocytes and leukocytes in UV light or magnetic field amplified the immunomodulating, hepatoprotective, and antioxidant activities of erythrocytic and leukocytic carriers, prepared from these cells, with the immobilized antibiotics. It seems that UV and magnetic exposure modulate the specific and nonspecific photoacceptors and iron-containing molecules by impairing their conformation, which results in modification of their paramagnetic characteristics. It was found that exposure to UV light and magnetic field led to transformation of deoxyhemoglobin into oxyhemoglobin [3]. Interactions of photoacceptors and molecules with modified paramagnetic characteristics determine the degree of physical energy absorption and provide its transformation in biophysical and biochemical processes. Modification of paramagnetic characteristics of iron-containing molecules, specifically, hemoglobin, improves the protein sensitivity to UV and magnetic exposure and modifies the patterns of their electrochemical reactions with integrated proteins of the inner surface of cell membrane, and, presumably, improves the efficiency of their reactions with incorporated antibiotics. In addition, membranes of cells and intracellular organelles also serve as the targets for UV and magnetic radiation, their conformation restructuring serving as the molecular basis for their antioxidant effect. Changes in the architecture and, presumably, composition of the membrane surface epitopes seem to improve the efficiency of their reaction with immunocytes.

Hence, the use of cell carriers for directed transport of antibiotics is a prospective technology for antibacterial drug delivery, and preliminary exposure to UV rays or magnetic field improves their efficiency.

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