BIOPHYSICS AND BIOCHEMISTRY

Stimulation of Peritoneal Macrophage Activity in Rats with Experimental Peritonitis during Transfusion of UV-Irradiated Blood

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Three transfusions of blood irradiated at 254 nm induce an increase of phagocytic activity of peritoneal macrophages in rats with peritonitis in comparison with infusion of intact blood. Addition of aspirin, an inhibitor of enzymatic peroxidation of lipids, to the irradiated portion of the blood before irradiation abolishes this stimulating effect.

Key Words: *ultraviolet irradiation; blood; peritoneal macrophages; peritonitis*

Transfusions of UV-irradiated blood (photohemotherapy) are used in the treatment of many diseases, among them peritonitis [4,7]. However, the mechanisms imparting therapeutic properties to the blood for its UV irradiation are still unknown. In a previous study we confirmed the therapeutic effect of transfusion of UV-irradiated blood in rats with acute experimental peritonitis. The state of the system of nonspecific resistance of the body to a great extent determines the outcome of a disease. In the present study we tried to assess the effects of transfusions of UV-irradiated blood on the phagocytic activity of rat peritoneal macrophages in peritonitis.

UV irradiation is known to induce activation of nonenzymatic and enzymatic lipid peroxidation in blood components [2]. As a result of enzymatic peroxidation bioactive products are formed. Elucidation of the possible significance of stimulation of enzymatic lipid peroxidation for transforming blood into a medicinal agent during UV irradiation was a further goal of this study.

MATERIALS AND METHODS

Experiments were carried out with male Wistar rats weighing 100 to 150 g. Chemically pure salts and Sigma aspirin (USA) were used.

Blood collection and UV irradiation. Blood for transfusion was collected in healthy animals of the same line with a syringe from the right heart ventricle; the rats were anesthetized with ether for this purpose. The blood was stabilized with sodium citrate (3.8% trisubstituted sodium citrate; 0.139 mM NaCl; pH 7.36) in a 9:1 ratio.

A BUV-30P low-pressure mercury lamp whose radiation is mainly confined to 254 nm was the source of UV light for blood irradiation. Total radiation intensity in the UV band, measured by ferrioxalate actinometry [6], was 5.6×10^{-5} einsteins/($m^2\times sec$). Blood samples were irradiated in open plastic cuvettes, poured in 3 mm layers, and constantly stirred. The incident exposure dose was 5×10^{-2} einsteins/m^2.

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TABLE 1. Integral Phagocytic Activity of Peritoneal Macrophages of Rats with Acute Peritonitis (mean±SEM)

Day	Group			
	Infusion of UV-irradiated blood	Infusion of intact blood	Infusion of UV-irradiated blood with aspirin	Infusion of intact blood with aspirin
3	129±8*	104±9	98±4	99±5
4	127±11*	99±30	99±5	105±5

Note. The data are presented in percent of the values of integral macrophagal phagocytic activity of intact animals. Asterisk: $p<0.05$ *vs.* the control.

The cyclooxygenase inhibitor aspirin was added to the irradiated portion of the blood as an ethanol solution with vigorous stirring. The final concentration of aspirin in irradiated infused blood was 10^{-5} mol.

Peritonitis induction. Acute fecal peritonitis was selected as an experimental disease. It was induced by intraperitoneal injection of 30% rabbit fecal suspension. The dose was selected so that by day 15 after peritonitis induction the lethality was not more than 20%. Irradiated or intact blood was injected in the rat caudal vein in a dose of 2 ml per kg b.w., 0.2 to 0.3 ml in all. The course of photomodified blood infusion consisted of three procedures. The first infusion was performed 0.5-2 h after intraperitoneal injection of fecal suspension, the other two after 1 and 2 days.

Peritoneal macrophages were isolated as described previously [5]. Macrophage content in the resultant suspension was at least 80%. Cell viability, assessed by trypan blue absorption, was 95-98%.

Acridine orange staining of E. coli cells. E. coli cells were incubated in a medium containing 0.01% acridine orange for 5 min at 22-23°C. The bacteria were then washed twice free of unused stain by centrifugation at 20,000 g for 30 min at 4°C. The supernatant was discarded after each centrifugation and the pellet resuspended in buffered saline (137 mM NaCl, 2.7 mM KCl, 5 mM Na_2HPO_4, 1.5 mM KH_2PO_4). The bacterial concentration was assessed turbidimetrically; it was 3×10^8 cells/ml in the final preparation. The resultant suspension of stained cells was stored on ice for up to 1 h before use.

Assessment of macrophage phagocytic activity. Assessment of the phagocytic activity of peritoneal macrophages was based on the fluorimetric method [8]. 0.5 ml of *E. coli* cells stained with acridine orange (3×10^8 cells/ml) and 0.5 ml of a peritoneal macrophage suspension (2.5×10^6 cells/ml) were mixed and the mixture was incubated for 40 min at 37°C with regular stirring. Bacteria uncaptured by macrophages were twice washed by centrifugation at 460 g for 10 min at ambient temperature. Supernatant with uncaptured bacterial cells was discarded. After the second centrifugation the pellet was resuspended in 0.5 ml of Hanks solution, 0.1 ml of a 1% aqueous solution of Triton X-100 was added, and the fluorescence spectrum was measured in the 500-600 nm range at stimulating light wavelength 450 nm. This spectrum was measured with a Perkin-Elmer-44A spectrofluorimeter. Control samples were those in which macrophages and bacteria were washed immediately after mixing, and the precipitate was similarly resuspended in 0.5 ml of Hanks solution with Triton X-100. The index of phagocytic activity was the difference between the fluorescence intensities of the experimental and control samples at 530 nm.

Macrophage phagocytic activity, assessed by the phagocytic number and phagocytic index, was studied as described previously [8]. Isolated macrophages were suspended in Hanks solution in a concentration of 2.5×10^6 cells/ml; 0.03 ml of this

TABLE 2. Phagocytosis Parameters and Macrophage Count in Peritoneal Exudate of Rats with Experimental Peritonitis on Day 3 after Its Induction (mean±SEM)

Parameter	Group			
	Healthy animals	Untreated animals with peritonitis	Infusion of UV-irradiated blood	Infusion of intact blood
Phagocytic index, %	32±3	40±4.2	57±2.6	41±2.2
Phagocytic number	2.9±0.07	3.0±0.14	3.1±0.08	3.1±0.19
Cell count	14±2.3	49±10.3	114±3.2	101±19.2

Note. Cell count in millions in the total volume of isolated peritoneal exudate.

suspension was layered onto a slide and incubated for 15 min at 37°C. Then the sample was washed free of nonadhering cells in buffered saline, and 0.5 ml of *E. coli* suspension (3×10^8 cells/ml) in Hanks solution was layered onto adhered macrophagal cells and incubated for 15 min at 37°C. The sample was then washed free of nonphagocytized bacterial cells, dried, and fixed for 10 min in ethanol. Fixed preparations were stained after Noht [3]. The phagocytic number and phagocytic index were calculated per 100 cells.

RESULTS

Peritoneal macrophage phagocytic activity was measured on the third and fourth days after peritonitis induction. Peritoneal macrophages were isolated from rats 2-3 h after infusion of UV-irradiated or intact blood if there were such infusions. Table 1 shows that infusion of UV-irradiated blood to animals with peritonitis leads to an increase ($p<0.05$) of integral phagocytic activity of peritoneal macrophages in comparison with that in animals administered no photohemotherapy. Infusions of intact blood did not increase peritoneal macrophage phagocytic activity.

The phagocytic number and phagocytic index were determined to elucidate the type of increase of macrophage integral activity after infusion of UV-irradiated blood. The phagocytic index of peritoneal macrophages in the animals infused UV-irradiated blood was much higher than in control animals (Table 2). If intact blood was infused, this parameter was the same as in control animals. In contrast to the phagocytic index, the phagocytic number was virtually unchanged.

Cell counts in peritoneal exudate were assessed. The data are presented in Table 2. On the third day after peritonitis induction the count of cells in peritoneal exudate from animals transfused irradiated or intact blood was noticeably higher than in control animals.

We may conclude that an infusion of UV-irradiated blood leads to an increase of phagocytic activity of peritoneal macrophages of rats with peritonitis and to an increase of their count. An increment of integral phagocytic activity is due mainly to an increased count of actively phagocytizing cells.

Exposure to UV irradiation results in many cells, including blood platelets and leukocytes, in enzymatic lipid peroxidation [2]. The key enzymes of the enzymatic systems of lipid peroxidation are cyclooxygenase (prostaglandinsynthetase) and lipoxygenase. The work of these enzymes results in the formation of such bioactive substances as prostaglandins of various classes and leukotrienes [1]. Another goal of our study was to elucidate the role played by activation of lipid cyclooxygenase oxidation during blood irradiation in the stimulating effect of photohemotherapy on macrophages. For this purpose, aspirin, a specific inhibitor of cyclooxygenase [1], was added to the irradiated portion before exposure.

Table 1 shows that infusion of UV-irradiated or intact blood with aspirin did not result in activation of peritoneal macrophage phagocytic activity. This proves that the main cause of the increased phagocytic activity of peritoneal macrophages in rats administered photohemotherapy is activation of cyclooxygenase oxidation in the irradiated portion of the blood.

Hence, induction of lipid cyclooxygenase oxidation in irradiated blood cells appears to be responsible for increased phagocytic activity of peritoneal macrophages after transfusion.

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