MICROBIOLOGY AND IMMUNOLOGY

Cholinomimetics Boost the Immunological Mechanisms of Radioresistance

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 541-543, May, 1996
Original article submitted May 5, 1995

Acetylcholine and aceclidine reduce the mortality of mice after irradiation. This effect is evidently due to activation of M-cholinoreactive structures leading to an increase of the number of colony-forming units in the spleen, migration of T and B lymphocytes from lymphoid organs, and boosting of antibody production and of antibody-dependent cell cytotoxicity.

Key Words: *ionizing radiation; immunocyte migration; antibody production; antibody-dependent cell cytotoxicity*

Radioresistance is known to appreciably increase under the effect of cholinergic stimulation prior to irradiation [10]. There are different interpretations of the mechanisms lying at the basis of the radioprotective effect of cholinomimetics, but the immunological mechanisms of this phenomenon, specifically, during the use of cholinergic stimulants after irradiation, have been little studied. On the other hand, we know that agents stimulating M-cholinoreactive structures can activate some immune reactions [1,2,13]. Methods aimed at increasing radioresistance are based on the use of immunotropic agents (interleukin-1, tumor necrosis factor, etc.), which are used both before and after exposure [3]. This study was carried out to investigate the immunological mechanisms of radioresistance boost under the effect of cholinomimetics used after ionizing radiation.

MATERIALS AND METHODS

Experiments were carried out with outbred and CBA mice weighing 18 to 22 g. Acetylcholine in a dose of 5 mg/kg three times a day and aceclidine in a single dose of 1 mg/kg were used as cholinergic stimulants. The outbred animals were totally irradiated in a dose of 7 Gy. The lethality was assessed on day 9. Colony-forming splenocytes were determined by the endogenous colony formation method [7,15] after total exposure of outbred mice in a dose of 7 Gy.

Migration of T cells from the thymus and of B lymphocytes from the bone marrow was assessed in CBA mice as described elsewhere [10], the index of migration being the content of antibody-producing cells in the spleens after 8 days, determined as described previously [12]. Cholinergic agonists were injected subcutaneously 30 to 60 min after exposure. Syngeneic T cells (2×10^7) or bone marrow cells (10^7) were administered simultaneously with sheep red cells (SRBC, 2×10^8) in a dose of 0.5 ml intravenously 1 day after irradiation. The migration of T and B cells was examined after exposure to 8 Gy with shielding of the thymus or hind limbs to the level of the knee joint, respectively. Antibody-producing cells in CBA mouse spleens were counted after 5 days as described previously [12] and antibody-dependent cell cytotoxicity (ADCC) was assessed in nonirradiated mice. These mice were immunized with sheep red cells (5×10^8 cells in 0.5 ml

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0007-4888/96/0005-0489$15.00

TABLE 1. Effects of Cholinomimetics on Lethality of Mice and Immune Reactions after Ionizing Irradiation

Parameter	Control	Acetylcholine	Aceclidine
Outbred mice irradiated in a dose of 7 Gy:			
Lethality, %	36.6±8.8 (30)	16.6±6.8 (30)	13.3±6.1 (30)*
Colony-forming splenocytes	6.4±1.3 (14)	15.9±3.4 (12)*	16.2±2.9 (12)*
CBA mice irradiated in a dose of 8 Gy with the respective organs shielded:			
Migration of T cells (number of antibody-producing cells in spleen)	245±45 (9)	399±50 (9)*	425±53 (9)*
Migration of B cells (number of antibody-producing cells in spleen)	321±59 (9)	532±63 (9)*	498±49 (9)*
No irradiation:			
Antibody-producing cells, 10^3	26.8±3.1 (9)	41.3±3.8 (9)*	38.9±4.9 (8)*
Antibody-dependent cell cytotoxicity, %	7.9±1.8 (12)	15.9±2.1 (8)*	14.2±2.4 (8)*

Note. The number of animals is in parentheses. *$p<0.05$ in comparison with the control.

intravenously) simultaneously with the injection of cholinergic antagonists. For determining ADCC, the spleen was removed after 5 days and a cell suspension in Hanks' solution was prepared, which was then filtered through a Kapron net. The cell suspension was washed twice for 10 min at 400 g. Cell viability was determined by supravital staining with 0.1% trypan blue solution. SRBC washed three times for 10 min each time at 400 g were the target cells. SRBC in a 2.5% suspension were mixed with an equal volume of hyperimmune rabbit antiserum in a subagglutinating dilution (1:500). The mixture was incubated 30 min at 37°C, then washed 3 times with Hanks' solution, and brought to the required concentration. The antiserum was preinactivated for 30 min at 56°C. Splenocytes were mixed with sheep red cells in a 20:1 ratio (absolute values corresponded to 20×10^6 and 1×10^6, respectively) in 2 ml of Hanks' solution without phenol red and incubated for 4 h at 37°C. After incubation, the cell mixture was centrifuged for 20 min at 200 g and the supernatant collected. Killer cytopathogenicity was assessed spectrophotometrically at wavelength 412 nm by the release of hemoproteins from lyzed red cells in the incubation medium. Samples containing effectors and intact sheep red cells were the control. The level of ADCC was assessed from the cytotoxicity index [6]. The data were processed using Student's *t* test.

RESULTS

Table 1 demonstrates that the lethality of mice assessed on day 9 after total irradiation is appreciably lowered under the effect of acetylcholine and the M-cholinomimetic aceclidine. This is a well-known phenomenon which has been interpreted in different ways. It may occur due to hypoxia caused by the cholinergic stimulation, activation of cGMP, etc. It is noteworthy that in our experiment cholinergic agonists were injected not before, but after the exposure (after 30-60 min).

It is possible that immune mechanisms determining the survival of animals during the initial period of acute radiation sickness (up to day 9) underlie the radioprotective action of cholinomimetics, along with other pathogenetic effects. The cholinergic agonists we used appreciably increased endocolonization of the spleen. On the one hand, this could have been due to increased migration of stem hemopoietic cells (SHC) from the bone marrow after total irradiation in a dose of 7 Gy, while on the other the growth of SHC colonies in the spleen might have been activated. In either case, myeloid, megakaryocyte, erythroid, lymphoid, and mixed colonies are formed in the spleen [8], where they perform different immune functions, one of them being the orchestration of a complex of mechanisms conducive to reducing mouse mortality after exposure to sublethal irradiation. The mechanisms related to T-cell migration from the thymus and B-lymphocyte migration from the bone marrow are essential during the inductive phase of cholinomimetic-effected immunogenesis [10]. Due to the migration, the spleen is the organ in which cooperative relationships between immunocytes predominantly take place. The extent of T- or B- cell migration may be assessed from the number of antibody-producing cells [10]. Increased migration of T cells from the thymus under the effect of cholinergic stimulation has been described; it is explained by expression of the muscarinic receptors on thymocytes at late stages of differentiation [14]. There is good reason to believe that

activation of M-cholinoreceptors on bone marrow B cells is one mechanism boosting their migration [1,4].

Acetylcholine and aceclidine stimulated antibody production and ADCC, which is clearly explained by the mechanisms analyzed by other scientists [1,2,13, 14] and confirmed in other experimental models [5]. One pathogenetic effect of ADCC activation may be an increased level of cGMP in these cells due to stimulation of their M-cholinoceptive structures [11].

Analysis of the immunotropic effects of acetylcholine and the M-cholinomimetic aceclidine during radioactive exposure and their effects on antibody production and ADCC suggest that the immune mechanisms of radioresistance increase by cholinergic agonists may be mainly due to stimulation of M-cholinoreactive structures.

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