Effects of Cholinotropic and Cytostatic Drugs on the Development of Arthus Reaction

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 12, pp. 654-656, December, 2008
Original article submitted May 6, 2008

Antimuscarinic effects of ipratropium bromide and atropine are associated with prevention of the development of Arthus reaction, while the cytostatic effects of cyclophosphamide and doxorubicin lead to involution of the thymus and spleen, suppression of antibody production, and aggravation of inflammation, which causes edema of the ankle joint (cyclophosphamide treatment). Apoptosis of tumor cell and inhibition of inflammation can be essential for chemotherapy of malignant and autoimmune diseases.

Key Words: *Arthus reaction; cholinergic agonists and antagonists; doxorubicin; cyclophosphamide; B lymphocytes*

Cyclophosphamide and doxorubicin used in breast cancer, multiple myeloma, Hodgkin's and non-Hodgkin's lymphomas [11], cyclophosphamide used also in juvenile arthritis, systemic lupus erythematosus, and other diseases [6] have little effect on inflammation accompanying the tumor process. However, chronization of inflammation is associated with accumulation of proinflammatory factors (TNF-α, IL-1, chemotoxins, antibodies, immune complexes). These factors promote the formation of free radicals directly damaging DNA and RNA, inhibiting DNA repair enzymes, antioxidant system, and proapoptotic proteins (caspase, p53 protein), and activating oncogenes and angiogenesis [8]. Modification of cell membrane potential in the focus of inflammation in tumors reduces the threshold sensitivity to endogenous (acetylcholine, histamine, glutamate, *etc.*) and exogenous substances [9]. Stimulation of muscarinic or nicotinic cholinoceptors on tumor cells with carbacholine [5] or nicotine [13], respectively, abolishes the apoptotic effect of cisplatin, taxol, and gemcitabine, inhibits tumor cell apoptosis, and stimulates the production of angiogenesis factors [4]. It is quite possible that classical nicotine antagonists and uncanonical cholinoceptor ligands can be used as antitumor agents [7]. Correction of activity of ionic channels in the plasma membrane as a result of metabotropic effect of cholinotropic drugs can be an effective method for prevention of inflammation, supplementing chemotherapy of tumors and treatment of autoimmune diseases.

We compared the effects of cholinergic and cytostatic drugs on the function of B cells and inflammation associated with the development of Arthus reaction (AR) or ankle joint edema.

MATERIALS AND METHODS

Male hybrid (CBA×C57Bl/6)F_1 mice (20-22 g; n=180) from Rappolovo Breeding Center served as the model for inflammation induction. The animals were kept under standard vivarium conditions on standard rations with free access to water and 12-h light day. The animals were sensitized by subcutaneous injection (20 μl) of complete Freund's adjuvant (CFA; Sigma) into the tail base; the drugs were

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0007-4888/08/1466-0747

injected intraperitoneally. The resolving dose of CFA (the same volume to the tail base) was injected 14 days after sensitization [14]. The following drugs were used: central-action drugs: choline alphoscerate (muscarinic and nicotinic receptor agonist; 130 mg/kg; *n*=8) or atropine sulfate (muscarinic receptor antagonist; 0.13 mg/kg; *n*=8), galantamine hydrobromide (anticholinesterase drug; 0.65 mg/kg; *n*=6); peripheral-action drugs: ipratropium bromide (muscarine receptor antagonist; 0.01 mg/kg; *n*=10) or metacin (2 mg/kg; *n*=6); benzohexonium (hexamethonium, nicotinic receptor antagonist; 10 mg/kg; *n*=6); antitumor drugs: cyclophosphamide (140 mg/kg; *n*=6) or doxorubicin (adriamycin; 6 mg/kg; *n*=6) [1]. The preparations were dissolved in water for injections. Controls received sensitizing and resolving doses of CFA (positive control; *n*=9) or incomplete Freund's adjuvant (IFA; Sigma) and the solvent (negative control; *n*=10). Inflammation reaction was evaluated within 1-7 days after injection of the resolving CFA dose.

The count of antibody-producing cells (per 10^6 splenocytes) was evaluated on days 5 and 14 after sensitization and drug injections. Controls were immunized with sheep erythrocytes and injected with saline. The thymus and spleen were weighed on day 14 after drug injection. The weight of the organs from intact mice served as the control.

The data were statistically processed using Student's *t* test. The differences were considered significant at $p<0.05$.

RESULTS

Cholinergic drugs and cytostatics significantly modified the development of AR (immunological reaction underlying inflammation [14]) caused by CFA (Table 1). Sensitization and injection of resolving dose of CFA aggravated edema at the tail base and led to the development of AR on day 3 (appearance of ulcers at the tail base in 50% cases) in comparison with mice injected with IFA and exhibiting no clinical signs of this kind. Cyclophosphamide (but not cholinergic drugs) augmented manifestations of inflammation and extended its location. Injections of metacin, hexamethonium, galantamine, and doxorubicin led to AR of different severity in response to resolving CFA dose on day 3 (Table 1), while injection of cyclophosphamide during this period was associated with not only AR development, but also with edema of the ankle joint. The diameter of the ankle joint edema (25% cases) reached 5.0±0.3 mm *vs.* 2.5±0.2 mm in the negative control group, which attested to failure of immunological control reactions. Injection of choline alphoscerate or atropine delayed the development of AR (Table 1). It is known that stimulation of central muscarinic cholinoceptors initiates cholinergic antiinflammatory response [10]. However, injection of atropine (but not choline asphoscerate, leading to the appearance of ulcers in 100% cases) led to minor AR (in 17% cases), which seemed to be caused by the cholinolytic effect of atropine at the periphery. It was indirectly confirmed by prevention of AR development by injection of muscarinic receptor antagonist ipratropium bromide (Table 1). It can be hypothesized that inefficiency of galantamine and hexamethonium in the antiinflammatory response is related to the fact that they can be allosteric modifiers of nicotinic (galantamine) [12] and muscarinic (hexamethonium) [3] cholinoceptors. Different effects of metacin and ipratropium bromide on the development of AR can be explained by different effects of their metabolites. It is confirmed by metabolic degradation of metacin after 40-60 min into acetic acid and choline

TABLE 1. Effects of Cholinergic and Antitumor Drugs on AR Development (*M*±*m*)

Drug	Day of ulcer appearance	Percentage of mice with ulcers at the tail base	Ulcer area, μ²
Control	3	50.0±0.3	10.5±1.0
Atropine, 0.13 mg/kg	7	17±0.4*	3.8±0.5*
Choline alphoscerate, 130 mg/kg	5	100±0.0*	5.5±0.9*
Ipratropium bromide, 0.01 mg/kg	—	—	—
Metacin, 2 mg/kg	3	38.0±0.6*	23.2±4.3*
Galantamine, 0.65 mg/kg	3	100±0.0*	11.0±1.5
Hexamethonium, 10 mg/kg	3	17±0.3*	12.4±1.1
Cyclophosphamide, 200 mg/kg	3	40±0.5	15.2±0.8*
Doxorubicin, 6 mg/kg	3	60±0.2*	16.9±1.0*

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Effects of Drug on Weights of Lymphoid Organs and B-Lymphocyte Function (*M*±*m*)

Drug	Lymphoid organ weight, mg		Count of antibody-producing cells per 10^6 splenocytes	
	thymus	spleen	on day 5	on day 14
Control	25±0.6	112±1.7	480±45	320±37
CFA	29±1.7	117±2.3	1020±138*	1076±79*
Atropine, 0.13 mg/kg	28±1.1	98±0.9	1389±83*	849±78*
Choline alphoscerate, 130 mg/kg	25±1.4	105±1.1	951±77*	1067±73*
Ipratropium bromide, 0.01 mg/kg	21±0.9	97±1.5	599±72	486±22
Metacin, 2 mg/kg	22±1.2	109±0.7	560±40	792±26*
Galantamine, 0.65 mg/kg	27±1.3	102±1.5	1082±71*	861±41*
Hexamethonium, 10 mg/kg	22±1.6	111±1.4	920±48*	890±36*
Cyclophosphamide, 200 mg/kg	17±1.4*	60.2±1.8*	158±54*	204±41*
Doxorubicin, 6 mg/kg	16±2.8*	60.5±1.5*	228±47*	234±32*

Note. Each group consisted of 5 mice.

residues [2]; due to cholinomimetic action, the latter presumably inhibits the development of this reaction (large ulcers appeared in 38% cases). Involvement of not only skin damages, but also autoimmune processes into the inflammation process seems to be due to the fact that alkylating metabolites of cyclophosphamide (phosphoramide mustard and acrolein) binding to nucleophilic centers of protein molecules induce the formation of new antigenic structures causing generation of autoreactive cytolytic cells [6] and leading to the development of arthritic reaction. It is known that the immunosuppressive effect of cytostatics manifests by suppressed proliferation of lymphocyte clones (mainly B cells) involved into the immune response [11]. This is indirectly confirmed by the fact that antitumor drugs inducing involution of lymphoid organs significantly suppress antibody production (Table 2). By contrast, cholinergic drugs (except ipratropium bromide) stimulated antibody production. These data indicate that ipratropium bromide little modified the functions of B-lymphocytes, presumably limiting their involvement in inflammation.

Hence, under certain conditions inflammation induces malignant transformation of cells and promotes their growth, which eventually can lead to tumor formation. Due to their antiinflammatory effects, muscarinic receptor antagonists can play an important role in combined therapy of malignant and autoimmune diseases.

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