Some Aspects of Immunomodulatory Effects of New Benzimidazole Derivatives

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Immunomodulatory activity of new condensed benzimidazole derivatives was studied in CBA mice. Some of these derivatives injected in a dose of 50 mg/kg on the day of immunization stimulated humoral and cellular elements of the primary immune response to sheep erythrocytes in mice.

Key Words: *benzimidazole derivatives; primary immune response; antibody-producing cells; delayed-hypersensitivity reaction*

Drug prevention and treatment of immunopathological diseases is a pressing problem. Increasing incidence of primary and secondary immunodeficiencies necessitates the search for new effective means for the correction of various states of the immune system [1,7].

During the latest 15 years great attention was paid to benzimidazole derivatives first synthesized by Hompper and Effenberger in 1959. The parent substance of this chemical structure, benzimidazole, is a purine analog, differing from purine by benzene (instead of pyrimidine) ring in its structure. Due to structural similarity to purine bases adenine and guanine, these substances can react with cell genome, which activates RNA production and hence, the production of various proteins [4].

This group includes highly effective immunomodulatory preparations. For example, dibasole increases phagocytic activity of leukocytes and induces proliferation of mature sensitized T- and B-lymphocytes, secretion of mutual regulatory factors by these cells, cooperative reaction and activation of the final effector function of cells. The immunostimulatory activity of the drug is due to regulation of the ratio of cGMP/cAMP concentrations in immune cells [5]. Bemethyl (actoprotector) and some thiethanil benzimidazoles also possess pronounced immunostimulatory activity [2,4].

We studied new condensed benzimidazole derivatives: imidazo(1,2α)benzimidazoles with the aim of finding compounds with immunomodulatory effects.

MATERIALS AND METHODS

The study was carried out on 3-4-month-old CBA mice (n=360) of both sexes of the same weight, kept under standard conditions.

Immunotropic activity of the test compounds was evaluated by primary immune response to sheep erythrocytes.

Two experimental series were carried out. The effects of benzimidazole derivatives RU-13, RU-36, RU-284, RU-354, RU-355 were studied in series I and in series II RU-64, RU-85, RU-254, RU-353, and RU-670 were studied. The substances were injected intraperitoneally in a dose of 50 mg/kg in 0.5 ml distilled water on the day of immunization. Immunized mice injected with 0.5 ml distilled water served as controls. The dose of 50 mg/kg, a 10-fold presumable therapeutic dose, was selected in order not to miss possible immunomodulatory activity of new benzimidazole derivatives.

The effects of benzimidazole derivatives on splenocyte proliferation and activity were studied by evaluating spleen weight and counts of nuclear (NC) and antibody-producing cells (APC) by local hemolysis in gel by the

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TABLE 1. Effects of Benzimidazole Derivatives on Cellular and Humoral Primary Immune Response in Mice (*M*±*m*)

Experimental series, preparation		Spleen weight, mg	NC count, $\times 10^6$	APC count, $\times 10^3$	DTH reaction index, %
Series I	control	127±6	1182±78	236±16	7.1±1.4
	RU-13	176±20*	1748±262	394±52	22.4±3.6**
	RU-36	139±12	1640±218*	351±43*	8.8±1.7
	RU-284	172±14*	2630±404**	526±80**	16.7±2.6**
	RU-354	289±22***	2233±146***	471±26***	10.2±1.02
	RU-355	208±25*	1818±134**	363±26**	29.8±2.8***
Series II	control	136±9	1743±167	348±33	7.1±1.4
	RU-64	166±15	1736±239	247±47	19.0±1.9**
	RU-85	266±33**	1942±133	388±26	13.2±1.7*
	RU-254	175±32	1276±299	280±59	26.77±2.60***
	RU-353	194±28	1281±194	238±48	13.4±2.6
	RU-670	192±17*	3083±381*	516±77	18.6±2.5**

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control.

method of Erne and Nordin [3]. The animals were immunized subcutaneously with a suboptimal dose of sheep erythrocytes (2×10^7) in 0.2 ml distilled water.

Cellular immunity was evaluated by the delayed-type hypersensitivity (DTH) reaction [6]. The optimal dose of sheep erythrocytes (2×10^8) in 0.2 ml and resolving dose (10^8 sheep erythrocytes) in 0.05 ml normal saline were used for immunization. The reaction index (RI) was calculated for each mouse by the formula:

$$IR=\frac{M_e-M_c}{M_c}\times100\%,$$

where M_e and M_c are the weights of "experimental" and "control" paws, respectively.

All manipulations with animals were carried out in accordance with the Helsinki Declaration.

The results were processed using Statgraph software.

RESULTS

Single injection of all benzimidazole derivatives increased the spleen weight in all mice, but significant differences from the control were found only for RU-13, RU-284, RU-354, RU-355, RU-85, and RU-670. Some substances increased splenocyte count and APC count among splenocytes. All three components of humoral immunity increased significantly only after injection of RU-354, RU-355, and RU-284 (Table 1).

The effects of the preparations on cellular component of the primary immune response was evaluated by the development of DTH reaction. Seven of 10 substances significantly increased RI (Table 1). RU-36 RI had virtually no effect on RI, while in mice receiving RU-354 and RU-353 this parameter increased 1.5-2-fold compared to the control, but this increase was not statistically significant.

When analyzing the effects of the test preparations on all studied parameters, we compared the experimental doses of preparations exhibiting immunotropic effects with their LD_{50}. Experimental dose of RU-354 significantly modulating humoral immunity virtually coincided with LD_{50}, while the dose of RU-670, which exhibited good results as regards DTH reaction and nuclear cell count, was one-half of LD_{50}, thus making these preparations not perspective for further practical studies because of their possible toxic effects. The doses of RU-355 and RU-284, stimulating all parameters, and of RU-254, markedly stimulating DTH reaction, were $^1/_3$, $^1/_4$, and $^1/_6$ of LD_{50}, respectively, thus making further studies perspective.

Hence, our results indicate that condensed benzimidazole derivatives RU-284, RU-355, and RU-254 are characterized by pronounced immunotropic effects and are promising as the base for new highly effective immunomodulators.

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