In Vitro Immunomodulating Activity of β-Heptylglycoside Muramyldipeptide

O. V. Kalyuzhin, N. S. Zakharova, M. V. Britsina, A. E. Zemlyakov, and V. V. Kalyuzhin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 11, pp. 553-554, November, 1999
Original article submitted July 31, 1998

The effects of β-heptylglycoside muramyldipeptide (glymuride) on antibody production and delayed type hypersensitivity reaction were studied in mice with weak and strong reactions to sheep erythrocytes. The drug exhibited high immunomodulating activity which depended on the initial genetically determined immunoreactivity of animals and drug dose.

Key Words: *β-heptylglycoside muramyldipeptide; antibody production; delayed type hypersensitivity*

Immunomodulators modifying the direction and intensity of immune reactions occupy a special place among modern immunotropic drugs. The type of biological effect (stimulation or suppression) is determined by a sum of factors: drug dose, number of administrations, initial immunity status, *etc.*

We investigated the dose-dependent effects of β-heptylglycoside muramyldipeptide (glymuride) on immunoreactivity of mice with genetically predetermined strong and weak reaction to sheep erythrocytes (SE).

MATERIALS AND METHODS

Experiments were performed on 6-8-week-old CBA and C57Bl/6 mice (Kryukovo Breeding Center, Russian Academy of Medical Sciences). β-Heptylglycoside-N-acetylmuramyl-L-alanyl-D-isoglutamine (glymuride) was synthesized as described previously [1]. The effect of glymuride on delayed type hypersensitivity (DTH) reaction was studied by a previously described method [4]. The effect on the production of antibodies to SE was evaluated as described elsewhere [5]. Glymuride was injected intravenously in doses of 0.2, 20, and 200 μg/mouse in 100 μl normal saline. Control mice were intravenously injected with 100 μl normal saline.

I. M. Metchnikov Institute of Vaccines and Sera, Russian Academy of Medical Sciences; Institute of Human Morphology, Russian Academy of Medical Sciences; Kristal-Med, Moscow

RESULTS

In *in vitro* test systems β-heptylglycoside-muramyldipeptide activated T- and B-cell immunity [2,3,7]. It stimulated lymphocyte proliferation in response to T- and B-cell mitogens and activated the generation of allospecific cytotoxic T lymphocytes in a mixed lymphocyte culture. We investigated the effect of glymuride on cell and humoral immunity *in vivo.* The effect on humoral immunity was evaluated by the intensity of the production of antibodies (agglutinins) to SE in mice of two oppositely reacting strains, CBA and C57Bl/6, injected with glymuride (Table 1). On day 4, anti-SE antibody titer increased in all mice. On days 4-7, serum concentrations of antibodies in CBA mice (strongly reacting) treated with glymuride were virtually the same as in the control. On day 14, antibody titers were lower than in controls. However, increased level of antibodies persisted up to day 21 of the experiment, while in CBA mice immunized with SE alone antibody titers dropped at this period.

In weakly reacting C57BL/6 mice a statistically significant ($p<0.05$) increase in agglutinin concentration was observed in groups injected with glymuride in doses of 200 (days 4 and 21) and 20 μg/mouse (day 7).

0007-4888/99/0011-1140$22.00

TABLE 1. Effect of Glymuride on Antibody Production (*M*±*m*, *n*=5)

Mouse strain	Drug dose, μg	Antibody titer			
		day 4	day 7	day 14	day 21
CBA	Normal	16±0			
	Control	170±86	170±86	426±170	16±0
	0.2	106±42	128±0	256±0	64±0*
	20	213±86	128±0	170±86	128±0*
	200	170±86	170±86	213±86	42±22*
C57Bl/6	Normal	4±0			
	Control	48±22	48±22	64±0	64±0
	0.2	51±14	48±20	57±38	70±32
	20	57±12	179±62*	64±36	48±20
	200	179±62*	115±74	89±46	153±52*

Note. Here and in Table 2: *$p<0.05$ *vs.* the control (Student's *t* test). Results of one typical out of three independent experiments are shown.

TABLE 2. Antiradical Activity of Baikaline and Ionol (*M*±*m*)

Mouse strain	Control	DTH index, % (glymuride dose, μg)		
		0.2	20	200
CBA	69±5.2	49±4.8*	36±5.2*	65±8
C57Bl/6	48±6.6	73±2.8*	73±4.8*	65±6.6*

It is noteworthy that in mice sensitive to SE the drug ensured a longer persistence of high antibody titers. In weakly reacting mice the level of antibodies notably increased only after high doses (20 and 200 μg/mouse) of glymuride. This increase persisted for 21 days only after injection of 200 μg glymuride.

The effect of glymuride on cell immunity was evaluated by the intensity of DTH (Table 2). In CBA mice, the DTH index decreased in a dose-dependent manner, while in C57BL/6 mice it increased in comparison with the control. Hence, the drug exerted various effects on immunoreactivity depending on genetically determined pattern of immune response. In weakly reacting mice glymuride stimulated T-cell immunity, while in doses of 0.2 and 20 μg it suppressed the immunity in strongly reacting mice.

Our findings confirm previous data that glymuride modifies T- and B-cell immunity *in vitro* [3,7]. Immunomodulating drugs are now widely used in therapy of many diseases, notably improving the treatment efficiency [6]. Therefore, the description of immunomodulating properties of a new muramyldipeptide derivative is of special interest.

REFERENCES

1. A. E. Zemlyakov and V. Ya. Chirva, *Khimiya Prirod. Soedinenii,* No. 5, 714-718 (1987).
2. O. V. Kalyuzhin and B. B. Fuks, *Byull. Eksp. Biol. Med.,* **122,** No. 12, 658-661 (1996).
3. O. V. Kalyuzhin, B. B. Fuks, N. V. Bovin, *et al., Ibid.,* **117,** No. 5, 510-513 (1994).
4. R. V. Petrov, R. M. Khaitov, A. N. Cheredeev, and E. V. Kozhinova, *Immunomodulators* [in Russian], Moscow (1987), pp. 3-25.
5. H. Frimel', B. Shirgel', and E. Bell, *Immunological Methods* [in Russian], Moscow (1979).
6. R. M. Khaitov and B. V. Pinegin, *Prakt. Vrach,* No. 9 (2), 5-13 (1997).
7. O. V. Kalyuzhin, A. E. Zemlyakov, and B. B. Fuchs, *Int. J. Immunopharmacol.,* **18,** No. 11, 651-659 (1996).