# EFFECT OF RETINOIC ACID ON SOME PARAMETERS OF HUMORAL IMMUNITY

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Among the nearest metabolites of vitamin A retinoic acid has the strongest biological activity. Proof of its epitheliotropic, growth-promoting, carcinoprotective, and cytostatic properties has been obtained [2, 10]. Clinical data on the use of retinoic acid in skin tumors, psoriasis, and acne vulgaris [7, 9, 10]. One aspect of the mechanism of action of retinoic acid is its ability to exert an immunomodulating action. The number and proliferative activity of cells of the lymphocyte series were increased in the red bone marrow of animals receiving retinoic acid [8], and the number and phagocytic intensity of the macrophages in the skin also were increased [6]. Under the influence of retinoic acid the ratio between the areas of cortex and medulla, of white and red pulp, of lymphoid follicles, medullary cords, and the paracortical zone, were changed in the thymus, spleen, and lymph nodes by the action of retinoic acid [2, 4, 7]. Retinoic acid stimulated accumulation of effector forms of T lymphocytes in experimental animals [2, 7]. Meanwhile the effect of retinoic acid on parameters of humoral and, in particular, of antiviral immunity has been the subject of only solitary investigations [2, 7].

The aim of this investigation was to study the effect of retinoic acid on antibody formation and on the level of inhibitors in the blood of mice hyperimmunized by injection of bacterial antigen (*Escherichia coli*) and influenza viruses.

## EXPERIMENTAL METHOD

In three experiments noninbred C57BL/6 and (C57BL/6 × CBA)$F_1$ mice of both sexes weighing 18-20 g were used. Retinoic acid was used in the form of all all-trans-methylretinoate (MR) and of 13-cis-methylretinoate. To compare the action of the test substance, vitamin A (retinyl palmitate — RP, retinyl acetate), and retinoid $C_{15}$ (a possible shortened metabolite of vitamin A and retinoic acid) structural and trivial names of the retinoids were given in [1]) were used. The substances were obtained from the Laboratory of Chemistry of Polyemic Compounds (Head, Professor G. I. Samokhvalov), "Vitaminy" Research and Production Combine, USSR.

Animals of group 1 were given 1, 3, and 9 injections, each of 0.1 ml of a 1% solution of MR and RP intraperitoneally, at intervals of 24 h. On the 2nd and 6th days after the last injection, the animals were immunized with a living 24-h culture of *E. coli* strain M-17. Next, a $2 \cdot 10^9$ suspension of this culture was used as antigen in the agglutination test. Animals of group 2 were given five injections of 0.05% solution of MR, 13-cis-methylretinoate, and retinoid $C_{15}$ intraperitoneally at intervals of 7 days. On the 40th and 46th days after the last injection the animals were immunized with inactivated influenza vaccine, containing viruses of the genus A($H_1N_1$) and A($H_3N_2$). Sera from the animals of this group were studied in the hemagglutination inhibition test with antigens identical to the vaccinal antigens, in order to detect antibodies and thermolabile inhibitors in them. Animals of group 3 were given intramuscular injections of 0.3% solution of MR and retinyl acetate in the course of 4 days. Allantoic fluid — infectious titer $10^{-9}$, hemagglutinin titer 1:640 of A($H_1N_1$) influenza virus, served as the interferon inducer. The interferon titer in the animals' sera was determined *in vitro* on mouse fibroblasts of the L-929 line against 100 $CPD_{50}$ of vesicular stomatitis virus. The results were subjected to statistical analysis.

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TABLE 1. Effect of Intraperitoneal Injection of MR and RP on Antibody Titer to Bacterial Antigens of *E. Coli* Strain M-17 (M ± m)

| Substance injected | Total dose | Survival rate of animals | Antibody titer (reciprocals) |
|---|---|---|---|
| S | 0,1 ml | 9/9 | 30±5 |
| RP | 0,001 g | 9/9 | 100±13* |
| MR | 0,001 g | 9/9 | 140±13* |
| S | 0,3 ml | 11/11 | 252±30 |
| RP | 0,003 g | 11/11 | 309±44 |
| MR | 0,003 g | 11/11 | 503±36* |
| S | 0,9 ml | 14/14 | 181±16 |
| RP | 0,009 g | 14/12 (86%) | 333±31* |
| MR | 0,009 g | 14/0 (0%) | — |

Legend. Here and in Table 2 results of experiments on male (CBA × C57BL/6)$F_1$ are given; solvent (S) was soy oil; *P < 0.001.

TABLE 2. Effect of Intraperitoneal Injection of MR on Titer of Serum Inhibitors and Antibodies against Influenza Viruses (M ± m, n = 15)

| Substance injected | Total dose | Titers of inhibitors and antibodies to influenza viruses of undermentioned strains (reciprocals) | | |
|---|---|---|---|---|
| | | $A(H_3N_2)$ — inhibitor-sensitive | $A(H_0N_1)$ — inhibitor-resistant | $A(H_3N_2)$ — inhibitor-resistant |
| S | 0,1 ml | 307±12 | 266±11 | 93±9 |
| MR | 0,001 g | 96±17* | 62±7* | 52±17* |
| S | 0,3 ml | 234±15 | 108±12 | 209±21 |
| MR | 0,003 g | 143±11* | 101±16 | 51±10 |

## EXPERIMENTAL RESULTS

The experiments showed that MR and RP stimulate antibody formation against *E. coli* bacterial antigen. The serum antibody titer rose with an increase in dose of the substances injected: it was higher in the case of MR than with RP, but this retinoid also proved to be more toxic (Table 1). The considerable toxicity of retinoic acid was mentioned previously [5]. As regards the parameters of natural immunity (serum inhibitors, interferon) to virus antigens, MR exhibited immunodepressive properties (Table 2). The interferon titer — the inducer was influenza virus $A(H_1N_1)$ — of noninbred female mice receiving 0.0012 g of MR and retinyl acetate intramuscularly, was 64 ± 0 and 256 ± 10 respectively, compared with 350 ± 58 (injection of the solvent — peach oil) and 267 ± 30 (injection of the interferon inducer) in the control. Each group contained 10 animals, all of which survived until the end of the experiment. MR, 13-cis-methylretinoate, and retinoid $C_{15}$, injected in a total dose of 0.0013 g, significantly lowered the level of specific antiviral antibodies. Antibody titers (reciprocals of values for viruses) of influenza $A(H_3N_2)$ were 320, for intraperitoneal injection of 2.5 ml of soy oil (solvent for the retinoids, control), 208 ± 40 for MR, 110 ± 25 for 13-cis-methylretinoate, and 210 ± 30 for retinoid $C_{15}$, all injected intraperitoneally. The survival rate of the animals in all the groups was 100% except for mice receiving MR: in this group four of the 19 mice died.

It can be concluded from these results that with an increase in the dose of retinoic acid its immunostimulating action relative to the parameters of antibacterial immunity and the immunodepressive properties relative to parameters of antiviral immunity likewise increased.

To explain the adjuvant effect of vitamin A and retinoids, it has been postulated that an excess of retinoids damages the blood cells, of which the most numerous are erythrocytes. The autoantigens formed are initiators of the immunostimulant action of compounds of this group [3]. The ways whereby vitamin A and its synthetic analogs influence antiviral immunity have not been studied.

Experiments on mice thus showed that retinoic acid, in the form of MR and 13-cis-isomers of methylretinoate, have a modulating action on certain parameters of humoral immunity. MR stimulates antibody production in response to the action of bacterial (*E. coli*) antigens and lowers the parameters of nonspecific antiviral defense — the serum inhibitor and interferon levels. Both isomers reduced the titer of anti-influenzal antibodies.

LITERATURE CITED


1. Yu. I. Afanas'ev, V. I. Nozdrin, O. I. Mikhailov, et al., Vopr. Onkol., No. 3, 77 (1983).
2. Yu. I. Afanas'ev, V. I. Nozdrin, and O. I. Mikhailov, Usp. Sovrem. Biol., 95, No. 3, 358 (1983).
3. Yu. I. Afanas'ev, V. I. Nozdrin, K. A. Fofanova, et al., Byull. Éksp. Biol. Med., No. 12, 20 (1982).
4. Yu. I. Afanas'ev (Yu. I. Afanasjev) and V. I. Nozdrin (V. I. Nosdrin), Folia Morphol., 28, 294 (1980).
5. Yu. I. Afanas'ev, V. I. Nozdrin, and A. A. Perilov, Vopr. Onkol., No. 12, 84 (1979).
6. N. Z. Bakhshinyan and V. I. Nozdrin, Biol. Zh. Arm., No. 5, 473 (1981).
7. V. I. Nozdrin and S. M. Subbotin, Vorp. Onkol., No. 9, 96 (1983).
8. V. I. Nozdrin, M. Z. Bakhshinyan, A. A. Aznauryan, and V. M. Padalko, Biol. Zh. Armenii, No. 2, 107 (1982).
9. C. N. Ellis, R. C. Gold, R. C. Grekin, et al., J. Am. Acad. Dermatol., 6, 699 (1982).
10. M. A. Zile and M. E. Cullum, Proc. Soc. Exp. Biol. (N.Y.), 172, 139 (1983).


# CENTRAL CHOLINOLYTIC EFFECT OF TROPANE DERIVATIVES: CORRELATION BETWEEN STRUCTURE AND ACTION


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Besides an aliphatic chain, in the structure of the known cholinolytics which are tropane derivatives (atropine, scopolamine, and so on), there are also an aromatic ring and a hydroxyl group. It is considered that both aryl and hydroxyl radicals are necessary for tropane derivatives to exhibit their cholinolytic properties [8, 9]. This conclusion was drawn from a study of peripheral cholinolytic properties of atropine and its analogs, the effect of which was estimated according to the degree of mydriasis. We know that the acetylcholine receptors of the neocortex are of the muscarinic type. The muscarinic cholinolytic atropine reduces the responses of cortical neurons to acetylcholine (ACh) [5, 7].

The aim of the present investigation was to study whether the presence of the aryl radical and hydroxyl groups is essential for tropane derivatives to exhibit their central cholinolytic activity. For this purpose two substances synthesized in the Department of Organic Synthesis, Institute of Pharmacology, Academy of Medical Sciences of the USSR, under the working name of LK-11 [dihydrochloride of the β-(N-morpholyl)priopionic tropine ester] and LK-14 [trihydrochloride of the β-(N-methylpiperazinyl)propionic ester of tropine], were studied. Neither compound has aryl and hydroxyl radicals, although the tropane structure and the ester grouping are preserved.

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