PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 298-300 (1976)

Comparison of the Suppression of Interferon Production and Inhibition of Its Action by Vitamin A and Related Compounds (39532)

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We have previously shown that vitamin A inhibited the antiviral action of interferon (1) and that this inhibition seemed to be due to an effect of vitamin A on the interferon molecule (2). Inhibition of interferon action showed a critical dependence on the form of vitamin A that was tested.

We subsequently reported that retinoic acid (vitamin A acid) suppressed the production of interferon (3). The suppression of interferon production was shown not to be due to inactivation of interferon. For instance, retinoic acid treatment of cells was required only prior to interferon production for suppression. Also, suppression of production occurred in the presence of 10% calf serum (CS) whereas interferon inactivation by vitamin A did not. Inactivation did not occur in 10% CS, presumably because of a competition of the serum proteins for the vitamin A. These observations suggested that suppression of interferon production and inhibition of its action might be dependent on different parts of the vitamin A molecule. Therefore, we have investigated the structural requirements for vitamin A suppression of interferon production and inhibition of interferon action.

Cells. Mouse L-929 cells were maintained by weekly passage in Eagle's minimal essential medium (MEM) supplemented with 10% calf serum (CS). This medium contained 125 μg of streptomycin and 150 units of penicillin/ml.

Virus. A large-plaque variant of vesicular stomatitis virus (VSV), Indiana strain, and a lentogenic strain of Newcastle disease virus (NDV) were grown in the allantoic cavity of developing chick embryos. The pool of VSV contained about 1×10^9 plaque forming units (PFU)/ml when assayed on L-929 mouse cells. The pool of NDV contained about 640 hemagglutination units/ml.

Production and assay of interferon. Mouse interferon was produced by infection of L-929 cells with a lentogenic strain of NDV. Supernatant fluids were harvested 24 hr after infection and dialyzed against pH 2 buffer for 5 days at 4° and then against Gey's balanced salt solution (BSS) to restore the pH to neutrality.

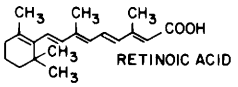
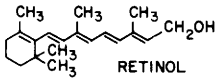
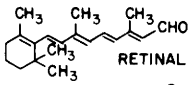
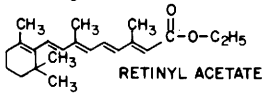
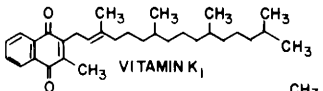
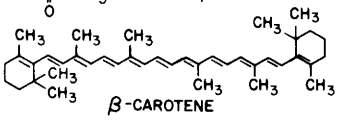
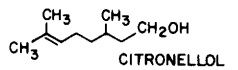
A plaque reduction assay using VSV and L-929 cells was employed for quantitating interferon. Confluent cell monolayers in 2-oz glass bottles were treated overnight with two fold serial dilutions of interferon preparations in triplicate or quadruplicate. Supernatant fluids were then aspirated and cells infected with 0.2 ml of a dilution of VSV containing about 300 PFU. After a 1-hr incubation at room temperature to permit adsorption of virus, each monolayer was overlaid with 5 ml of MEM containing 1% methyl cellulose, 5% calf serum, 25 m*M* Hepes buffer, 125 μg/ml of streptomycin, and 250 units/ml of penicillin (overlay medium). The cultures were incubated for 48 hr at 37° and monolayers were then stained with crystal violet. Plaques were enumerated after ×6.5 magnification of the monolayers by use of a photographic enlarger. The 50% plaque depressing dose (PDD_{50}) was defined as the amount of an interferon preparation, in microliters, that inhibited 50% of the plaques from developing as compared to the controls.

Chemicals. All *trans* forms of retinoic acid (vitamin A acid), retinol (vitamin A alcohol), retinyl acetate (acetate ester of vitamin A), and retinal (vitamin A aldehyde) were obtained from Sigma Chemical Company, St. Louis, Mo. Vitamin K_1 was obtained from Schwarz/Mann, Orangeburg, N.Y. *Trans*-β-carotene and citronellol were obtained from Aldrich Chemical Company, Milwaukee, Wis. Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific Company, Pittsburgh, Pa.

Stock solutions were made by dissolving each compound in DMSO. For experimental purposes, the compounds were made 6.7×10^{-5} *M* in medium (equivalent to 20 μg/ml of retinoic acid).

Results and discussion. Different forms of vitamin A and related compounds dissolved in DMSO were tested to determine their effect on the antiviral activity of interferon. Retinoic acid, retinol, retinal, retinyl acetate, vitamin K_1, β-carotene, and citronellol were each made 6.7×10^{-5} *M* in an interferon preparation diluted 1 to 10 in MEM. Controls received an equivalent amount of DMSO (1%). After 24 hr at 37°, the mixtures were further diluted 1 to 100 in MEM with 5% CS and assayed for residual interferon activity. Table I shows that retinoic acid and retinol were similarly effective at inhibiting interferon activity while retinal and retinyl acetate were considerably less inhibitory. These data suggested that the terminal group on the vitamin A molecule was important for the inhibitory effect on interferon activity. This concept is supported by the low level of inhibitory activity of β-carotene on interferon action. β-Carotene is a dimer of vitamin A with a ring on both ends of the molecule. Since β-carotene was not very inhibitory for interferon action, it

TABLE I. COMPARISON OF THE SUPPRESSION OF INTERFERON PRODUCTION AND INHIBITION OF ITS ACTION BY VITAMIN A AND RELATED COMPOUNDS.

Compound tested[a]	Percentage of suppression of interferon					
	Antiviral activity			Production		
	Replicate experiments[b]	Mean ±SD	*P*	Replicate experiments	Mean ±SD	*P*
 RETINOIC ACID	60,64,65,65	63 ± 3	<0.001	75,67,61	68 ± 7	<0.001
 RETINOL	57,100	79 ± 21	<0.01	67,47	57 ± 14	<0.01
 RETINAL	24,17	21 ± 5	NS[c]	75,68	72 ± 5	<0.001
 RETINYL ACETATE	14,0	7 ± 10	NS	51,40	46 ± 8	<0.01
 VITAMIN K_1	2,0	1 ± 1	NS	19,0	10 ± 13	NS
 β-CAROTENE	29,24	27 ± 4	<0.05	57,48	53 ± 6	<0.01
 CITRONELLOL	0,0	0	NS	0,0	0	NS
DMSO (CONTROL)	0[d]	0	–	0	0	–

[a] Compounds were tested at 6.7×10^{-5} *M* which is equivalent to 20 μg/ml of retinoic acid.

[b] Each value represents a separate interferon assay compared to a control interferon assay, both of which were performed in triplicate or quadruplicate. Cultures were challenged with about 300 PFU of VSV.

[c] Statistically not significant (all *P* values >0.3). Statistical analysis was done by Student's *t* test.

[d] By definition 0% suppression. DMSO had no effect on interferon action or production at the concentration employed (1%). Control interferon levels were from 8000 to 16,000 PDD_{50} units/ml.

seems that the ring portion of the vitamin A molecule was not very important in the inhibition of interferon action. Furthermore, citronellol, an analog of the side chain of retinol, was not inhibitory, suggesting that the conjugated double bond system was also important in the interaction of vitamin A with the interferon molecule. Another fat-soluble vitamin, vitamin K_1, did not inhibit interferon activity.

The same compounds were tested for their effect on interferon production. NDV was adsorbed to L-929 cells for 1 hr at room temperature. Cell cultures were then washed and received either retinoic acid, retinol, retinal, retinyl acetate, vitamin K_1, β-carotene, or citronellol at 6.7×10^{-5} *M* in MEM with 10% CS. Ten percent CS was employed because vitamin A does not inhibit interferon activity under these conditions (2). In contrast to the marked dependence on the form of vitamin A required for inhibition of interferon action, all forms of vitamin A tested suppressed interferon production (Table I). This observation suggested that the ring portion of the vitamin A molecule was of primary importance for the suppression of interferon production. That β-carotene, a dimer of vitamin A with a ring group at each end of the molecule, was suppressive for interferon production is consistent with this idea. Also, the lack of a suppression by citronellol, an analog of the side chain of retinol, supports this concept. Vitamin K_1 was also not very suppressive.

These results have illustrated that vitamin A can suppress both interferon action and production. The inhibitory effects, however, appear to result from different mechanisms. The inhibition of interferon activity by vitamin A seems to be due to an effect of vitamin A on the interferon molecule (2), while the suppressive effect on interferon production is clearly due to an effect of vitamin A on the cell which has been induced to make interferon (3). It is interesting that the different mechanisms of action of vitamin A on interferon action and production apparently occur by different moieties of the vitamin A molecule (Table I). Inhibition of interferon action seems to be most dependent on the side chain of the vitamin A molecule. The side chain apparently requires a conjugated double bond system and a hydrophilic terminal group (hydroxy group for retinol and carboxy group for retinoic acid) to inhibit interferon action. In contrast, the ring portion of the vitamin A molecule seems to be most important for the suppression of interferon production.

Summary. We have investigated the structural requirements for vitamin A suppression of interferon production and inhibition of interferon action. Inhibition of interferon action seems to be most dependent on the side chain of the vitamin A molecule. The side chain apparently requires a conjugated double bond system and a hydrophilic terminal group to inhibit interferon action. In contrast, the ring portion of the vitamin A molecule seems to be most important for the suppression of interferon production.

This study was supported by United States Public Health Service Grant AI-10900.

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Received March 11, 1976. P.S.E.B.M. 1976, Vol. 153.