Virucidal Activity of Retinal

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Herpes simplex virus type 2 and simian virus 40 were rapidly inactivated by retinal at micromolar concentrations. Other fat-soluble vitamins, particularly vitamin A derivatives, were also active against herpes simplex virus type 2 and several lipid-containing bacteriophages.

Vitamin A derivatives have attracted much attention recently due to their abilities to inhibit the growth of some benign and malignant tumors (12, 14), to induce differentiation in carcinoma cells (13), and to cause dramatic improvement in serious cases of acne (4).

We are investigating the potential antiviral and virucidal activities of a wide variety of fat-soluble and membrane-active compounds, using lipid-containing bacterial viruses as an initial screening system for detecting compounds that might have potent virucidal effects against mammalian viruses. We reported several years ago that the common food additive butylated hydroxytoluene is a potent inactivator of the lipid-containing bacteriophages φ6 and PM2 and human herpes simplex virus type 1 (HSV-1) (10). More recently, we have reported on the virucidal effectiveness of fatty acids and fatty acid derivatives (6, 7, 11), finding that 12- to 18-carbon alkyl chain length unsaturated fatty acids, unsaturated and saturated alcohols, and unsaturated monoaoclyglycerides exhibit potent virucidal effects against lipid-containing viruses. None of these compounds, however, was effective against several non-lipid-containing viruses tested. We report here that vitamin A derivatives have potent virucidal effects. Most notably, retinal (vitamin A aldehyde) is an extremely potent inactivator of HSV-2 and the non-lipid-containing tumor virus simian virus 40 (SV40).

Initial experiments were carried out with bacteriophage φ6, which has an external envelope similar to that of several mammalian viruses and which has a susceptibility to membrane perturbers comparable to that of HSV (6). We treated φ6 for 30 min at 25°C with a variety of fat-soluble vitamins and found φ6 to be extremely susceptible (Fig. 1). The results show that retinal, retinol, and retinyl acetate at 0.35 μM inactivated φ6 by more than 99% and are thus among the most potent virucidal compounds known.

Vitamin K1, α-tocopherol, and retinoic acid inactivated φ6 by more than 99% at concentrations between 10 and 20 μM. Retinyl palmitate was the least active of the compounds tested, requiring a concentration of 45 μM to inactivate φ6 by 99%. The fat-soluble vitamins all showed a sharp rise in virucidal activity over a narrow concentration range.

The lipid-containing marine bacteriophage PM2, which has a lipid bilayer surrounded by a protein coat (8), was less susceptible than φ6 to the fat-soluble vitamins. Table 1 shows the vitamin concentrations required for 50% inactivation of PM2. Vitamin K1, α-tocopherol, and the vitamin A derivatives retinoic acid and retinyl palmitate were the most potent compounds against PM2. Retinal and retinol, which were the most potent inactivators of φ6, were among the least active against PM2.

The antiviral activities of the vitamins against bacteriophages φ6 and PM2 indicated that these compounds may be active against enveloped animal viruses. We tested the vitamins against HSV-2, an enveloped mammalian virus transmitted venereally and linked with cervical cancer (5). The vitamin concentrations required for 50% inactivation of HSV-2 are presented in Table 1. Retinal was by far the most potent compound tested, giving inactivation at concentrations as low as 0.45 μM. Approximately 25 μM retinal was required to give detectable cytopathic effects in host human embryonic lung cells, corresponding to an in vitro therapeutic index of 56. HSV-2 was susceptible to the other vitamin A derivatives, α-tocopherol (vitamin E alcohol), and vitamin K1, but only at much higher concentrations.

SV40, a non-lipid-containing virus that can induce tumor production in hamsters (2), and several non-lipid-containing bacteriophages were also inactivated by retinal. Figure 2 shows a comparison between the inactivation of HSV-
2 and SV40 by retinal. Retinal was more effective against the enveloped virus (HSV-2) than against the nonenveloped virus (SV40), but nevertheless it did inactivate SV40 (50%) at 8 μM. Retinal is the first membrane-active compound found to exhibit virucidal activity against non-lipid-containing viruses at very low concentrations. The non-lipid-containing bacteriophages T4, PRR1 (3), and P3 (1) were inactivated (50%) at concentrations of retinal between 60 and 175 μM, whereas even higher concentrations of the other compounds were needed to inactivate these viruses. For all of the viruses tested, inactivation by more than 99.9% could be achieved by exposure for 60 min to retinal at the concentration that resulted in approximately 99% inactivation in 30 min.

The findings reported here, coupled with the very recent report (15) that retinoic acid inhibits induction of a herpesvirus (Epstein-Barr) by some tumor promitigators, suggest that one or several forms of vitamin A may be useful as therapeutic agents against some enveloped and possibly nonenveloped virus infections. It is possible that the combined effect of inhibition of virus induction and potent virucidal activity could result in significant in vivo antiviral effects. The mechanism(s) of inactivation by retinal is not known but probably involves the destabilization of lipid-protein (in lipid-containing viruses) and partially hydrophobic protein-protein interactions. Further studies on the antiviral activities of retinal and the other fat-soluble vitamins seem to be warranted and are in progress.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PM2*</th>
<th>HSV-2*</th>
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<tbody>
<tr>
<td>Retinal</td>
<td>&gt;175</td>
<td>0.45</td>
</tr>
<tr>
<td>Retinol</td>
<td>&gt;175</td>
<td>10</td>
</tr>
<tr>
<td>Retinyl acetate</td>
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<td>36</td>
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<tr>
<td>Retinoic acid</td>
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<td>33</td>
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<tr>
<td>Retinyl palmitate</td>
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<td>100</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>Vitamin K&lt;sub&gt;1&lt;/sub&gt;</td>
<td>16</td>
<td>210</td>
</tr>
</tbody>
</table>

*The compounds were dissolved in ethanol at 100 times the desired final concentrations and added to the virus suspensions to give a final ethanol concentration of 1%. The viruses were not inactivated by 1% ethanol.
*Bacteriophage PM2, at 5 × 10<sup>5</sup> plaque-forming units per ml in ND medium (9), was incubated with the compounds for 30 min at 25°C. After incubation, samples were diluted and assayed for plaque-forming units by using Alteromonas espejiana (formerly designated Pseudomonas BAL-31).

Fig. 1. Inactivation of bacteriophage φ6 by fat-soluble vitamins. The fat-soluble vitamins were dissolved in ethanol at 100 times the desired final concentration and added to the virus suspension in LS medium (7) to give a final ethanol concentration of 1%, which does not inactivate the viruses used in this study. Bacteriophage φ6 was added to the LS-vitamin mixture at 25°C to give approximately 5 × 10<sup>5</sup> plaque-forming units per ml. After 30 min of incubation, samples were diluted and assayed for plaque-forming units with Pseudomonas phaseolicola HB10Y as the host cell. Data are presented as percentage of inactivation compared with control samples in the absence of the vitamins (0% inactivation).

Fig. 2. Inactivation of HSV-2 and SV40 by retinal. Viruses at 10<sup>5</sup> plaque-forming units per ml were treated for 30 min at room temperature in TBS buffer. HSV-2 and SV40 were assayed for plaque-forming units on monolayers of HEL cells and CV-1 cells, respectively. The data shown are an average of two experiments, for which all data points fell within 10% of the average value.
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LITERATURE CITED