Failure of 1-β-d-Ribofuranosyl-1,2,4-Triazole-3-Carboxamide (Virazole, ICN 1229) to Stimulate Interferon

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In cultured cells, Virazole failed to stimulate either interferon or a cellular resistance which continued in its absence. Serum from Virazole-treated mice likewise contained no demonstrable interferon.

The broad-spectrum in vitro and in vivo activity of Virazole (1, 2, 4, 6), combined with the observation that the nucleoside appeared more virus-inhibitory when added prior to the virus (2, 4), suggested that the compound was perhaps an inducer of interferon. The following experiments were performed to elucidate this possible mechanism of antiviral action.

To determine whether Virazole stimulated an interferon-like resistance which would continue in its absence, we added the drug, in concentrations varying from 1,000 to 1 µg/ml, to L-929, KB, and RK-13 cells in plastic panels (5). Three time schedules were compared. (i) Uninfected cells were exposed for 24 h to the drug dissolved in Eagle minimal essential medium (Grand Island Biological Co., San Francisco, Calif.) supplemented with 5% fetal bovine serum, penicillin (100 units/ml), and streptomycin sulfate (100 µg/ml). The compound was removed and the cells were washed three times with Hanks balanced salt solution (HBSS) before addition of virus. (ii) The cells were exposed to the drug for 24 h, after which the drug-containing medium was removed prior to the addition of virus and drug was added again to the cells simultaneously with the virus. (iii) The cells were exposed to the drug and virus simultaneously. Vesicular stomatitis virus (VSV), strain Indiana, was used at a concentration of 1,000 CCID₆₀/ml as an indicator of interferon activity. Inhibition of viral cytopathogenic effect (CPE) in the drug-exposed cells compared with control (untreated) infected cells was determined after a 72-h incubation at 37 C.

The second treatment schedule yielded the greatest degree of antiviral effect (Table 1), suggesting the importance of the continued presence of Virazole within the cell during virus infection. Pretreatment only was not protective to the cells under these conditions, indicating that the drug did not stimulate a resistance comparable to an interferon-mediated resistance.

To determine whether Virazole stimulated the cell to release interferon into the medium, we treated L-929 cells for 3, 6, 9, or 12 h with Virazole, after which the cells were washed three times and then incubated at 37 C in Virazole-free medium. After 24 h, the undiluted medium from these cells was removed and incubated at 37 C for 24 h with other L-929 cells prior to addition of VSV. When these latter cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Lowest Virazole concn (µg/ml) inhibiting 50% virus CPE</th>
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<tbody>
<tr>
<td></td>
<td>Pretreatment only*</td>
</tr>
<tr>
<td>L-929</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>KB</td>
<td>&gt;1,000</td>
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<tr>
<td>RK-13</td>
<td>&gt;1,000</td>
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* Pretreatment with Virazole for 24 h, with the cells washed immediately prior to addition of virus.

* Pretreatment with Virazole for 24 h, with the drug removed just prior to addition of virus and then re-added to the cells simultaneously with virus.

* Drug added to the cells simultaneously with the virus.
were observed 3 days later, no inhibition of CPE was seen, indicating that Virazole did not trigger the release of a virus-inhibitory material from the cells.

Using similar techniques in experiments with polynosinic-polycytidylic acid (poly I:C), statolon, or Newcastle disease virus, other investigators have readily demonstrated the in vitro interferon-stimulating capacity of these compounds (3, 7). In the same cell system, we have demonstrated the efficacy of poly I:C as an interferon inducer (5).

Male Swiss Webster mice weighing 18 to 22 g were injected intraperitoneally with 1,000 mg of Virazole/kg dissolved in sterile saline. Serum samples were collected 2, 4, 8, 12, 18, and 24 h later, and twofold dilutions from 1:20 to 1:1,280 were incubated with L-929 cells for 24 h prior to challenge with 1,000 CCID₅₀ of VSV/ml. No CPE inhibition was seen at any serum level, indicating that Virazole also fails to stimulate interferon production in vivo.

LITERATURE CITED