Statolon-induced Resistance of Mice to Mengovirus

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Mice receiving statolon intraperitoneally were 1,000 times more resistant to intraperitoneal challenge with mengovirus than were untreated controls. Protection was afforded when statolon was administered 1 day before or 1 day after intraperitoneal inoculation with the virus. No therapeutic effect was observed when treatment with statolon was delayed for 2 days or more after virus infection. Exposure of mice to a simulated space cabin environment did not increase their susceptibility to the lethal effects of mengovirus infection or eliminate the protective effect of statolon.

Statolon is a complex derivative obtained from cultures of Penicillium stoloniferum (7). Among its most outstanding properties are relative non-toxicity to an intact host and ability to induce interferon release both in vivo and in vitro (6). These characteristics suggest that statolon might be not only a useful tool in studies on the interaction of host cells and viruses but possibly also of value in prevention of certain viral diseases. We selected this compound for a study of its activity against the lethal effect of mengovirus in mice. Its protective effect in animals maintained under normal conditions was compared with that observed in mice exposed to a simulated space cabin environment.

Materials and Methods

Animals. Male Swiss albino mice (22 to 26 g) were quarantined for 2 weeks in an air-conditioned facility at 20°C after their arrival from a commercial source. No deaths or illness was observed during this period. They were then randomly divided into two groups. One group was maintained in the animal room while the other was transferred to a biosimulator for a 2-week period of acclimatization. This instrument and its supporting equipment have been described previously (3). Conditions maintained in the biosimulator were: atmospheric pressure corresponding to 18,000-ft elevation (380 mm of Hg); oxygen concentration, 43% (equivalent to partial pressure at ground level); carbon dioxide, less than 1%; temperature, 20°C; relative humidity, approximately 70%.

Treatment with statolon. Statolon (obtained through the courtesy of W. J. Kleinschmidt of the Lilly Research Laboratories) was administered to groups of acclimatized and normal mice by intraperitoneal injection of 250 μg of active ingredient in 0.2 ml of sterile phosphate-buffered saline (PBS) at pH 7.4. Groups of mice were treated with statolon 24 hr or 5 min before and 1, 2, or 3 days after the virus challenge.

Virus. The large plaque-forming variant of mengovirus, propagated in monolayers of L cells and appropriately diluted in PBS, was inoculated intraperitoneally in 0.2-ml amounts. Groups of normal mice and those treated with statolon 24 hr previously received virus dilutions ranging from 10⁻⁴ to 10⁻². All those mice which were given statolon on the same day or on any day after the virus inoculation received the 10⁻⁴ virus dilution. The mice were observed daily for illness or death.

Results

Among the nonprotected controls (no statolon), the earliest deaths occurred on the 5th day after inoculation and were limited to animals receiving the highest concentrations of virus. The greatest number of deaths was recorded between the 6th and 8th days. Animals surviving 10 days postinoculation without visible signs of illness were considered to be refractory to the infectious challenge. The experiment was terminated on the 10th day.

The LD₅₀ (10 days) dose was determined from data presented in Table 1, by use of probit analysis. Results with this method were in essential agreement with those obtained by use of the Reed and Muench or Dragstedt and Behrens methods. Statistical analysis has shown that, among the nonprotected animals, there was no significant difference between the resistance of mice at ground-level conditions and those kept in the biosimulator. The respective LD₅₀ doses were 10⁻⁴.ₜ.ₖ and 10⁻⁴.ₛ₁.

When comparable groups of mice were given 250 μg of statolon 24 hr before inoculation with the virus, only 2 mice died in the biosimulator (on the 7th and 8th days) and 1 mouse died under the ground-level conditions (on the 10th day).
Table 1. Effect of statolon on susceptibility of mice to mengovirus

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Nonprotected mice</th>
<th>Statolon-protected mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent survival</td>
<td></td>
</tr>
<tr>
<td>Ground-level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>conditionsa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-4</td>
<td>5.6</td>
<td>94.4</td>
</tr>
<tr>
<td>10-5</td>
<td>16.7</td>
<td>100.0</td>
</tr>
<tr>
<td>10-6</td>
<td>94.4</td>
<td>100.0</td>
</tr>
<tr>
<td>10-7</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2. Therapeutic effect of statolon against mengovirus in mice kept at ground-level conditions or in the biosimulator

<table>
<thead>
<tr>
<th>Statolon administered</th>
<th>Ground-level conditions</th>
<th>Biosimulator conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mice</td>
<td>Per cent survival</td>
</tr>
<tr>
<td>1 day before virus</td>
<td>18</td>
<td>100.0a</td>
</tr>
<tr>
<td>5 min before virus</td>
<td>24</td>
<td>100.0a</td>
</tr>
<tr>
<td>1 day after virus</td>
<td>18</td>
<td>77.8a</td>
</tr>
<tr>
<td>2 days after virus</td>
<td>18</td>
<td>38.9</td>
</tr>
<tr>
<td>3 days after virus</td>
<td>18</td>
<td>55.6</td>
</tr>
</tbody>
</table>

Discussion

The work of Powell and co-workers (11), dealing with chemophrophylactic properties of a filtrate from a Penicillium culture against MM and Semliki Forest viruses, stimulated further search for compounds which could elicit protection against viruses similar to that offered by antibiotics in bacterial infections. These investigations dealt with crude filtrates of mold cultures and led to a more purified and concentrated preparation called "statolon." This preparation was described as a complex anionic polysaccharide derived from P. stoloniferum (7). When injected intraperitoneally or intravenously, it induces the release of a viral inhibitor with biological properties consistent with those of interferon (5). In mice, a maximal interferon level is attained at 12 hr, and the level remains relatively high for an additional 12 hr. By 72 hr, however, the interferon level has decreased to a lower plateau which is maintained for at least another 4 days. The protective effect of a single injection of statolon against MM virus may persist for 30 days or longer (W. J. Klienschmidt and E. B. Murphy, Bacteriol. Rev., in press).

The production of interferons in many in vitro and in vivo systems may be induced by such diverse agents as viruses (13), bacterial endotoxins (15), mold filtrates, and phytohemagglutinin (14). Interferons are known to render the cells incapable of reproducing a wide spectrum of viruses without having a direct effect on the virus particles themselves. However, aside from the demonstrated species specificity (9), certain differences among interferons are known to exist, i.e., their mode of release (15), molecular weight (8), heat (4, 14), and pH stability (8, 14). These differences warrant rather detailed investigations of the particular interferons in regard to their viral spectrum as well as the time elapsing between the introduction of the viral agent and the substance affecting the release of the interferon. Since statolon appears to be nontoxic at the effective dosages and may be introduced subcutaneously (2), it seems to offer certain advantages over other compounds capable of eliciting a similar effect.

In addition to other viruses already reported (1, 2, 6, 10, 12), our results show that statolon is...

*Eighteen mice per group at each dilution level. Statolon given 24 hr before virus inoculation.

\[ L_{D_{50}} = 10^{-2.0} \times 0.12 \]

\[ L_{D_{50}} = 10^{-4.1} \times 0.19 \]

\[ L_{D_{50}} \text{ could not be determined with dilution range employed.} \]

\[ L_{D_{50}} = 10^{-2.3} \pm 1.49 \]
effective against mengovirus infection in mice when injected intraperitoneally 24 hr preceding the virus challenge. Equal protection was obtained when the statolon was given just before the virus inoculation, and a significant sparing effect was also observed when statolon was injected 1 day after the virus challenge. In the last instance, a release of interferon could not have taken place before the virus gained access to susceptible cells, but its appearance following the delayed administration of statolon may have been sufficient to curb further spread of the virus and thus prevent the development of the disease. This finding indicates that statolon might be useful not only as a prophylactic but also as an immediate therapeutic agent in cases of known exposure to a virulent virus. The duration of its prophylactic effectiveness as well as determinations of the maximal possible delay for its administration after infection is the subject of current studies.

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LITERATURE CITED