Efficacy of Exogenous Interferon
Treatment Initiated After Onset of Multiplication of Vesicular Stomatitis Virus in the Brains of Mice

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SUMMARY

Initiation of treatment with potent interferon preparations (6.4 × 10⁸ units per dose) 4 days after intranasal inoculation of VSV (at a time when virus has already multiplied in the brains of most mice) resulted in a marked increase in mouse survival. In a series of 6 experiments only 11 to 18% of control mice survived whereas 52% of interferon treated mice survived. These results suggest the usefulness of exogenous interferon even when treatment is begun late in the course of an acute virus disease.

Although exogenous interferon affords protection in experimental animals when inoculated prior to, or shortly after, virus infection (Finter, 1973) it is of obvious clinical importance to determine the extent to which a beneficial therapeutic effect may be obtained when treatment is initiated after virus has already multiplied in a crucial organ such as the brain. De Clercq & De Somer (1971) were able to demonstrate a slight but significant increase in mouse survival when interferon was administered at a time when signs of vesicular stomatitis virus (VSV) meningoencephalitis had appeared, whereas in a similar type of study, Worthington, Levy & Rice (1973) found that interferon did not alter mortality when treatment was initiated 2 days after infection of mice with Semliki Forest virus. Since relatively small amounts of interferon were used in both studies it was considered of interest to determine the efficacy of far more potent interferon preparations than had previously been available in the treatment of an established virus infection. Accordingly, in the experiments to be reported, interferon treatment was initiated 4 days after intranasal infection with VSV, at a time when virus has already multiplied in the brains of most inoculated mice. The efficacy of this treatment suggest the clinical usefulness of exogenous interferon therapy even after virus has multiplied in crucial target organs.

Five- to six-week-old male, Institut du Cancer (IC) mice weighing 17 to 25 g were used throughout this study. Mice were lightly anaesthetized with ether. As they emerged from anaesthesia, 0.1 ml of a dilution of VSV was slowly dropped onto the external nares. Only mice having inhaled the inoculum were included in the experiment. The Indiana strain of VSV was propagated and assayed in mouse L cells or by intranasal (i.n.) inoculation of mice. 1 LD₅₀ in mice (i.n. inoculation) was found to be approx. the equivalent of 100 TCD₅₀ for L cells. Virus was titred on 4 occasions in mice, and, in the experiments to be described, mice were inoculated with approx. 25 LD₅₀. In each experiment four days after virus infection, mice were distributed into different groups. Deaths were scored daily. Most mice died between the 6th and 10th days and those surviving 21 days after infection without signs of illness were considered to have survived. In the experiments to be presented we have tried to keep as uniform as possible a number of experimental variables (age, sex, weight of mice, virus inoculum, potency of interferon). An adjusted chi-square test was used to eliminate inter-experimental variables (Lellouch & Lazar, 1974).
Mouse interferon was prepared from mouse C-243 cells cultivated in suspension culture and infected with Newcastle disease virus (NDV) according to techniques previously described (Tovey, Begon-Lours & Gresser, 1974). After concentration and partial purification by selective precipitation with ammonium sulphate (Tovey et al. 1974) the activity of the interferon preparation was assayed on mouse L cells. In the experiments to be described, mice were inoculated by the intraperitoneal (i.p.) and intravenous (i.v.) routes with 0·2 ml interferon containing $6\times10^6$ interferon reference units (sp. act. $3\cdot2 \times 10^6$ units to $6\cdot4 \times 10^6$ units/mg protein).

In a series of 6 experiments, mice were inoculated i.n. with approx. 25 LD$_{50}$ of VSV and then sacrificed at given days thereafter. The brain of each mouse was harvested, weighed, ground in a mortar and diluted 1:10 (by weight), and assayed for virus content. As can be seen from Fig. 1, (the results of the 6 experiments are pooled) virus was first detected 3 days after virus infection (2 brains were positive out of 6 tested). Four days after infection, virus was recovered from the brains of 8 out of 11 mice. The finding that virus was recovered from the brains of 24 of 25 mice on the 6th day emphasizes the reliability of the intranasal route of inoculation in these experiments.

Since it is apparent from the previous experiments (Fig. 1) that VSV has multiplied in the brains of most mice 4 days after infection, treatment with interferon was initiated at this time. The results of 6 experiments are presented in Table 1. Despite virus infection, all mice do not succumb and paralysis can regress with ultimate survival. Thus 11% of untreated mice and 18% of mice treated with phosphate buffered saline (PBS) survived (the difference is not significant). A delay in death and an increased survival was observed for interferon-treated mice compared to control groups of mice in 5 of 6 experiments (the exception being Expt. 5, Table 1). The difference between the survival of untreated mice or mice treated with PBS and the survival of mice treated with interferon was highly significant ($P < 0\cdot0001$).
Table 1. Survival in mice when interferon treatment was initiated four days after intranasal infection with VSV

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Total no. of injections*</th>
<th>Days of treatment after infection</th>
<th>Route of injection</th>
<th>Survival in treatment group† (no. surviving/no. inoculated with VSV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>4-7</td>
<td>i.p. and i.v.</td>
<td>1/12</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>4-8</td>
<td>i.p.</td>
<td>2/14</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4-8</td>
<td>i.p.</td>
<td>—</td>
</tr>
<tr>
<td>4‡</td>
<td>22</td>
<td>4-9</td>
<td>i.p. and i.v.</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>4-8</td>
<td>i.p.</td>
<td>0/4</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>4-8</td>
<td>i.p.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Total 5/48</td>
</tr>
</tbody>
</table>

* Each injection (0.2 ml) contained 6.4 × 10⁶ mouse reference units.
† Adjusted chi-square test and significance: the difference in survival was not significant (χ² = 0.88) between the groups of mice without treatment and those treated with PBS in experiments 2 to 5. The difference in survival was significant (χ² = 23.9, P < 0.0001) between the groups of mice treated with PBS and those treated with interferon in experiments 2 to 6.
‡ In this experiment, interferon treatment was also initiated in another group of mice 5 days after infection. No increased survival was observed in this group.

Our results do not permit us to draw any conclusions as to the minimal amount of interferon necessary. In one of the experiments, groups of virus infected mice were treated with tenfold dilutions of interferon. A marked increase in survival was observed when mice were repeatedly inoculated with 6.4 × 10⁶ units, but only a slight effect was observed in another group of mice injected with 6.4 × 10⁵ units and no increase in mouse survival was observed in mice treated with 6.4 × 10⁴ units.

To determine the effect of interferon treatment on the amount of virus present in the brain, mice treated with interferon (beginning 4 days after infection) and untreated virus infected mice were sacrificed on the 6th day. In one such experiment no difference was observed in the amount of virus recovered from the brains of 8 control and 8 interferon treated mice—but in this experiment (Expt. 5, Table 1) no therapeutic effect was observed. In another experiment interferon treatment was associated with a delay in death and an increased survival (Expt. 6, Table 1) and a significant difference in the amount of VSV recovered, was observed. Thus, the mean virus titres in the brains of 10 control mice was 5.74 ± 0.64/log (TCD₅₀)/ml of brain homogenate whereas the mean virus titres in the brains of 10 interferon treated mice was 4.82 ± 0.73 (using t test ρ < 0.005).

The results presented herein show that repeated inoculation of large amounts of interferon is associated with a clear cut increase in the survival of mice, even when treatment is initiated 4 days after infection with VSV, at which time virus has already multiplied in the brains of most mice. In one experiment less virus was recovered on the 6th day from the brains of interferon treated mice than from the brains of control mice which would appear to suggest that interferon is exerting a direct inhibitory effect on virus replication. However, interferon has been shown to exert multiple effects on cells and in particular to affect lymphocyte division and function (Lindahl-Magnusson, Leary & Gresser, 1972, Lindahl, Leary & Gresser, 1972, Cerottini et al. 1973; Gisler, Lindahl & Gresser, 1974). Thus it seems to us conceivable that increased survival in our experiments may result in part from effects of interferon other than a direct intracellular inhibition of viral multiplication. In this regard,
Short communications

Billiau, Muyembe & De Somer (1970) noted inhibition of lung consolidation in mice infected with influenza virus and treated with the interferon inducer chlorite-oxidized oxylamylose. Despite an increase in the survival rate of treated mice, no difference in the amount of virus recovered from the lungs was observed between control and treated mice. This appears therefore to be an example of increased survival in a virus disease not mediated by a direct effect of virus replication.

The point we should like to emphasize regardless of the mode of action of interferon is that it is still possible to alter the outcome of an acute virus disease even at a time when virus has multiplied in a crucial target organ such as the brain. As in the case of bacterial diseases there is clearly a point beyond which therapy is of no avail. It may well be that this point is not reached until even late in the course of some virus diseases.

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REFERENCES


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