Post-exposure Local Treatment of Mice Infected with Rabies with Two Axonal Flow Inhibitors, Colchicine and Vinblastine

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SUMMARY

Post-exposure protection of rabies-infected mice was observed by proximal application of axonal flow inhibitors, particularly vinblastine, to the local nerve(s). These observations indicate that rabies virus is transported by the axonal flow of the peripheral nerves to the central nervous system. Both a fixed virus (CVS) and a street (sylvatic) virus were used.

This model in mice could be used to develop an additional post-exposure local treatment of rabies infection in man, by infiltrating local nerves or ganglions with axonal flow inhibitors, with the advantage that it would not interfere with subsequent vaccination as is the case with the administration of hyperimmune serum or immunoglobulin.

A number of studies of the pathogenesis and local treatment of rabies infection have shown that the spread of the infection from the exposure site to the central nervous system (CNS) takes place via the peripheral nerves. However, the actual mechanism of transport in those nerves is not known (Kaplan et al. 1962; Dean et al. 1963; Wiktor & Koprowski, 1963; Baer et al. 1965; Schneider, 1969; Murphy et al. 1973).

During the last decade a vast literature has appeared in the field of neurophysiology, dealing with the axonal flow in neurons and by which flow exogenous and endogenous materials are being transported. The axonal transport can be inhibited in both directions by local application to nerves of alkaloids such as colchicine (Kristensson et al. 1971; Kristensson & Sjöstrand 1972; Hendry et al. 1974) and vinblastine (Edström & Hanson, 1973). In the study of herpes simplex virus in mice Kristensson et al. (1971) showed that the intra-plantarily inoculated virus was transported by the axonal flow of the peripheral nerve in the direction of the CNS. Our recent experiments with the axonal flow inhibitors colchicine and vinblastine (Heaney et al. 1976) using the CVS fixed strain of rabies virus, were based on the study with herpes simplex virus and have given similar evidence of transport of the virus or its infectious subunit by the axonal flow in the peripheral nerve.

We report here additional experiments with one of these axonal flow inhibitors, vinblastine, using a rabies virus isolated from the salivary gland of a fox. These findings give further support to the idea that the transport of rabies virus after infection follows the axonal flow of the local peripheral nerves to the CNS.

The two alkaloids colchicine and vinblastine were applied locally to the sciatic nerves by means of small pieces of blotting paper impregnated with the alkaloids, a method based on other experiments (Dahlström, 1968). In previous experiments (Heaney et al. 1976) we showed that a saturated solution of colchicine applied 3 days before intraplantar infection with the CVS strain of rabies virus was the most effective in arresting the infection. A saturated solution of colchicine has a concentration of 115 mM. Knowing the amount of the saturated solution impregnated in blotting paper with a size of 3 to 4 mm², we
Table 1. Protective effect in mice of alkaloids applied either simultaneously or at different times after intraplantar infection in the hind limb with CVS or sylvatic rabies virus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time of treatment (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVS rabies infection</td>
<td></td>
</tr>
<tr>
<td>Colchicine</td>
<td>0/10* 4/10 2/10 6/10 8/10 10/10 10/10</td>
</tr>
<tr>
<td>Vinblastine</td>
<td>0/10 2/10 0/10 2/10 7/10 6/10 8/10</td>
</tr>
<tr>
<td>Neurectomy</td>
<td>0/10 0/10 0/10 0/10 10/10 9/10 10/10</td>
</tr>
<tr>
<td>Saline treated</td>
<td>10/10 — — — — — —</td>
</tr>
<tr>
<td></td>
<td>0 12 24 30 36 42 48 60</td>
</tr>
<tr>
<td>Sylvatic rabies infection</td>
<td></td>
</tr>
<tr>
<td>Vinblastine</td>
<td>4/10† 2/10 2/10 5/10 5/10 6/10 9/10 7/10</td>
</tr>
<tr>
<td>Controls (untreated)</td>
<td>9/10 — — — — — —</td>
</tr>
<tr>
<td>Neurectomy</td>
<td>3/10† 0/10 1/10 6/10 7/10 6/10 6/10 7/10</td>
</tr>
<tr>
<td>Controls (untreated)</td>
<td>8/10 — — — — — —</td>
</tr>
</tbody>
</table>

* Number of dead mice/number of treated mice.
† These mice died approximately one week later than the mice of the other groups, including the controls.
estimated that the total amount of colchicine applied locally to the nerve was between 36 and 64 \(\mu g\) as contained in two blotting papers. The toxic dose of colchicine for three week-old mice by the intramuscular route was 50 \(\mu g\). However, no toxic reaction was ever observed in the mice treated locally on the nerve by means of the blotting paper impregnated with colchicine. This is due to the fact that 15 min after the local application to the nerve, it was washed carefully with physiological saline. Vinblastine was more effective than colchicine and the best results were obtained at a concentration of 10 mM. Here again, knowing the amount of this solution impregnated in the blotting papers, the approximate amount locally administered was between 9 and 12 \(\mu g\). The acute toxicity of vinblastine is difficult to evaluate due to the fact that the drug provokes a leukocytopenia which leads to bacteremia and death. The toxic dose obtained (Johnson et al. 1963) by the intraperitoneal route is approximately 30 \(\mu g\) for three week-old mice (10 g), 330 \(\mu g\) by the oral route and 175 \(\mu g\) intravenously.

In order to determine the efficiency of the drugs in arresting rabies infection, mice were infected and treated with the alkaloids either simultaneously or at different times after infection (Table I). Swiss albino mice of 3 to 5 weeks old employed were inoculated intraplantarily for this purpose with 0.02 ml in the left hind pad with either the CVS strain of rabies virus (10 LD\(_{50}\) by this route; the virus titre by the intracerebral route of undiluted material was \(10^{2.3}\) LD\(_{50}\)/0.03 ml) or the sylvatic rabies virus. Because of the limited amount (0.02 ml) applied intraplantarily, it was not possible to kill all mice with the latter virus. This is due to the fact that street virus is not a fixed type of virus and does not usually reach a high titre after a few intracerebral mouse passages. Only three such passages were made in order to obtain a working pool of this sylvatic virus which still has the characteristics of the original virus and yet kills mice by the peripheral route. The intracerebral titre of this virus was \(10^{5.8}\) LD\(_{50}\)/0.03 ml.

The two blotting papers, 4 mm\(^2\) each, impregnated with either colchicine or vinblastine were applied to the sciatic nerve, one on top and the other underneath. As the papers were stored dry, physiological saline was added at the moment the papers were applied to the nerve in order to activate local action of the alkaloids. After 15 min the blotting papers were removed and the skin was sutured. Care was taken during the whole surgical procedure, using a binocular dissecting microscope and small glass tools to avoid any possible injury to the nerve. In control groups, mice were treated similarly but the blotting papers were impregnated with physiological saline, in order to check any possible effect of the surgery. In other control groups a neurectomy (2 to 3 mm of the sciatic nerve) was performed at the same location as the alkaloids application. In Table I it can be seen that infection with the CVS strain of rabies virus was arrested by both drugs up to 6 h after infection. Similar results were obtained with neurectomized control mice. By 8 h the CVS virus had apparently already passed through the local axons of the nerve and infection could not be prevented. Similar results were found for neurectomized mice by other authors (Dean et al. 1963; Baer et al. 1965). As vinblastine appeared to be more effective and less toxic than colchicine, further experiments were carried out using only vinblastine. If applied up to 24 h after the sylvatic rabies infection, mice were still protected. This result was again comparable with neurectomized mice (Table I).

We observed that in mice infected with sylvatic rabies virus and simultaneously treated at 0 h with vinblastine or by neurectomy, respectively, 4 and 3 mice died approximately one week later than the control mice or those in the other groups treated at later times. This may be explained by a difference in neurotropism (viral adherence to susceptible cells) between fixed and sylvatic rabies virus. In other words, more free (non-adhered) sylvatic virus...
Table 2. Neutralizing antibody levels in the sera of mice surviving infection with rabies virus after treatment with vinblastine

<table>
<thead>
<tr>
<th>Number of sera and survivors in:</th>
<th>Titres expressed in International Units (i.u.) and classified in four groups:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–1</td>
</tr>
<tr>
<td>CVS group</td>
<td>17 (3)*</td>
</tr>
<tr>
<td>Sylvatic rabies group</td>
<td>16 (10)</td>
</tr>
</tbody>
</table>

* Mice which had been treated successfully with the drugs were bled 4 weeks after infection and one week later challenged intraplantarily in the right footpad of the hind limb with 150 LD₅₀ of the CVS virus. The sera were examined for neutralizing antibodies using the plaque reduction method (Bijlenga & Joubert, 1974). In each test the International Reference Serum (IRS) was included to express titres in International Units. One i.u. is equivalent to a 1/16₀ dilution of the IRS. The number of mice in parentheses died after this challenge.

is present locally for a short period which could be spread by a lympho-haematogenous route activated by the surgical procedure. The longer incubation period of one week could be further explained by the fact that a very small amount of sylvatic rabies arrives finally in the CNS by the lympho-haematogenous route, where sufficient multiplication and spread of the virus has to take place before nervous symptoms develop.

The alkaloids did not appear to have a virucidal effect as serum specimens of surviving mice contained specific neutralizing antibodies (Table 2). It is interesting to observe that the antigenicity of the sylvatic rabies virus is comparable or even higher than that of the CVS strain, as theoretically 100 times more CVS virus than the sylvatic virus had been inoculated intraplantarily. The 100-fold lower amount of CVS virus hardly produced any antibody response by the intraplantar route.

On the basis of these results in mice, one could speculate on a post-exposure treatment of human beings with vinblastine. The surgical procedure in man is much easier to perform due to the larger size of the local nerves and/or ganglions. Here peripheral infiltration of vinblastine, which has been shown to be very effective, would probably be the method of choice. A possible treatment after an accidental CVS virus infection in the laboratory seems to be excluded as the time lapse to perform a surgical procedure would be too short (in mice only 6 h are available). In the case of a street virus infection much more time is available (30 h in the experimental mice), but one should not immediately perform any surgery to avoid the possible spread of the virus.

The advantages of treatment with the alkaloid vinblastine as compared with serum treatment and subsequent vaccination are: (a) the infecting virus stimulates the production of interferon, specific rabies antibody and cell-mediated immunity (natural primary vaccination) which adds to the post-exposure vaccination treatment; (b) no blocking or interference with the post-exposure vaccination occurs as has been observed with local or systemic treatment with specific hyperimmune serum or immunoglobulin (WHO, 1973).

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REFERENCES


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