Chemoprophylaxis of scrapie in mice

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Three applications of the polyanion pentosanpolysulphate about 2 months before infection of mice with scrapie completely protected animals infected with up to 100 LD₅₀, and considerably prolonged the lifespan of those infected with 100 to 10000 LD₅₀. The clinical diagnosis was confirmed by immunoblot analysis for the protein of scrapie-associated fibrils.

Immune prophylaxis of the slow virus diseases caused by unconventional viruses (e.g. Creutzfeldt-Jakob disease in man and scrapie or other spongiform encephalopathies in animals) has not been achieved, simply because unconventional viruses do not evoke an immune response in their hosts (for review, see Gajdusek, 1990). Chemotherapeutic effects have been demonstrated with several polyanions in models of scrapie in mice when these compounds were administered around the time of infection (Kimberlin & Walker, 1983, 1986; Ehlers et al., 1984; Ehlers & Diringer, 1984; Farquhar & Dickinson, 1986) and with amphotericin B in hamsters (Pocchiari et al., 1989). Here we report an effect of a polyanion on scrapie disease in mice which is novel not only for chemotherapy of unconventional virus diseases but for viruses in general. Treatment of mice with a polyanion for a limited period of time months before infection not only increases the lifespan of every single animal but also protects a considerable number of animals against an otherwise fatal disease.

Earlier observations on dextran sulphate 500 [(DS 500), polysulphated (1,6)-D-glucopyranoside (M₉ 500000)] showed that (i) a single application of the drug administered 72 days before infection still had a slight but statistically significant positive effect on the survival time of mice challenged with the 139A strain of scrapie (Ehlers & Diringer, 1984), (ii) DS 500 resided in the cytoplasm of macrophages within spleen and lymph nodes for more than 100 days (Ehlers et al., 1984), (iii) DS 500 inhibited the replication of the virus in spleen (Ehlers & Diringer, 1984) and (iv) other polyanions with lower toxicity elicited similar life-prolonging effects when administered around the time of infection (Ehlers & Diringer, 1984).

The less toxic drug pentosanpolysulphate [SP54 or HOE/BAY946, polysulphated (1,4)-D-xylopyranoside (M₉ 3500 to 5000)], which is tolerated at intraperitoneal (i.p.) doses of more than 50 mg per mouse (Ehlers & Diringer, 1984), was then tested. We performed two successive experiments in which groups of between six and 10 NMRI mice were infected i.p. with 100 µl of 10⁻² to 10⁻⁶ dilutions of scrapie (strain 139A) mouse brain homogenate in phosphate-buffered saline (PBS), covering a range of 1 to 10⁴ LD₅₀ per inoculum.

At the age of about 6 weeks, mice were treated with 10 mg SP54 dissolved in 1 ml PBS on days 84, 77 and 71 (Expt. 1) or days 70, 60 and 50 (Expt. 2) before infection. A control group was mock-treated with PBS only. Mice were observed for signs of disease twice weekly until clinical symptoms became apparent and daily thereafter.

The results based on clinical diagnosis are summarized in Table 1. In both experiments, all animals receiving the greatest amount of virus (10⁻² dilutions of brain homogenate) died of scrapie, except one in the SP54-treated group of Expt. 2. However, the groups receiving treatment showed a highly significant increase in survival time (Student's t-test: < 0.001) of about 80 and 50 days in Expt. 1 and 2, respectively. In Expt. 1 this was also true for the group receiving a 10⁻³ dilution in which, again, one out of six treated animals survived; in Expt 2, five out of eight mice survived. Of the mice infected with lower doses of virus, i.e. 10⁻⁴ and 10⁻⁵ dilutions of scrapie brain, almost all animals survived in the drug-treated groups. Animals that did not survive the infection consistently lived much longer than control animals. According to these results, treatment with SP54 2 months before infection reduced the effective titre of a scrapie inoculum by about 100-fold (for calculation see Kimberlin & Walker, 1978).

The pathogenesis of scrapie has been ascribed to a virus-induced amyloidosis of the central nervous system (Czub et al., 1986; Diringer et al., 1988). During the process of amyloidosis a phosphoinositol-linked glycoprotein (the pre-amyloid) of the nerve cell membrane
Table 1. Effect of SP54 on scrapie in mice

<table>
<thead>
<tr>
<th>Inoculum (dilution of brain homogenate)</th>
<th>Incubation period (days ± s.e.) and ratio of survivors to animals infected</th>
<th>Expt. 1*</th>
<th>Expt. 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated†</td>
<td>Control</td>
</tr>
<tr>
<td>10⁻²</td>
<td>155 ± 1</td>
<td>239 ± 9</td>
<td>167 ± 2</td>
</tr>
<tr>
<td></td>
<td>0/7</td>
<td>0/6</td>
<td>0/6</td>
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<tr>
<td>10⁻³</td>
<td>160 ± 2</td>
<td>248 ± 9</td>
<td>174 ± 3</td>
</tr>
<tr>
<td></td>
<td>0/7</td>
<td>1/6</td>
<td>0/7</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>184 ± 5</td>
<td>187 ± 3</td>
<td>267</td>
</tr>
<tr>
<td></td>
<td>0/7</td>
<td>6/6</td>
<td>1/7</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>187 ± 4</td>
<td>186 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/6</td>
<td>6/6</td>
<td>4/7</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>-</td>
<td>-</td>
<td>7/7</td>
</tr>
</tbody>
</table>

* Expt. 1 and 2 were terminated at days 384 and 304 post-infection, respectively.
† Animals were treated as described in the text.
‡ Mean for three animals only. One of the five animals scored as survivors died at day 295 with ascites but without clinical signs of scrapie. See also legend of Fig. 1.

(Stahl et al., 1987) aggregates to form scrapie-associated fibrils (SAF; Merz et al., 1981; Diringer et al., 1983), the amyloid. The aggregation into SAF is the most specific (Merz et al., 1984; Bode et al., 1985; Farquhar et al., 1989), as well as a pathogenetically very important (Diringer et al., 1988), step in the development of clinical symptoms in all the unconventional virus diseases. After biochemical concentration (Multhaup et al., 1985), the SAF protein can be sensitively detected by antisera raised against the fibril protein (Diringer et al., 1984). Therefore, we performed an immunoblot analysis on the individual mouse brains to confirm our results based on clinical diagnosis.

SAF amyloid was isolated by a time-saving modification of a standard procedure (Multhaup et al., 1985). Brain homogenate (5 ml, 10%) was processed to pellet P22 as published (Multhaup et al., 1985). This pellet was dissolved by sonicating in 4 ml Tris-buffered saline containing 1% of the detergent sulphobetain 3,14. After centrifugation for 1 h at 100000 g (swinging bucket rotor TST54; Kontron), pellet P100 was redissolved in 0.5 ml Tris-HCl pH 8.5, 0.0005 M-CaCl₂, 0.1 % sulphobetain 3,14 and 1.5 M-NaCl. After subsequent treatments with 1 µg micrococcal nuclease and 1 µg proteinase K at 37 °C for 30 min each, remaining proteins were precipitated with 1:5 ml ethanol at −20 °C for 1 h and pelleted by centrifugation. The final pellet was dissolved in Laemmli buffer and a sample equivalent to 50 mg of brain was analysed for SAF protein by immunoblotting (Bode et al., 1985).

As an example of this technique, the analyses on brains of all mice in Expt. 2 receiving a 10⁻³ dilution of scrapie-infected brain are given in Fig. 1 (for comparison, see Table 1). All control animals developing clinical disease between days 166 and 183 post-infection (p.i.) contained the amyloid. The same was true for the SP54-treated animals developing clinical symptoms between days 246 and 295 after infection. In contrast, the remaining animals, which were killed at day 304 p.i. without clinical symptoms, had not accumulated any SAF in their brains. The immunoblot analysis, therefore, confirmed our results based on clinical diagnosis that the polyanion SP54, a chemical drug, can prevent a viral disease when administered over a limited time period months before infection.

Such long lasting protection has not been obtained so far with any chemical compound in any viral disease. Nucleoside analogues in human immunodeficiency virus (HIV) and herpesvirus infections, or amantidine in influenza virus infections, are among the best known examples of chemotherapy of virus infections but they elicit a prophylactic effect only when administered at the time of infection or afterwards (for reviews, see Bauer, 1985; Hovi, 1988).

It is unlikely that SP54 exhibits its effect via a known response of the host’s immune defence system although the polyanion DS500 has been reported to stimulate antibody production (Diamantstein et al., 1971), is a B cell mitogen (Dories et al., 1974) and an adjuvant for cell-mediated immune responses (McCarthy et al., 1977). However, unconventional viruses do not elicit an immune reaction and immunostimulatory substances used to alter scrapie pathogenesis have been shown to shorten rather than to lengthen incubation periods (Dickinson et al., 1978; Kimberlin & Cunnington, 1978). Furthermore, evidence that the inhibitory effects of DS500 on scrapie infection are not due to its action as a B cell mitogen has been published (Kimberlin & Walker, 1986).

SP54 is also known to inhibit the replication of HIV (Biesert et al., 1988; Baba et al., 1988). AIDS is another slow virus disease in which the virus replicates in macrophages during the early pathogenetic process (Gendelman et al., 1989). In this case, binding of the virus to susceptible cells is thought to be inhibited by the drug (Baba et al., 1988). In scrapie pathogenesis, however, such a mechanism is inconsistent with the long lasting effect that the drug exhibits.

SP54 has been shown to inhibit in vitro not only reverse transcriptase activity (Biesert et al., 1988; Baba et al., 1988) but also, even more extensively, RNase H activity (Moelling et al., 1989). Both enzymes are essential for the replication of retroviruses and exhibit their activities in the cytoplasm of the infected cells exactly where the polyanion DS500 has been shown to accumulate in vivo.
SAF, which appears as three major protein bands between 29K and 20K. (a) Controls of mock-treated, scrapie-infected mice. The animals positive and killed on days 166, 166, 173, 176, 176, 180 and 183, dilution of scrapie-infected brain homogenate (E×pt. 2). The animals from which the extracts shown in lanes 1 to 7 were diagnosed scrapie-diagnosed scrapie-positive and killed on day 295 with ascites and those from which samples in lanes 1 to 3 were taken died on days 246, 262 and (Ehlers of scrapie. represented in lanes 5 to 8 were killed on day 304 with no clinical signs pathogenesis through an inhibitory effect on enzymes in the cytosol of those cells capable of phagocytosis, which also play a role in the uptake and multiplication of scrapie virus. Trials to verify the accumulation of SP54, in analogy to DS 500, produces its effect on scrapie also play a role in the uptake and multiplication of cells of the spleen by histological staining with toluidine blue failed (data not shown), although the same procedure readily detected DS500 up to 100 days after administration (Ehlers et al., 1984). If technical problems are not the reason for our failure to find SP54 in phagocytosing cells in vivo then there may be a possibility that the results presented here indicate the existence of a cellular defence mechanism of a virus-infected host that hitherto has escaped detection.

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References


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