Sulphate polyanions prolong the incubation period of scrapie-infected hamsters

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The effect of the organic sulphated polyanions, pento-san sulphate (SP54), dextran sulphate 500 (DS500) and suramin, have been tested on golden Syrian hamsters infected with the 263K strain of scrapie by the intraperitoneal (i.p.) or the intracerebral route. SP54 had the greatest effect in prolonging the incubation period of the disease when administered within 2 h of the i.p. inoculum. The same amount of SP54 given 24 h after scrapie inoculation had a potent effect in some animals and no effect in others. This result suggests that SP54 inhibits the uptake of the scrapie agent into the nerve endings and/or carrier cells at the site of the inoculum, i.e. the peritoneum, and that this event occurs in about 24 h. DS500 had a similar although less potent effect (22-4 days delay during the incubation period) than SP54 (54.4 days) when administered within 2 h of scrapie injection by the i.p. route, and suramin had only a minimal effect (10 days). This study suggests that treatment of scrapie and related spongiform encephalopathies of animals and man is possible only before the agent has reached the clinical target areas of the brain.

Introduction

The antiviral property of sulphated polyanionic substances on several enveloped RNA and DNA viruses has been known for almost 2 decades (Baba et al., 1988). Recently, these compounds have received increasing attention since the discovery of their in vitro effect on AIDS-related viruses (Ito et al., 1987; Moelling et al., 1989).

Some polyanions also prolong the incubation period of scrapie disease in experimental animal models (Ehlers & Diringer, 1984; Kimberlin & Walker, 1983a, 1986a; Farquhar & Dickinson, 1986; Diringer & Ehlers, 1991). Polyanions are taken up by cells of the reticuloendothelial system (RES) (Ehlers et al., 1984); this may prevent the entrance and/or the replication of the infectious agent in these cells and delay the appearance of scrapie disease (Ehlers & Diringer, 1984).

Scrapie is a central nervous system (CNS) disease naturally occurring in sheep and goats which is caused by a transmissible, as yet not characterized, agent (Brunori et al., 1988). In mice, the RES is essential for scrapie pathogenesis when the agent is injected by peripheral routes [e.g. intraperitoneally (i.p.)]. Scrapie replicates in the spleen before it becomes detectable in the CNS (Kimberlin & Walker, 1979) and as a consequence, the removal of the spleen before or soon after scrapie injection lengthens the incubation period of the disease (Fraser & Dickinson, 1970, 1978). This does not occur in i.p. infected hamsters where the replication of scrapie in the RES is believed not to be essential for the progression of the virus from the periphery to the CNS (Kimberlin & Walker, 1986b). The simultaneous rise of infectivity in the spleen and in the brain after i.p. injection of the scrapie agent (Casaccia et al., 1989), and the failure of splenectomy to prolong the incubation period of the disease (Kimberlin & Walker, 1977, 1986b), support this hypothesis.

Thus, it has been postulated that polyanions would be less effective in scrapie-infected hamsters than in scrapie-infected mice (Kimberlin & Walker, 1986b). In order to verify this hypothesis we have investigated the effect of three sulphated organic polyanions on scrapie-infected hamsters.

Methods

Scrapie strain and animals. Golden Syrian hamsters (from a colony bred at the Catholic University, Rome) were injected either intracerebrally (i.c., left hemisphere) or i.p. (lower left quadrant) with 0.05 ml of a 10% (w/v) suspension of pooled brains derived from hamsters clinically affected with the 263K strain of scrapie (Kimberlin & Walker, 1977). For the titration experiment after i.p. injection, serial 10-fold dilutions of the 10% brain homogenate were prepared in sterile PBS. Each sample was sonicated three times for 15 s in an Ultratip Labsonic System (Lab Line) prior to dilution, and stored at -70 °C.
show the ED_{50} of the drug. The experimental points were fitted using the computer program ALLFIT.

Before use, samples were thawed, vigorously vortexed and 0.1 ml of each dilution was injected i.p. into six animals. Animals were housed three per cage with food and water (Pocchiari et al., 1987). Data are reported as mean incubation period ± S.E.M. The titre estimate was calculated by the method of Reed & Mench (1938).

Polysulphates. Dextran sulphate 500 (DS500; Fluka) and pentosan polysulphate (SP54; Sigma) are polymers of sugars (glucose and xylose, respectively) with an approximate polysulphate (SP54; Sigma) are polymers of sugars (glucose and xylose, respectively) with an approximate

value of 500K and 5K, respectively, containing approximately two sulphate groups per sugar unit. DS500 and SP54 were dissolved and diluted in physiological saline immediately before use, and 0.5 ml was injected i.p. into the lower right quadrant of the abdomen at different times after scrapie inoculation (see Results). The dose–response curve of DS500 shown in Fig. 1 was obtained using the weighted non-linear least square computer curve-fitting program ALLFIT (De Lean et al., 1978).

Suramin, a symmetrical trisodium salt of a sulphonated organic acid (benzamido-benzamido-naphthalene trisulphonic acid) of urea, contains six sulphate groups per molecule. Vials (1 g) of suramin (Germanin; Bayer) were divided into 50 mg samples and kept in the dark under vacuum. Immediately before use, each sample of suramin was dissolved in 5 ml of sterile pyrogen-free distilled water, diluted in physiological saline, and 0.5 ml was injected i.p. following the schedule reported in Table 4.

Control groups were scrapie-infected hamsters inoculated by the same route and on the same day as treated animals, but i.p. injected with 0.5 ml of physiological saline.

Results

DS500

The effect of single doses of DS500 administered within 2 h of i.p. scrapie injection was dose-dependent up to 32 mg/kg (the average weight of a hamster was about 100 g), after which there was no further increase in the incubation period of treated animals (Table 1). The effect of injection of high concentrations (80 to 100 mg/kg) of DS500 was difficult to assess because only a few animals survived the acute toxicity of the drug (Table 1). The TD_{50} of DS500 calculated by the method of Spearman and Kärber (Dougherty, 1964) was 85.4 mg/kg of body weight. The maximal efficacy of DS500 in delaying the appearance of scrapie disease was 22.0 days and the ED_{50} was 16.8 mg/kg (Fig. 1). The therapeutic index of DS500 (i.e. the TD_{50}/ED_{50} ratio) in prolonging the incubation period of scrapie disease induced by the i.p. route was 5.08.

The effectiveness of a single injection of 40 mg/kg of DS500 was then tested in i.c. scrapie-infected hamsters. This dose was chosen because it caused the greatest lengthening of the incubation period in i.p. scrapie-infected hamsters without producing any toxic consequence (Table 1). The drug was given the same day or the week prior to i.c. scrapie inoculation and in both cases it produced a short but significant delay (6 days, P < 0.001, and 5.3, P < 0.05, respectively) in the appearance of scrapie disease (65.7 ± 1.0 days, n, 21, and 65.0 ± 0.9 days, n, 9, respectively) compared with untreated scrapie-infected hamsters (59.7 ± 0.9 days, n, 21).

SP54

Single doses of 50 and 100 mg/kg of SP54 similarly delayed the appearance of scrapie disease induced by the i.p. route, regardless of the time of drug administration (Table 2). However, there was always a great variability in the incubation periods of treated animals in comparison with controls (Fig. 2). Moreover, the administration of the drug 24 h after scrapie inoculation (Fig. 2) resulted in some animals having an exceptionally long incubation period (two of them developed scrapie after 200 days...
Polyanions in scrapie-infected hamsters

Table 1. Effect of DS500 in i.p. scrapie-infected hamsters

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Incubation period (days; mean ± S.E.M.) n</th>
<th>Delay (days)</th>
<th>Acute toxicity of DS500 (injected/dead)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0†</td>
<td>103.8 ± 1.6 51</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>105.7 ± 3.9 12 1-9 NS 12/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>116.5 ± 4.6 11 12-7 &lt;0.005 11/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>123.8 ± 7.3 9 20-0 &lt;0.001 9/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40†</td>
<td>126.3 ± 3.0 17 22-5 &lt;0.001 17/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60†</td>
<td>128.3 ± 4.2 18 21-2 &lt;0.001 21/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>128.3 ± 5.7 32 24-5 &lt;0.001 9/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>123.5 ± 7.1 42 19-7 &lt;0.005 12/7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Determined by two-tailed t-test; NS, not significant.
† Data are the mean of two separate experiments.
‡ One further animal died during the experiment for reasons unrelated to either acute toxicity of DS500 or scrapie.

Table 2. Effect of SP54 in i.p. scrapie-infected hamsters

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose (mg/kg)</th>
<th>Incubation period (days; mean ± S.E.M.) n</th>
<th>Delay (days)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>–</td>
<td>105.2 ± 2.9 9</td>
<td>67.2 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>2†</td>
<td>50</td>
<td>105.3 ± 2.9 12</td>
<td>73.5 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>3‡</td>
<td>100</td>
<td>147.7 ± 10.2 10</td>
<td>36.4 &lt;0.005</td>
<td></td>
</tr>
</tbody>
</table>

* Determined by two-tailed t-test.
† SP54 was administered 24 h after scrapie injection.
‡ SP54 was administered within 2 h of scrapie injection.

Table 3. Effect of SP54 on the titre of 263K scrapie injected i.p. into hamsters

<table>
<thead>
<tr>
<th>Dilution of scrapie brain</th>
<th>Control</th>
<th>SP54*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected/scrapie</td>
<td>Injected/scrapie period (days)</td>
<td>Injected/scrapie period (days)</td>
</tr>
<tr>
<td>10^-5</td>
<td>6/6</td>
<td>188 ± 14.8 5/3</td>
</tr>
<tr>
<td>10^-5</td>
<td>6/2</td>
<td>219, 219 4/1</td>
</tr>
<tr>
<td>10^-6</td>
<td>5/2</td>
<td>335, 319 6/1</td>
</tr>
<tr>
<td>Titre†</td>
<td>5-0</td>
<td>44</td>
</tr>
</tbody>
</table>

* A single dose of SP54 (100 mg/kg) was administered i.p. (left quadrant) within 2 h of scrapie i.p. injection (right quadrant).
† Log10 LD50/0.1 g brain. Animals were observed up to 365 days.

post-inoculation), whereas in others (eight out of 24) there was no effect; the incubation period of these eight animals fell within 2 S.D. (18-6 days) of the mean of pooled controls (107-0 days, n, 30). On the other hand, treatment with SP54 within 2 h of scrapie injection always resulted in disease with an incubation period longer than that of controls (Fig. 2). In the experiment shown in Table 3, SP54 significantly prolonged the incubation periods of treated hamsters but had only a minimal effect on the titre (less than 10-fold).

A single i.p. administration of SP54 (100 mg/kg) also lengthened (14.1 days, P < 0.001) the mean incubation period of i.c. scrapie-infected hamsters (62-1 ± 2 days; n, 7) compared with controls (62-2 ± 1.4 days; n, 16). However, in this experiment, we did not observe the great variability in the appearance of the disease which is found after treatment with SP54 among the i.p. scrapie-infected animals.

Table 4. Effect of suramin in i.p. scrapie-infected hamsters

<table>
<thead>
<tr>
<th>Day of suramin treatment</th>
<th>Incubation period before (–) or after (+) scrapie infection*</th>
<th>Delay (days)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>–</td>
<td>108.7 ± 1.7 30</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>0, +7, +14, +21</td>
<td>113.0 ± 3.9 5</td>
<td>43</td>
</tr>
<tr>
<td>15</td>
<td>–7, –4, –1</td>
<td>117.0 ± 2.4 6</td>
<td>83</td>
</tr>
<tr>
<td>15</td>
<td>0, +3, +6</td>
<td>118.7 ± 2.7 6</td>
<td>104</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
<td>112.4 ± 4.7 8</td>
<td>37</td>
</tr>
<tr>
<td>40</td>
<td>0, +6</td>
<td>118.0 ± 5.0 6</td>
<td>93</td>
</tr>
</tbody>
</table>

* Hamsters treated the day of scrapie inoculation (0) received suramin within 2 h after the inoculum.
† Determined by two-tailed t-test; NS, not significant.

Suramin

In Table 4 the results of different schedules of suramin treatment in i.p. scrapie-infected hamsters are reported. The drug had a beneficial effect only when two or three doses were administered the week before or the week after the scrapie inoculation. There was no significant difference between the 15 and 40 mg/kg doses. No notable delay in the incubation period was observed when only a single injection of suramin was given within the week after scrapie injection. The administration of suramin three times/week at a dose of 40 mg/kg produced extensive necrosis of the lower abdomen at the site of injection, and this necessitated the sacrifice of the animals.

The effect of suramin therapy (15 and 40 mg/kg) was also investigated in i.c. scrapie-inoculated hamsters. Treatment was started the week before scrapie inoculation with either two (40 mg/kg) or three (15 mg/kg) doses and it was continued (once a week for both doses) until the appearance of clinical signs of disease. Notwithstanding the length of the treatment, no benefit was observed in challenged animals compared to controls.
The results of this study suggest that in hamsters polyanions may prevent the entry of the scrapie agent into peripheral nerve endings or carrier cells at the site of the inoculation, i.e. the peritoneum, and that this event occurs within 24 to 48 h after the injection of the infectious agent.

These drugs might also increase the aggregation of the scrapie agent at the site of the inoculum, facilitating its removal by phagocytic cells (Kimberlin & Walker, 1986a). Although this mechanism may have been responsible for some anti-scrapie effect when the drug and the agent were injected by the same route (i.e. in the peritoneum), it is unlikely that this was the only mode of action. In fact, the i.p. administration of SP54 and DS500 was still effective when the agent was injected by the i.c. route.

DS500 was less potent than SP54, and its effect, but not that of SP54, is dose-dependent as found in scrapie-infected mice (Ehlers & Diringer, 1984). However, it is possible that the dose dependence of SP54 was not noticed as the two doses tested (50 and 100 mg/kg) were already above the ED50 of the drug. The anti-scrapie effect of suramin was negligible.

It has been suggested that the 263K strain of scrapie in hamsters is taken up by nerve endings (or carrier cells) directly in the peritoneum and thereafter transported to the CNS without passing through the obligatory stage in the spleen as is seen in mice (Kimberlin & Walker, 1986b). If this happens about 24 h after the i.p. injection of scrapie, the SP54 treatment given 24 h after the inoculum may be critical; SP54 was found to be ineffective in animals where the scrapie had already been taken up by nerve endings, whereas it was effective in animals where the agent had not yet been taken up (Fig. 2). This was further confirmed by the uniformity of the beneficial effect of SP54 (Fig. 2) and DS500 in animals treated within 2 h of the scrapie injection.

A similar type of result is obtained in mice treated with DS500 when it is administered 1 to 2 months after the i.p. injection of the scrapie agent (Farquhar & Dickinson, 1986). Farquhar & Dickinson (1986) proposed an interaction between DS500 and peripheral nerves which may delay the spread of scrapie from the spleen and visceral lymph nodes along autonomic nerve fibres to the mid-thoracic cord, and then to the brain (Kimberlin & Walker, 1988). The higher neuroinvasiveness of the 263K strain compared with the mouse-adapted scrapie strains (Kimberlin & Walker, 1986b) may explain why, in hamsters, the effect of polyanions occurs at the site of the inoculum as early as 24 h after scrapie injection. Thus, in hamsters, infection is established within minutes of injection as in mice (Kimberlin & Walker, 1990), but, unlike in mice, the scrapie agent does not need to replicate in the RES before entering into the CNS.

When SP54 was administered to i.c. infected hamsters, it prolonged the incubation period by about 2 weeks in all the animals tested. It is of interest that in some of the i.p. infected animals not even this delay occurred. A possible explanation is that once the agent was injected into extraneural routes, it was quickly taken up by peripheral nerves/carrier cells and from here it directly reached the clinical target areas (CTA) of the CNS (Kimberlin & Walker, 1983b) leaving no possibility of altering the progression of the disease with drugs. In contrast, injection of the agent by the i.c. route into a non-CTA allowed, for a short period of time before the infectious agent was taken up by cells projecting into the CTA, interference in the pathogenesis of the disease. This mechanism differs from that of the anti-scrapie drug amphotericin B (Pocchiari et al., 1987, 1989), which works also during the transport of the scrapie agent to the CTA (Pocchiari et al., 1991). The anti-scrapie effect of DS500 in i.c. inoculated hamsters was minimal and this is likely to be due to its high Mr, preventing passage through the blood–brain barrier.

We conclude that in the hamster, sulphate polyanions exert their effect in the very early events of pathogenesis by inhibiting the uptake of the infectious agent from nerve endings and/or carrier cells. This mechanism of action differs from that found in mice where polyanions delay scrapie replication in non-neural permissive cells (i.e. splenocytes or other RES cells) during the weeks that precede the entrance of the infectious agent into the CNS (Ehlers & Diringer, 1984; Farquhar & Dickinson, 1986; Kimberlin & Walker, 1986a; Diringer & Ehlers, 1991). This explains why in mice, but not in hamsters, these drugs are efficacious even when administered weeks after scrapie inoculation (Ehlers & Diringer, 1984; Farquhar & Dickinson, 1986).

The authors wish to thank Dr R. Filippetti for his skilful assistance in hamster breeding. This study was supported by grants from MURST, CNR Progetto Finalizzato Chimica Fine II and CNR Progetto Finalizzato Invecchiamento (INV-91084).

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(Received 9 September 1991; Accepted 6 November 1991)