Amphotericin B Delays the Incubation Period of Scrapie in Intracerebrally Inoculated Hamsters

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SUMMARY

The scrapie-infected hamster has been considered an excellent model for the study of slow virus diseases of man (Creutzfeldt-Jakob disease) and animals. At the moment no therapy is available for the cure of these fatal central nervous system diseases, although several drugs have been tested. We found that amphotericin B (AmB), a polyene antibiotic, increased the incubation time of scrapie disease in animals infected by either the intraperitoneal or intracerebral route. Hamsters inoculated with a 10% brain suspension of the 263K strain of scrapie showed clinical signs of disease in 54.6 ± 4.7 days. Under AmB treatment (1 mg/kg for 6 days a week) the incubation time increased with the length of treatment, up to a maximum delay of 45 days. AmB may interact with the scrapie agent on cell plasma membranes and may thereby decrease the rate of scrapie replication. However, AraB did not have any effect when administered after the clinical onset of scrapie disease.

Experimental scrapie in hamsters is an excellent and convenient model to study unconventional slow viral infections naturally occurring in animals (scrapie), and man (Creutzfeldt-Jakob disease, CJD) (Gajdusek, 1977).

At the moment no therapy is available for the cure of these fatal central nervous system (CNS) diseases, although several drugs have been tested: antivirals, immunosuppressants, immunostimulants, interferon and interferon inducers, and others (for review, see Brown, 1984). However, two groups of drugs have been shown to increase the mean incubation period in rodents experimentally infected with scrapie by peripheral routes, i.e. (i) inorganic (HPA23: Kimberlin & Walker, 1979a, 1983) and organic (dextran sulphate: Ehlers & Diringer, 1984; Farquhar & Dickinson, 1986) polyanions and (ii) amphotericin B (AmB) (Amyx et al., 1984). The conclusion of these studies was that polyanions interact with the lymphoreticular system, inhibiting scrapie replication before the agent reaches the CNS. Dextran sulphate is ineffective when the animals are intracerebrally (i.e.) inoculated with the scrapie agent (Ehlers & Diringer, 1984). In contrast, AmB may increase the incubation period of scrapie disease by interacting with the agent on cell plasma membranes (Brown, 1984). Thus, it is reasonable to presume that AmB can also interfere with the structure of the plasma membranes of CNS cells and therefore may be active against scrapie infection even after i.c. inoculation of the agent.

We therefore studied the efficacy of AmB by determining the incubation period of scrapie disease in hamsters inoculated either i.c. or intraperitoneally (i.p.) with the 263K strain of scrapie in comparison with saline solution-treated scrapie-inoculated hamsters. We also determined the effects of an antiviral immunomodulator, methisoprinol, and two CNS metabolically active compounds, L-carnitine and L-acetylcarnitine, in i.c. scrapie-inoculated hamsters.

Weanling golden Syrian hamsters were inoculated either i.c. in the left hemisphere or i.p. with 0.05 ml of a 10% phosphate-buffered saline suspension of hamster brains infected with the 263K
Table 1. Mean incubation periods of intracerebrally scrapie-infected hamsters after intraperitoneal administration of different drugs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days (mean ± S.D.)</th>
<th>Animals (n)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>54.6 ± 4.7</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Saline solution (0.5 ml/6 days a week)</td>
<td>56.6 ± 1.9</td>
<td>14±</td>
<td></td>
</tr>
<tr>
<td>L-Carnitine (50 mg/kg/6 days a week)</td>
<td>55.6 ± 1.1</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>L-Acetylcarnitine (25 mg/kg/6 days a week)</td>
<td>56.3 ± 2.3</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Methisoprinol (500 mg/kg/6 days a week)</td>
<td>56.0 ± 1.4</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Amphotericin B for 50 days (1 mg/kg/6 days a week)</td>
<td>82.7 ± 5.1</td>
<td>7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amphotericin B for 75 days (1 mg/kg/6 days a week)</td>
<td>95.9 ± 3.5</td>
<td>8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amphotericin B for 100 days (1 mg/kg/6 days a week)</td>
<td>101.6 ± 2.2</td>
<td>7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Determined by t-test; NS, not significant.
† Hamsters were eight in the first experiment and six in the second.

strain of scrapie (Kimberlin & Walker, 1977). Animals were housed two or three per cage with water and food ad libitum. Hamsters were observed 6 days a week and scored from 1 to 5 as follows: 1, normal animals; 2, animals which showed an increased alertness to noise or were irritable and aggressive when handled; 3, animals which showed evident clinical signs of disease such as tremor of the head and wobbling gait; 4, as 3 but with spontaneous and frequent backrolls; and 5, animals which were no longer able to stand up in the cage. Hamsters were considered positive for scrapie infection when they reached a score of 3. Animals in the advanced stage of disease (score 5) were killed with chloroform for neuropathological evaluation, which always confirmed the clinical diagnosis.

Hamsters were treated with the following drugs: L-carnitine (50 mg/kg), L-acetylcarnitine (25 mg/kg), methisoprinol (500 mg/kg) and AmB (the dose was gradually increased from 0.1 to 1 mg/kg over 2 weeks). Treatment was started on the same day as scrapie inoculation unless otherwise indicated. L-Carnitine, L-acetylcarnitine and methisoprinol were obtained from the Sigma-tau Pharmaceutical Company, Pomezia, Italy, and AmB (Fungizone) from E. R. Squibb & Sons, Rome, Italy. Once dissolved, the drugs were kept in the dark at 4 °C for no longer than 1 week. Drugs were diluted appropriately and 0.5 ml was injected 6 days a week by the i.p. route using the lower left and right quadrant alternatively. Controls were scrapie-inoculated animals of the same age which received 0.5 ml saline solution (0.9% NaCl).

In our experience hamsters inoculated i.c. with a 10% brain suspension of the 263K strain of scrapie showed clinical signs of disease (score 3) in 54.6 ± 4.7 days (mean ± s.d., n = 62) and advanced clinical signs (score 4) in the subsequent 13.0 ± 5.1 days.

In the first experiment five groups of eight hamsters were inoculated i.c. with the 263K strain and treated with saline solution, L-carnitine, L-acetylcarnitine, methisoprinol or AmB. The animals were treated until they showed clinical signs of disease, with the exception of AmB treatment which was stopped 75 days after inoculation when the animals were still clinically normal (Table 1). AmB was able to delay the incubation period by 39.3 days compared to the control group, whereas the other drugs were ineffective. Thus, we planned a second experiment to evaluate whether the increase in the incubation period was related to the duration of treatment. A group of seven i.c. scrapie-inoculated animals was treated for 50 days with the same dose of AmB used in experiment 1 and another group of seven hamsters was treated with AmB until they showed clinical signs of disease (score 3). Controls (six animals) received saline solution as in experiment 1. As shown in Table 1, 50 days of AmB treatment increased the incubation period of the disease by 26.1 days whereas those animals receiving continuous
administration of the drug manifested the disease in $101.6 \pm 2.2$ days, which corresponded to a delay of $45.0$ days.

If we take into consideration the half-life of AmB (about $15$ days; Atkinson & Bennett, 1978) there is a perfect linear correlation ($r = 0.99$) between the period of pharmacological effectiveness of AmB and the increase in the incubation period in comparison with the control animals (see Fig. 1).

In another group of hamsters ($n = 10$) AmB was given only after the onset of scrapie disease (score $3$) and the time taken to reach the advanced clinical stage of the disease (score $4$) was $14.1 \pm 2.5$ days. When saline solution was administered to control animals ($n = 7$) the time interval was $14.3 \pm 3.7$ days. Thus, AmB did not modify the clinical course of scrapie disease.

When AmB was administered to hamsters inoculated i.p. with the $263K$ scrapie strain, the incubation period of the disease was significantly delayed. Indeed, hamsters ($n = 5$) receiving AmB treatment for $50$ days showed clinical signs of disease at $162.8 \pm 31.3$ days but those ($n = 5$) receiving saline solution showed scrapie disease at $107.2 \pm 6.9$ days. In another group of five i.p. infected hamsters we decided to administer AmB until the appearance of the disease. However, three animals died at $108, 133$ and $145$ days after inoculation with no sign of disease while in the other two animals the treatment was stopped after $147$ days; these hamsters showed clinical signs of disease (stage $3$) at $204$ days after inoculation. To exclude the possibility that AmB had interacted with the inocula when they were injected by the same route, we mixed $0.05$ ml of inoculum with $100 \mu g$ of AmB dissolved in $0.5$ ml saline solution for $2$ h at room temperature. This mixture was injected i.p. in the lower left quadrants of five animals which were then treated with saline solution for $50$ days as were the controls. The incubation period in these animals was $107.4 \pm 7.8$ days which showed that AmB did not directly interact with the scrapie agent.

We report for the first time a delay in the mean incubation period of scrapie disease in i.c. inoculated hamsters which were treated daily with AmB. Moreover, we confirm previous data showing a beneficial effect of AmB in i.p. scrapie-inoculated animals (Amyx et al., 1984; Brown, 1984). Besides AmB, other drugs, inorganic and organic polyanions (Kimberlin & Walker, 1983; Ehlers & Diringer, 1984; Farquhar & Dickinson, 1986), have been shown to increase the incubation period of scrapie disease following extraneural infection. These drugs modify the cells of the reticuloendothelial system, interfering with the early replication of the scrapie agent in spleen and lymph nodes. Pathogenetic studies of scrapie disease in mice (Kimberlin & Walker, 1979 $b$) and in hamsters (Kimberlin & Walker, 1986) show that with peripheral routes of infection the agent replicates in lymphoid tissues before entering the CNS. This is not an
obligatory step in pathogenesis when the i.c. route of infection is used. This might explain why the polyanions are ineffective on scrapie disease when the agent is inoculated i.c. in animals (Ehlers & Diringer, 1984). Therefore it is likely that AmB is effective on scrapie disease by a different mode of action.

AmB is a polyene antibiotic used for more than 25 years in the treatment of systemic fungal infections. Its peculiar structure (it has both a hydrophilic and a hydrophobic group) allows it to interact readily with the sterols present in the membranes of fungi, altering the ionic balance of the cells and causing their death. The effect on membranes is not limited to fungi but extends also to animal cell membranes (for review, see Medoff et al., 1983). Thus, it has been proposed (Brown, 1984) that AmB-altered membranes of host cells could, by an unknown mechanism, interfere with the replication of the scrapie agent. Our study shows that this mechanism, proposed for extraneural infections, is also valid for i.c. infected animals. Thus, it is reasonable to suppose that AmB crosses the blood–brain barrier and interacts with the scrapie agent on the plasma membranes of the CNS target cells. These cells are likely to be neurons (Masullo et al., 1984; Pocchiari et al., 1985; Kretzschmar et al., 1986). However, we can not rule out the possibility that AmB is active on scrapie disease, especially when obtained by extraneural infection, through a mechanism mediated by the activation of macrophages (Lin et al., 1977), in this case, AmB and polyanion drugs might have a similar mode of action.

The linear correlation between the delay in the onset of disease and the activity of AmB suggests that the drug is effective during the whole incubation period of the disease, and that the clinical appearance of scrapie disease depends on the length of AmB treatment. On the other hand, AmB is ineffective when administered during the clinical stage of the disease and this supports the hypothesis (Braig & Diringer, 1985) that the replication of the scrapie agent terminates before the onset of clinical disease. Therefore we agree with Braig & Diringer (1985) that any treatment of clinical cases of CJD with drugs directed against the replication of the agent(s) would be ineffective. Clinical data on the treatment of CJD (Brown, 1984) confirm this hypothesis although there are some reports of a prolongation of the disease after treatment with amantadine (Terzano et al., 1983), vidarabine (Furlow et al., 1982) or methisoprinol (Villa et al., 1982). However, experimental data on scrapie and CJD (Kimberlin & Walker, 1979a; Tateishi, 1981; and the present data) fail to show any effect of these drugs on slow virus diseases.

In conclusion, the study of the interaction between AmB and the scrapie agent is a valuable tool in the understanding of the pathogenesis of the slow viral infections of the CNS irrespective of the usefulness of AmB in CJD therapy.

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REFERENCES


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