

## MS-8209, a New Amphotericin B Derivative, Provides Enhanced Efficacy in Delaying Hamster Scrapie

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**To test the efficacy of a new amphotericin B derivative, MS-8209, in delaying scrapie, hamsters were infected intracerebrally with the 263K scrapie agent and treated with MS-8209 either early in the course of the disease or continuously. The results show that (i) all treatments lengthened the incubation period of hamster scrapie, (ii) continuous treatment with MS-8209 doubled the length of the incubation period compared with that observed in infected, untreated animals, and (iii) all treatments delayed the accumulation of a proteinase-resistant prion protein and glial fibrillary acidic protein in the brain. These findings suggest that MS-8209 is a powerful tool for investigating the pathogenesis of transmissible subacute spongiform encephalopathies.**

Experimental scrapie in hamsters is a simple, reproducible model of transmissible spongiform encephalopathies (TSE) which have been described for many mammalian species. TSE include Creutzfeldt-Jakob disease in humans and scrapie in sheep and goats. These fatal diseases are characterized by a long incubation period and histopathological changes such as neuronal vacuolization and astrogliosis in the central nervous system. TSE are caused by as-yet-unknown infectious agents named transmissible spongiform agents, or prions, and characterized by the accumulation of an abnormal proteinase-resistant isoform (PrP-res) of the host-encoded prion protein (PrP), derived from PrP posttranslational modifications (2). The transcriptional accumulation of glial fibrillary acidic protein (GFAP), a specific marker of astrocytes, has also been observed (5).

At present, no treatment is available for TSE. Many unsuccessful therapeutic trials have been performed in experimental animal models; the trials have included the use of hormones, antibiotics, antiviral drugs, and antifungal agents (3). Three groups of drugs exhibited relative efficacy against scrapie: polyanions (6), the amyloid-binding dye Congo red (7), and amphotericin B (AmB) (1, 12). AmB belongs to the group of polyene antibiotics with a ring structure that includes a hydrophobic region of conjugated double bonds and a hydrophilic region of hydroxyl groups with a mycosamine sugar moiety. AmB is an effective antifungal agent that is widely used for the treatment of systemic fungal infections such as candidiasis, histoplasmosis, and aspergillosis (9). It has been reported to delay the appearance of experimental scrapie in hamsters infected intracerebrally or intraperitoneally with the 263K scrapie strain (11). AmB also delayed PrP-res accumulation in the brain (15), but its effect on scrapie agent replication remains unclear (8, 15). The efficacy of AmB is limited by the absolute need to start the treatment in the early stages of scrapie infection and by its acute toxicity. MS-8209 is a polyene macrolide antibiotic derived from AmB which exhibits identical antifungal properties but in addition possesses greater water solubility and is at least five times less toxic (14). MS-8209

is thus an antiviral drug that might be useful for experimental chemotherapeutic studies. We previously observed that it is efficient in delaying the incubation period in mouse scrapie (4). In view of this, we investigated the pharmacological effects of MS-8209 in hamster scrapie. The experiments reported here were designed (i) to obtain the maximum protective effect of MS-8209 in continuous treatment of hamster scrapie, (ii) to compare the efficacy of MS-8209 with that of AmB, and (iii) to investigate, by comparing the effects of early and continuous treatments, the action of MS-8209 on PrP-res and GFAP accumulation in the central nervous system.

Outbred, weanling female golden Syrian hamsters (René Janvier, Centre d'élevage, Le Genest-St-Isle, France) were injected intracerebrally with the 263K scrapie agent, a gift from H. Fraser, Edinburgh, United Kingdom (titer of the stock suspension,  $4.4 \times 10^8$  50% lethal doses/g of brain). Fifty microliters of 1% (wt/vol) brain homogenate was injected into the right cerebral hemisphere. All animals were over 8 weeks of age at the time of infection. Parenteral desoxycholate AmB (Fungizone; Squibb) was obtained from the Squibb Laboratory. MS-8209 is the *N*-methylglucamine salt of 1-deoxy-1-amino-4,6-*O*-benzylidene-*D*-fructosyl-AmB (Mayoly Spindler Laboratories, Chatou, France). Drugs were diluted in 5% glucose (wt/vol) sterile solution. Animals were treated by the intraperitoneal route 6 days a week, either throughout the duration of the disease until death or for 1 week before, after, or at the time of scrapie infection. They were regularly monitored for the onset of clinical symptoms. Control groups were either untreated or injected intraperitoneally with the same volume of *N*-methylglucamine. Three hamsters of each group were killed by cervical column disruption to estimate PrP-res and GFAP accumulation in the brain. The central nervous system (including the cerebellum) was then removed, rapidly frozen in liquid nitrogen, and kept at  $-80^\circ\text{C}$  until use. Brain homogenates were suspended at 20% (wt/vol) in a 5% glucose solution. Aliquots (50  $\mu\text{l}$ ) were digested with 50  $\mu\text{g}$  of proteinase K per ml for 1 h at  $37^\circ\text{C}$ . Denaturation buffer containing 150  $\mu\text{l}$  of Tris-glycine, 2% sodium dodecyl sulfate (SDS), 2% 2-mercaptoethanol, and 5% sucrose was added, and the samples were heated at  $100^\circ\text{C}$  for 5 min. They were then sonicated and centrifuged for 5 min at 15,000 rpm at  $20^\circ\text{C}$  and separated by SDS-polyacrylamide gel electrophoresis at 100 V for 2 h. Proteins were then transferred onto nitrocellulose membranes

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TABLE 1. Efficacy of continuous treatment with AmB and MS-8209 in hamsters infected intracerebrally with the 263K scrapie agent and intraperitoneal treatment against scrapie for 6 days a week

Treatment (mg/kg) <sup>a</sup>	Survival time (mean d.p.i. ± SEM) <sup>b</sup>	Delay in scrapie onset (days) <sup>c</sup>
None	86.6 ± 1.34	
AmB (2.5)	144.0 ± 1.73	57.4
MS-8209 (2.5)	151.5 ± 0.71	64.9
MS-8209 (10)	171.0 ± 1.00	84.4
MS-8209 (25)	171.1 ± 1.22	84.5

<sup>a</sup> Each treatment group contained six hamsters.

<sup>b</sup> d.p.i., days postinfection.

<sup>c</sup> All treatment values are significantly different from the control at a *P* value of <0.005 (Mann-Whitney test).

by electrotransfer. The membranes were saturated with a milk buffer (5% [wt/vol] milk, 0.1% Tween 20, and 0.1% NaN<sub>3</sub> in phosphate-buffered saline [PBS]), immunostained for 263K PrP-res with 3F4 monoclonal antibody (kindly provided by R. J. Kascsak, Institute for BRDD, New York, N.Y.), washed three times in PBS containing 0.1% Tween 20, and incubated for 30 min at room temperature with monoclonal antibodies to mouse immunoglobulin conjugated with peroxidase. Immunodetection was carried out with an enhanced chemiluminescence kit (Amersham). Signals were quantified by laser densitometry.

The first experiment was designed to determine the optimum pharmacological effect of continuous treatment with MS-8209 or AmB. Hamsters were treated from the day of inoculation and throughout the course of the disease until death. As shown in Table 1, the pharmacological action of MS-8209 is dose dependent, and all tested doses were effective. Both AmB and its derivative significantly increased the incubation time of the disease. Animals treated with 2.5 mg of AmB or MS-8209 per kg of body weight had mean incubation periods prolonged by 57.4 and 64.9 days, respectively (Table 1). Doses of 10 and 25 mg of MS-8209 per kg induced delays in scrapie onset of 84.4 and 84.5 days, respectively. These delays correspond to 100% of the incubation period for hamster scrapie in infected, untreated animals.

The second experiment was designed to determine whether 6 consecutive days of treatment initiated before, after, or on the day of experimental infection would also be effective.

(i) **Hamsters treated from 0 to 6 days after infection.** Daily doses of 2.5 mg of MS-8209 and AmB per kg increased the mean incubation time of scrapie by 15 and 17 days, respectively (Fig. 1A, treatment a). The highest dose of MS-8209 (25 mg/kg) prolonged survival time by 36.5 days (Fig. 1A, treatment a).

(ii) **Hamsters treated with a daily dose for 6 days, either 2 weeks before infection or 1 week before and 1 week thereafter (Fig. 1A, treatments b and c).** Low doses of 1 mg of AmB per kg and 2.5 mg of MS-8209 per kg prolonged survival time by 18.6 and 16.6 days, respectively (Fig. 1A, treatment b). For hamsters given the highest dose of MS-8209 (25 mg/kg) 2 weeks before infection, survival time was prolonged by 31 days, and for those given this dose 1 week before and 1 week after infection, survival time was prolonged by 36 days (Fig. 1A, treatment c).

(iii) **Hamsters given a single high dose of MS-8209 or AmB given shortly after infection.** As shown in Fig. 1A, treatment d, administration of 50 or 100 mg of MS-8209 per kg or 50 mg of AmB per kg 2 h after scrapie infection increased the mean incubation periods by 20, 22.5, and 21.6 days, respectively.

However, in this experiment, two of six hamsters in each group died of acute toxicity.

To examine the effects of MS-8209 and AmB on PrP-res and GFAP accumulation in the central nervous system, three hamsters in each group of animals were killed at 72 days postinfection, which is when clinical signs appear in untreated, infected hamsters. The study was carried out both in hamsters treated early (0 to 6 days postinfection) and in those given continuous treatment. In hamsters given early treatment, brain PrP-res levels were markedly lower in the treated groups than in the controls (Fig. 1B). These levels were identical to those found in untreated, infected hamsters at 50 days postinfection (Fig. 1B). In hamsters given continuous treatment, the PrP-res and GFAP levels in the brain also dropped (data not shown). However, at the terminal stage of the disease, all brains from all groups contained similar levels of PrP-res and GFAP (data not shown).

AmB has been shown to prolong the clinical course of scrapie in hamsters infected intracerebrally and intraperitoneally with the 263K scrapie strain (11–13). Our previous studies showed that MS-8209, a new AmB derivative, was effective in lengthening the incubation period in mouse scrapie (C506M3 strain) (4). The beneficial effect of MS-8209 was observed after both intracerebral and intraperitoneal inoculation, and it differed from the action of mepartricin, another AmB analog,

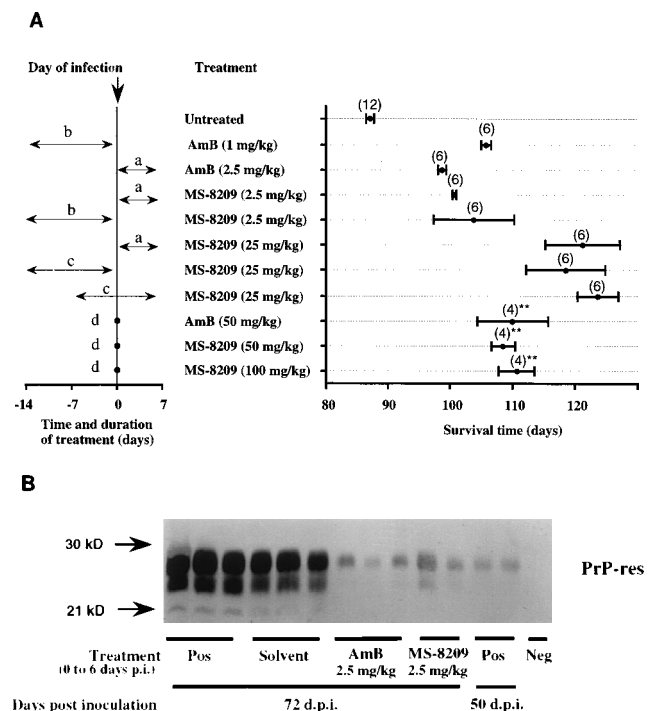


FIG. 1. (A) Effects of AmB and MS-8209 administered before or after intracerebral inoculation of hamsters with the 263K scrapie strain. Hamsters were treated daily 6 days a week by the intraperitoneal route. Survival times are shown as means ± standard errors (horizontal bars). Values in parentheses are numbers of hamsters used. \*\*, Two of the six animals in each group died of acute toxicity within 48 h of drug administration. (B) PrP-res detection by Western blotting (immunoblotting) in brains from untreated hamsters and hamsters treated with AmB or MS-8209 from 0 to 6 days postinfection and killed at 72 days postinfection, which is when clinical symptoms appear in untreated, infected hamsters. Brain homogenates were digested with 50 µg of proteinase K per ml for 1 h at 37°C. AmB- and MS-8209-treated animals at 72 days postinfection exhibited PrP-res accumulation similar to that in controls at 50 days postinfection. p.i., postinfection; Pos, positive control; Neg, negative control; Solvent, *N*-methylglucamine.

which was effective only after intraperitoneal scrapie injection (11). The mechanism of action of AmB and MS-8209 in experimental scrapie is still unknown.

In the present study, we demonstrated that MS-8209 had a strong effect on hamster survival whatever treatment protocol was used. It was as efficient as AmB in prolonging survival time, but its lower toxicity allowed the use of higher doses, and it delayed hamster scrapie longer than AmB. In fact, when MS-8209 was administered as a long-term treatment at high doses (10 and 25 mg/kg), it delayed the appearance of the first clinical symptoms by 84.4 and 84.5 days, respectively; i.e., it doubled the incubation periods relative to those in infected, untreated hamsters. The longest delay reported until now for hamsters infected intracerebrally and given AmB as a long-term treatment (1 mg/kg 6 days a week) was only 45 days (13).

Treatments carried out close to the moment of experimental scrapie infection were effective. Furthermore, we observed that treatments administered a few days before infection and stopped on the day of infection (day 0) were as efficient as those performed after infection. These results prove that drug administration near the time of infection is crucial for successful therapy. A likely explanation is that soon after infection MS-8209 interferes with the scrapie agent or its hypothetical receptor molecule on the target cells in the central nervous system. This mechanism might reduce the infectivity titer of the inoculum by a clearance process and then increase the incubation period of the experimental disease. If true, this would explain why the administration of treatment a few hours before or after infection is of crucial importance, because its effectiveness would depend on whether the scrapie agent had already been directed towards the target cells in the nervous system. Moreover, it was reported that the antiscrapie effect of AmB is mainly limited to the period of treatment covering the early stages after infection (13), suggesting that its mechanism of action is similar to that of MS-8209.

At the molecular level, analyses were carried out to evaluate the efficacy of MS-8209 therapy in terms of PrP-res and GFAP accumulation in the brain. The present comparison of the accumulation of these markers in the brains of hamsters treated with MS-8209 or AmB and in the brains of untreated animals at 72 days postinfection (when clinical symptoms appear in untreated, infected hamsters) indicates that both early and continuous treatments significantly delayed PrP-res and GFAP accumulation. These results are in agreement with those of previous studies (4, 8, 15) and suggest that the delay in the onset of clinical symptoms in treated hamsters is linked to the delay in PrP-res accumulation. They also support the hypothesis that PrP-res has a neurotoxic effect (10).

In conclusion, MS-8209 is a new hydrosoluble AmB analog which is more effective than AmB in prolonging survival time in experimental scrapie. Since the apparent toxicity of MS-

8209 is at least five times lower than that of AmB, the use of higher doses might improve the action of the drug on scrapie agent replication in the brain. Although it is important to stress that at the present time this derivative does not constitute a realistic treatment for human TSE, we believe that MS-8209 could constitute a potent drug for studying the pathogenesis of and therapeutic strategies in transmissible subacute spongiform encephalopathies.

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#### REFERENCES

1. Amyx, H., A. M. Salazar, D. C. Gajdusek, and C. J. Gibbs. 1984. Chemotherapeutic trials in experimental slow virus disease. *Neurology* **34**:149.
2. Bolton, D. C., M. P. McKinley, and S. B. Prusiner. 1982. Identification of a protein that purifies with the scrapie prion. *Science* **218**:1309-1311.
3. Brown, P. 1990. A therapeutic panorama of the spongiform encephalopathies. *Antivir. Chem. Chemother.* **1**:75-83.
4. Demaimay, R., K. T. Adjou, C. I. Lasmézas, F. Lazarini, K. Cherifi, J. P. Deslys, M. Seman, and D. Dormont. 1994. Pharmacological studies of a new derivative of amphotericin-B, MS-8209, in mouse and hamster scrapie. *J. Gen. Virol.* **75**:2499-2503.
5. Dormont, D., B. Delpech, A. Delpech, M. N. Courcel, J. Viret, P. Markovits, and L. Court. 1981. Hyperproduction de la protéine gliofibrillaire acide (GFAP) au cours de l'évolution de la tremblante expérimentale de la souris. *C. R. Acad. Sci.* **293**:53-56.
6. Ehlers, B., and H. Diringer. 1984. Dextran sulphate 500 delays and prevents mouse scrapie by impairment of agent replication in spleen. *J. Gen. Virol.* **65**:1325-1330.
7. Ingrosso, L., A. Ladogana, and M. Pocchiari. 1995. Congo red prolongs the incubation period in scrapie-infected hamsters. *J. Virol.* **69**:506-508.
8. McKenzie, D., J. Kaczkowski, R. Marsh, and J. Aiken. 1994. Amphotericin B delays both scrapie agent replication and PrP-res accumulation early in infection. *J. Virol.* **68**:7534-7536.
9. Medoff, G., J. Brajtburg, G. S. Kobayashi, and J. Bolard. 1983. Antifungal agents useful in therapy of systemic fungal infections. *Annu. Rev. Pharmacol. Toxicol.* **23**:303-330.
10. Müller, W. E. G., H. Ushijima, H. C. Schröder, J. M. S. Forrest, W. F. H. Schatton, P. G. Rytik, and M. Heffner-Lauc. 1993. Cytoprotective effect of NMDA receptor antagonists on prion protein (PrionSc)-induced toxicity in rat cortical cell cultures. *Eur. J. Pharmacol.* **246**:261-267.
11. Pocchiari, M., P. Casaccia, and A. Ladogana. 1989. Amphotericin B: a novel class of antiscrapie drugs. *J. Infect Dis.* **160**:795-802.
12. Pocchiari, M., S. Schmittinger, A. Ladogana, and C. Masullo. 1986. Effects of Amphotericin B in intracerebrally scrapie inoculated hamster, p. 314-323. *In* L. A. Court, D. Dormont, P. Brown, and D. T. Kingsbury (ed.), *Unconventional virus diseases of the central nervous system*. C.E.A. Diffusion, Fontenay aux Roses, France.
13. Pocchiari, M., S. Schmittinger, and C. Masullo. 1987. Amphotericin B delays incubation period of scrapie in intracerebrally inoculated hamsters. *J. Gen. Virol.* **68**:219-223.
14. Saint-Julien, L., V. Joly, M. Seman, C. Carbon, and P. Yeni. 1992. Activity of MS-8209, a nonester amphotericin B derivative, in treatment of experimental systemic mycoses. *Antimicrob. Agents Chemother.* **36**:2722-2728.
15. Xi, Y. G., L. Ingrosso, A. Ladogana, C. Masullo, and M. Pocchiari. 1992. Amphotericin B treatment dissociates *in vivo* replication of the scrapie agent from PrP accumulation. *Nature (London)* **356**:598-601.