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Prion Strain- and Species-Dependent Effects of Antiprion Molecules in Primary Neuronal Cultures[▽]

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Transmissible spongiform encephalopathies (TSE) arise as a consequence of infection of the central nervous system by prions and are incurable. To date, most antiprion compounds identified by in vitro screening failed to exhibit therapeutic activity in animals, thus calling for new assays that could more accurately predict their in vivo potency. Primary nerve cell cultures are routinely used to assess neurotoxicity of chemical compounds. Here, we report that prion strains from different species can propagate in primary neuronal cultures derived from transgenic mouse lines overexpressing ovine, murine, hamster, or human prion protein. Using this newly developed cell system, the activity of three generic compounds known to cure prion-infected cell lines was evaluated. We show that the antiprion activity observed in neuronal cultures is species or strain dependent and recapitulates to some extent the activity reported in vivo in rodent models. Therefore, infected primary neuronal cultures may be a relevant system in which to investigate the efficacy and mode of action of antiprion drugs, including toward human transmissible spongiform encephalopathy agents.

Transmissible spongiform encephalopathies (TSE), which include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy in cattle, and scrapie in sheep, are fatal, incurable neurodegenerative disorders caused by prions, a class of unconventional agents that target predominantly the central nervous system (14, 39). The only known, specific molecular marker of prion diseases is the abnormal prion protein (PrP^{Sc}), a misfolded form of the cellular PrP (PrP^{C}). Transmissibility is believed to stem from the ability of the prion isoform to promote the conformational transition from PrP^{C} to PrP^{Sc} (39, 40). Biologically distinct prion strains can propagate in the same host (for a review, see reference 9), presumably through the perpetuation of different, specific PrP^{Sc} conformers (47).

The search for drugs able to impede infection or prion-induced neuropathology currently relies on various experimental models, including an acellular PrP transconformation assay (27, 46), yeast prion systems (2), PrP^{Sc} accumulation in chronically infected mammalian cell lines (28), and assay in TSE animal models (for a review, see reference 51). Among many compounds selected for their ability to prevent PrP^{Sc} accumulation in cultured cells, only some of the most potent inhibitors significantly delay disease onset in prion-infected rodents. A few of them showed a therapeutic activity sensu stricto, and none was effective in clinically affected human patients (56). The reasons for these discrepancies remain unclear but probably include pharmacokinetic limitations (reviewed in reference 51). However, compounds known to cross the blood-brain barrier such as quinacrine and chlorpromazine proved to be ineffective in vivo (4, 5, 21). It is conceivable that biological differences between the available permissive cell lines and postmitotic neurons, the primary target of prions, may account for the disparity between in vitro and in vivo results. In addition, there is evidence to suggest that drug efficacy may depend upon the infecting prion strain (18, 28). Thus, there is a need for in vitro screening systems able to replicate different strains in a congruent cellular context and to predict more accurately the in vivo potency of antiprion drugs.

Dissociated primary neurons can be explanted from various brain regions from a wide range of organisms, thus allowing the growth of highly differentiated neuronal subtypes. These systems have several advantages for in vitro studies. They make an individual living cell with a phenotype very close to the in vivo one accessible for local application of pharmacological compounds or neurotropic infectious agents and allow morphological studies of, for example, neuronal connectivity and viability. As such, primary neuronal cultures are valuable tools routinely used for neurotrophic and antiapoptotic drug evaluation in neurodegenerative as well as infectious diseases (15, 35, 52, 57).

The propagation of sheep prions in primary nerve cell cultures derived from transgenic mice overexpressing ovine PrP has been recently reported (16). We show here that it is feasible to propagate rodent and human prions in cultures derived from transgenic mouse lines expressing the cognate PrP^{C}. Using this cell system, we assayed the antiprion activity of three generic compounds that are known to cure prion-infected cell lines and for which efficacy has been evaluated in vivo (see Discussion). In primary cell culture, clear differences in the efficacy of these compounds were observed depending on the prion strain and/or species combination.

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MATERIALS AND METHODS

Mouse lines. Primary neuronal cultures were established from the following transgenic mouse lines: $PrP^{0/0}$ (PrP knockout mice) (Zurich I) (10), tga20 (mouse *Prnp*^{a} allele [20]), tg7 (hamster PrP [40]), tg338 (ovine PrP, V^{136} R^{154} Q^{171} allele [31]), tg650 (human PrP, M^{129} allele [unpublished data]). The tg7 line used in this study was kindly provided by CSL-Behring (Marburg); it originates from the S. Prusiner laboratory, where subsequent to publication (40) the line has been bred onto a $PrP^{0/0}$ background. All mouse lines are homozygous for the transgene array and have been established on the same Zurich I $PrP^{0/0}$ background. All experiments were performed according to national guidelines.

Primary cell cultures. Primary cultures of cerebellar granule neurons (CGN) were established as previously described (16). Briefly, CGN cells were extracted from 6- or 7-day-old mice by enzymatic and mechanical dissociation. They were plated at a density of ~2,000 cells/mm^2 in 12- or 48-well plastic culture plates coated with 10 μg/ml poly-D-lysine and cultivated in Dulbecco's modified Eagle's medium-glutamax I high glucose (Gibco) containing 10% fetal calf serum (BioWhittaker), 20 mM KCl, penicillin and streptomycin (Gibco), and completed with N2 and B27 supplement (Gibco). The medium was complemented weekly with 1 mg/ml glucose and 10 μM concentrations of the antimitotics uridine and fluorodeoxiuridin (Sigma) to control astrocyte proliferation.

Prion infection. Primary neuronal cultures were exposed to prion as previously described (16). Briefly, brains of terminally ill prion-infected mice were homogenized and adjusted to 20% (wt/vol) with 5% (wt/vol) glucose and stored at −80°C until use. Brain homogenates were then sonicated and added at final concentrations between 0.002% and 0.1% to primary cultures 2 or 3 days after plating (unless stated otherwise) and left for the whole experiment without washes. In one series of experiments performed with human CJD agent (see Results), PrP^{Sc} was purified from brain homogenate by sodium phosphotungstic acid precipitation (55), further diluted in phosphate-buffered saline as necessary, and applied to cultures similarly to homogenate. The final concentration of PrP^{Sc} was calculated as an equivalent of the initial homogenate. Prion infectious sources consisted of sheep scrapie strain 127S (54); the mouse strains 139A, 22L, ME7 (originating from the R. Carp Laboratory, Staten Island, NY) and Fukuoka-1 (originating from the S. Katamine Laboratory, Nagasaki, Japan); the hamster strains Sc237 (subclone of 263K strain, [24]) and 139H (26) (provided by R. Carp); and human type 1 CJD (WHO reference sporadic CJD brain sample NHBY0/0001; National Institute Biological Standards and Control, Potters Bar, United Kingdom). All strains were propagated in transgenic mice expressing PrP^{C} of the corresponding species.

PrP^{Sc} detection. The accumulation of proteinase K (PK)-resistant PrP (PrP^{res}) was assessed by immunoblotting in cells lysed at various times postexposure as described previously (16). In short, lysates were clarified with a rapid centrifugation of 2,400 × *g* for 1 min. Then 50 μg of cell lysate proteins (measured by the bicinchoninic protein assay; Pierce) was treated with PK (7.5 μg/mg of protein; Euromedex) for 30 min at 37°C, and digestion was stopped by addition of 1 mM Pefabloc. Proteins were methanol precipitated for 1 h at −20°C and then centrifuged for 30 min at 16,000 × *g*. Pellets were resuspended in sample buffer, denatured, and loaded on 12% acrylamide precast gels (Invitrogen). Proteins were then electrotransferred on nitrocellulose membranes, and PrP was detected by incubation with biotinylated monoclonal anti-PrP antibodies ICSM18 (0.2 μg/ml) or Sha31 (0.1 μg/ml), followed with horseradish peroxidase-conjugated streptavidin (0.8 μg/ml; Pierce). Immunoreactivity was visualized by enhanced chemiluminescence (ECL kit; Pierce). Densitometric image analysis was performed by using Scion Image software.

Drug treatment. The following drugs were used at the indicated final concentrations: chlorpromazine (1 and 5 μM), Congo red (1.5 and 7 μM), and MS-8209 (3.5 and 7.5 μM). Stock solutions were aliquoted and stored at −20°C until use. Unless stated otherwise, the drug or vehicle (0.1% dimethyl sulfoxide) was first added to the cultures at 3 days after prion exposure and then twice a week. Two or three independent experiments were performed for each treatment, as specified in the Results section. Each experiment was performed using duplicate or triplicate cultures.

RESULTS

Primary neuronal cultures transgenic for PrP allow the propagation of prions from various species. As reported in our previous study (16), CGN primary cultures derived from transgenic mice expressing ovine PrP^{C} (CGN^{Ov}) and exposed to infectious inoculum at 2 to 3 days after plating are able to replicate the sheep scrapie agent. In such cultures, infection is effective at an inoculum dilution up to 0.0001% of brain homogenate, corresponding to a multiplicity of infection of ~1 infectious dose/500 cells (Fig. 1A). The permissiveness to infection of CGN^{Ov} cultures exposed at 8 days postplating, i.e., after phenotypical and functional differentiation of the neurons (38), was also examined. Such cultures were composed of 85% MAP2- and β3 tubulin-positive cells and less than 1% nestin-positive cells (data not shown). As shown in Fig. 1B, PrP^{res} accumulated at levels approaching those in cultures exposed at 2 days postplating (despite a 6-day-shorter incubation). This observation is of interest as it suggests that prion replication may initiate and take place in postmitotic, differentiated neurons.

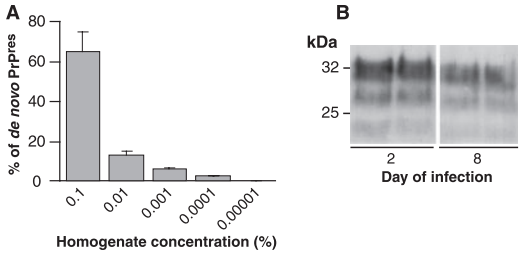

FIG. 1. High sensitivity of postmitotic neurons to prion infection. (A) Accumulation of PrP^{res} in CGN^{Ov} cultures following infection with serial dilutions of 127S brain homogenate (final concentrations as indicated). De novo PrP^{res} was quantified at 28 days postexposure by densitometric image analysis of immunoblots in two independent experiments (mean ± standard deviation) and is expressed as a percentage of total PrP^{res} detected by immunoblotting of 1 mg of mouse brain from terminally ill 127S-infected tg338 mice. (B) CGN^{Ov} cultures were exposed to infectious prions either 2 or 8 days after plating, i.e., before or after their full differentiation. PrP^{res} accumulation was assessed at 30 days postplating by immunoblotting of PK-treated lysate using biotinylated monoclonal antibody ICSM18 (see methods).

Deriving primary cell cultures from transgenic mice provided access to various PrP genotypes and allowed us to assess whether prions from other species could be propagated in CGN cells expressing the cognate PrP^{C}. Figure 2 shows the results obtained with well-characterized strains of rodent-adapted prions. Cultures of CGN cells expressing mouse and hamster PrP (CGN^{Mo} and CGN^{Ha}, respectively) were established from the mouse lines tga20 and tg7 overexpressing mouse and Syrian hamster PrP, respectively (see Materials and Methods). Neuronal differentiation and survival as well as PrP^{C} expression levels were found to be similar to levels in CGN^{Ov} cultures (data not shown). To infect the CGN^{Mo} cells, we used four mouse scrapie strains, 139A, 22L, ME7, and Fukuoka-1, which kill tga20 mice within 60 to 80 days postinfection (20; also our own data). For CGN^{Ha} cells, we used the hamster strain Sc237, a subclone of the 263K strain that kills tg7 mice within 50 days (40). Following exposure to diluted brain homogenate, a steady increase in PrP^{res} was consistently observed in PrP-expressing CGN cultures but not in CGN cultures established from nonpermissive $PrP^{0/0}$ mice ($CGN^{0/0}$) (Fig. 2A and B) (n = 3 independent experiments). PrP^{res} accumulation was detected at 2 weeks postexposure except with the ME7 strain, which multiplied less efficiently than the other mouse strains, as previously observed in different neuronal cell lines (3, 8, 44).

There is currently no available cellular model in which pri-

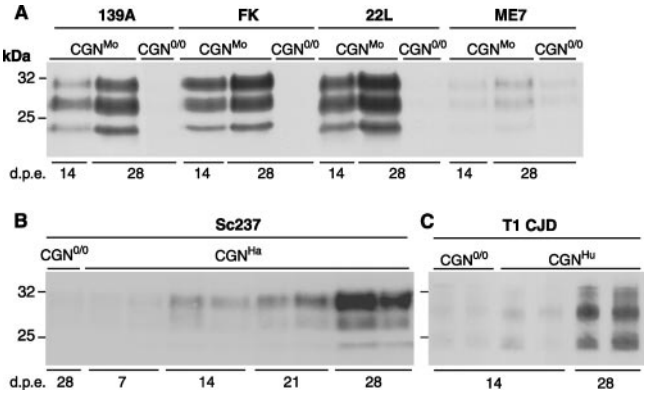

FIG. 2. Accumulation of PrP^{res} in CGN cultures upon exposure to rodent and human prions. CGN cultures were established from transgenic mice expressing mouse (A), hamster (B) or human (C) PrP and were exposed to brain homogenates (A and B) or purified PrP^{Sc} (C) from terminally ill mice infected with prions. CGN cultures established from $PrP^{0/0}$ transgenic mice ($CGN^{0/0}$) were also exposed to infectious prions in parallel. (A) CGN^{Mo} cultures exposed to mouse strain 139A, Fukuoka-1 (FK), 22L, or ME7 at a final concentration of 0.1% (wt/vol). (B) CGN^{Ha} cultures exposed to hamster strain Sc237 at a final concentration of 0.002%. (C) CGN^{Hu} cultures exposed to type 1 CJD (T1 CJD) at a final concentration equivalent to 0.1% of brain homogenate. The data shown in panels B and C correspond to duplicate culture wells within a representative experiment. In all PrP-expressing cultures, PrP^{res} accumulation increased from 14 to 28 days postexposure (d.p.e.) and was weak or absent in nonpermissive $CGN^{0/0}$ cultures. Cell lysates were PK treated, and PrP^{res} was detected by immunoblotting using biotinylated monoclonal antibody ICSM18 (A and C) or Sha31 (B).

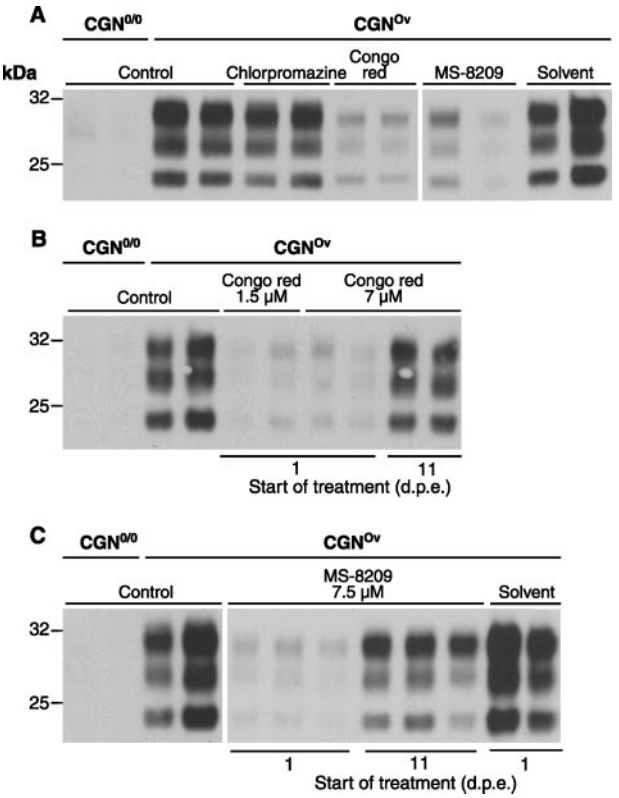

FIG. 3. Effect of chlorpromazine, Congo red, and MS-8209 on sheep prion propagation in CGN^{Ov} cultures at an early or advanced stage of infection. (A) CGN^{Ov} cultures were infected on day 2, two treatments (chlorpromazine, 5 μM; Congo red, 7 μM; MS-8209, 7.5 μM; or solvent, 0.1%) were performed on days 5 and 8, and cells were lysed on day 12. Control wells of CGN^{Ov} and nonpermissive $CGN^{0/0}$ cultures were infected similarly and left untreated (Control). (B and C) CGN^{Ov} cultures were infected on day 3, drugs (B, Congo red at 1.5 μM and 7 μM; C, 7.5 μM MS-8209 or 0.1% solvent) were added 1 day or 11 days after prion exposure and then twice a week; cells were lysed on day 21 (respectively, after 5 or 2 treatments). PrP^{res} was revealed by immunoblotting of PK-treated lysates using biotinylated monoclonal antibody ICSM18. d.p.e., days postexposure.

ons affecting humans can be propagated. To examine whether CGN cells would give access to a system permissive to a human agent, cultures derived from tg650 transgenic mice expressing human PrP Met^{129} were exposed to human CJD agent type 1. tg650 mice develop a TSE disease within ~150 days when inoculated with this TSE agent (unpublished data). As a result, a specific and reproducible ($n = 4$) accumulation of PrP^{res} was detected in CGN cells expressing human PrP (CGN^{Hu}) at 28 days postexposure (Fig. 2C) and as early as 14 days postexposure in half of the experiments (see also Fig. 4C). Collectively, these data demonstrate that primary cultured, postmitotic neurons are permissive to infection by prions from various species.

Congo red and MS-8209 efficiently inhibit PrP^{Sc} formation in sheep scrapie-infected neuronal cultures. Next, we asked whether this novel TSE cell model would be suitable to assess the effect of compounds with antiprion activities. To this end, we selected three molecules known to be effective in prion-infected cell lines: chlorpromazine, Congo red, and MS-8209, a less-toxic derivative of amphotericin B. After their neurotoxicity levels in noninfected CGN^{Ov} cultures were estimated through a cell survival assay (data not shown), the following concentrations were retained for subsequent experiments: 5 μM chlorpromazine (subtoxic concentration), 7 μM Congo red, and 7.5 μM MS-8209; these concentrations are close to the effective concentrations for 50% inhibition of PrP^{res} formation in chronically infected cell lines (4, 7, 29, 45). In a first series of experiments, drug treatments were started 3 days after exposure of CGN^{Ov} cultures to infectious brain homogenate (Fig. 3A). Both Congo red and MS-8209 reproducibly impaired prion propagation, based on the markedly lowered PrP^{res} accumulation observed in treated versus untreated cultures (inhibition ≥80% as quantified by densitometric image analysis; $n = 3$). In contrast, chlorpromazine treatment had no effect on PrP^{res} accumulation in infected CGN^{Ov} cells.

In the brain of prion-infected individuals, propagation of the infectious agent is likely to induce infection of new neurons while PrP^{Sc} accumulation continues in already infected neurons. Therefore, we questioned whether Congo red or MS-8209 would show a curative activity in infected CGN^{Ov} cultures once PrP^{res} had accumulated in substantial amounts. CGN^{Ov} cultures were treated 11 days after exposure to infectious inoculum (Fig. 3B and C). Contrary to early postexposure treatment, late treatment with Congo red was inefficient. However, a moderate inhibitory effect could be observed with MS-8209 (approximately 40% PrP^{res} decrease in comparison to untreated, infected cells).

Drug efficiency in primary neuronal cultures can vary according to the infecting prion. The cell lines currently used for

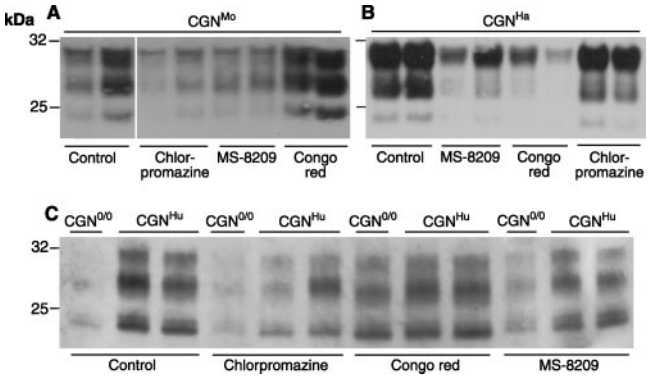

FIG. 4. Inhibition of PrP^{res} accumulation by chlorpromazine, Congo red, and MS-8209 in CGN cultures infected by prions from different species. CGN cultures expressing mouse (A), hamster (B), or human (C) PrP^{C} were infected by brain homogenates on day 2 with prions from the corresponding species: 139A (0.01% final concentration), Sc237 (0.01%), and type 1 CJD (T1 CJD; 0.05%). (C) Nonpermissive $CGN^{0/0}$ cultures were also exposed to T1 CJD prions. Three treatments were performed on days 5, 8, and 11 with chlorpromazine at a concentration of 1 μM (B) or 5 μM (A and C), Congo red (7 μM), or MS-8209 at a concentration of 3.5 μM (B) or 7.5 μM (A and C), and cells were lysed on day 15. PrP^{res} was revealed by immunoblotting of PK-treated lysates using biotinylated monoclonal antibody Sha31 (A and B) or ICSM18 (C).

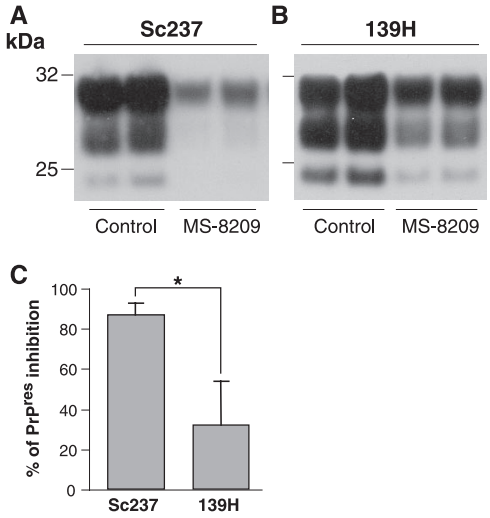

FIG. 5. Comparison of antiprion activity of polyene antibiotic MS-8209 according to hamster prion strains. CGN cultures expressing hamster PrP were infected with hamster prion strains Sc237 (A; 0.01% final concentration) or 139H (B; 0.002%) on day 2. Three treatments with MS-8209 (3.5 μM) were performed, starting at the early stage of infection, and cells were lysed shortly after the last treatment. PrP^{res} was revealed by immunoblotting of PK-treated lysates using biotinylated monoclonal antibody Sha31. (C) PrP^{res} inhibition was quantified by densitometric image analysis of immunoblots ($n = 3$ independent experiments) and is expressed as a percentage of controls (mean ± standard error of the mean). MS-8209 treatment was significantly more effective toward Sc237 hamster prions than the 139H strain. *, $P < 0.05$ (Student's t test).

antiprion drug screening propagate only mouse-adapted strains or sheep scrapie agent (28), and the data thus generated might not be fully transposable to prions infecting other species. Since primary cultured neurons are able to propagate prions from different species within a comparable environment, we sought to investigate whether any species-related effect of antiprion drugs would be observed. CGN^{Mo}, CGN^{Ha}, and CGN^{Hu} cultures were exposed to diluted homogenates from brains infected with one of the above-mentioned prion strains and then submitted to early postexposure treatment with one each of the three antiprion compounds (Fig. 4). PrP^{res} accumulation levels reproducibly showed clear disparities of drug efficacy according to the host PrP species ($n = 2$). Congo red markedly inhibited PrP^{res} accumulation in CGN^{Ha} cultures (Fig. 4B), as in CGN^{Ov} cultures (Fig. 3A), but had little or no effect in CGN^{Mo} and CGN^{Hu} cultures compared to untreated, control cultures (Fig. 4A and C). Notably, the PrP^{res} levels in CJD-exposed CGN^{Hu} and nonpermissive $CGN^{0/0}$ cultures were similar following Congo red treatment (Fig. 4C), indicating that input PrP^{Sc} present in the inoculum was actually stabilized by the drug. Chlorpromazine presented a relatively modest antiprion efficacy overall, except in CGN^{Mo} cultures. MS-8209 proved to be the only drug to be efficient across the range of prion species in these experiments, although with variable efficacy.

Earlier studies mainly in hamster TSE models have revealed a variable effect of amphotericin B and its analogue MS-8209 on the survival of the infected host according to the strain of prion (18, 33, 58). To see whether such a differential effect could also be visualized in cell culture, we compared the effects of MS-8209 in CGN^{Ha} cultures infected in parallel with either Sc237 or 139H, another well-characterized Syrian hamster strain. Upon early postexposure treatment of these cultures, a weaker inhibitory effect on PrP^{res} accumulation was consistently found with 139H than with Sc237 prion (Fig. 5) ($n = 3$), in accordance with in vivo observations (see Discussion).

DISCUSSION

Despite the efforts of many research groups, only a few cell systems susceptible to prion infection have been developed, offering limited genetic diversity and susceptibility to a small number of strains. While several cell lines currently provide robust systems in which a few mouse-adapted strains (3, 48) or sheep scrapie (1, 54) and, recently, a deer TSE agent (42) can be cultivated, no cell system enabling routine propagation of prions affecting other species is available. Although a hamster TSE-infected hamster cell line and a CJD-infected human cell line were reported in earlier publications (30, 50), they have not been mentioned for more than a decade. More recently, there have been descriptions of alternative approaches utilizing mouse brain fetal stem cells or neurospheres that enabled the propagation of mouse-adapted prions (22, 34).

In this study, we report that primary cultured, differentiated neurons are susceptible to infection by a range of prion strains from four different species: sheep, mouse, hamster, and human. Modeling our previously described approach (16), we employed CGN cultures established from transgenic mice expressing PrP proteins of different species. Upon exposure, these cells were found to be susceptible to infection by prions propagated in the corresponding species, based on the accumulation of abnormal PrP. This newly introduced cell system thus offers a common, biologically relevant cellular environ-

ment in which prion agents from different species can be studied comparatively. While CGN cells may not provide a universal, susceptible system, one can expect that extending this approach to other neuron populations—as done successfully with cortical cells expressing ovine PrP (11)—might enlarge the spectrum of permissiveness. This would also pave the way for future studies of the determinism of the apparent, strain-dependent neuronal selectivity exhibited by these agents (17).

Primary nerve cell cultures have proved their usefulness for the evaluation of therapeutic compounds in neurodegenerative or infectious diseases (15, 52, 57). Here, we addressed the relevance of scrapie-infected primary nerve cell cultures as a potential model for the evaluation of antiprion molecules by using three different compounds that were previously described to clear PrP^{res} in chronically infected cell lines but presented inconsistent prophylactic and therapeutic activities in vivo (51). As a main finding, these compounds markedly differed in their antiprion efficacy according to the species and/or prion strain, based on their effect on the accumulation of PrP^{res}. In several instances, the differential effects observed in CGN cultures paralleled those documented in vivo for the same compound.

As a striking example, Congo red was shown here to exhibit a marked inhibitory activity toward ovine and hamster prions but not human and mouse prions. Such a result was not particularly expected in the case of the mouse prion, since this compound manifested clear antiprion activity when tested in the mouse cell lines ScN2a (12, 13) and ScSMB (45) infected by the Chandler strain, which is closely related to the 139A strain used in this study. The reason for these apparent discrepancies is uncertain but might reflect the limited effect of Congo red on preexisting PrP^{res}, since several subpassages under treatment (12, 13) or high doses of the drugs (100 μM) (45) were required to inhibit PrP^{res} accumulation in these cell lines. Of interest, the results obtained in CGN cultures infected with either mouse or hamster prions were in line with previous observations in both cell-free and animal models, where the effects of Congo red appear to differ markedly between these two rodents. In hamster, it was reported to disrupt inoculum-associated PrP^{res} and to delay disease onset in scrapie-infected animals (6, 25), whereas it overstabilized murine PrP^{res} and did not prevent PrP^{res} accumulation in the spleen (6) or increase mouse survival time in vivo (6; also R. Race, unpublished data). Our experiments revealed a persistence of the inoculum-derived PrP^{res} signal in both permissive and nonpermissive CJD-exposed cultures following Congo red treatment, thus suggesting similar interactions with the drug of the human and mouse prions.

Chlorpromazine was identified as an inhibitor of PrP^{res} accumulation after screening on chronically infected ScN2a cells (29); it also proved active in prion clearance in mouse ScGT1 cells (4), as well as in a *Saccharomyces cerevisiae* prion model (2). However, this drug failed to delay significantly the outcome of the disease in compassionate treatments of human TSE (5), despite its ability to cross the blood-brain barrier. In primary cultured neurons, long-lasting application of chlorpromazine was more toxic than in stable cell cultures, which is consistent with its reported neurotoxic activity in cerebellum cell culture (43). At the maximal drug concentration tolerated in CGN cultures, this compound hampered PrP^{res} accumulation to various degrees according to the prion agent. While chlorpromazine showed some inhibitory effect in CGN^{Mo} and in CGN^{Hu} cultures, it scarcely prevented PrP^{res} accumulation in CGN^{Ha} and CGN^{Ov} cultures. In Rov cell cultures (53) infected by the same sheep strain as CGN^{Ov} cells, this drug showed a clear PrP^{res}-inhibitory effect (data not shown). In a clinical trial on scrapie-infected ewes, however, it failed to produce any therapeutic benefit in combination with quinacrine, another tricyclic derivative (21). Altogether, these observations suggest that this antiprion drug assay involving primary neuronal cells could lead to a more accurate prediction of the in vivo effect than in a cell line, possibly due to a greater phenotypic proximity with postmitotic neurons. In such cells, the dynamics of PrP^{Sc} synthesis or accumulation differs from that in actively dividing cells. Moreover, the biogenesis of PrP^{C} in primary and immortalized neuronal cells exhibits notable differences in terms of turnover and endocytosis (37, 49), which might also affect drug activity.

Of the three compounds assayed in our CGN cell model, the amphotericin B derivative MS-8209 showed the broadest spectrum of antiprion efficacy in terms of species or prion strain, yet variations in its PrP^{res} inhibitory potency were noticeable. In CGN cells expressing hamster PrP, MS-8209 was found to be more effective against the Sc237 than the 139H strain. This is in keeping with previous in vivo observations showing an apparent strain-dependent effect of polyene antibiotics in the absence of PrP sequence variation. In hamsters, amphotericin B could significantly delay clinical phase onset following inoculation with the prion strain 263K (equivalent to Sc237) but not with the strains 139H (58) and DY (33), which have a longer incubation period. MS-8209 also exhibited some specificity, extending the life spans of transgenic mice with neuron-restricted hamster PrP expression when infected with the 263K strain but not with the DY strain (18). Thus, the strain-specific effect observed in CGN cells recapitulated to some extent those reported in vivo for this class of compounds. Such cells may therefore provide a relevant tissue culture system in which to investigate the mechanisms underlying the antiprion activity.

The cell system developed in this study made it possible for the first time to test the relative potency of several drugs toward a human prion. Thus far, intraventricular infusion of pentosan polysulfate in a variant CJD patient has been the only treatment that might slow the disease progression in human (41). Failure of compassionate treatments with other compounds was attributed to the late intervention and/or a poor penetration of the blood-brain barrier (19, 23, 32, 36). Another issue that is also supported by our findings is that screening in rodent TSE models might be inaccurate and select molecules that may not be particularly active against human agents. In this regard, this ex vivo assay against a CJD agent in primary nerve cells derived from transgenic mice expressing human PrP may provide new opportunities for the selection of compounds active against human prions.

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