

Quinacrine promotes replication and conformational mutation of chronic wasting disease prions

Jifeng Bian, Hae-Eun Kang, and Glenn C. Telling¹

Prion Research Center and Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523

Edited by Charles Weissmann, The Scripps Research Institute, Jupiter, Florida, and approved March 6, 2014 (received for review November 30, 2013)

Quinacrine's ability to reduce levels of pathogenic prion protein (PrP^{Sc}) in mouse cells infected with experimentally adapted prions led to several unsuccessful clinical studies in patients with prion diseases, a 10-y investment to understand its mechanism of action, and the production of related compounds with expectations of greater efficacy. We show here, in stark contrast to this reported inhibitory effect, that quinacrine enhances deer and elk PrP^{Sc} accumulation and promotes propagation of prions causing chronic wasting disease (CWD), a fatal, transmissible, neurodegenerative disorder of cervids of uncertain zoonotic potential. Surprisingly, despite increased prion titers in quinacrine-treated cells, transmission of the resulting prions produced prolonged incubation times and altered PrP^{Sc} deposition patterns in the brains of diseased transgenic mice. This unexpected outcome is consistent with quinacrine affecting the intrinsic properties of the CWD prion. Accordingly, quinacrine-treated CWD prions were comprised of an altered PrP^{Sc} conformation. Our findings provide convincing evidence for drug-induced conformational mutation of prions without the prerequisite of generating drug-resistant variants of the original strain. More specifically, they show that a drug capable of restraining prions in one species/strain setting, and consequently used to treat human prion diseases, improves replicative ability in another and therefore force reconsideration of current strategies to screen antiprion compounds.

prion therapeutics | prion enhancing drugs

Primions are protein-based infectious agents that cause an increasing variety of fatal, infectious neurodegenerative disorders, frequently as epidemics. Variant Creutzfeldt–Jakob disease (CJD) resulting from bovine spongiform encephalopathy exemplifies prion zoonosis, and the continued, unpredictable emergence of additional epidemics in other species, particularly chronic wasting disease (CWD) in deer, elk, and moose, raises additional concerns. Its unprecedented contagious spread, widening host range, and conflicting evidence surrounding its zoonotic potential place CWD at the forefront of public health concerns. Although the notion, that prion replication occurs by corruption of host-encoded cellular prion protein (PrP^C) by abnormally conformed PrP^{Sc}, is now widely accepted, the details of this mechanism remain enigmatic.

Except under certain experimental conditions, treatments capable of arresting or of effectively modifying the course of disease do not exist for prion disorders. Compounds targeting prevention of PrP misfolding have been aggressively pursued as therapeutics. Cells chronically infected with rodent prions are used as a means either to screen compounds inhibiting PrP^{Sc} accumulation (1–5) or to confirm the proposed antiprion effects of compounds discovered by other means (6, 7). Such strategies rest on the assumption that compounds with efficacy against experimentally adapted rodent prions will be effective against naturally occurring prions, in particular those causing human diseases. Exemplifying this approach, quinacrine, a compound that has been repeatedly shown to reduced PrP^{Sc} levels in rodent cells chronically infected with experimentally adapted mouse prions (8, 9), was used to treat patients with human prion diseases in several settings (10–15), albeit unsuccessfully.

Our development of cultured cells in which CWD infection is stably and indefinitely maintained (16) provided a unique and convenient means of exploring CWD therapeutics. Our first approach was to assess the efficacy of compounds with known effects on rodent prions. Consistent with its effect on mouse prion infectivity, sustained treatment of Elk21⁺ cells with dextran sulfate 500 (DS-500) resulted in PrP^{Sc} clearance, which did not reemerge in the resulting Elk21⁻ cells after more than 40 passages, and inoculation of susceptible transgenic (Tg) mice showed that Elk21⁻ cells were cured of CWD prion infection (16). The present study was initially designed to assess the effects of quinacrine on CWD. We found that, far from inhibiting PrP^{Sc} accumulation and curing infection, quinacrine augmented PrP^{Sc} levels and stimulated CWD prion replication in cells expressing elk or deer PrP. We performed additional studies to address the properties of quinacrine-enhanced CWD prions.

Results

We confirmed previous observations (8, 9, 17–21) that treatment of ScN2A or SMB cells chronically infected with rodent prions with 1 μM quinacrine for 6 d resulted in 5- to 14-fold reductions of PrP^{Sc} depending on cell type ($n = 3$ independent comparisons, $P \leq 0.05$ in both cases) (Fig. 1 *A* and *C*). Surprisingly, when Elk21⁺ cells chronically infected with CWD prions (16) were treated with the same amount of quinacrine for the same time, PrP^{Sc} levels, assessed by Western blotting, increased by more than twofold compared with untreated Elk21⁺ ($n = 11$ comparisons, $P \leq 0.05$) (Fig. 1 *A* and *C*). To address whether these effects were cell-specific, we produced RK13 cells expressing mouse PrP^C persistently infected with mouse RML prions, referred to as RKM⁺. Treatment of RKM⁺ with 1 μM quinacrine for 6 d reduced mouse PrP^{Sc} levels more than threefold ($n = 3$ comparisons, $P \leq 0.05$) (Fig. 1 *A* and *C*). We also produced

Significance

Searching for drugs to prevent conversion of host-encoded prion protein (PrP^C) to its infectious conformation (PrP^{Sc}) is a key strategy in the pursuit of therapies for prion disorders: fatal, transmissible epidemic diseases of unpredictable occurrence and uncertain zoonotic potential. Despite quinacrine's ability to reduce mouse PrP^{Sc} in cell models, its use to treat patients has been unsuccessful. Here, we show that quinacrine augments PrP^{Sc} and intensifies replication of prions, causing chronic wasting disease of deer, elk, and moose, and that the resulting prions have altered conformational and transmission properties. Our finding, that a drug capable of restraining PrP^{Sc} in one species acts to improve replicative ability and induce mutation in another, forces reexamination of current strategies to combat these diseases.

Author contributions: J.B. and G.C.T. designed research; J.B. and H.-E.K. performed research; J.B., H.-E.K., and G.C.T. analyzed data; and J.B., H.-E.K., and G.C.T. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹To whom correspondence should be addressed. E-mail: glenn.telling@colostate.edu.

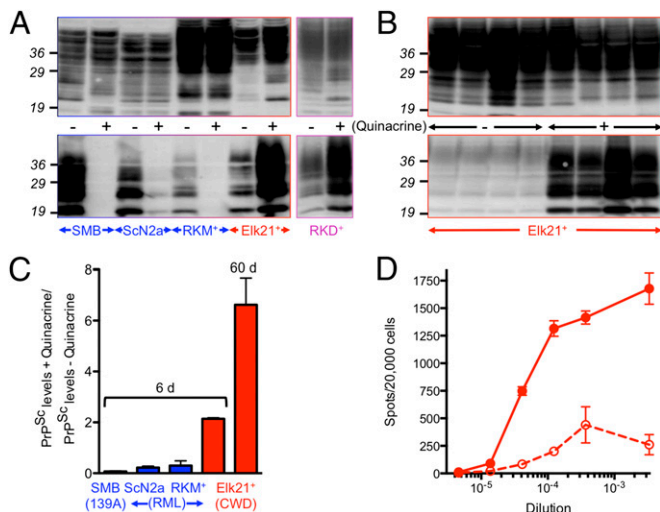


Fig. 1. Discrepant effects of quinacrine on mouse and cervid PrP^{Sc} accumulation and prion replication. (A and B) Equal amounts of proteins from quinacrine treated (+) and untreated (-) cells were either not treated (Upper) or digested with PK to detect PrP^{Sc} (Lower). Blots were probed with mAb 6H4. (A) Cells were treated with 1 μ M quinacrine for 6 d, except RKD⁺ were treated for eight passages. (B) Replicate plates of Elk21⁺ were treated with 1 μ M quinacrine for 10 passages compared with untreated cells maintained for the same time. (C) Densitometric analysis of PrP^{Sc} levels in quinacrine-treated and untreated SMB ($n = 3$ pairs), ScN2a ($n = 3$ pairs), RKM⁺ ($n = 3$ pairs), and Elk21⁺ ($n = 11$ pairs) for 6 d, or Elk21⁺ for 60 d ($n = 5$ pairs). (D) Numbers of prion-infected Elk21⁺ cells following quinacrine treatment (solid line, filled circles) or no treatment (dashed line, open circles) determined by CPCA. Mouse, elk, or deer PrP indicated in blue, red, and magenta respectively.

RK13 cells stably expressing deer PrP^C, referred to as RKD, that were readily and stably infected with elk CWD isolate 012-09442 from diseased Tg(DeerPrP)1536^{+/-} mice (22), to produce RKD⁺ cells. Similar to Elk21⁺, treatment of RKD⁺ with 1 μ M quinacrine increased PrP^{Sc} levels, assessed by Western blotting, by more than twofold compared with untreated RKD⁺, albeit after longer treatment times (Fig. 1A). Enhancement of PrP^{Sc} was more pronounced when Elk21⁺ were treated for 10 passages (~60 d) with 1 μ M quinacrine, after which time PrP^{Sc} levels increased approximately sevenfold ($n = 5$ comparisons, $P \leq 0.001$) (Fig. 1B and C).

We previously developed a cell-based assay for quantifying CWD prions with sensitivity comparable with the CWD bioassay in susceptible Tg mice (16). We used this cervid prion-cell assay (CPCA) to compare levels of CWD prions in Elk21⁺ with quinacrine-treated cells. Untreated Elk21⁺ accumulated 1.38×10^6 CPCA units/g whereas treatment with 1 μ M quinacrine for 60 d resulted in a greater than 13-fold increase of 1.86×10^7 CPCA units/g (Fig. 1D).

Treatment of Elk21⁺ with various quinacrine concentrations for 6 d produced a dose-dependent increase in the number of CWD-infected cells compared with untreated cells, when equivalent cell numbers were applied to enzyme-linked immunosorbent spot (ELISpot) plates. A maximal fourfold increase occurred at 1.2 μ M ($P \leq 0.0001$) (Fig. 2A). We also observed an ~60% increase in the number of CWD-infected RKD⁺ after eight passages in the presence of 1 μ M quinacrine ($n = 4$ comparisons, $P \leq 0.0001$) (Fig. 2A). In accordance with previous reports describing cytotoxicity or inhibition of cell growth of N2a-derived and SMB cells at drug concentrations ranging from 1.0 μ M to ≥ 2.0 μ M (8, 17), we observed lower numbers of adherent cells and significant reductions in cell viability when Elk21⁺ were treated with quinacrine concentrations of 2 μ M and higher (Fig. 2B). During

protein-misfolding cyclic amplification (PMCA), quinacrine failed to enhance or inhibit elk PrP^{Sc} amplification at these, or higher, drug concentrations (Fig. 2C).

To address whether the enhancing effects of quinacrine on PrP^{Sc} were permanent, we treated Elk21⁺ continuously for up to 10 passages and compared PrP^{Sc} levels in untreated, continuously treated, and cells maintained for a further five passages after withdrawal of drug. Although PrP^{Sc} levels in quinacrine-treated Elk21⁺ gradually increased compared with untreated cells, after withdrawal of drug, PrP^{Sc} returned to original levels within five passages, regardless of the duration of quinacrine treatment (Fig. 3).

We compared the conformational stability of PrP^{Sc} produced in Elk21⁺ exposed to 1 μ M quinacrine for 6 d, with that of PrP^{Sc} produced in untreated cells. To do so, we established a sensitive, quantitative cell-based conformational stability assay (CSA), referred to as C-CSA, where numbers of PrP^{Sc}-producing cells were assessed by ELISpot analysis following exposure to various concentrations of guanidinium hydrochloride (GdnHCl). The C-CSA denaturation curves of PrP^{Sc} from untreated and quinacrine-treated Elk21⁺ were reproducibly different. The 2.58-M concentration of GdnHCl at the midpoint of the sigmoidal transition (GdnHCl_{1/2}) for quinacrine-treated Elk21⁺ was 13% higher than the 2.29-M value of untreated Elk21⁺ ($n = 16$ replicates, $P \leq 0.0001$) (Fig. 4A). We confirmed the differences in conformational stabilities of PrP^{Sc} produced in the presence and absence of 1 μ M quinacrine, by adapting standard procedures used to assess the conformational stabilities of PrP^{Sc} in infected brains (23) to our cell-culture system. When we treated cell extracts with increasing GdnHCl concentrations and compared conformational stabilities following proteinase K (PK) treatment of materials applied to PVDF membranes, according to previously published approaches

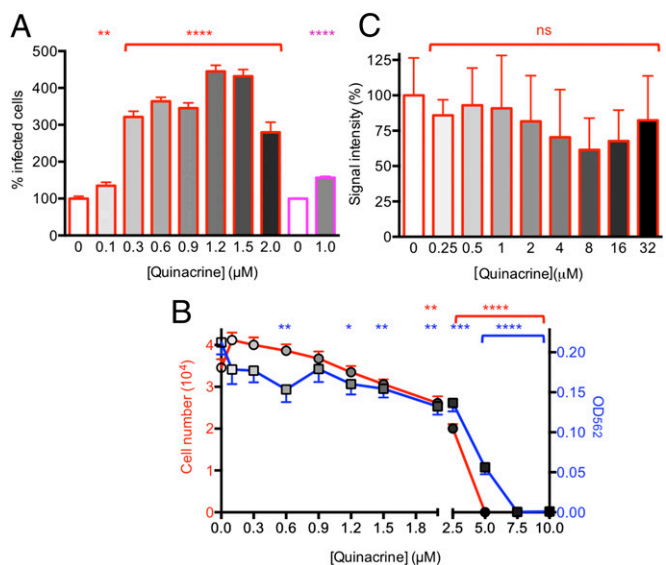


Fig. 2. Effects of various quinacrine concentrations on CWD propagation and cell viability in Elk21⁺ and RKD⁺ cells, and during protein misfolding cyclic amplification. (A) Dose-response effects in Elk21⁺ (red) treated with quinacrine for 6 d, and RKD⁺ cells treated with 1 μ M quinacrine for 60 d (magenta). (B) Effect of quinacrine on numbers of adherent Elk21⁺ (left y axis; red line, circles), and cell viability assessed by MTT assay (right y axis; blue line, squares). For each treatment, 16 replicates were assessed. The statistical difference of means was assessed by one-way ANOVA. (C) Densitometric analysis of western blotted PrP^{Sc} following PMCA performed in the absence or presence of quinacrine ($n = 3$ samples for each concentration). Error bars refer to standard errors of the mean (SEM). ns, $P > 0.05$; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$.

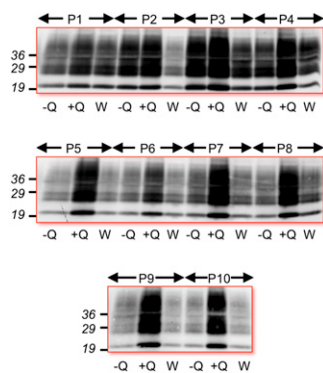


Fig. 3. Continuous drug treatment is required to sustain enhanced CWD PrP^{Sc} levels in Elk21⁺. Western blots of PrP^{Sc} in Elk21⁺ treated with 1 µg/mL quinacrine (+Q) for up to 10 passages (P1–P10) compared with untreated cultures (–Q) and treated cells withdrawn from drug and subsequently maintained for a further five passages (W).

(24, 25), the 1.57-M GdnHCl_{1/2} of PrP^{Sc} isolated from quinacrine-treated Elk21⁺ reflected a significantly greater conformational stability than the 0.94-M GdnHCl_{1/2} of PrP^{Sc} from untreated Elk21⁺ ($n = 8$ replicates, $P \leq 0.0001$) (Fig. 4B). We also performed C-CSA on quinacrine-treated and untreated RKD⁺. In accordance with our previously published results showing differences in the unfolding characteristics of PrP^{Sc} from CWD-infected Tg mice expressing elk or deer PrP (26), C-CSA showed that the 3.61-M GdnHCl_{1/2} of deer PrP^{Sc} in RKD⁺ was considerably higher than the 2.29-M GdnHCl_{1/2} of elk PrP^{Sc} in Elk21⁺ ($P \leq 0.0001$) (Fig. 4A and B). Consistent with its effect on increasing the stability of elk PrP^{Sc}, quinacrine treatment of RKD⁺ resulted in an additional 10% increase in conformational stability of deer PrP^{Sc} (GdnHCl_{1/2} = 3.97 M; $P \leq 0.0001$) (Fig. 4A). We refer to prions formed from the more stable quinacrine-treated conformers as Q-CWD.

The mean 172 ± 2 d (\pm SEM) time to disease onset in Tg(ElkPrP)5037^{+/-} mice inoculated with Q-CWD prions from quinacrine-treated Elk21⁺ was 54% longer than the 112 ± 1 d mean time to disease onset in mice receiving prions from untreated Elk21⁺ ($P \leq 0.001$) (Fig. 4C). Similarly, mean incubation times were 27% longer in Tg(DeerPrP)1536^{+/-} mice inoculated with elk Q-CWD prions (403 ± 12 d compared with 318 ± 10 d, $P \leq 0.001$) (Fig. 4C). Immunohistochemical and histoblot analyses revealed reproducibly distinct differences in PrP^{Sc} distribution, particularly in the cerebellum of mice receiving elk Q-CWD or elk CWD prions (Fig. 5). Although PrP^{Sc}-containing plaques in the granular layer of the cerebellum were observed in Tg(ElkPrP)5037^{+/-} mice receiving Elk21⁺ extracts that had not been exposed to quinacrine, this feature was not reproduced in Q-CWD-infected Tg(ElkPrP)5037^{+/-} mice. In contrast, although elk Q-CWD prions produced relatively large PrP^{Sc}-containing plaques in the molecular layer of the cerebellum in diseased Tg(DeerPrP)1536^{+/-} mice, the same sections of diseased Tg(DeerPrP)1536^{+/-} mice inoculated with untreated Elk21⁺ extracts contained only occasional deposits in these areas, but relatively intense PrP^{Sc} staining in the medulla.

To determine whether quinacrine affected disease outcome when directly administered to CWD-infected mice, Tg(ElkPrP)5037^{+/-} mice were orally dosed with 30 mg/kg quinacrine per day, 2 wk before inoculation with elk CWD prions. Animals continued to receive quinacrine daily until manifesting terminal signs of prion disease. Quinacrine-treated mice developed disease with a mean onset of 153 ± 3 d, which was similar to the 150 ± 2 d incubation time in Tg(ElkPrP)5037^{+/-} mice that did not receive drug (Fig. 4D). Levels and distribution of PrP^{Sc} in the brains of diseased mice from both groups were indistinguishable.

Patterns of deposition in the cerebellum in both groups of mice were similar to Tg(ElkPrP)5037^{+/-} mice infected with prions from untreated Elk21⁺, but distinct from Tg(ElkPrP)5037^{+/-} mice infected with Q-CWD prions (Fig. 5).

Discussion

In stark contrast to its well-documented inhibitory effects on PrP^{Sc} accumulation in cells chronically infected with experimentally adapted rodent prions (8, 9, 17–21), the same range of quinacrine concentrations increased elk and deer PrP^{Sc} accumulation in cells persistently infected with CWD prions. This paradoxical dose- and time-dependent enhancement was associated with a greater than order of magnitude increase in CWD prion titers in drug-treated Elk21⁺. Even though the enhancing effects of 2 µM quinacrine on the number of CWD-infected Elk21⁺ cells are extremely ($P \leq 0.0001$) significant (Fig. 2A), we speculate that the decreases at this and higher concentrations may correspond to detrimental effects of the drug on cell viability.

Although quinacrine is reported to inhibit PMCA of mouse PrP^{Sc} (17), it failed to increase levels of CWD PrP^{Sc} during PMCA. Augmentation of PrP^{Sc} in cells, but not during cell-free amplification, suggests that quinacrine-enhanced CWD propagation depends on processes linked to cellular integrity. The contradictory effects of quinacrine on mouse and CWD prions seem unrelated to effects specific to RK13 cells because we show that quinacrine reduces mouse PrP^{Sc} in RK13 cells expressing mouse PrP^C infected with RML prions, while improving CWD replication in RK13 cells expressing either elk or deer PrP^C. The

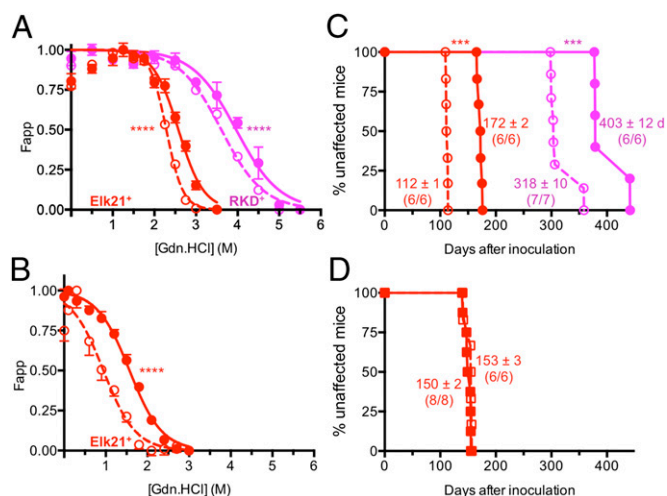


Fig. 4. Quinacrine alters the conformation of elk and deer PrP^{Sc} and the transmission properties of elk CWD prions. (A) Conformational stability of elk PrP^{Sc} in Elk21⁺ treated with 1 µg/mL quinacrine (solid line, filled red symbols) or untreated (dashed line, open red symbols) for 6 d ($n = 16$), assessed by C-CSA. RKD⁺ were treated with 1 µg/mL quinacrine (solid line, filled magenta symbols) or untreated (dashed line, open magenta symbols) for 60 d ($n = 4$). (B) Conformational stability of elk PrP^{Sc} extracted from Elk21⁺ treated with 1 µg/mL quinacrine (solid line, filled red symbols) or untreated (dashed line, open red symbols) for 6 d ($n = 8$), assessed by densitometric analysis of PrP^{Sc} applied to dot blots. **** $P \leq 0.0001$ refers to differences between GdnHCl_{1/2} from best-fitted curves. (C) Incubation times of Q-CWD prions are prolonged compared with CWD prions. Times to disease onset in Tg(DeerPrP)1536^{+/-} and Tg(ElkPrP)5037^{+/-} mice (red and magenta lines/symbols, respectively) inoculated with Elk21⁺ either with 1 µg/mL quinacrine (filled circles) or untreated Elk21⁺ (open circles). (D) Quinacrine treatment of Tg(ElkPrP)5037^{+/-} mice fails to affect time to onset of CWD. CWD-inoculated Tg(ElkPrP)5037^{+/-} mice orally dosed with quinacrine, filled squares; animals similarly inoculated, but not receiving quinacrine, open squares. *** $P \leq 0.001$. Incubation times (mean \pm SEM) of the numbers of diseased mice are shown for each transmission.

several subsequent clinical studies invested considerable efforts to assess its effects in patients. Tragically, in no case did treatment provide discernible benefits (10–15). Possibly related to this failure, studies in mice lacking the multiple drug resistant gene inoculated with RML prions and orally dosed with quinacrine resulted in selection and propagation of quinacrine-resistant prions (28). Similarly, IND24, although effective at prolonging the lifespan of RML-infected mice, also produced drug-resistant prions but had no effect in CJD-infected Tg mice (37). Our findings are distinct from such cases because they show that a compound with demonstrated, albeit transient, efficacy against a strain in one species acts to directly enhance prion replication in a different species and produce prions with altered biological properties without the prerequisite of generating drug-resistant variants of the original strain.

Several practical repercussions arise from quinacrine's prion-enhancing and mutagenic effects. First, the effects of "antiprion" compounds are clearly species/strain-dependent, in that drugs found to restrain prions in one situation may improve replicative ability in another. Based on initial reports of quinacrine's effects on mouse prion propagation in cell culture, a plethora of studies were designed to discern the mechanism of its presumed antiprion effects (19, 20, 29–31, 38–45), to assess its pharmacokinetics (21, 36, 42, 46) and to derive similar compounds with improved antiprion efficacy (18, 20, 47–52, 53–59). These studies continue to this day, despite multiple failed clinical studies in patients with human prion diseases (10–15). The findings reported here force a reconsideration of current strategies to screen antiprion drugs. In particular, it seems imperative that cells with susceptibilities to cervid, ovine, bovine, and human prions be used to identify drugs capable of treating prions affecting those species. To date, no such resources have been reported for human prions, and the reasons for this drawback remain enigmatic, given the relatively facile production of cell lines with susceptibilities to ovine, murine, bank vole, and cervid prions.

Second, to the best of our knowledge, the effect of quinacrine on CWD represents the first reported example of a drug capable of facilitating cellular prion replication. On a positive note, our results raise the possibility of using prion-enhancing compounds, in addition to previously described effects of retroviral GAG expression on cellular prion susceptibility (16), as a strategy to heighten the vulnerability of otherwise resistant cell cultures to novel prion infections: for example, to improve cellular susceptibility to human prions.

Materials and Methods

Cell Culture. SMB and ScN2a cells were cultured in DMEM containing 10% (vol/vol) FBS and 1 µg/mL penicillin/100 U/mL streptomycin. Rabbit kidney epithelial (RK13) cells were obtained from the American Type Culture Collection (Cat. no. CCL-37; ATCC). Mouse, deer, and elk PrP coding sequences were cloned into pIRESpuro3 vector. HIV-1 GAG precursor protein coding sequence was cloned into pcDNA3.1-neomycin or pcDNA3.1-hygromycin. RKD⁺ expressing deer PrP and HIV-1 GAG were cultured in the presence of 1 µg/mL puromycin and 250 µg/mL hygromycin; Elk21⁺ were cultured in the presence of puromycin and 1 mg/mL G418; RKM⁺ were cultured in the presence of puromycin. RK13 cells stably transfected with pIRESpuro3 vector, referred to as RKV, were used as negative controls for prion infections. Cells were passaged at a ratio of 1:10 every 5 d. Quinacrine (Sigma-Aldrich) was dissolved in PBS and filtered with a 0.22-µm filter. Cell culture medium was replaced every other day.

Cell Viability. A 12-mM solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Life Technologies) was prepared in PBS. Cell viability was assessed as described previously (60). Cell numbers were assessed with a TC20 Automated Cell Counter (Bio-Rad).

Western Blotting. Western blotting was performed and analyzed as previously described (24).

Protein Misfolding Cyclic Amplification. PMCA reactions were performed as described previously (32) at a seed to substrate ratio of 1:4,000, digested with PK at a final concentration of 0.33 µg/µL, and analyzed by Western blotting using anti-PrP mAb 6H4 (Prionics AG) or PRC5 (61).

Cervid Prion Cell Assay. The CPCA using Elk21⁺ cells was performed as previously described (16), with the following modifications. Images were scanned with CTL Immunospot S6UNIV equipment, and spot numbers were determined using Immunospot 5.0 Analysis and 5.1 Counting software (Cellular Technology).

Cell-Based Conformational Stability Assay. Twenty thousand cells were filtered onto ELISpot plates (EMD Millipore) and air-dried at 50 °C for 2 h. Cells were denatured with 100 µL of GdnHCl at concentrations ranging from 0 to 3.5 M in 0.25-M increments. Following removal of denaturant, plates were washed twice with PBS, followed by a 5 µg/mL PK digestion for 90 min at 37 °C, and then denaturation in guanidinium thiocyanate. PrP^{Sc}-producing cells were detected as described for the CPCA (16), except that a semiautomated approach was used, in which the operator manually adjusted software sensitivity to optimize detection of infected cells at each GdnHCl concentration. Spot numbers were plotted against the concentration of applied guanidine hydrochloride using Prism 6.0d for Mac OS X (GraphPad Software). Datasets were normalized to a common scale, and the sigmoidal dose–response was best fitted by a variable-slope four-parameter algorithm and nonlinear least-squares fit using Prism 6.0d for Mac OS X, with maxima and minima constrained. Results are presented as fractions of the apparent spot counts (F_{app}). Statistical differences between curves deriving from the various GdnHCl treatment series were assessed and reported as differences between the concentrations of GdnHCl at the midpoint of the sigmoidal transitions.

Conformational Stability Assay of PrP^{Sc} from Cell Extracts. Cell extracts from quinacrine-treated and untreated Elk21⁺ cells containing 50 µg and 150 µg of total protein, respectively, were incubated with various concentrations of GdnHCl for 1 h at room temperature as previously described for brain samples (24) except membranes were treated with 1 µg/mL PK, and membranes were blocked with Odyssey Blocking Buffer (LI-COR Biosciences) for 1 h, probed with mAb 6H4 overnight at 4 °C, followed by IRDye 800 CW goat anti-Mouse IgG secondary antibody (LI-COR Biosciences) for 1 h. After three washes with Tris-buffered (pH 7.6) saline and 0.05% Tween 20, the membrane was rinsed with PBS and scanned with the Odyssey CLx infrared imaging system (LI-COR Biosciences), and signals were analyzed with Odyssey CLx Image Studio software (LI-COR Biosciences).

Preparation of Brain and Cell Homogenates. Preparation of brain and cell homogenates was performed as previously described (24).

In Vivo Studies. Tg(DeerPrP)1536^{+/-} and Tg(ElkPrP)5037^{+/-} mice have been described previously (22, 62). Tg(ElkPrP)5037^{+/-} mice were orally dosed with quinacrine (30 mg·kg⁻¹·d⁻¹) by gavage, 2 wk before intracerebral inoculation with elk CWD prions. Animals continued to receive quinacrine daily until manifesting terminal signs of prion disease. Ten percent brain or cell homogenates in PBS lacking calcium and magnesium ions were diluted a further 10-fold before inoculation. Incubation time assays were performed as described (24). Following diagnosis, animals were euthanized by CO₂ asphyxiation. All work with animals was performed in compliance with University of Kentucky and Colorado State University Institutional Animal Care and Use Committees.

Histoblotting. Histoblots were produced and analyzed according to previously described protocols (32). Images were captured with a NikonDMX 1200F digital camera and analyzed using MetaMorph imaging software (Molecular Devices).

Immunohistochemistry. Immunohistochemistry was performed as previously described (32) using mAb 6H4 as primary antibody and IgG1 biotinylated goat anti-mouse as secondary antibody (SouthernBiotech). Digitized images were obtained by light microscopy using a Nikon Eclipse E600 microscope equipped with a Nikon DMX 1200F digital camera.

ACKNOWLEDGMENTS. The authors acknowledge technical assistance from Tanya Seward and Dana Napier. We thank Dr. Charles Merrill for critically reading the manuscript and providing advice for improvements. We thank Prionics for mAb 6H4. This work was supported by National Institutes of Health Grants R01 NS040334 and P01 AI077774.

1. Lu D, et al. (2013) Biaryl amides and hydrazones as therapeutics for prion disease in transgenic mice. *J Pharmacol Exp Ther* 347(2):325–338.
2. Li Z, et al. (2013) Discovery and preliminary SAR of arylpiperazines as novel, brain-penetrant antiprion compounds. *ACS Med Chem Letters* 4(4):397–401.
3. Kimata A, et al. (2007) New series of antiprion compounds: Pyrazolone derivatives have the potent activity of inhibiting protease-resistant prion protein accumulation. *J Med Chem* 50(21):5053–5056.
4. Geissen M, et al. (2011) From high-throughput cell culture screening to mouse model: Identification of new inhibitor classes against prion disease. *ChemMedChem* 6(10):1928–1937.
5. Ghaemmaghami S, May BC, Renslo AR, Prusiner SB (2010) Discovery of 2-aminothiazoles as potent antiprion compounds. *J Virol* 84(7):3408–3412.
6. Nicoll AJ, et al. (2010) Pharmacological chaperone for the structured domain of human prion protein. *Proc Natl Acad Sci USA* 107(41):17610–17615.
7. Kuwata K, et al. (2007) Hot spots in prion protein for pathogenic conversion. *Proc Natl Acad Sci USA* 104(29):11921–11926.
8. Doh-Ura K, Iwaki T, Caughey B (2000) Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. *J Virol* 74(10):4894–4897.
9. Korth C, May BC, Cohen FE, Prusiner SB (2001) Acridine and phenothiazine derivatives as pharmacotherapeutics for prion disease. *Proc Natl Acad Sci USA* 98(17):9836–9841.
10. Mead S, et al. (2011) PRION-1 scales analysis supports use of functional outcome measures in prion disease. *Neurology* 77(18):1674–1683.
11. Collinge J, et al. (2009) Safety and efficacy of quinacrine in human prion disease (PRION-1 study): A patient-preference trial. *Lancet Neurol* 8(4):334–344.
12. Benito-León J (2004) Combined quinacrine and chlorpromazine therapy in fatal familial insomnia. *Clin Neuropharmacol* 27(4):201–203.
13. Nakajima M, et al. (2004) Results of quinacrine administration to patients with Creutzfeldt-Jakob disease. *Dement Geriatr Cogn Disord* 17(3):158–163.
14. Kobayashi Y, Hirata K, Tanaka H, Yamada T (2003) [Quinacrine administration to a patient with Creutzfeldt-Jakob disease who received a cadaveric dura mater graft: An EEG evaluation]. *Rinsho Shinkeigaku* 43(7):403–408.
15. Geschwind MD, et al. (2013) Quinacrine treatment trial for sporadic Creutzfeldt-Jakob disease. *Neurology* 81(23):2015–2023.
16. Bian J, et al. (2010) Cell-based quantification of chronic wasting disease prions. *J Virol* 84(16):8322–8326.
17. Barret A, et al. (2003) Evaluation of quinacrine treatment for prion diseases. *J Virol* 77(15):8462–8469.
18. Mays CE, et al. (2012) Prion inhibition with multivalent PrPSc binding compounds. *Biomaterials* 33(28):6808–6822.
19. Fasano C, et al. (2008) Gene expression profile of quinacrine-cured prion-infected mouse neuronal cells. *J Neurochem* 105(1):239–250.
20. Klingenstein R, et al. (2006) Tricyclic antidepressants, quinacrine and a novel, synthetic chimera thereof clear prions by destabilizing detergent-resistant membrane compartments. *J Neurochem* 98(3):748–759.
21. Ryou C, et al. (2003) Differential inhibition of prion propagation by enantiomers of quinacrine. *Lab Invest* 83(6):837–843.
22. Browning SR, et al. (2004) Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. *J Virol* 78(23):13345–13350.
23. Peretz D, et al. (2001) Strain-specified relative conformational stability of the scrapie prion protein. *Protein Sci* 10(4):854–863.
24. Saijo E, et al. (2013) Epigenetic dominance of prion conformers. *PLoS Pathog* 9(10):e1003692.
25. Makarava N, et al. (2011) Genesis of mammalian prions: From non-infectious amyloid fibrils to a transmissible prion disease. *PLoS Pathog* 7(12):e1002419.
26. Angers RC, et al. (2010) Prion strain mutation determined by prion protein conformational compatibility and primary structure. *Science* 328(5982):1154–1158.
27. Collins SJ, et al. (2002) Quinacrine does not prolong survival in a murine Creutzfeldt-Jakob disease model. *Ann Neurol* 52(4):503–506.
28. Ghaemmaghami S, et al. (2009) Continuous quinacrine treatment results in the formation of drug-resistant prions. *PLoS Pathog* 5(11):e1000673.
29. Kamatari YO, Hayano Y, Yamaguchi K, Hosokawa-Muto J, Kuwata K (2013) Characterizing antiprion compounds based on their binding properties to prion proteins: Implications as medical chaperones. *Protein Sci* 22(1):22–34.
30. Georgieva D, et al. (2006) Interactions of recombinant prions with compounds of therapeutical significance. *Biochem Biophys Res Commun* 344(2):463–470.
31. Vogtherr M, et al. (2003) Antimalarial drug quinacrine binds to C-terminal helix of cellular prion protein. *J Med Chem* 46(17):3563–3564.
32. Green KM, et al. (2008) Accelerated high fidelity prion amplification within and across prion species barriers. *PLoS Pathog* 4(8):e1000139.
33. Telling GC, et al. (1996) Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* 274(5295):2079–2082.
34. Li J, Browning S, Mahal SP, Oelschlegel AM, Weissmann C (2010) Darwinian evolution of prions in cell culture. *Science* 327(5967):869–872.
35. Ghaemmaghami S, et al. (2011) Conformational transformation and selection of synthetic prion strains. *J Mol Biol* 413(3):527–542.
36. Yung L, et al. (2004) Pharmacokinetics of quinacrine in the treatment of prion disease. *BMC Infect Dis* 4:53.
37. Berry DB, et al. (2013) Drug resistance confounding prion therapeutics. *Proc Natl Acad Sci USA* 110(44):E4160–E4169.
38. Zawada Z, et al. (2013) Quinacrine reactivity with prion proteins and prion-derived peptides. *Amino Acids* 44(5):1279–1292.
39. Dearmond SJ, Bajsarowicz K (2010) PrPSc accumulation in neuronal plasma membranes links Notch-1 activation to dendritic degeneration in prion diseases. *Mol Neurodegener* 5:6.
40. Spilman P, et al. (2008) A gamma-secretase inhibitor and quinacrine reduce prions and prevent dendritic degeneration in murine brains. *Proc Natl Acad Sci USA* 105(30):10595–10600.
41. Huang Y, et al. (2006) Quinacrine is mainly metabolized to mono-desethyl quinacrine by CYP3A4/5 and its brain accumulation is limited by P-glycoprotein. *Drug Metab Dispos* 34(7):1136–1144.
42. Gayraud V, et al. (2005) A possible pharmacological explanation for quinacrine failure to treat prion diseases: Pharmacokinetic investigations in a ovine model of scrapie. *Br J Pharmacol* 144(3):386–393.
43. Sandberg MK, Wallén P, Wikström MA, Kristensson K (2004) Scrapie-infected GT-1 cells show impaired function of voltage-gated N-type calcium channels (Ca_v2.2) which is ameliorated by quinacrine treatment. *Neurobiol Dis* 15(1):143–151.
44. Farrelly PV, et al. (2003) Quinacrine blocks PrP (106–126)-formed channels. *J Neurosci Res* 74(6):934–941.
45. Turnbul S, Tabner BJ, Brown DR, Allsop D (2003) Quinacrine acts as an antioxidant and reduces the toxicity of the prion peptide PrP106–126. *Neuroreport* 14(13):1743–1745.
46. Dohgu S, et al. (2004) Uptake and efflux of quinacrine, a candidate for the treatment of prion diseases, at the blood-brain barrier. *Cell Mol Neurobiol* 24(2):205–217.
47. Chung E, et al. (2011) Styryl-based and tricyclic compounds as potential anti-prion agents. *PLoS ONE* 6(9):e24844.
48. Nguyen TH, et al. (2008) Antiprion activity of functionalized 9-aminoacridines related to quinacrine. *Bioorg Med Chem* 16(14):6737–6746.
49. Nguyen T, Sakasegawa Y, Doh-Ura K, Go ML (2011) Anti-prion activities and drug-like potential of functionalized quinacrine analogs with basic phenyl residues at the 9-amino position. *Eur J Med Chem* 46(7):2917–2929.
50. Villa V, et al. (2011) Efficacy of novel acridine derivatives in the inhibition of hPrP90–231 prion protein fragment toxicity. *Neurotox Res* 19(4):556–574.
51. Tran HN, Bongarzone S, Carloni P, Legname G, Bolognesi ML (2010) Synthesis and evaluation of a library of 2,5-bis(diamino)-benzoquinone derivatives as probes to modulate protein-protein interactions in prions. *Bioorg Med Chem Lett* 20(6):1866–1868.
52. Csuk R, et al. (2009) Synthesis of monomeric and dimeric acridine compounds as potential therapeutics in Alzheimer and prion diseases. *Arch Pharm (Weinheim)* 342(12):699–709.
53. Doh-ura K, et al. (2007) Chelating compound, chrysoidine, is more effective in both antiprion activity and brain endothelial permeability than quinacrine. *Cell Mol Neurobiol* 27(3):303–316.
54. Dollinger S, Löber S, Klingenstein R, Korth C, Gmeiner P (2006) A chimeric ligand approach leading to potent antiprion active acridine derivatives: Design, synthesis, and biological investigations. *J Med Chem* 49(22):6591–6595.
55. Sebestik J, Safarik M, Stibor I, Hlaváček J (2006) Acridin-9-yl exchange: A proposal for the action of some 9-aminoacridine drugs. *Biopolymers* 84(6):605–614.
56. Kocisko DA, Caughey B (2006) Mefloquine, an antimalaria drug with antiprion activity in vitro, lacks activity in vivo. *J Virol* 80(2):1044–1046.
57. Murakami-Kubo I, et al. (2004) Quinoline derivatives are therapeutic candidates for transmissible spongiform encephalopathies. *J Virol* 78(3):1281–1288.
58. May BC, et al. (2003) Potent inhibition of scrapie prion replication in cultured cells by bis-acridines. *Proc Natl Acad Sci USA* 100(6):3416–3421.
59. May BC, et al. (2006) Structure-activity relationship study of 9-aminoacridine compounds in scrapie-infected neuroblastoma cells. *Bioorg Med Chem Lett* 16(18):4913–4916.
60. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of radiosensitivity. *Cancer Res* 47(4):943–946.
61. Kang HE, et al. (2012) Characterization of conformation-dependent prion protein epitopes. *J Biol Chem* 287(44):37219–37232.
62. Angers RC, et al. (2009) Chronic wasting disease prions in elk antler velvet. *Emerg Infect Dis* 15(5):696–703.