Only a few years ago, pharmacotherapy of Creutzfeldt-Jakob disease was inconceivable. The enigmatic prion agent causing Creutzfeldt-Jakob disease, consisting solely of a misfolded conformational isoform, the scrapie prion protein, of the normal cellular prion protein was considered hard to treat by routine drug development. However, huge progress has been achieved in recent years, demonstrating principal reversibility of the neuropathological features and protection from clinical symptoms in animal models and introducing potential pharmaceutical agents. Among the most promising ones, antibodies have been shown to be protective against prion disease and heterocyclic small-molecule compounds have been proposed as antiprion lead compounds, initiating clinical trials. Arch Neurol. 2006;63:497-501

Human prion diseases are relatively rare disorders, with a worldwide average prevalence around 0.1 per 100,000 persons; however, their infectious nature makes them a health threat for humans and domestic and wildlife animals. The most common human prion disease is Creutzfeldt-Jakob disease (CJD). Approximately 85% of CJD cases occur as a sporadic disease and 15% as a genetic disease caused by mutations in the prion protein (PrP) gene (PRNP); fewer than 1% of human prion diseases are of infectious origin. Rare human prion diseases, like Gerstmann-Straussler-Scheinker disease or familial fatal insomnia, are genetic diseases. Prominent symptoms in the most prevalent form of sporadic CJD are cerebellar ataxia, myoclonic attacks and cognitive decline rapidly progressing to akinetic mutism, and complete disability within months after disease onset.

Prion diseases are more prevalent in ruminants like cattle, sheep, and US mule deer or elk. Scrapie, the endemic prion disease in sheep and goats, has been known for more than 200 years, and transmission of sheep prions to humans is thought to be impossible because of the existence of a species barrier between sheep and humans. In contrast, a species barrier between cattle and humans was abolished by prions of cattle affected by bovine spongiform encephalopathy causing variant CJD. Variant CJD is considered a novel human prion disease characterized by distinct clinical symptoms, including younger age of onset, more prevalent psychiatric symptoms initially, and a longer average time of disease (2 years instead of 6 months for sporadic CJD). Histopathologically, there is abundance of case-proving so-called florid plaques, microscopically visible protein aggregates consisting of the prion agent. An estimated 150 cases of variant CJD have been reported mainly in Great Britain, the country foremost affected with bovine spongiform encephalopathy. Concerns have been raised that blood or organ donors with asymptomatic disease might spread this seemingly more infectious strain of CJD among blood or organ recipients, which has already led to deferral of blood donations from high-risk individuals. It is not clear if a species barrier exists between cervid prions (chronic wasting disease) from Rocky Mountain mule deer or elk and humans, and how the chronic wasting disease prion agent compares with...
bovine spongiform encephalopathy. The advent of a domestic animal’s prion crossing the human species barrier in bovine spongiform encephalopathy has already alerted public health control, and—apart from introducing highly efficient prevention measures like the bone meal feeding ban to ruminants—has put a medical focus on prophylaxis and pharmacotherapy of human prion disease. As for all other neurodegenerative diseases, there are no causal therapies yet, but there have been several major advances in identifying lead compounds, some of which are undergoing clinical evaluations.

CELL BIOLOGY OF PrP CONVERSION DEFINES PHARMACOLOGICAL TARGETS

The uniqueness of prion diseases with regard to their pathogenesis, namely, their existence as sporadic, genetic, and infectious diseases, is explained by the cell biology of prions: the normal PrP, PrPC (where C indicates cellular), derives from a gene that is expressed by most cells of the body, and in particularly high levels by cells of nervous and lymphatic organs. After protein synthesis, PrPC is posttranslationally processed to receive up to 2 N-linked carbohydrate side chains and a glycosyl phosphatidylinositol membrane anchor before it is secreted to the plasma membrane (Figure), where it sorts into cholesterol-rich detergent-resistant membrane (DRM) domains or so-called lipid rafts.1 This membrane-anchored PrP can adopt a pathological and infectious conformation, PrPSc (where Sc indicates scrapie), by a mutation in its amino acid sequence due to either germline or somatic mutations, the latter explaining an increased likelihood in getting CJD with advanced age. Alternatively, it is assumed that a spontaneous switch into the “wrong” conformation occurs in sporadic CJD and that simultaneously cellular or subcellular clearance mechanisms that would normally rapidly degrade these misfolded proteins are impaired.

Once a critical amount of PrPSc is present—corresponding to the “infectious dosage”—PrPSc induces normal PrPC molecules to adopt the PrPSc conformation.1 It has been proposed1 that cofactors, like an assumed protein X, assist in this conversion. Sorting of PrPC into DRMs is mandatory for conversion because trafficking of PrPC out of DRMs or abolishing DRMs prevents conversion. Once the chain reaction of PrPSc converting membrane-resident PrPC into PrPSc has started, signals involving yet unidentified pathways are transduced, resulting in neuronal dysfunction and, ultimately, neuronal death. Accumulation of PrPSc in microscopically visible plaques and intensive vacuolation of central nervous system tissue accompanied by massive gliosis are neuropathological hallmarks of prion diseases.1

In a seminal experiment, Mallucci and coworkers1 were able to show, in transgenic mice in which neuron-
specific PrP could be knocked out during incubation of prions, that neuronal vacuolation could be reversed and neurological symptoms halted while PrPSc was still replicated by nonneuronal cells and accumulating in plaques. The PrP expression by neurons was, thus, critical for mediating toxic effects, whereas PrP replication also occurred independent of neurons. These experiments reversed the dogma that neuronal vacuolation and clinical disease progression are irreversible and give hope that pharmaceutical antiprion therapies—if targeting the right cells and molecules—may be effective.

From this short outline of prion cell biology, pharmacological targets can clearly be defined (Figure):

1. Gene silencing by small interfering RNA targets PrP expression as the substrate of prion conversion and the mediator of neurotoxicity. The feasibility of small interfering RNA–mediated PrP knockout has only been demonstrated in cells, but successful small interfering RNA–based therapies in other human central nervous system diseases suggest that a future therapy in CJD patients might be promising. Because no clear and obvious phenotypes have been described in PrP knockout mice, adverse effects through PrRN gene silencing are believed to be minor, if present at all.

2. Protecting or shielding PrPSc from conversion by antibodies is a powerful strategy that has been proved in cell and animal models of prion disease. The problem is how to engineer antibodies or fragments thereof such that they cross the blood-brain barrier.

3. Because the intactness of cholesterol-rich DRMs is mandatory for PrP conversion, its destabilization can prevent PrPSc replication. Depletion of cholesterol by the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, the statins, has been demonstrated to have antiprion effects in cell models of prion disease, but translation of this strategy into clinical medicine remains to be shown; interference with DRMs that execute essential cellular functions may cause adverse effects. Antiprion effects of quinacrine are possibly mediated by destabilization of DRMs (Ralf Klingerstein, PhD, and Dr Korth, unpublished data, 2005).

4. The misfolded form of PrP, PrPSc, is the only molecular component identified in prions. Whether there are other cellular cofactors assisting in PrP conversion has been hypothesized but not demonstrated. Inactivating these cofactors for preventing conversion would be a valuable pharmacological strategy. A potential binding site of PrP to an assumed conversion cofactor termed protein X has been used to construct a competitor pharmaceutical by molecular design that was effective in a cell model of prion disease.

5. Inactivating or shielding PrPSc such that it is rendered conversion incompetent is the most obvious antiprion strategy. Polyanions, like Congo red, dextran, heparin sulfate, and pentosan polysulfate, have been described as possessing antiprion activity in cell and animal models. The presumed mechanism of action is a direct interaction with PrPSc.

Because pentosan polysulfate is used for the treatment of interstitial cystitis and other urological disorders, its clinical use for treating CJD is considered. The major shortcoming of all strongly charged molecules, including the polyanions, is their lack of blood-brain barrier permeability. This problem was circumvented by intrathecal application of pentosan polysulfate in a mouse model of prion disease, which led to a significant increase in incubation time of treated mice. Whether this approach will be feasible and efficient in CJD patients is discussed.

6. To some extent, the cell can degrade misfolded PrPSc because in a cell model of prion disease, the half-life of PrPSc has been calculated to be around 28 hours. That would make stimulation of cellular endogenous degradation mechanisms a potential pharmacological target. However, because the identity of specific PrPSc-degrading proteins is unknown, these thoughts are purely conceptual.

7. Ultimately, the neurotoxic effects of PrPSc are responsible for the clinical symptoms of CJD. Preventing these effects has, therefore, paramount importance in treating CJD. So far, the exact cellular signals involved in transducing the neurotoxicity of PrPSc are not known. Fluoxetine has been used in treating CJD patients in, to our knowledge, the only double-blind placebo-controlled clinical trial for CJD treatment. The rationale for this study was that the N-methyl-D-aspartate antagonist properties of fluoxetine might limit accompanying glutamate-induced neurotoxicity in progressing CJD. Mild positive effects on cognitive functions, but no prolongation in survival time, were observed in this study.

HETEROCYCLIC ANTIPRION COMPOUNDS AND CURRENT CLINICAL TRIALS

A major disadvantage of the early antiprion compounds like the polyanions was their inability to cross the blood-brain barrier and their relative failure to show antiprion effects when applied late in the incubation period in animal models of prion disease. In an effort to identify antiprion compounds with high blood-brain barrier permeability, approved drugs used in treating central nervous system diseases were screened for antiprion effects in a cell model of prion disease. This search resulted in identification of the heterocyclic compounds phenothiazine and acridine derivatives, with clear structure-activity relationships, as lead compounds for antiprion drug development. Quinacrine, the acridine derivative with the highest antiprion activity (median effective concentration, 300 nm) in cell culture, was 10 times more active than chlorpromazine, the highest antiprion phenothiazine derivative. Originally an antiparasitic drug, quinacrine was used for the treatment of malaria during World War II and, more recently, for treating giardiasis. The fact that quinacrine was an approved drug led to its immediate use for compassionate treatment of CJD patients. In parallel, animal experiments were started to further elucidate its mechanisms of action. In mice, results were ambiguous, with one group reporting extension of survival time by up to 25% with quinacrine therapy at a dosage of 37 mg/kg per day, while others reported failure to see antiprion effects or reported toxic effects after intrathecal application. Application of a combination therapy of quinacrine (150 mg/d intramuscularly) and chlorpromazine (100 mg/d intramuscularly) for 7 to 30 days...
in symptomatic ewes that had been naturally infected with scrapie did not show a significant extension of average survival times; the authors of this study, however, raised the issue that intracerebral quinacrine concentrations might not have been high enough, even at subtoxic dosages, to reach antiprion-effective concentrations.

Application of quinacrine in dosages of 300 mg/d to symptomatic CJD patients based on compassionate treatment has been reported in single cases. In some quinacrine-treated CJD patients, a transient improvement of aural and akinetic symptoms was described. No outstanding extensions of survival time that would differ from the usual rapid course of CJD were reported. However, in the absence of a controlled study with a sufficient number of patients, modest differences in disease course between quinacrine- and placebo-treated CJD patients will not be detected, an outcome that would still justify quinacrine’s role as a lead compound. The rarity of patients is a major problem for evaluating the therapeutic potential of lead compounds in CJD pharmacotherapy. Three groups are evaluating quinacrine as a treatment for CJD patients in trials.

In a stratified, randomized, double-blinded, placebo-controlled clinical trial, Michael Geschwind, MD, PhD, and Bruce Miller, MD, from the Memory and Aging Clinic at the University of California, San Francisco, are investigating survival as a primary outcome and the results of magnetic resonance imaging, electroencephalography, and neuropsychological testing and neurological examination changes as secondary outcomes; for this study, the plan is to enroll more than 60 patients over 3 years.

At the National Prion Clinic at the National Hospital for Neurology and Neurosurgery, London, England, Stephen Wroe, MD, and John Collinge, MD, and colleagues are conducting a partially randomized patient preference trial to evaluate quinacrine’s effects on the treatment of CJD patients. Randomization includes either quinacrine or delayed quinacrine at 24 weeks; alternatively, patients or their relatives may choose to receive quinacrine immediately or just be monitored without specific treatment. The plan is to recruit 160 patients into the trial.

An open study that will evaluate the clinical benefits of quinacrine in combination with flupirtine is under way by Markus Otto, MD, at the University of Ulm, Ulm, Germany. Flupirtine as a specific CJD monotherapy was previously shown to improve cognitive symptoms in CJD patients in a randomized, double-blind, clinical trial.

The Table provides a description of the pharmaceutical agents that have been used in patients with CJD or those who are in clinical trials.

### OUTLOOK: A 2-HIT STRATEGY FOR COMBATTING CJD?

We hope that ongoing clinical trials will come up with a conclusive result regarding the role of quinacrine as an antiprion agent and, possibly, as a model substance for future drug development. The rapid progression and devastating clinical and histopathological consequences of prion propagation will also require the definition of realistic outcomes of clinical trials. Specifically, an average survival of 6 months after symptom onset for the most prevalent sporadic CJD case will make it difficult to detect subtle improvements after pharmacological intervention unless a substance has truly dramatic biological effects. However, neuropathological and clinical symptoms may be reversible to a greater degree than previously thought, which should encourage further developments in CJD therapy. Two aspects will be paramount for the immediate future of CJD pharmacotherapy: (1) developing strategies to counteract PrPSc-induced neurotoxicity and (2) developing diagnostic tests for early CJD, or even asymptomatic CJD.

An aspect that has been neglected in the development of experimental pharmacotherapies in cell or animal models of prion disease is the necessity of preventing neurotoxicity in parallel with preventing prion replication. For example, even if conversion of new PrPSc can be prevented by replication-targeting drugs like the heterocyclic compounds, extracellular prion plaques or PrPSc deposits that have accumulated in the asymptomatic phase of CJD—possessing a half-life much longer than the cellular half-life—serve as a reservoir of PrPSc-induced neurotoxicity by constantly shedding PrPSc oligomers into the extracellular space. Destroying these PrPSc deposits and preventing their neurotoxicity simultaneous to preventing prion replication might, therefore, lead to a successful future 2-hit strategy.

It is also clear that early detection of CJD, at best identification of asymptomatic “incubating” CJD, may greatly improve the prospects of a “cure” from prions before structural and irreversible damage has occurred. This strategy has been applied successfully in other disorders, including neoplastic diseases, in which a routine preventive screening often leads to medical advice for prophylactic therapy. Because high-sensitivity tests for asymptom-
atic CJD are not yet available, their development should be another major effort in the improvement of pharmacotherapy for CJD.

Accepted for Publication: September 1, 2005.
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Author Contributions: Study concept and design: Korth and Peters. Acquisition of data: Korth. Analysis and interpretation of data: Korth. Drafting of the manuscript: Korth. Critical revision of the manuscript for important intellectual content: Korth and Peters. Obtained funding: Korth. Administrative, technical, and material support: Korth. Study supervision: Korth and Peters.

Funding/Support: This study was supported by grant 01KO0107 from the Federal Ministry of Education and Research, Germany (Dr Korth), and grant QLK2-CT-2002-81628 from the European Union (Dr Peters).

Acknowledgment: We acknowledge the work and numerous articles on prion pharmacotherapy that could not be cited in this short review because of space constraints.

REFERENCES


Correction

Error in Text. In the Original Contribution by Hodapp et al titled “Double Trouble in Hereditary Neuropathy: Concomitant Mutations in the PMP-22 Gene and Another Gene Produce Novel Phenotypes,” published in the January issue of the ARCHIVES (2006;63:112-117), on page 113 in the “Family 1” subsection of the “Case Reports” section, the genetic analysis showing the missense mutation in the GJB1/Connexin-32 gene should be Arg220Gly instead of arg200gly.