Quinacrine: Therapeutic or apoptotic? Certainly double-edged.

Michael Janeczek
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Abstract
A new drug has demonstrated some success in tackling deadly prion diseases. But, it turns out that it only treats experimentally developed, mouse-specific strains—and it may have a much darker side to it.

Prion diseases—sneaky killers that always get the job done, stripping their victims of dignity, as well as of mental and physical capabilities—have been in the crosshairs of neuroscientific endeavor for the past five decades. The name “prion,” originally coined by Stanley B. Prusiner in 1982, simply refers to the infectious nature of the proteins involved in a number of neurodegenerative diseases, including “mad cow disease” (formally known as bovine spongiform encephalopathy, or BSE), Creutzfeldt-Jakob disease (CJD), scrapie, and kuru. Although sporadic CJD affects only about one person per million,2 the risk of a worldwide epidemic reminiscent of “mad cow disease” continues to haunt us like a skeleton in the closet, waiting to come out and wreak havoc. The remarkable, yet chilling, common denominator of all the above pathologies is the method by which an infectious version of a prion molecule (PrPSc) hijacks the protein synthesis machinery of the host organism and thereby converts non-harmful proteins (PrPC) into their deadly counterparts (PrPSc). This elusive crystallization-like process that turns the innocuous into the deadly has been the target of many therapeutic compounds, the most promising of which appears to be “quinacrine.”

Indeed, research conducted by Bian and colleagues (2010) has demonstrated that quinacrine is capable of reducing levels of harmful PrPSc in mouse cells infected with experimentally adapted rodent prions.3-7 Such findings, however, might lead the scientific community to prematurely celebrate quinacrine as the panacea for prion diseases. Bian and colleagues (2010) remind us that efforts to replicate quinacrine’s success in human patients, even across a number of settings, have been unsuccessful.8-12 Thus, in spite of a decade-long investment into researching quinacrine as a potential therapy for prion diseases, its controversial results13 stimulated Bian and colleagues (2010) to re-examine quinacrine’s effectiveness in naturally occurring prion diseases.

Their findings are startling to say the least. Upon administering quinacrine to transgenic mice that had variant cervid CJD (vCJD results from BSE), their condition significantly worsened and they developed an advanced form of prion disease: chronic wasting disease, or CWD. In addition, Western blotting, a technique used to qualitatively assess atomic mass, demonstrated a twofold increase of harmful PrPSc in said transgenic mice treated with quinacrine. With findings that represent the exact opposite of a therapeutic effect, Bian and colleagues (2010) questioned not only the viability of quinacrine, but also the viability of using experimentally adapted prions for research, since they originally hinted at quinacrine’s neuroprotective potency.

Initially, the researchers intended to assess the effects of quinacrine on CWD. In light of their results, however, Bian and colleagues (2010) subsequently focused on examining quinacrine-enhanced CWD, or Q-CWD. In order to test naturally occurring CWD in mice, Bian and colleagues (2010) had previously developed a cell culture that would allow for the expression and maintenance of CWD in mice.14 The culture was tested to ensure a response to drugs (dextran sulfate 500) consistent with drugs that reduce PrPSc levels in mice. Next, the researchers modified the scrapie cell assay to allow for the quantification of CWD in mice. Afterward, five strains of prion disease, all introduced into transgenic mice, were treated with 1 µM quinacrine for six consecutive days. Three of these strains were experimental rodent scrapie-based prions (SMB, ScN2a, and RKM+); one was an elk strain (Elk21+); and one was a deer strain (RKD+), which served as another cervid prion to eliminate the possibility that whatever happened with the elk strain was cell-specific. After the previous studies, upon administering quinacrine, mice with experimentally developed rodent prion disease had their levels of harmful PrPSc reduced by five to fourteen times.

However, as foreshadowed, when the same dose of quinacrine was administered to mice inoculated with the cervid cell cultures that allowed for the maintenance of CWD, the vCJD in those mice turned into CWD. Moreover, the number of harmful PrPSc in mice exhibiting CWD more than doubled in the elk strain and significantly increased in the deer strain, supporting the hypothesis that quinacrine is ineffective against naturally occurring prion disease—in this case, CWD. In other words, quinacrine intensified the replication of the deadly prions in cervid strains and even altered the structure of the harmful molecules, essentially producing brand new ones. These unsettling results were replicated at different time frames and at various dosages, which led Bian and colleagues (2010) to examine their structural basis.

As the dosage of quinacrine increased (from 1 µM up to 10 µM), the mice that developed CWD had increased numbers of PrPSc and suffered from reduced cell viability. The most dramatic decrease in cell viability was between 2.5 µM and 5 µM, both of which lead to apoptosis. Interestingly, when quinacrine was withdrawn, the levels of PrPSc returned to normal values, comparable with other vCJD-infected mice. These findings led Bian and colleagues to hypothesize that quinacrine altered the conformational stability of PrPSc, which was later confirmed.

The researchers devised a cell-based conformational stability assay (CSA) and discovered that PrPSc isolated from quinacrine-treated, elk-strain infected mice had a significantly greater conformational stability than the proteins without quinacrine. Similarly, the deer strain exhibited a ten percent increase in conformational stability when treated with quinacrine. Thus, the researchers called such quinacrine-enhanced prions “Q-CWD.” After inoculating mice with the new Q-CWD prions, the researchers noticed that mice with quinacrine-enriched prions had a later disease onset at about 172 ± 2 days, compared to 112 ± 1 day in untreated mice. Moreover, the incubation period was 27 percent longer in Q-CWD infected mice.

In addition to an altered physical conformation of the Q-CWD prions, Bian and colleagues (2010) compared the distribution of Q-CWD and CWD plaques using immunohistochemical and histoblot analyses, finding reproducibly distinct patterns. For instance, the Q-CWD plaques were denser in the cerebellum, but relatively scarce in the medulla. On the other hand, non-treated misfolded prions followed the opposite pattern: they were more prominent in the medulla, but less visible in the cerebellum (see Figure 1 for a comparison of the pattern in the cerebellum).

Figure 1. A chart summarizing the effects of identical dosage of quinacrine in transgenic mice infected with natural (left) and experimental (right) PrPSc. The contra-dictory effect is even more pronounced in Q-CWD mice, since the physical conformation of the prion changes, producing a different disease (as seen on the cerebellar plaque pattern—adapted from Bian et al.).
To conclude, Bian and colleagues (2010) have demonstrated the ineffectiveness of quinacrine in naturally-occurring prion disease (CDW) while re-affirming its effectiveness in experimentally designed rodent prions. This inconsistency poses some serious questions regarding animal testing and its applicability to human clinical trials. The researchers speculate that the variable effects of quinacrine in mice and cervid prions have two possible explanations: 1) differential binding of quinacrine to species-specific structures, or 2) particular prion conformation. The researchers mention that one of the binding sites for quinacrine, called glutamine, is shared by cervid and human prions but not shared by mice, suggesting a possible explanation for the inefficiency of quinacrine in human trials. Ultimately, prion diseases are clearly species and strain dependent, a finding which future researchers ought to take into consideration.

In the future, more research should target murine, ovine, and cervid prions, as opposed to relying on experimentally derived strains. In addition, research suggests that quinacrine's usefulness may have been over-estimated, which should encourage individuals to focus on other therapeutic means.

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References


