Inhibition of Protease-Resistant Prion Protein Accumulation In Vitro by Curcumin

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Received 15 November 2002/Accepted 3 February 2003

Inhibition of the accumulation of protease-resistant prion protein (PrP-res) is a prime strategy in the development of potential transmissible spongiform encephalopathy (TSE) therapeutics. Here we show that curcumin (diferoylmethane), a major component of the spice turmeric, potently inhibits PrP-res accumulation in scrapie agent-infected neuroblastoma cells (50% inhibitory concentration, ∼10 nM) and partially inhibits the cell-free conversion of PrP to PrP-res. In vivo studies showed that dietary administration of curcumin had no significant effect on the onset of scrapie in hamsters. Nonetheless, other studies have shown that curcumin is nontoxic and can penetrate the brain, properties that give curcumin advantages over inhibitors previously identified as potential prophylactic and/or therapeutic anti-TSE compounds.

Transmissible spongiform encephalopathies (TSE) or prion diseases are untreatable, fatal neurodegenerative diseases that include bovine spongiform encephalopathy, chronic wasting disease, scrapie, and Creutzfeldt-Jakob disease. A central event in TSE diseases is the conversion of the normal, protease-sensitive isoform of prion protein (PrP-sen or PrP\(^{\text{C}}\)) to an abnormal, protease-resistant form, PrP-res or PrP\(^{\text{Sc}}\). Numerous compounds have been identified as inhibitors of PrP-res formation in scrapie agent-infected murine neuroblastoma (ScNB) cells (1–3, 5). The most potent of these inhibitors can also protect rodents against scrapie if they are administered near the time of infection (7, 8, 10, 11, 14). Unfortunately, none of these compounds are known to be both safe and effective for use in humans and animals (8, 10, 11). One therapeutic weakness of most of these compounds is likely an inability to penetrate the brain where most of the PrP-res accumulates and TSE pathogenesis occurs.

Curcumin, the major component of the spice turmeric and the yellow pigment in curry powder, has several properties that make it of interest as a possible anti-TSE drug. First, its structure resembles Congo red (6). Second, unlike Congo red, curcumin is uncharged and is thought to have at least limited bioavailability to the brain after consumption. Indeed, recent studies with a rat model of Alzheimer’s disease reported that dietary curcumin reduces β-amyloid deposition in the brain as well as associated neuropathology and cognitive deficits (9, 12). Third, curcumin has antioxidant activity, a factor that may be important given that oxidative damage is a feature in TSE neuropathogenesis (13). Fourth, humans consume curcumin in large amounts with no apparent toxicity. Toxicology studies have indicated that rodents can tolerate for a long period up to 5% of their diet being turmeric oleoresin (∼80% curcumin) without their life spans being shortened (http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr427.html). These considerations prompted us to test whether curcumin could inhibit the formation and accumulation of PrP-res.

Curcumin was added to the culture medium of ScNB cells seeded at a density of 1/10 confluence and grown to near confluence for 3 to 4 days. Approximately 90% of the ScNB cells used to seed these cultures were infected, as was indicated by the fact 9 out of 10 single-cell subclones from concurrent passes of this ScNB cell line were highly positive for PrP-res (data not shown). The accumulation of PrP-res during the growth of the ScNB cultures was assayed by detergent extraction, proteinase K (PK) treatment, and immunoblotting by previously described methods (5). A curcumin concentration-dependent reduction of PrP-res accumulation was observed with a concentration giving half maximal inhibition of ∼10 nM (Fig. 2A and B). This 50% inhibitory concentration rivals that of Congo red (∼10 nM) (2, 3) and is 2,500-fold lower than the concentration of curcumin (25 μM) that began to show evidence of cytotoxicity in the ScNB cells (not shown). The curcumin-induced reduction in PrP-res was long-lasting because the PrP-res levels in cultures treated with 1 μM curcumin for 4 days (one pass) remained low after four subsequent passes in the absence of curcumin (Fig. 2C). The observed effects of curcumin were not due to artifactual interference with the detection of PrP-res or an enhancement of the protease sensitivity of PrP-res after lysis of the cells, because addition of 1 μM curcumin to the otherwise untreated control cell lysates prior to PK digestion did not affect the amount of PrP-res detected (Fig. 2D). Furthermore, incubation of purified hamster 263K strain PrP-res (0.13 μM) with curcumin concentrations of up to 5 mM in 50 mM Tris-HCl–150 mM NaCl, pH 8, for 16 h at room temperature did not enhance its sensitivity to PK or reduce its detection by immunoblotting (data not
The inhibition of PrP-res accumulation in the ScNB cells by curcumin was not due to a reduction of protein synthesis generally or PrP-sen biosynthesis specifically because there was no reduction in the metabolic labeling of cellular proteins (Fig. 3A) or PrP-sen (Fig. 3B) after either 1-h or 4-day treatments with 1 μM curcumin, a concentration that fully inhibited PrP-res accumulation in the intact cells. Taken together, these results provide evidence that curcumin inhibits PrP-res accumulation potently, irreversibly, and selectively in live ScNB cells.

To test the direct effects of curcumin on the conversion of PrP-sen to PrP-res, we used cell-free conversion reaction mixtures in which purified hamster PrP-res (263K strain) induces the conversion of hamster 35S-PrP-sen to 35S-PrP-res. Partial mean inhibition of up to 41% (at 10 μM curcumin) was observed under previously described conditions with guanidine hydrochloride and detergents (6) (Fig. 4). A similar inhibition of PrP conversion reactions was observed under other conditions, including those without guanidine hydrochloride (data not shown). These results indicated that curcumin partially inhibits the direct interactions between PrP-sen and PrP-res that lead to conversion. Whether this direct inhibition accounts mechanistically for the more complete inhibition observed in ScNB cells is not clear.

Tests of the prophylactic anti-TSE effects of curcumin in...
vivo are in progress with hamsters inoculated with the 263K strain of scrapie. Hamsters were fed ad libitum diets containing 0.2% (wt/wt) curcumin beginning 14 days before infection, on the day of infection, or 45 days postinfection (dpi) by the intracerebral route with 50 μL of 1% (vol/vol) brain homogenate (107 50% lethal doses) from clinically ill hamsters infected with the 263K strain of scrapie. Incubation periods to end-stage clinical disease (recumbent animals) of the hamsters fed a control diet (no curcumin) or curcumin at days −14 and 0 and 45 dpi were 82.6 ± 3.9, 85.3 ± 4.0, 84.1 ± 4.3, and 83.5 ± 4.1 days (means ± standard deviations [SD]), respectively, with 10 to 12 animals in each group. A 10-fold-higher dose of curcumin (2% [wt/wt]) beginning at 45 dpi gave an average incubation period of 85.4 ± 4.7 days and, thus, did not substantially improve the efficacy of the late curcumin treatment.

Similar relative effects were observed in the incubation periods to the first detectable clinical signs. Thus, curcumin had little or no protective effect against a large intracerebral dose of scrapie.

Further experiments are under way to determine whether curcumin is more effective against lower oral, intraperitoneal, and intracerebral doses of scrapie agents of different strains and in different host species. Furthermore, it is possible that other routes of administration of curcumin may be more effective than mixing it with feed. Although curcumin has been demonstrated to exert some beneficial effects in the brains of rats with Alzheimer’s disease (9, 12), it remains to be determined how well it accumulates in the brain after oral administration to other species (e.g., hamsters and mice). Disease state might also impact curcumin’s blood-brain-barrier permeability in a species-dependent manner.

In conclusion, curcumin is among the smallest and most potent inhibitors of PrP-res formation to be identified in vitro. Our initial tests have not indicated that dietary curcumin is effective against high intracerebral doses of scrapie in vivo. However, continued study of curcumin as a potential lead compound for TSE prophylaxis and therapeutics seems warranted because of the major advantages that it has over other known PrP-res inhibitors, namely, lack of toxicity and carcino-
genicity, apparent oral bioavailability to the brain (9, 12), and antioxidant activity.

REFERENCES