Enhanced Antiscrapie Effect Using Combination Drug Treatment

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The transmissible spongiform encephalopathies (TSEs), or prion diseases, include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy, chronic wasting disease of deer and elk, and scrapie of sheep and goats. The appearance of variant CJD, linked to consumption of bovine spongiform encephalopathy-infected cattle, has increased awareness of TSEs. These diseases are characterized by the accumulation of an abnormal protease-resistant form of prion protein (PrP-res), derived from normal prion protein (PrP-sen) (2). Considerable evidence indicates that PrP-res is either the infectious agent or a critical component (8).

Some compounds have been able to delay scrapie onset in rodents when administered at or near the time of peripheral infection, but few have helped after intracerebral (i.c.) inoculation. Two compounds effective after i.c. scrapie inoculation in rodents when administered at or near the time of peripheral infection, but few have helped after intracerebral (i.c.) inoculation of an abnormal protease-resistant form of prion protein (PrP-res), derived from normal prion protein (PrP-sen) (2). Considerable evidence indicates that PrP-res is either the infectious TSE agent or a critical component (8).

Some compounds have been able to delay scrapie onset in rodents when administered at or near the time of peripheral infection, but few have helped after intracerebral (i.c.) inoculation. Two compounds effective after i.c. scrapie inoculation include pentosan polysulfate (PPS) (5) and Fe(III)meso-tetra(4-sulfonatophenyl)porphine (FeTSP) (7), which, due to poor blood-brain barrier penetration, must be administered directly to the brain. PPS, a semisynthetic carbohydrate polymer approved as an oral therapy for interstitial cystitis (Elmiron), is being infused into the brains of CJD patients as an experimental therapy (11). FeTSP, a porphyrin, recently demonstrated antiscrapie activity when administered via i.c. injections to mice with established brain infections (7). Here, we report significant antiscrapie activity by using the combined formulation of PPS and FeTSP.

Increased survival time after scrapie inoculation is a common measure of antiscrapie activity. Here, transgenic mice overexpressing hamster prion protein (Tg7) were used because of their relatively short scrapie incubation period (9). All mice were inoculated i.c. with 50 µl of 1% (wt/vol) brain homogenate from 263K scrapie-infected hamster brains. The first of five weekly i.c. drug injections was initiated 14, 28, or 35 days later. Tg7 mice in this study were euthanized when they showed obvious scrapie clinical symptoms, which in this strain is usually within 1 day of death (5). Animal procedures were approved by the guidelines of the Rocky Mountain Laboratory Animal Care and Use Committee. FeTSP and Fe(III)meso-

tetra(4-N,N,N,N-trimethylanilinium)porphine (FeTAP) were purchased from Porphyrin Products (Logan, UT), and PPS was a gift from Biopharm Australia (Bondi Beach, Australia). Statistical calculations were made using GraphPad Prism 4 software.

Scrapie-infected mice injected i.c. separately with either PPS or FeTSP beginning 14 days after inoculation had an average increased survival time of 26.5 or 16.9 days, respectively (Fig. 1A). Treatment with a combination of PPS and FeTSP by the same dosing regimen increased survival time by an average of 52.4 days (Fig. 1A). This delay was 9 days or 21% more than the survival times induced by the drugs individually (26.5 days + 16.9 days = 43.4 days). Using two-way analysis of variance (ANOVA) (10), the combined use of PPS and FeTSP produced a statistically significant positive interaction effect (P = 0.0004). In contrast to combined FeTSP and PPS treatment, FeTAP, an iron-substituted porphyrin without antiscrapie activity under these circumstances, did not result in an increased antiscrapie effect when combined with PPS (Fig. 1B). Consequently, although FeTSP and PPS treatment resulted in an enhanced antiscrapie effect, this is not a characteristic of all porphyrins.

Testing of PPS, FeTSP, and their combination was also started at 28 or 35 days after inoculation or at the onset of clinical symptoms (~40 to 50 days). Treatment starting at 28 days postinoculation was less effective than at 14 days. FeTSP increased survival time by an average of 3.4 days, marginally significant by an unpaired t test (P = 0.057), but PPS treatment extended life span by an average of 12.4 days (Fig. 2A). The combination extended life span by an average of 29.0 days, which is 13.2 days or 84% more than the sum of the single-compound treatment extensions. As with treatment starting at 14 days, two-way ANOVA showed a statistically significant positive interactive effect for the combined use of PPS and FeTSP (P = 0.03). Treatment starting at 35 days postinoculation demonstrated no significant benefit with either single-treatment group or the combination (Fig. 2B). To investigate PPS and FeTSP as a possible therapy for late-stage treatment, animals were treated with one dose of PPS and FeTSP intracerebrally and 10 mg PPS/kg of body weight intraperitoneally at the onset of clinical symptoms. Even with the additional intraperitoneal dose of PPS, no benefit was observed.
Determination of the antiscrapie mechanism of the FeTSP- and PPS combination treatment in vivo is hindered by an incomplete understanding of TSE infection and disease mechanisms. However, two-way ANOVA of the results from combination treatment at 14 and 28 days postinoculation suggests synergy rather than a simple additive effect (10). One possible explanation is that the presence of PPS or FeTSP might increase the half-life of the other compound by inhibiting an enzyme important in that compound’s metabolism. Alternatively, each may differentially bind PrP and/or other molecules which might slow PrP-res accumulation or its pathological consequences. PPS and FeTSP individually inhibit the formation of PrP-res in chronically scrapie-infected cell cultures (3, 4); however, combinations of PPS and FeTSP were additive, and not synergistic, in this in vitro PrP-res inhibition model (Fig. 3). Also, PPS treatment alone has been shown to vastly reduce PrP-res in scrapie-infected mouse brains (5). This suggests that the in vivo effects seen may involve more-complex biological interactions than the inhibition of PrP-res accumulation seen in cell culture or in vivo.

Regardless of the mechanism of action, on a practical level, the combination therapy was more effective than separate treatments. As PPS is being infused into the brains of CJD patients, the initial results reported here suggest that the addition of FeTSP to the treatment might be beneficial. Because the results from weekly i.c. dosing were so encouraging, further experiments are planned to continuously deliver PPS, FeTSP, and PPS/FeTSP to the brain by an infusion pump. It is hoped that brain infusion will be a more effective route of administration by providing a more constant concentration of drug over a longer period of time and that it will also allow a greater total dose of the combination to be safely administered. Finally, toxicology studies of PPS/FeTSP are needed, but a number of other porphyrins and porphyrin analogs have been approved for clinical use (1). Based on this finding, combination therapy for TSE treatment may lead to more-effective intervention for neurodegenerative diseases in general.

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