MAJOR ARTICLE

Prophylactic and Therapeutic Effects of Phthalocyanine Tetrasulfonate in Scrapie-Infected Mice

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The transmissible spongiform encephalopathy (TSE) diseases are rare, neurodegenerative diseases that include scrapie in sheep, bovine spongiform encephalopathy, and Creutzfeldt-Jakob disease in humans. There are no effective treatments available for clinical use in humans. We now demonstrate that, in 2 different rodent models of scrapie, multiple pretreatments with the cyclic tetrapyrrole phthalocyanine tetrasulfonate (PcTS) were as effective at delaying disease as multiple treatments starting at the time of infection. At low doses of scrapie infectivity, PcTS also protected some mice from peripheral scrapie infection, even if treatment was initiated several weeks after infection. Furthermore, PcTS completely inactivated low levels of scrapie infectivity when incubated with the infectious inoculum. Thus, PcTS has a broad range of antiscrapie activities. These findings suggest that cyclic tetrapyrroles may be useful both prophylactically and therapeutically against TSE diseases in vivo, as well as for inactivation of TSE infectivity suspended in solution.

The transmissible spongiform encephalopathy (TSE) diseases are a group of rare, fatal neurodegenerative diseases that include scrapie in sheep, Creutzfeldt-Jakob disease (CJD) in humans, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (BSE). After the onset of clinical signs, TSE diseases are uniformly fatal. Although many different compounds have been shown to at least partially inhibit TSE disease in rodent models of scrapie [1, 2], there are currently no effective prophylactic or therapeutic treatments for humans afflicted with CJD. Development of treatment strategies for TSE diseases is of particular importance, given that BSE has crossed a species barrier to cause variant CJD (vCJD) in humans [3]. There are similar concerns in North America that CWD could also cross species barriers and either directly infect humans or pose a threat to human health by infecting livestock animals, such as sheep and cattle.

A consistent finding in TSE diseases is the accumulation in the brain of an abnormal form of the host prion protein, PrP. Normal PrP (PrP-sen), a glycoprotein that is expressed on the cell surface in a wide variety of tissues, is both soluble and sensitive to digestion with proteinase-K. PrP-sen is the precursor to the protease-resistant abnormal form of PrP associated with disease (PrP-res). There is a significant amount of data demonstrating that PrP-res formation plays a key role in TSE disease pathogenesis [4]. Thus, PrP-res is an obvious target for therapeutic intervention, and multiple studies have shown that many compounds that inhibit PrP-res formation in vitro also are capable of inhibiting TSE disease in vivo [5–8].

The cyclic tetrapyrroles (cTPs) are a class of compounds that include biologically relevant porphyrins (e.g., heme and chloryphyll) [9], as well as phthalocyanines, many of which are commercially used as pigments and dyes. A distinguishing characteristic of the cTPs is their aromatic planar ring structure (figure 1). This structure has a polarizable, delocalized pi-electron system that, in heme proteins, is an important factor in the bonding between heme and protein. The pi-pi

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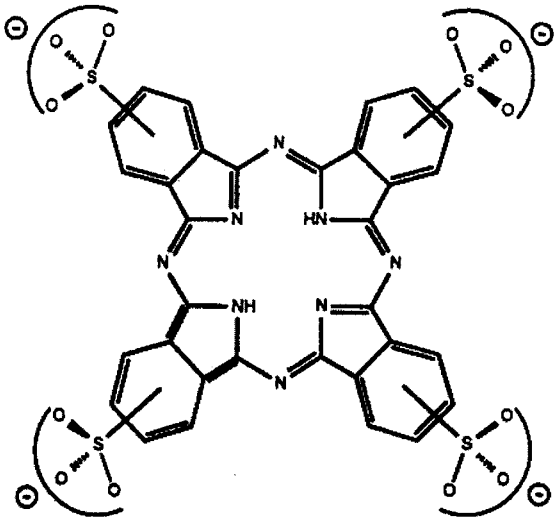

Figure 1. Structure of phthalocyanine tetrasulfonate anion

interactions between cTPs and aromatic amino acid residues contribute importantly to the ability of porphyrins to induce marked changes in heme-protein conformation [5]. This ability to induce changes in protein conformation suggests that the cTPs might be potential inhibitors of diseases of protein conformation, such as TSE diseases.

Recently, we demonstrated that several structurally distinct cTPs could inhibit PrP-res formation in vitro [5] and significantly delay TSE disease in vivo [8]. The compounds tested were effective either when administered at the time of infection or when mixed with a high-titer infectious inoculum but were ineffective if administered several weeks after infection [8]. Although the data demonstrated that these cTPs could be potent anti-TSE inhibitors, they suggested that the compounds would be most useful if given either prophylactically or used as a sterilizing agent.

We now demonstrate that pretreatment with the cTP phthalocyanine tetrasulfonate (PcTS) several weeks before infection delayed disease in 2 different models of rodent scrapie. Against lower doses of infectivity, PcTS also demonstrated a therapeutic effect. Furthermore, incubation of PcTS with different dilutions of the infectious inoculum inactivated several logs of infectivity and prevented clinical disease. Our data suggest that certain cTPs may be useful either prophylactically in individuals at risk of developing CJD, such as patients with familial TSE, or therapeutically against accidental exposure to a low dose of TSE agent. In addition, they may be useful for inactivation of low levels of TSE infectivity that may be present in medically relevant materials, such as blood products.

MATERIALS AND METHODS

Mice. Transgenic mice (Tg7) were derived by use of a hamster PrP-sen gene under the control of the native PrP promoter, as described elsewhere for the transgenic mouse line Tg10 [10], and were crossed onto a mouse $Prnp^{0/0}$ background [11]. Thus, Tg7 mice express high levels of hamster PrP-sen in a wide variety of tissues, including brain, but do not express endogenous mouse PrP-sen. Because Tg7 mice have significantly shorter disease incubation times than Syrian hamsters when infected with hamster 263K scrapie either intraperitoneally (~82 days vs. 120 days) or intracranially (~45 days vs. 75 days), they were used as a rapid assay for inhibition of scrapie disease. RML mice are an outbred strain of mice that are susceptible to high doses of scrapie and develop clinical disease ~220 days after intraperitoneal infection or 170 days after intracranial infection. All animal experimentation was performed according to the experimentation guidelines of the National Institutes of Health Animal Research Advisory Committee.

Tg7 scrapie infection and PcTS treatment. Weanling Tg7 mice were infected intraperitoneally with 0.05 mL of a stock 10% brain homogenate of hamster 263K scrapie that was diluted as indicated in the figure and table legends. The stock had an intraperitoneal (IP) lethal $dose_{50}$ (IP LD_{50}) in Tg7 mice of $1 \times 10^{6.2}$/0.05 mL.

A solution of PcTS (Porphyrin Products; figure 1) was freshly prepared in sterile PBS at a concentration of 5 mg/mL. Animals were injected intraperitoneally with 0.05 mL of the PcTS solution 3 times/week for 4 weeks, starting 28 days before infection, or for 3 times/week for 2 weeks, starting 14 days before infection. For both the 28- and 14-day pretreatment groups, animals were infected 3 days after the pretreatment regimen had been terminated. In postinfection-treatment groups, animals were treated intraperitoneally with PcTS 3 times/week over the course of 4 weeks, starting at the time of infection (0 days postinfection [dpi]), or at 1,14, 28, or 56 dpi. When treatment was initiated at 0 dpi, mice were first infected intraperitoneally with scrapie, followed immediately (within 5 min) by intraperitoneal administration of PcTS.

For intracranial infections, weanling Tg7 mice were inoculated with 0.05 mL of a 1:10 dilution of the same stock hamster 263K brain homogenate used for the IP inoculations (intracranial [IC] LD_{50}, 1×10^{9}/0.05 mL). PcTS treatment of Tg7 mice infected intracranially with 263K hamster scrapie was started either at the time of infection using the same regimen as for mice inoculated intraperitoneally or consisted of a single IC administration at the onset of clinical scrapie (0.05 mL of 5 mg/mL PcTS ~40 dpi).

RML scrapie infection and PcTS treatment. Weanling RML mice were infected with a 1:10 dilution of a stock 10% brain homogenate of RML mouse scrapie. The PcTS treatment regimen was the same as for the Tg7 mice, except that 0.1 mL of 5 mg/mL PcTS was administered intraperitoneally, because RML mice weigh approximately twice as much as Tg7 mice.

Treatment was started either 14 days before infection, at the time of infection (0 dpi), or 50, 100, and 150 dpi.

For IC infections, weanling RML mice were inoculated intracranially with 0.05 mL of a 1:10 dilution of the same stock of RML scrapie brain homogenate used in the IP inoculations (IC LD_{50}, 1×10^9/0.05 mL). Intraperitoneal PcTS treatment was done 3 times/week for 4 weeks and was initiated at the time of infection or at 14 and 70 dpi.

For all experiments, mice were monitored and sacrificed when deemed to have clinically advanced scrapie. Animals that survived scrapie infection were monitored until they became sick because of intercurrent disease or old age (up to 1000 dpi) and then were sacrificed. To confirm the diagnosis of scrapie, brain and spleen were removed and analyzed by Western blot, using the hamster PrP-specific monoclonal antibody 3F4 (Tg7 mice) or the rabbit polyclonal antiserum R.30 (RML mice [12]) for the presence of PrP-res, as described elsewhere [10].

Inactivation of infectivity in suspension. PcTS (10 mg/mL) was mixed with a 10^{-2}, 10^{-4}, or 10^{-6} dilution of a stock 263K hamster scrapie brain homogenate. The final concentration of PcTS was 5 mg/mL. The mixture was incubated at room temperature for ~30 min. For each dilution, 0.05 mL was injected intraperitoneally into weanling Tg7 mice, and the animals were monitored for signs of clinical scrapie.

Statistical analysis. When a single experimental group was compared to a control group, the unpaired Student's *t* test was used to assess the significance of the data. If multiple experimental groups were compared to a single control group within 1 experiment, statistical significance was determined by use of 1-way ANOVA with Dunnett's posttest.

RESULTS

PcTS can protect Tg7 mice against low level scrapie infection. Our previous results demonstrated that, in animals infected peripherally with a high dose of scrapie, PcTS (figure 1) was capable of increasing scrapie incubation times 2–3 fold if treatment was initiated at the time of infection [8]. To determine whether the protective effect of PcTS would be greater against lower doses of scrapie infectivity, we infected transgenic mice overexpressing hamster PrP-sen (Tg7 mice) intraperitoneally with increasing dilutions of hamster 263K scrapie. Infected animals then were treated with PcTS at various times after infection. For all infectious doses tested, incubation times significantly increased if treatment with PcTS was initiated at the time of infection (figure 2*A*–2*C*). At the highest dilution tested, 60% of the animals treated with PcTS starting at the time of infection survived scrapie challenge, compared to none of the untreated animals (10^{-4} dilution; figure 2*C*). Although 1 animal survived infection, PcTS treatment was generally ineffective when initiated 56 dpi, regardless of the infectious dose.

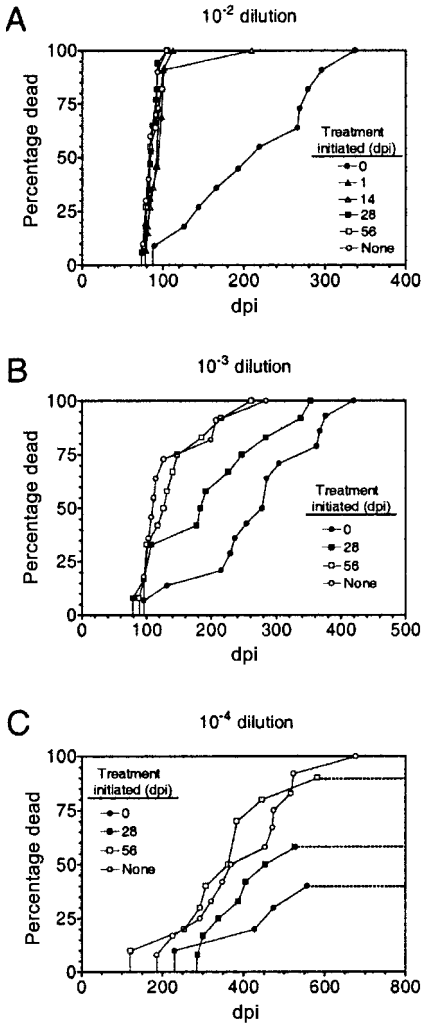

Figure 2. Antiscrapie effect of phthalocyanine tetrasulfonate (PcTS) treatment increases as the scrapie infectious dose decreases. Tg7 mice were infected intraperitoneally with 10^{-2} *(A),* 10^{-3} *(B),* or 10^{-4} *(C)* dilution of a 10% brain homogenate from hamsters infected with the hamster scrapie strain 263K. Intraperitoneal treatment with PcTS was initiated at the time of infection (0 days postinfection [dpi]) or from 1–56 dpi. Each panel shows the percentage of animals that died of scrapie over time. Each group consisted of 9–17 animals, and a single point may represent >1 animal. The dashed line indicates that, for the remainder of the experiment, no further scrapie deaths occurred in the groups indicated.

By contrast, if treatment was initiated at 28 dpi, a slight increase in disease incubation times was observed with decreasing infectious dose (figure 2; 28-dpi groups) and, at the lowest infectious dose, some animals survived scrapie challenge (figure 2*C*). PrP-res was not detected in the brains of these surviving animals (data not shown). Thus, PcTS has a therapeutic effect against low doses of TSE infectivity. Overall, the data demonstrate that the antiscrapie effect of PcTS was increased in animals exposed to lower levels of scrapie infectivity.

The fact that sheep transfused with blood from preclinical BSE-infected sheep developed TSE disease has heightened con-

Table 1. Preincubation of infectious inoculum with phthalocyanine tetrasulfonate (PcTS) eliminates low-level scrapie infectivity.

PcTS + inoculum[a]	No. dead/ total no.	Survivors, %	Time to death, dpi		P^b
			Mean ± SEM	Last death	
None + 10^{-2}	8/8	0	92.5 ± 4	119	NA
10 mg/mL + 10^{-2}	6/6	0	233 ± 28.9	310	.0001
None + 10^{-4}	7/7	0	257.1 ± 28.9	366	NA
10 mg/mL + 10^{-4}	10/13	23	436.4 ± 24.8	636	.002
None + 10^{-6}	3/8	63	517.3 ± 33.7	558	NA
10 mg/mL + 10^{-6}	0/13	100	691.5 ± 56.9	1007	.04

[a] PcTS was mixed with a 10^{-2}, 10^{-4}, or 10^{-6} dilution of a stock 263K hamster scrapie brain homogenate. Final concentration of PcTS was 5 mg/mL. After a 30-min incubation at room temperature, 0.05 mL of the mixture was injected intraperitoneally into weanling Tg7 mice.

[b] Significance of the survival time of treated vs. untreated animals using the unpaired Student's *t* test. NA, not applicable.

cerns that TSE diseases could be transmitted via contaminated blood products [13, 14]. Compounds capable of inactivating low levels of TSE infectivity in suspension could alleviate these concerns. We have previously shown that PcTS partially inactivates TSE infectivity when mixed with a highly infectious brain homogenate, although all animals eventually succumbed to disease [8]. To determine whether PcTS could completely eliminate low levels of scrapie infectivity in solution, PcTS was mixed with increasing dilutions of hamster 263K-infected brain homogenate. The mixtures then were injected intraperitoneally into Tg7 mice. For each dilution tested, there was a significant increase in disease incubation times in mice inoculated with the PcTS/brain homogenate mixture, compared with those inoculated with infected brain homogenate alone (table 1). As PcTS was mixed with increasing dilutions of the 263K positive brain homogenate, significantly more mice survived infection versus control mice that were injected with a diluted, but untreated, brain homogenate (table 1; 10^{-4} and 10^{-6} dilutions). The brains of surviving animals were either negative for PrP-res or contained ≤1% of the PrP-res levels detected in animals with clinically advanced scrapie (data not shown). These results clearly demonstrate that PcTS can inactivate low level TSE infectivity in suspension and protect some mice from clinical scrapie infection.

Pretreatment with PcTS delays disease in scrapie-infected Tg7 mice. PcTS is present in peripheral tissues of mice for months after multiple IP administrations [8], which suggests that pretreatment with PcTS might be as effective at inhibiting scrapie as treatments initiated on the day of infection. Tg7 mice were given PcTS intraperitoneally 3 times/week starting at 14 or 28 days before IP infection. Three days after the final treatment, mice were inoculated IP with a high dose of the 263K hamster scrapie strain. Disease incubation times in pretreated animals were significantly increased, compared with that in untreated animals, and were almost identical to those obtained when animals were treated with PcTS starting at the time of infection (figure 3*A*); thus, PcTS can be used prophylactically to delay disease onset in scrapie-infected animals.

It was possible that the antiscrapie effect of prophylactic PcTS was caused solely by the final PcTS injection administered 3 days before challenge with hamster scrapie. To determine whether single or multiple PcTS pretreatments were required to delay disease in Tg7 mice, mice were injected once intraperitoneally with PcTS at 28, 14, or 3 days before infection. Other than 1 mouse that survived >300 days after infection, a single treatment with PcTS before scrapie infection had no significant effect on disease incubation times (figure 3*B*). This result is in agreement with previous data that demonstrated that a single dose of PcTS at the time of infection was significantly less effective than multiple PcTS treatments (figure 3*B* and [8]). Thus, the prophylactic effect of PcTS was dependent on multiple IP treatments over an extended period of time.

PcTS can delay disease in an outbred mouse model of scrapie. Some anti-TSE compounds are effective against only certain TSE agents [1, 2, 15]. To determine whether PcTS could inhibit disease in a different TSE model, we tested whether it could affect disease in nontransgenic RML mice infected with the RML/Chandler strain of mouse scrapie. RML mice were treated intraperitoneally with PcTS starting before infection, at the time of infection, or at different timepoints after infection. Mice then were infected intraperitoneally with a high dose of the mouse scrapie strain RML/Chandler and were monitored for disease. As in the Tg7 model of hamster scrapie, initiation of PcTS treatment at the time of infection significantly delayed disease in mouse scrapie-infected RML mice, compared with untreated mice (figure 4). Pretreatment with PcTS starting at 14 days before infection also led to an increase in disease incubation times, with 1 animal surviving scrapie challenge. However, there was no significant difference between untreated and treated animals when PcTS treatment was initiated after infection. Overall,

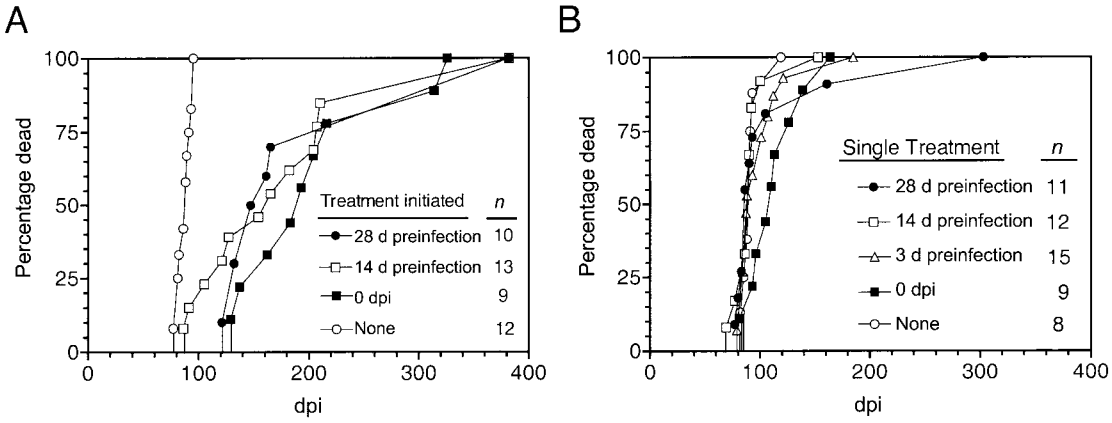

Figure 3. Pretreatment with phthalocyanine tetrasulfonate (PcTS) delays disease in scrapie-infected Tg7 mice. Tg7 mice were treated intraperitoneally with PcTS starting either at the time of infection (0 days postinfection [dpi]) or 3, 14, and 28 days before intraperitoneal infection with hamster 263K scrapie. Mice were treated either multiple times *(A)* or with a single dose *(B)* of PcTS, as described in Materials and Methods. Each panel shows the percentage of animals that died of scrapie over time. Each group consisted of 10–15 animals, and a single point may represent >1 animal.

these results are consistent with those seen in the Tg7 hamster scrapie model. Thus, PcTS has a potent anti-TSE activity against TSE agents in at least 2 different species (mouse and hamster), which suggests that some cTPs may be effective against multiple animal species and different strains of TSE agent.

PcTS has no effect on mice infected intracranially with scrapie. The fact that PcTS is most effective if given before or at the time of infection suggests that it is inhibiting replication of the TSE agent at a point before entry of the agent into the central nervous system. If this is the case, then IP administration of PcTS should have no effect on animals inoculated intracranially with the scrapie agent, because peripheral replication is not required for IC infection. Tg7 and RML mice were infected intracranially with a high dose of 263K hamster scrapie or RML/Chandler mouse scrapie, respectively, and then were treated with PcTS intraperitoneally, starting either at the time of infection or at different times after infection. PcTS did not significantly delay disease in animals infected intracranially, regardless of when treatment was initiated (table 2). Direct IC injection of PcTS into animals showing early clinical scrapie signs also had no effect, which suggests that the inability of PcTS to inhibit disease in animals infected intracranially was not solely caused by an inability of PcTS to cross the blood-brain barrier (table 2). These data are consistent with the hypothesis that PcTS acts to interfere with agent replication in peripheral tissues before entry of the TSE agent into the central nervous system.

DISCUSSION

A variety of compounds have been identified that delay TSE disease in rodent scrapie models, including polyene antibiotics [16, 17], tetracycline [18], sulfonated dyes [6], and polyanions [7, 19–23]. However, their anti-TSE effects are dependent on the manner of administration. One of the strongest anti-TSE compounds, dextran sulfate 500, can strongly inhibit scrapie when given at or near the time of infection [20, 21], but has no effect when mixed with the infectious inoculum [20]. By contrast, tetracycline has no effect in vivo [24] but can inactivate TSE infectivity in suspension [18]. PcTS is an unusual compound, because it is equally effective when given either prophylactically, at the time of infection, or when mixed with an infectious inoculum. In each of these cases, PcTS inactivated 2–3 logs (>99%) of the starting infectivity. This broad range of activities makes PcTS, and, by extension, PcTS analogs and other cTPs particularly attractive as potential anti-TSE compounds.

Our current results provide the first demonstration that a cTP can be therapeutically effective against a TSE disease. In

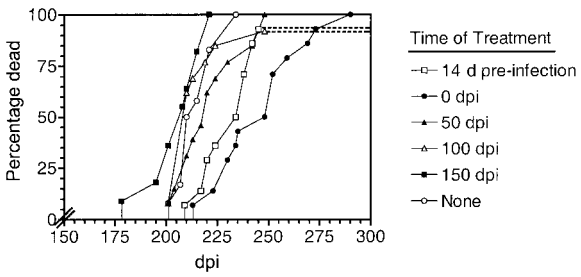

Figure 4. Treatment with phthalocyanine tetrasulfonate (PcTS) inhibits disease in nontransgenic RML mice infected with the mouse scrapie strain RML/Chandler. RML mice were treated intraperitoneally 3 times/week with PcTS starting at the time point indicated. Mice then were infected intraperitoneally with a high dose of the RML/Chandler mouse scrapie strain. Percentage of animals that died of scrapie over time is indicated. Each group consisted of 11–14 animals, and a single point may represent >1 animal. Dashed line indicates that, for the remainder of the experiment, no further scrapie deaths occurred in the groups indicated.

Table 2. Effect of phthalocyanine tetrasulfonate (PcTS) treatment on mice infected intracranially with scrapie.

Strain/agent, treatment[a]	No. dead/ total no.	Time to death, dpi		Delay of death, days	
		Mean ± SEM	Last death	Mean ± SEM[b]	Significance[c]
Tg7/263K					
None	10/10	46.3 ± 1.4	57	NA	NA
0 dpi (IP)	10/10	49.6 ± 0.8	54	3.3 ± 1.6	NS
Clinical (IC)	12/12	45.8 ± 0.8	51	−0.5 ± 1.5	NS
RML/RML					
None	10/10	174.7 ± 3.5	201	NA	NA
0 dpi (IP)	7/7	171.1 ± 1.8	175	−3.6 ± 4.5	NS
14 dpi (IP)	11/11	169.5 ± 7.2	191	−5.2 ± 8.3	NS
70 dpi (IP)	10/10	179.1 ± 4.3	201	4.4 ± 5.6	NS

[a] Strain of mouse infected/strain of scrapie agent; intracranial (IC) or intraperitoneal (IP) treatment indicated within parentheses. Tg7 mice were inoculated intracranially with a 1:10 dilution of a stock hamster 263K scrapie brain homogenate, whereas weanling RML mice were inoculated intracranially with a 1:10 dilution of a stock RML scrapie brain homogenate. Treatment with 5 mg/mL of PcTS was started at the indicated days postinfection (dpi). For IP administered PcTS, treatment was administered 3 times/week for 4 weeks. For IC administered PcTS, a single dose was given at the first indication of clinical signs (~40 dpi).

[b] Mean difference in time to death of treated vs. untreated animals.

[c] Significance of the survival time of treated vs. untreated animals using a 1-way analysis of variance, with Dunnett's posttest. NA, not applicable; NS, not significant.

animals infected peripherally with low doses ($\leqslant 10^3$IP LD_{50}) of scrapie, PcTS treatment initiated 1 month after infection increased disease incubation times and enabled some animals to survive scrapie challenge (figure 2). Because it is likely that natural exposure to TSE infectivity occurs peripherally and involves doses of infectivity significantly lower than those present in concentrated infectious brain homogenates, our data suggest that PcTS might be useful therapeutically against acquired TSE infections. However, when administered ~3 months after infection, PcTS treatment had no effect against any dose of infectivity. Thus, there may be a limited window of opportunity, in which PcTS can be used therapeutically. Alternatively, it may be that a longer treatment regimen and/or a higher dose of PcTS is needed as the interval after infection increases. Nevertheless, our data demonstrate that, under certain conditions, PcTS can act therapeutically against a TSE infection.

Although PcTS significantly delayed disease in both Tg7 and RML mouse models of scrapie, it did not inhibit disease as strongly in RML mice as it did in Tg7 mice. The increase in incubation time in RML mice treated before or at the time of infection was 7%–14%, whereas disease incubation times in similarly treated Tg7 mice increased 100%–300%. It may be that the length of the disease incubation time relative to the length of the treatment time influences the anti-TSE activity of PcTS. Scrapie incubation times in RML mice infected intraperitoneally with a high dose of scrapie are ~2–3 times longer than similarly infected Tg7 mice (216.2 ± 3 days vs. 86.4 ± 3 days, respectively). Therefore, the 4-week PcTS treatment regimen in Tg7 mice covered one-third of the disease incubation time. By contrast, the 4 weeks of treatment in RML mice covered only one-tenth of the disease incubation time. Thus, maximizing the protective effect of PcTS in RML mice may require a longer treatment regimen and/or a higher dose of PcTS to accommodate the increased disease incubation time in these mice versus Tg7 mice.

PcTS was most effective when given before or at the time of infection but, at least at low dilutions of the infectious inoculum, had no effect when given as quickly as 1 day after infection (figure 2). This suggests that PcTS is interfering with events that occur very early during infection. The fact that PcTS is ineffective against intracranially administered infectivity, in which peripheral agent replication is not necessary before entry of the agent into the CNS, is consistent with this hypothesis. Therefore, PcTS is similar to another TSE inhibitor, dextran sulfate, which also has been proposed to interfere with peripheral agent replication [7, 20]. Unlike dextran sulfate, however, PcTS also inactivates infectivity in suspension. Previous studies have demonstrated that PcTS and other cTPs can block PrP-res formation in both scrapie-infected tissue culture cells and cell-free systems [5], which strongly suggests that they can bind to PrP. In vivo, binding of PcTS to PrP could prevent uptake of infectivity into the central nervous system or other cells that are capable of replicating the TSE agent.

The ability of PcTS and other cTPs to inhibit the conversion of PrP-sen to PrP-res [5], as well as their ability to inhibit TSE disease in animals, provides strong support for a specific cTP receptor site on either PrP-sen or PrP-res. The striking structural differences between the cTPs and other anti-TSE com-

pounds further suggest that the cTP-PrP binding site is different from those used by other compounds. Indeed, previously described structure-function relationships provide strong support for the aromatic ring structure being a critical feature in the ability of cTPs to interact with proteins. In PcTS, the array of negatively charged sulfonate groups at the periphery of the aromatic ring (figure 1) also might be expected to influence its ability to bind PrP, perhaps by allowing it to interact with suitably positioned positive centers on the PrP surface. However, these sulfonate groups are not essential to the anti-TSE activity of PcTS (S.P., data not shown), which suggests that other surfaces of PcTS may be more important.

Using 2 different rodent models of scrapie, we have shown that the cyclic tetrapyrrole PcTS can significantly inhibit disease when given before scrapie infection. In Tg7 mice, the prophylactic effect of PcTS is as potent as its previously described anti-TSE effect when administered at the time of infection [8]. In both instances, disease incubation times are at least doubled. We have further shown that at low doses of scrapie infectivity PcTS can have a therapeutic effect, in that some mice survive infection when treatment is initiated 4 weeks after infection, as well as a sterilizing effect in that it can inactivate low levels of TSE infectivity in suspension. Thus, PcTS has several properties that are important for a potential anti-TSE drug: it has an effect in different models of TSE disease and can work both prophylactically and therapeutically.

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