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NOTES

Congo Red Prolongs the Incubation Period in Scrapie-Infected Hamsters

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In scrapie-infected cells, Congo red inhibits both the replication of the infectious agent and accumulation of the protease-resistant form of PrP (PrP-res). In this report, we show that Congo red prolongs the incubation periods of hamsters experimentally infected with two different strains of scrapie.

Experimental scrapie in hamsters is a useful model for the naturally occurring transmissible spongiform encephalopathies of humans (Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia) and animals (scrapie in sheep and goats and bovine spongiform encephalopathy) (18). Transmissible spongiform encephalopathies are fatal neurologic diseases characterized by the formation and accumulation of the partially proteinase-resistant protein (PrP-res), which is derived from a modification, not yet known, of a normal host cellular protein (PrP-sen) (7, 17). The delay in PrP-res formation in the brains of scrapie-infected hamsters, as shown by treatment with the polyene antibiotic amphotericin B, resulted in a prolonged incubation period (22). Moreover, recent findings that sulfated polyanions, another class of antiscrapie drugs (8–11, 14–16), also inhibit the formation of PrP-res in scrapie-infected mice (8) and neuroblastoma cells (6) emphasize the importance of controlling PrP-res formation for transmissible spongiform encephalopathy therapy.

Recently, Caughey and colleagues had shown that the amyloid-binding dye Congo red (13) inhibits both PrP-res accumulation (6) and scrapie replication (4) in persistently scrapie-infected mouse neuroblastoma cells (2, 5) using the same mechanism as sulfated polyanions. Given these observations, it seems possible that Congo red would prolong the incubation period of scrapie-infected animals, similar to effects observed with sulfated polyanions. Therefore, we investigated this possibility by treating scrapie-infected hamsters with Congo red.

Weanling Golden Syrian hamsters (Charles River, Calco, Como, Italy) were injected either intracerebrally (i.c. [0.05 ml]) or intraperitoneally (i.p. [0.1 ml]) with a 10% (wt/vol) suspension of pooled brains derived from hamsters clinically affected with the 263K or 139H (i.c. only) strain of scrapie. Congo red (Sigma) was dissolved and diluted in distilled water immediately before use, and 0.5 ml was injected i.p. alternately into the lower right and left quadrants of the abdomen. Control groups were untreated scrapie-infected hamsters inoculated by the same route and on the same day as treated animals. Groups of 8 to 11 hamsters were housed in each cage with food and water ad libitum and were scored 5 days a week for early (i.e., tremor of head and wobbling gait) and late (i.e., spontaneous and frequent backrolls) clinical signs of scrapie (21) by two independent observers. Neither the hamsters nor cages were coded, so the observers knew which treatment each hamster had received. In our experience, the time lag between the appearance of early and late signs of scrapie disease is about 2 weeks (21).

In the first group of independent experiments, treatment (expressed in milligrams of Congo red per animal) was started either before (pretreatment) or on the same day as scrapie inoculation and maintained until the animals developed clinical signs of scrapie (Table 1). Treatment of i.c. scrapie (strain 263K)-infected hamsters with 0.1 and 1 mg of Congo red did not elicit any beneficial effect. However, treatment with 10 mg of Congo red, given once a week or divided into two weekly doses of 5 mg each, produced a small increase in the mean incubation period, which was further enhanced by a weekly treatment with 75 mg of Congo red (12.5 mg given six times per week).

In order to have a better perception of the relationship between the dose of Congo red and the magnitude of its antiscrapie effect, we fitted the data to a hyperbolic dose-response curve (Fig. 1), which yields an estimated 50% effective dose of 19.2 ± 1.4 mg per animal per week. The chi-square goodness of fit revealed that the curve did not deviate from the experimental points ($P > 0.75$) and that the equation is therefore appropriate for the data.

Moreover, a further delay in the incubation period was obtained when treatment with 5 mg of Congo red twice per week was preceded, 1 week before inoculation, by a single dose of 10 mg (Table 1). The clinical progression of scrapie disease (from early to late signs) was not affected by the treatment, even at the highest dose of Congo red (time interval of 13.2 ± 0.9 days compared with 14.4 ± 0.5 days in control animals).

A prolonged incubation period (10.6 days; $P < 0.005$) was also observed in hamsters infected i.c. with the 139H strain of scrapie after treatment with 5 mg of Congo red twice per week (146.4 ± 2.4 days; $n = 11$) compared with untreated scrapie-inoculated hamsters (135.8 ± 1.2 days; $n = 8$).

In i.p.-infected hamsters, either 1 and 10 mg of Congo red given once per week or 5 mg given twice per week elicited an increase in the incubation period compared with that in control animals (Table 1). No further increase in the incubation period was seen in pretreated animals (Table 1). The lowest tested dose of 0.1 mg once per week was ineffective.

In a second experiment, we tested the effectiveness of a

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TABLE 1. Effect of Congo red in hamsters infected with the 263K scrapie strain

Expt	Route of scrapie inoculation	Dose (mg/wk)[a]	Mean incubation period ± SE (days)	Hamsters (*n*)	Delay (days)
1–3	i.c.	Control[b]	58.3 ± 0.6	30	
1	i.c.	0.1	58.6 ± 0.8	10	0.3
		1	59.5 ± 1.1	10	1.2
		10	64.5 ± 0.9	10	6.2[c]
2	i.c.	10[d]	64.3 ± 1.0	10	6.0[c]
		10[d,e]	68.4 ± 1.3	11	10.1[c]
3	i.c.	75[f]	72.6 ± 1.0	9	13.7[c]
4–5	i.p.	Control[b]	100.9 ± 4.2	18	
4	i.p.	0.1	105.4 ± 4.7	10	4.5
		1	122.3 ± 5.5	10	21.4[c]
		10	134.6 ± 11.0	5	33.7[c]
5	i.p.	10[d]	120.4 ± 7.3	8	19.5[c]
		10[d,e]	119.0 ± 5.8	8	18.1[c]

[a] Doses of Congo red per animal. Therapy was protracted until early clinical signs of scrapie disease.
[b] Pooled data from three (i.c.) or two (i.p.) independent experiments.
[c] Statistically different from control at $P < 0.05$ (two-tailed Student's *t* test).
[d] Given in two doses of 5 mg each.
[e] Treatment was preceded, 1 week before inoculation, by a single dose of 10 mg of Congo red. These data are not plotted in Fig. 1.
[f] Given in six doses of 12.5 mg each.

single week of administration of Congo red (25 mg per animal for 6 consecutive days) at different time intervals before and after i.c. scrapie (strain 263K) injection. As shown in Table 2, the maximum effect was observed when treatment was started on the same day of i.c. scrapie inoculation. The beneficial effect of Congo red decreased when the drug was given 2 weeks

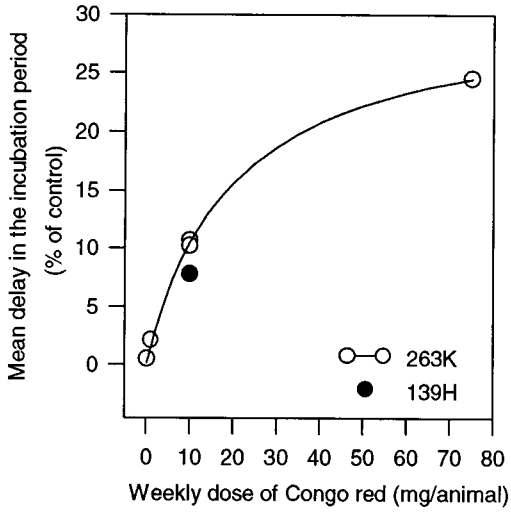

FIG. 1. Dose effect of Congo red in i.c. scrapie-infected hamsters. Treatment was started on the same day of scrapie inoculation and was continued until the animals showed clinical signs of disease. The experimental points (strain 263K) were fitted to a dose-response hyperbolic curve by using a weighted nonlinear least-squares computer curve-fitting program (Sigmaplot; Jandel Scientific).

TABLE 2. Efficacy of Congo red administered before or after i.c. inoculation with the 263K scrapie strain

Time of treatment before (−) or after (+) i.c. scrapie injection[a]	Mean incubation period ± SE (days)	Hamsters (*n*)	Delay (days)
Control	58.6 ± 1.7	8	
Wk			
−2	66.3 ± 1.1	8	7.7[b]
−1	65.8 ± 0.9	8	7.2[b]
Time zero	73.0 ± 0.9	11	14.4[b]
Wk			
+1	66.8 ± 1.0	8	8.2[b]
+2	66.8 ± 1.9	8	8.2[b]
+3	62.4 ± 1.4	10	3.8
+4	64.1 ± 2.0	8	5.5

[a] Congo red was administered daily at 25 mg per animal for 6 days.
[b] Statistically different from control at $P < 0.05$ (two-tailed Student's *t* test).

before or 2 weeks after scrapie injection, and it was essentially ineffective at 3 and 4 weeks.

These data show that Congo red treatment has an anti-scrapie effect in hamsters. This effect is independent of the strain of the agent and route of the inoculum. The finding that Congo red treatment has the same potency in delaying the mean incubation periods of 263K and 139H scrapie-infected hamsters (10.3 and 7.8% increases in the mean incubation period with respect to controls, respectively [Fig. 1]) suggests that the mechanism of action of this drug is not strain dependent, and this effect differentiates Congo red from amphotericin B (19, 22).

The increased effect of Congo red therapy observed in hamsters that received treatment close to the day of scrapie inoculation suggests that the timing of drug administration is a key issue, as it is for sulfated polyanions (9, 14–16, 20). Moreover, similar to sulfated polyanions, Congo red also inhibits the binding of glycosaminoglycans to PrP (3, 6, 12), suggesting that these compounds delay the appearance of scrapie disease through a common mechanism of action. Other sulfated substances with affinity to amyloid fibrils may therefore turn out to be effective against scrapie and possibly useful for preventive treatment of Creutzfeldt-Jakob disease in individuals at high risk for acquiring the disease (1, 20). At present, none of these compounds offers an effective treatment for these diseases, and much more work will be required before effective therapeutic compounds are developed.

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