ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1995, p. 1442–1444 Vol. 39, No. 7
0066-4804/95/$04.00+0
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Response of Rat Model of *Pneumocystis carinii* Pneumonia to Continuous Infusion of Deferoxamine

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Received 27 January 1995/Returned for modification 28 March 1995/Accepted 24 April 1995

The iron-chelating drug deferoxamine mesylate (DFO) is active against *Pneumocystis carinii* in vitro and in rat and mouse models of *P. carinii* pneumonia. Because DFO has a short half-life, daily divided or continuous dosage was expected to improve the dose response, as is the case with DFO treatment of malaria. Therefore, results of single daily intraperitoneal injections were compared with results of an evenly divided four-times-daily dosage and the efficacy of delivery with implanted infusion pumps. The highest bolus dosage (1,000 mg kg^{-1} of body weight day^{-1}) was as effective as the standard combination of trimethoprim with sulfamethoxazole. Unexpectedly, very little improvement was observed with the divided or continuous dosage, and several mechanisms that could account for this are discussed.

Pneumocystis carinii is a fungus that causes *P. carinii* pneumonia (PCP), an opportunistic infection of considerable importance for AIDS and other immunosuppressive conditions (9). Although the standard agents for treatment, pentamidine and a combination of trimethoprim and sulfamethoxazole (TMP-SMZ), have high response rates, they often produce severe side effects which necessitate cessation of therapy in more than 60% of AIDS patients (5). Better-tolerated modes of treatment are needed. Deferoxamine mesylate (DFO) is a very potent hexadentate iron chelator used routinely since the 1960s to treat iron overload in β-thalassemia patients (12). It is also used to remove aluminum from kidney dialysis patients (3). In both of these types of patients DFO has been well tolerated for prolonged periods. DFO is also active against *P. carinii* in vitro (17) and is therapeutic in animal models of PCP, although very high dosages, i.e., up to 1,000 and 400 mg kg^{-1} of body weight day^{-1}, are required for rats (4) and mice (17), respectively. In contrast, the usual clinical dose ranges from 40 to 60 mg kg^{-1}. Since the half-life ($t_{1/2}$) of DFO is about 10 to 20 min (10, 15) and the daily bolus dosage was used in the previous studies, administration by divided doses or by continuous infusion was expected to improve markedly the dose response, as has been observed for treatment of malaria (13, 18).

MATERIALS AND METHODS

Materials. The following materials were used: DFO, available as Desferal, Ciba Pharmaceuticals Co., Summit, N.J.; a pediatric suspension of TMP-SMZ, Barre-National, Baltimore, Md.; a water-soluble preparation of dexamethasone sodium phosphate for injection (10 mg ml^{-1}), Elkins-Sinn, Inc., Cherry Hill, N.J.; penicillin G plus procaine (Wycillin tubex), Wyeth Laboratories, Philadelphia, Pa.; a Gram stain kit, Fisher Scientific, Springfield, N.J.; penicillin-streptomycin for tissue culture, Mediatec, Washington, D.C.; a Giemsa stain supplied as Harleco Azure B type, Baxter Healthcare Corporation, Edison, N.J.; and toluidine blue O, Sigma Chemical Company, St. Louis, Mo.

Preparation of immunosuppressed rats. Specific-pathogen-free Sprague-Dawley rats were acquired from Taconic Farms (Germantown, N.Y.), placed in a barrier colony, and given multiple antibiotics to avoid other opportunistic infections as previously described (14) except that on arrival the rats were also given a single injection of penicillin G (6,000 IU) plus procaine. The animals were immunosuppressed by the addition of dexamethasone to the drinking water (1.5 mg liter^{-1}), a regimen which was continued for all animals throughout the experiment.

Induction of PCP. By using a sterile technique, a *P. carinii* inoculum was prepared from frozen lungs taken from *P. carinii*-infected animals which showed no sign of other infections. The lungs used for the inoculum were tested at the time of collection by Gram staining an impression smear and testing the lung tissue for colony-forming fungi or bacteria with a blood agar plate. The inoculum was prepared just prior to use by homogenizing a set of thawed lungs in a Dounce homogenizer with 10 ml of SPB buffer (58.5 mM disodium phosphate, 1.5 mM monopotassium phosphate, 43.5 mM sodium chloride, 10 mM trisodium citrate, 10 mM dithiothreitol, and 2.7 mM potassium chloride [pH 7.4]) containing 5,000 IU of penicillin and 5,000 mg of streptomycin ml^{-1}. Large debris was removed by centrifugation at $32 \times g$ for 10 min. All steps were carried out at 0 to 4°C. Homogenates were considered adequately infectious only if examination of a Giemsa-stained preparation indicated at least 50,000 *P. carinii* trophozoites per ml. A 0.2-ml inoculum was instilled into the trachea as previously described (14). Three weeks after inoculation, typical signs of PCP appeared in the rats, including weight loss of approximately 30%, hunched-back posture, listlessness, rapid shallow breathing, and cyanosis around the noses of some animals.

Administration of drugs. Drug treatment was always initiated 3 weeks after inoculation and was always continued for 3 weeks. TMP-SMZ was administered by addition of 25 ml of a pediatric suspension to each liter of drinking water, producing final concentrations of 0.2 mg of TMP ml^{-1} and 1.0 mg of SMZ ml^{-1}. Water consumption indicated that mean dosages of 47 and 233 mg of TMP and SMZ kg^{-1} day^{-1}, respectively, were administered. DFO was reconstituted by injecting 2.0 or 4.0 ml of water into 500-mg vials, producing 2.4 or 4.4 ml of solution, respectively, with 208 or 113 mg of DFO ml^{-1}. The animals were weighed weekly, the most recent weights being used to calculate the volume of reconstituted DFO to be used.

Esox pumps used for continuous drug delivery were from Harvard Apparatus (Boston, Mass.). These pumps had a reservoir volume of 1.0 ml and a mean delivery rate of 0.23 ml day^{-1}, although individual pumps varied. The pumps were implanted in the suprascapular region with the delivery catheter inserted into the peritoneum as directed by the manufacturer. The pumps were filled with DFO solution percutaneously with a 26-gauge infusion set to minimize damage to the silicone rubber of the fill port, which also serves as the spring mechanism for drug delivery. To avoid possible drug degradation, every second day the pumps were drained (the remaining volume was measured), flushed with 3 ml of fresh drug solution, and refilled.

Evaluation of infection. The total numbers of *P. carinii* cysts in the lungs of rats were calculated from light microscope counts of cysts in lung homogenates as previously described (14) except that toluidine blue O was used to stain the cysts

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TABLE 1. Efficacy of DFO when administered i.p. as a bolus or equally divided in equally spaced doses

Treatment[a]	Total daily dosage (mg kg^{-1})	Results for all animals		Results for animals dying during treatment		Results for sacrificed animals	
		No. of cysts/animal (10^6)[b]	Suppression (%)	No. of cysts/animal (10^6)	Suppression (%)	No. of cysts/animal (10^6)	Suppression (%)
None		18.62 ± 25.7 (20)	—[c]	35.63 ± 27.2 (10)	—	1.60 ± 5.9 (10)	—
TMP-SMZ	47/233	1.24 ± 3.7 (20)	93	11.91 ± 5.5 (2)	67	0.06 ± 0.1 (18)	96
DFO	1,000 q.d.[d]	0.62 ± 1.6 (20)	97	2.37 ± 2.1 (5)	93	0.04 ± 0.1 (15)	98
	500 q.d.	1.57 ± 2.95 (20)	92	5.59 ± 5.06 (4)	84	0.57 ± 0.8 (16)	64
	250 q.d.	9.40 ± 16.0 (20)	50	20.28 ± 19.2 (9)	43	0.50 ± 0.6 (11)	69
	250 q.i.d.[e]	6.8 ± 11.6 (20)	63	10.5 ± 13.1 (13)	71	0.01 ± 0.02 (7)	99

[a] Treatment was initiated 3 weeks postinoculation and was continued for 3 weeks.
[b] The data are means ± standard deviations. Numbers in parentheses are numbers of animals.
[c] —, the control value equals 0% suppression.
[d] q.d., once a day.
[e] q.i.d., four times a day.

(8). Slides were coded and read with the microscopist unaware of the sample identification.

RESULTS

To confirm the previous daily bolus dose response and to provide a basis for a comparison of the effects of the single daily intraperitoneal (i.p.) bolus and administration of the drug four times a day, six groups of 20 infected animals each were studied. Negative controls were not given anti-PCP treatment, while the positive controls were treated with a standard agent for PCP therapy, TMP-SMZ. Three groups were given single daily i.p. injections of 1,000, 500, or 250 mg of DFO kg^{-1} day^{-1}. The sixth group was treated with 250 mg of DFO kg^{-1} day^{-1} administered as 62.5 mg kg^{-1} every 6 h. Initially, a total of 150 animals were immunosuppressed and inoculated to permit a nonrandom selection of the 120 most closely matched animals (as judged by weight loss) for inclusion in treatment and control groups. The 120 animals selected were randomly assigned to groups. The results are presented in Table 1.

Because the distribution of cysts deviated from the normal distribution, nonparametric statistical methods were used for judging outcomes (6). The total numbers of cysts in the groups treated with 500 and 1,000 mg of DFO kg^{-1} were significantly less than the numbers of cysts in the untreated control group and in the animals given DFO at a dose of 250 mg kg^{-1}, there being no significant difference between the latter two groups. A significant dose-response relationship ($P < 0.0001$) was observed among the three groups of animals receiving single i.p. injections of DFO, confirming the results of our previous study (4). The animals treated with DFO four times a day that survived the full treatment protocol had significantly fewer cysts than either the negative controls or those animals receiving 250 mg of DFO kg^{-1} as a single dose ($P < 0.05$). While this suggests that dividing the daily dosage is more effective in reducing the number of cysts, it must be recognized that the rats which survived treatment may have been a biased sample; i.e., those that had fewer cysts before treatment began were better able to withstand the increased number of i.p. injections (84 versus 21). Consequently, the observed differences cannot be assumed to be due to treatment.

A higher number of deaths than we usually observe occurred during the treatment period, no doubt because of the intensity of infection overall, since the number of deaths was highest in the groups given the least amount of the drug. The only exception was that the animals treated with the divided dose of DFO had a higher rate of premature death than those not given any anti-PCP treatment, likely because of the low drug dose and multiple injections.

To maximize the steadiness of drug administration and to avoid the problems caused by the stress of multiple daily injections, we investigated the effectiveness of DFO continuously infused by an implanted pump. A group of 120 animals was inoculated, and 80 were selected for random division into four treatment groups of 20 animals each: a negative control group with no anti-PCP therapy, a positive control group treated with TMP-SMZ, and groups infused with solutions of 206 and 113 mg of DFO ml^{-1}. To control for stress, pumps were implanted in all animals. The dosages (means ± standard deviations) calculated from observed individual pump delivery rates were 358 ± 130 and 195 ± 46 mg kg^{-1} day^{-1}, respectively. One animal assigned to the group to receive pumps filled with 206 mg of DFO ml^{-1} died after pump implantation but before

TABLE 2. Efficacy of DFO administered as a continuous infusion

Treatment[a]	Total daily dosage (mg kg^{-1})	Results for all animals		Results for animals dying during treatment	Results for sacrificed animals	
		No. of cysts/animal (10^6)[b]	Suppression (%)	No. of cysts/animal (10^6)	No. of cysts/animal (10^6)	Suppression (%)
None		104.68 ± 48.4 (20)	—[c]	None	104.68 ± 48.4 (20)	—
TMP-SMZ	47/233	1.85 ± 4.1 (20)	98	4.28 ± 5.88 (5)	1.03 ± 3.1 (15)	99
DFO	358 ± 130	13.45 ± 12.0 (19)	87	10.94 (2)	13.73 ± 12.1 (18)	87
	195 ± 46	21.08 ± 18.4 (20)	80	None	21.08 ± 18.4 (20)	80

[a] Treatment was initiated 3 weeks postinoculation and was continued for 3 weeks.
[b] The data are means ± standard deviations. Numbers in parentheses are numbers of animals.
[c] —, the control value equals 0% suppression.

therapy began; this animal was excluded from the data set. The results are presented in Table 2.

The differences between all the treated groups and the untreated controls were highly significant ($P < 0.0001$). Analysis did not reveal any statistical difference between the two DFO treatments. The differences between the positive control group and both DFO treatment groups were not significant when ranked scores were used but were significant when the original scores were used.

DISCUSSION

The response to the daily bolus dosages of 250, 500, and 1,000 mg of DFO kg^{-1} day^{-1} confirmed our previous results (4). The 98% suppression of cyst count response observed with the highest daily bolus DFO dosage was equivalent to the 96% suppression observed with TMP-SMZ. However, the conservative conclusion regarding the effect of divided dosage was that this did not provide a significantly improved dose response, contrary to what was expected. Therefore, the experiment with implanted pumps was performed to provide continuous DFO administration. Continuous infusion of DFO did seem to provide a somewhat improved response in that dosages of 358 and 195 mg kg^{-1} day^{-1} delivered by implanted pumps produced 87 and 80% suppression of the cyst count, respectively, while dosages of 500 mg kg^{-1} day^{-1} given by daily bolus produced only a 64% suppression. However, this improvement is insignificant compared with that observed for DFO treatment of malaria. In monkeys with malaria, a dosage of 60 mg kg^{-1} day^{-1} was effective when given by implanted pump but totally without effect when given subcutaneously in two 30-mg kg^{-1} doses (13). In a rat model of malaria, a shift from treatment three times a day to treatment twice a day greatly reduced the efficacy of DFO despite the fact that the total daily dosage remained the same (18).

There are several mechanisms which could account for the minimal improvement observed when DFO was infused continuously. First, the reported $t_{1/2}$ values may not be relevant because these data represent only the $t_{1/2}$ during the distribution phase of the drug (12). Recent papers report $t_{1/2}$ values during the elimination phase ranging from 3 to 18 h (1, 2, 10). However, this does not account for the difference between the responses to DFO treatment of malaria and PCP. A possibility is that high concentrations of DFO are rapidly lethal for *P. carinii*, whereas a steady low dose is only suppressive. Another possibility is that DFO is sequestered in the lungs, thereby creating high concentrations at the site of infection despite a short $t_{1/2}$ in plasma. Support for the latter possibility comes from often-overlooked observations in six animal species that DFO is metabolized rapidly by plasma and at variable rates in the liver, pancreas, small intestine, brain, muscle, and spleen while being essentially unchanged by the kidney and lungs (11). It is also possible that a metabolite of DFO is responsible for the activity against PCP, but this seems unlikely since the drug is also active in tissue culture, where metabolism should be minimal. Finally, it is possible that this drug interacts with these pathogens in entirely different manners; the general consensus is that DFO acts against the malaria parasite by interacting with an internal iron pool (7), but this may not be the case for *P. carinii.*

ACKNOWLEDGMENTS

This work was partially supported by NIH grant R01 AI 27685.

Statistical analyses were performed by Mimi Kim and Roy E. Shore of the New York University Medical Center Department of Environmental Medicine.

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