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Trophozoite Elimination in a Rat Model of *Pneumocystis carinii* Pneumonia by Clinically Achievable Plasma Deferoxamine Concentrations

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In a rat model of *Pneumocystis carinii* pneumonia, a 3-week infusion of deferoxamine producing concentrations in plasma of ≥1.5 μg ml^{-1} eliminated the trophozoite life cycle stage. Since this concentration is well below that routinely achieved in patients treated for iron overload, deferoxamine has promise as a therapy for AIDS-associated *P. carinii* pneumonia.

The iron chelator deferoxamine (DFO) is used for lifelong treatment of iron overload resulting from transfusions required for β-thalassemia patients as well as iron overload from other causes (9). It is also used to treat aluminum toxicity in kidney dialysis patients (6). Recently, DFO has been found to be active against the AIDS-associated opportunistic pathogen *Pneumocystis carinii* in vitro (17), in a weekly prophylactic regimen in an animal model (3), and against animal models of acute *P. carinii* pneumonia (PCP) when it is given either as a daily bolus dosage (5, 13, 16) or by continuous infusion (13). Significantly, we found the response to continuous infusion in a rat model of PCP to be associated with steady-state plasma DFO concentrations lower than those observed in patients with routine DFO treatment (12).

Although often ignored, the trophozoite life cycle stage of *P. carinii* is thought to be the proliferative form and is the form that adheres closely to and damages type I pneumocytes, the site of gas exchange in the lungs (1, 2, 4, 15, 18). Thus, trophozoites can be considered key for both disease progression and morbidity. Nevertheless, in the past we have measured the response to DFO by determining the number of cysts remaining in the lungs after treatment, because our experience with other drugs has been that cysts provide a reasonable surrogate for trophozoites, counting of which is more problematic. After examining material collected from earlier experiments, we report here that the effect of DFO against the trophozoite life cycle stage is strong and that the complete elimination of trophozoites is associated with a plasma drug concentration well below that achieved in humans administered DFO for treatment of iron overload.

Measurements of trophozoites in rats treated with daily bolus injections of DFO were made from Giemsa-stained smears of lung homogenates prepared at the time of sacrifice, about 2.5 years before the trophozoites were counted. For animals treated with infused DFO, a complete set of microscope slides of lung homogenates had been prepared at the time of sacrifice and had been fixed with methanol; these slides were stored at −80°C until they were thawed and stained just before evaluation. Details of the rat model, drug administration protocol, and sample collection methods have been reported previously (13). Briefly, immunosuppression to allow for the development of PCP was achieved by the addition of dexamethasone to the drinking water at 1.5 μg ml^{-1} for the duration of the experiment; *P. carinii* inoculation was by intratracheal instillation of a lung homogenate taken from a rat with PCP; other opportunistic infections were prevented by using antibiotics and isolation in a barrier colony. The experiments in which DFO was administered by bolus injection and by continuous infusion each included a positive control group treated with trimethoprim-sulfamethoxazole (TMP-SMZ), which was added to the drinking water, and a negative control group given no anti-*P. carinii* therapy. Calculation of the total number of *P. carinii* trophozoites in the lungs was based on counting the number of Giemsa-stained organisms in 20 fields of a lung homogenate smear made by spreading 2 μl of homogenate over a 1-cm^2 marked area (13). The concentrations of DFO in plasma were measured as we described earlier (12).

Table 1 presents a summary of the previously reported data on the numbers of cysts remaining after treatment (13), together with a summary of the new data on the numbers of trophozoites remaining in the same animals. Initially, each group consisted of 20 animals, but only data for animals surviving the full treatment period are included in Table 1. Because readable slides were not available for all animals treated with a daily bolus injection, data for three animals are missing from Table 1. All other data sets are complete. DFO had a greater effect on trophozoites than on cysts, as judged both by the percentage of animals completely cleared of *P. carinii* after 3 weeks of DFO treatment and by the mean number of *P. carinii* organisms remaining in the lungs. This was true for animals treated with a daily bolus injection and by continuous infusion. The overall intensity of infection was lower in the animals used in the bolus administration experiment than in the animals used in the continuous infusion experiment; for this and other reasons, the efficacy of bolus administration cannot be compared with that of continuous infusion. Although the mean number of trophozoites remaining in animals treated by DFO infusion at both doses was lower than the corresponding value in animals treated with TMP-SMZ, there were not statistical differences among these groups due to the large variance in the scores. However, the difference between the response to the higher DFO infusion dose and that to TMP-SMZ approached significance ($P = 0.08$; Wilcoxon test of ranked values).

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TABLE 1. *P. carinii* in rats after completion of 3 weeks of treatment

Expt and treatment	Cysts as % of control (mean ± SD no. of cysts/lung [10^6])	% of animals with cysts remaining in lungs	Trophozoites as % of control (mean ± SD no. of trophozoites/lung [10^6])	% of animals with trophozoites remaining in lungs
Deferoxamine delivered by daily bolus intraperitoneal injection (21 days)				
Negative control (n = 10)[a]	100 (1.60 ± 0.6)	100	100 (143.0 ± 0.1)[b]	100
47 mg of TMP kg^{-1}, 233 mg of SMZ kg^{-1} (n = 18)	4 (0.06 ± 0.1)	56	0 (0)	0
1,000 mg of DFO kg^{-1} (n = 15)	3 (0.04 ± 0.1)	33	0 (0)	0
500 mg of DFO kg^{-1} (n = 16)	36 (0.57 ± 0.7)	88	4.2 (6.0 ± 0.1)	13
250 mg of DFO kg^{-1} (n = 11)	31 (0.50 ± 0.6)	73	3.6 (5.2 ± 0.1)[c]	67
Deferoxamine delivered by implanted pump (22 days)				
Negative control (n = 20)	100 (104.68 ± 48)	100	100 (8,300 ± 10,900)	100
47 mg of TMP kg^{-1}, 233 mg of SMZ kg^{-1} (n = 15)	1 (1 ± 3)	20	1.9 (160 ± 370)	53
335 ± 130[d] mg of DFO kg^{-1} (n = 18)	14 (14 ± 12)	89	0.2 (19 ± 49)	22
195 ± 46 mg of DFO kg^{-1} (n = 20)	20 (21 ± 18)	85	1.4 (119 ± 196)	45

[a] The number of animals surviving the treatment period and in the database.
[b] Data on trophozoites were obtainable for only 9 of the 10 animals in this group.
[c] Data on trophozoites were obtainable for only 9 of the 11 animals in this group.
[d] Standard deviation of the mean dose (12).

Figure 1 presents the DFO concentrations in the plasma of individual rats grouped according to whether trophozoites remained at the end of the 3-week treatment period. Within the left grouping in Fig. 1, the animals are ordered according to the number of trophozoites in the lungs. Within the right grouping in Fig. 1, the animals are ordered according to plasma DFO concentrations. The range of plasma DFO concentrations among the animals can be attributed to metabolism and clearance variations among individual animals and to the range of pump delivery rates, indicated by the standard deviations of the mean dosages. As indicated by the horizontal line, no trophozoites were remaining in any animal that had a plasma DFO concentration of ≥1.5 μg ml^{-1} at the end of the treatment period. Of the animals with plasma DFO concentrations of between 1 and 1.5 μg ml^{-1}, 38% were cured with respect to trophozoites. No animal with a plasma DFO concentration of less than 1 μg ml^{-1} was cured. Considering that in this rat model of PCP the concentrations of DFO in lungs ranged from two to four times the plasma concentrations in plasma (12), these data correlate well with in vitro studies which show that a DFO concentration of between 1 and 5 μg ml^{-1} fully inhibits the growth of *P. carinii* in tissue culture over a 7-day period (17). A plasma DFO concentration of ≥1.5 μg ml^{-1} is a reasonable clinical target, since humans attain a steady-state concentration in plasma of 4.15 μg ml^{-1} when DFO is infused at a rate of 50 mg of DFO kg of body $weight^{-1}$ (10) and a steady-state concentration of 10 μg ml^{-1} when DFO is infused at a rate of 100 mg kg^{-1} (14).

In examining the Giemsa-stained slides from animals treated with DFO, empty cysts with no intracystic bodies could frequently be seen. No such empty cysts were seen in either the untreated controls or the animals treated with TMP-SMZ.

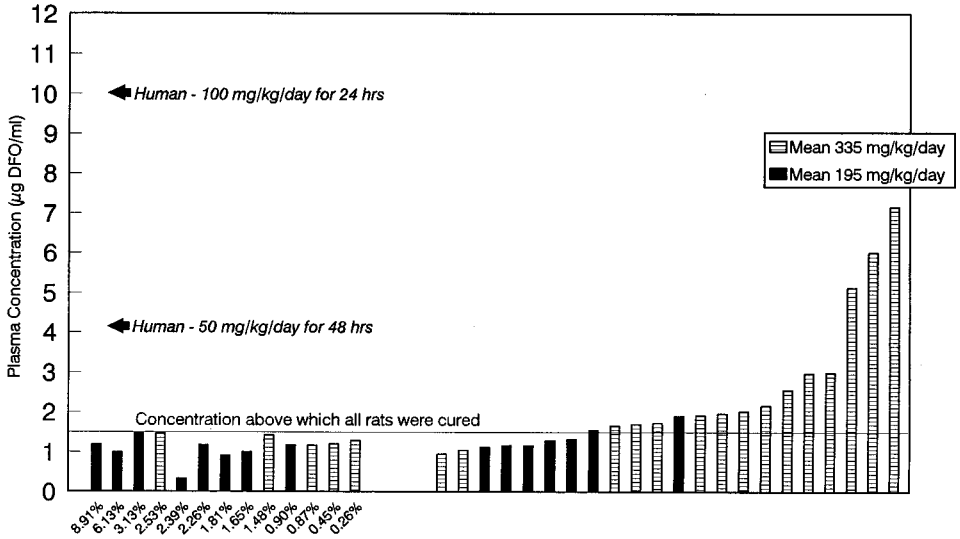

FIG. 1. *P. carinii* trophozoites and plasma DFO concentrations in rats. The bars represent the concentrations of DFO in the plasma of individual rats. The solid bars represent animals to which a mean dose of 195 mg of DFO kg of body $weight^{-1}$ day^{-1} was administered, and the shaded bars represent animals to which a mean dose of 335 mg kg^{-1} day^{-1} was administered. The data on the left indicate the trophozoites remaining in the lungs expressed as a percentage of those in untreated controls. The data on the right indicate rats in which no trophozoites were remaining at the end of treatment.

Since iron is involved in free-radical production associated with inflammatory immune mechanisms (7, 8), it is possible that DFO retarded the clearance of cyst remnants because of the inhibition of macrophage function. In patients with acute PCP, treatment outcome is improved by concomitant steroid administration, presumably because of the prevention of an exacerbation of pneumonia by an inflammatory response to killed *P. carinii* organisms (11). Therefore, if the slower removal of empty cysts indicates that DFO interferes with lung inflammation, this would be an additional benefit.

In summary, the prospects for DFO being useful as a treatment for PCP are enhanced by the discovery that this drug is effective against the important trophozoite life cycle stage and that effective plasma DFO concentrations in the rat model are below those routinely and safely achieved in patients given lifetime DFO treatment for chronic iron overload (12).

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