ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1996, p. 2318–2320 Vol. 40, No. 10
0066-4804/96/$04.00+0

Continuous Infusion of DL-α-Difluoromethylornithine and Improved Efficacy against a Rat Model of *Pneumocystis carinii* Pneumonia

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Received 1 March 1996/Returned for modification 29 May 1996/Accepted 31 July 1996

The rapid depletion of *Pneumocystis carinii* polyamines caused by in vitro exposure to DL-α-difluoromethylornithine (DFMO; also called eflornithine or Ornidyl) and the rapid repletion following removal of this drug suggested that the in vivo efficacy of DFMO against *P. carinii* pneumonia (PCP) may be limited by troughs in drug concentration resulting from the schedule of administration. This led to the prediction that, compared with the response to the standard animal protocol of administering DFMO in drinking water, the response of a rat model of PCP to DFMO would be lessened by bolus administration and improved by continuous infusion. These predictions were confirmed. Intraperitoneal bolus administration of up to 3 g of DFMO kg of body weight^{-1} was completely ineffective, although this dose has been shown to be effective when given in the drinking water. Conversely, continuous infusion improved the response against PCP seven- to ninefold over the response to drinking water administration. These findings suggest that, compared with the standard clinical investigational protocol for treatment of PCP with DFMO given in four divided daily doses, continuous infusion combined with monitoring of drug concentrations in plasma may improve efficacy and/or reduce the already low rate of adverse effects.

Pneumocystis carinii is the causative agent of *P. carinii* pneumonia (PCP), an opportunistic, life-threatening disease associated with AIDS and other immunocompromising conditions. PCP remains common despite recent improvements in prophylaxis (2). Drugs currently used for treatment are less than ideal because of either toxicity or lack of efficacy, or both (20). Among the investigational new drugs for PCP is DL-α-difluoromethylornithine (DFMO; also called eflornithine or Ornidyl), an irreversible, enzyme-activated inhibitor of ornithine decarboxylase (ODC), the enzyme which catalyzes the first committed step in polyamine biosynthesis. Following the successful initial compassionate use of DFMO to treat PCP in AIDS patients (7), other instances of compassionate use and open clinical trials confirmed the activity of this compound (15, 16, 18, 21, 22). The only controlled clinical trial (22) found a 39% rate of response to DFMO, which was equal to the 40% response rate in the control group treated with the standard combination of trimethoprim with sulfamethoxazole (TMP-SMZ). The 12% frequency of serious adverse effects associated with DFMO was lower than the 38% frequency associated with TMP-SMZ. However, the failure rate for DFMO was 58% versus 26% for TMP-SMZ.

Very little is known about the interaction of DFMO with *P. carinii*. DFMO was initially reported to be inactive in an animal model of PCP (10). Subsequently, in vitro activity (5) and then animal model activity (3, 4) were reported. ODC, the target of DFMO, was initially reported to be absent in *P. carinii* (17); then presumptive evidence was found (11), and finally enzyme activity was measured (13, 19). Recent data led to the hypothesis that rapid turnover of polyamines in *P. carinii* may underlie the selective effect of DFMO against *P. carinii* (13). These data showed that DFMO inhibition of ODC causes a much more rapid and complete depletion of polyamines in *P. carinii* than has been observed in mammalian cells. Also, removal of the drug resulted in a much more rapid repletion of polyamines than was observed in mammalian cells. The fact that the half-life of elimination of DFMO is short, about 1 h in rats (8) and 3 h in humans (1, 9), led us to the hypothesis that the optimal protocol for DFMO treatment of PCP would be one which maintains a steady and continuous presence of the drug so that *P. carinii* does not have the opportunity to recover from the effect of polyamine deprivation. The work reported here evaluated this prediction by testing the efficacy of large doses of DFMO administered to a rat model of PCP as daily intraperitoneal bolus injections, a moderate dose administered in the drinking water, and a small dose administered as a continuous infusion by means of an implanted pump.

MATERIALS AND METHODS

The methods of inducing PCP in rats and measuring the intensity of infection were as previously described (12, 19) and are briefly summarized as follows. Upon arrival from the supplier (Taconic Farms, Germantown, N.Y.), ~175-g rats were placed in a barrier colony and maintained on multiple antibiotics to avoid other opportunistic infections. After 7 days of antibiotic treatment, immunosuppression was begun by addition of 1.5 mg of dexamethasone to each liter of drinking water. Inoculation with *P. carinii*, at 7 and again at 10 days after initiation of immunosuppression, was done by intratracheal instillation of 0.2 ml of a lung homogenate prepared from an animal with PCP; the inoculum had been screened to exclude the presence of other pathogens. Data presented in Table 1 were collected by a procedure which involved counting the number of cysts in 250 microscopic fields of slides made of lung homogenates stained with cresyl echt violet (19). Data presented in Table 2 were collected by a newer and slightly different procedure, in which slides were also made from lung homogenates but were stained with toluidine blue O and were prepared in a manner that allowed an estimate of the total number of cysts per set of lungs (12). Both methods are adequate for measurement of drug response. Negative controls were given no other treatment. Positive controls were treated by adding 0.2 mg of TMP and 1.0 mg of SMZ for each ml of drinking water (3). DFMO was provided by the Merrell Dow Research Institute, and a 20% (wt/vol) solution in water was used for intraperitoneal bolus injections. Calculations of the actual dosages of TMP-SMZ and DFMO delivered were based on observations of amounts of water consumed per group of rats, as previously described (3).

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TABLE 1. Response of PCP to DFMO treatment by daily intraperitoneal bolus injection

Daily DFMO dose (n)[a]	No. of cysts in 250 fields ± SD
0.0 (4)	27.5 ± 14.7
0.75 (4)	48.8 ± 30.2
1.5 (2)	78.5 ± 65.8
3.0 (3)	45.7 ± 19.7

[a] Doses are in grams per kilogram. DFMO was given intraperitoneally for 21 days. n, number of animals receiving each dose.

Implantable Esox continuous-infusion pumps with 1.0-ml reservoir volumes were obtained through Harvard Apparatus (Boston, Mass.). The implantation and percutaneous filling procedures for these pumps were as previously described (12). Since the nominal 0.25-ml day^{-1} pump delivery volume was small and the previous dosages of DFMO used to treat PCP in rats were large (3.3 to 5.2 g kg of body weight^{-1} day^{-1} [3]), the pumps were filled with a solution containing as high a DFMO concentration as was possible without causing an increase in viscosity which would reduce the delivery rate. This concentration was found to be a 26% (wt/vol) solution of DFMO in water. Measurements of the actual dosages delivered were as previously described and involved determination of the amount of drug remaining in the pump prior to refilling after each 3-day interval (12). t tests were used for comparisons of the data presented in Table 2. When the variances of two groups being compared were unequal, t tests were based on the use of separate variances and adjusted degrees of freedom rather than the pooled variance (24).

RESULTS

All treatments were administered for 21 days. Daily bolus administration of up to 3 g kg^{-1} day^{-1} was ineffective against *P. carinii* infection (Table 1). Results for animals treated with DFMO administered in the drinking water, those treated with DFMO administered by implanted infusion pumps, untreated negative controls, and controls treated with a standard drug for PCP, TMP-SMZ, are presented in Table 2. Initially each group contained five animals, but there were deaths during the treatment period. On day 10 of treatment, one animal in the untreated control group died and one in the group treated with TMP-SMZ died. On day 14 two animals in the group treated with 1% DFMO in the drinking water died. These four animals had very high cyst counts (4.88×10^9, 0.68×10^9, 10.01×10^9, and 4.14×10^9 cysts lung^{-1}, respectively). Other than the inflamed lungs, postmortem examination revealed no obvious gross pathology; these animals presumably died of PCP. There were no deaths among the animals receiving DFMO by continuous infusion. Data for animals not surviving the full treatment period are not included in Table 2. For the animals treated with 1% DFMO in the drinking water, a dose of 1,527 mg kg^{-1} day^{-1} was calculated from measurements of the water consumed. Since rats absorb 83% of ingested DFMO (13a), the corrected dosage is 1,267 mg kg^{-1} day^{-1}. The animals treated with implanted pumps received a mean dosage of 418 mg kg^{-1} day^{-1}. The TMP-SMZ dosage was 47 and 233 mg kg^{-1} day^{-1}, respectively. The group of animals treated with 1% DFMO in the drinking water had about 1/3 as many cysts in the lungs at the end of treatment as the untreated controls, but the difference in the scores of the two groups was not statistically significant. Cyst counts for the group treated with TMP-SMZ and the group treated with DFMO by implanted pumps were >99 and 91% reduced, respectively, compared with those for the untreated controls. These were significantly different from the counts for the untreated controls ($P = 0.03$ and 0.04, respectively) but not from each other ($P = 0.16$). Cyst counts for the animals treated with DFMO by implanted pumps were significantly different from those for the animals treated with DFMO added to the drinking water ($P = 0.008$). When similar analyses were performed on data sets including both those animals dying during the treatment period and those surviving, the pattern was the same.

Table 2 also includes a pneumonitis index which reflects the degree of pneumonia, measured by lung weights. The pneumonitis index of an animal was defined as the ratio of the lung weight to the body weight, divided by the equivalent mean parameter for the TMP-SMZ control group. Thus, the TMP-SMZ-treated group had a pneumonitis index defined as 1.0. The pneumonitis indices of the animals treated with DFMO by infusion pumps were similar, while other groups had raised indices.

DISCUSSION

Our previous studies showed that *P. carinii* cells exposed to 1 mM DFMO in vitro for 3 h contained 18, 29, and 16%, respectively, of the preexposure concentrations of putrescine, spermidine, and spermine and that these cells were able to restore their polyamine content to 78, 93, and 49%, respectively, of the pretreatment concentrations within 1 h of removal of the DFMO (13). The combination of this observation with knowledge of the short (1-h) half-life in plasma in rats (8) led to the prediction that single daily bolus administration would be the least effective means of treatment, administration in the drinking water would be more effective, and continuous infusion of DFMO would be most effective. Daily bolus administration of DFMO up to 3,000 mg kg^{-1} day^{-1} had no effect on PCP in the animal model. An absorbed dosage of 1,267 mg kg^{-1} day^{-1} from administration of a 1% solution in the drinking water was associated with a reduction in cyst count, but this reduction was not statistically significant. In contrast, a low dosage administered by implanted pumps (418 mg kg^{-1} day^{-1}) produced a strong, statistically significant response as judged by the count of *P. carinii* cysts remaining in the lungs, the pneumonitis index, and the number of animals surviving for the full treatment period. The response to continuous infusion, 91% cyst suppression, was similar to the pre-

TABLE 2. Response of PCP to DFMO administered by addition to drinking water and by continuous-infusion pumps

Treatment (n)[a]	Mean daily dose (mg kg^{-1})[b]	No. of cysts (10^9)/set of lungs (mean ± SD)	Pneumonitis index[c] (mean ± SD)
None (control) (4)		3.40 ± 1.83	2.22 ± 0.53
TMP-SMZ (4)	150/748	0.01 ± 0.02	1.00 ± 0.14
1% DFMO in drinking water (3)	1,527	1.01 ± 0.05	1.50 ± 0.43
DFMO by implanted pumps (5)	418	0.29 ± 0.30	1.08 ± 0.26

[a] Within the untreated control group and within the TMP-SMZ group, one of a total of five animals died during the treatment period. Within the group of five animals receiving 1% DFMO in the drinking water, two died during the treatment period.

[b] Administered for 21 days.

[c] Means of ratios of individual lung weights to body weights, each divided by the equivalent mean parameter for the TMP-SMZ control group.

viously observed responses to 2, 3, and 4% solutions of DFMO in the drinking water, i.e., 88, 97, and 96% cyst suppression, respectively (3). Since these oral dosages are calculated to be equivalent to absorbed dosages of 2,820, 3,693, and 4,341 mg kg^{-1} day^{-1}, respectively, the response to continuous infusion is approximately seven- to ninefold better than the response to administration in the drinking water. Although a 91% reduction in lung cyst burden is not a cure of PCP, cures are obtained for patients treated with this drug, as discussed above. Considering that the plasma half-life of elimination in humans is three times longer than that in rats (1, 8, 9), the DFMO concentration in the plasma of patients given the standard 400 mg kg^{-1} day^{-1} (23) is likely to be considerably higher than that achieved for rats treated with 418 mg kg^{-1} day^{-1}.

The standard clinical investigational protocol for DFMO treatment involves intravenous administration of 100 mg kg^{-1} over at least 20 min, repeated at 6-h intervals for 10 to 14 days, followed by 75 mg kg^{-1} every 6 h for 4 to 6 weeks (23). The data presented above suggest that a better response may be achieved if the drug is administered by continuous infusion. Reviews and primary papers covering the use of DFMO as a salvage therapy for PCP patients for whom other therapies have failed have included the following numbers of patients: 345 (18), 42 (15), 31 (21), and 33 (16). On an intent-to-treat basis, the overall response rates were 36, 55, 45, and 68%, respectively. All these studies used the four times-a-day treatment schedule except the study which produced the 68% response rate (21), which used continuous infusion. In the only clinical study using DFMO as the initial therapy (22), which was also the only controlled study with patients randomly assigned to the DFMO treatment group (51 patients) or to a control group receiving standard TMP-SMZ therapy (47 patients), administration was by continuous infusion and the response rate observed was 39%, equal to the response to TMP-SMZ in that study (40%).

The ability of *P. carinii* to rapidly restore polyamine concentrations after exposure to DFMO (13) suggests that the limitation of effectiveness relates to the trough drug concentration rather than to either the peak or the mean. Administration of 100 mg kg^{-1} intravenously every 6 h to patients treated for PCP produced peak concentrations in plasma ranging from 196.6 to 317.9 μg ml^{-1} and troughs ranging from 71.3 to 113.3 μg ml^{-1} (6). Administration of 200 mg kg^{-1} every 12 h, the same total daily dose, to adult sleeping-sickness patients produced trough concentrations in plasma ranging from 4.2 to 46.4 μg ml^{-1} (14). Therefore, the cause for some of the variability in the response of PCP to DFMO could reside in variable drug troughs. Consequently, it is possible that continuous infusion at rates appropriate for individual patients would produce either an enhanced response or fewer adverse effects, or possibly both.

ACKNOWLEDGMENTS

This work was partially supported by NIH grant R01 AI 27685 to A. B. Clarkson. S. Merali is the recipient of a DAIDS/NIAID fellowship, training grant 2T32AI07382.

We are grateful to Roy Shore of our Department of Environmental Medicine for statistical analyses of the results and to Laura Del Angel for technical assistance.

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