Deferoxamine and Eflornithine (DL-α-Difluoromethylornithine) in a Rat Model of *Pneumocystis carinii* Pneumonia

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The iron chelator deferoxamine and the polyamine biosynthesis inhibitor eflornithine (DL-α-difluoromethylornithine) were examined for anti-*Pneumocystis carinii* activity in the rat model of *P. carinii* pneumonia. The activity of deferoxamine at 250, 500, and 1,000 mg/kg given intraperitoneally provides evidence that iron chelation is a promising novel approach to *P. carinii* chemotherapy. Results with eflornithine at 2, 3, and 4% in drinking water confirm and extend previously reported activity in the rat model.

*Pneumocystis carinii*, a ubiquitous lung-dwelling commensal organism, causes *P. carinii* pneumonia in immunocompromised individuals (14, 24, 28) and is one of the most important opportunistic infections seen in patients with acquired immunodeficiency syndrome (7, 11). It has been considered a protozoan, but recent evidence indicates that it may be a fungus (6, 15). The standard treatment is either trimethoprim-sulfamethoxazole (TMP/SMZ) or pentamidine. Both are generally effective although significantly more toxic in acquired immunodeficiency syndrome-related *P. carinii* pneumonia than in other cases of *P. carinii* pneumonia (9, 17). This toxicity and the nonresponsiveness of some individuals require the development of new therapies. Agents active against *P. carinii* pneumonia which are unrelated to existing drugs, both structurally and mechanistically, are especially important.

**Drugs.** Deferoxamine (desferrioxamine; Desferal) is the chelator currently used to treat patients with iron overload. It is active against *Plasmodium* spp., the malaria parasite (8, 20), and in the rat model of *P. carinii* pneumonia, as indicated by a preliminary experiment in this laboratory (unpublished data). Eflornithine (DL-α-difluoromethylornithine; Ornidyil), an inhibitor of polyamine biosynthesis currently being tested in clinical trials as an anti-*P. carinii* pneumonia agent (23), has been reported to be active in the rat model of *P. carinii* pneumonia when administered as a 3% solution in the drinking water (4). To extend and compare both observations, dose responses were measured.

**Induction of *P. carinii* pneumonia.** *P. carinii* pneumonia was induced in 200-g female Sprague-Dawley rats by immunosuppression with twice weekly cortisone acetate injections (25 mg/kg subcutaneously) and an 8% protein diet for 5 weeks before 3 weeks of anti-*P. carinii* pneumonia therapy. The cortisone protocol was maintained throughout the 8 weeks with normal rat chow being given at the beginning of the treatment period. Oxytetracycline was added to the drinking water (250 mg/liter) to suppress bacterial infections.

**Experimental and control therapies.** The rats were randomly assigned to groups at the beginning of the treatment period. Experimental therapies were continued until the animals died or were sacrificed. Controls consisted of one group given no anti-*P. carinii* pneumonia therapy and another given TMP/SMZ, 25 ml of a pediatric Septa suspension (Burroughs Wellcome Co., Research Triangle Park, N.C.) per liter of drinking water. Solutions of desferrioxamine (CIBA Pharmaceutical Co., Summit, N.J.) were prepared as directed in the package insert and administered intraperitoneally. Eflornithine, donated by the Merrell Dow Research Institute (Cincinnati, Ohio), was added to the drinking water at the concentrations indicated in Table 1.

**Evaluation of *P. carinii* pneumonia.** Infection was judged by counting the total number of cysts in a smear made from a known volume of total lung homogenate, as follows. After being removed and weighed, the lungs were minced with a stainless steel garlic press, then suspended (wt/vol) 1 part lung to 5 parts buffer (0.1 M Na2HPO4, 0.073 M NaCl, 100 mM EDTA [pH 7.4]), and forced through a stainless steel tea strainer. A smear was prepared from 5 μl of homogenate and stained with cresyl echt violet (3).

**Response to drugs.** Table 1 presents the results. The untreated controls had high cyst counts at the end of the treatment period, while the TMP/SMZ-treated rats responded to these drugs and had very low counts.

Desferrioxamine proved to be effective in this model. There was a dose-related suppression of cyst formation for the three doses tested. Compared with the usual human dose (40 to 80 mg/kg) used in treating transfusion-induced iron overload (1,16), high doses of desferrioxamine were given to these rats. This was done because desferrioxamine is rapidly cleared from the bloodstream (tl/2 = 10 to 30 min in humans [21,26]) and because technical considerations precluded administering the drug as a continuous infusion, the route of administration used clinically. Thus, to maintain a minimum effective concentration of drug in lung tissues for sufficient time to obtain a response, high doses were required.

Eflornithine was active in the rat model, and a dose response was observed. There was an increased response to eflornithine as the concentration was increased from 2% (3,390 mg/kg) to 3% (4,440 mg/kg), but a further increase to 4% (5,220 mg/kg) did not improve the response. The doses were calculated from the water consumed, and the lack of linearity of dose with drinking water concentration reflects the fact that at higher eflornithine concentrations the rats drank less water. These results confirm a previous report from this laboratory (4) showing responsiveness to eflornithine in the rat model after other laboratories had found no response (12,27). They also clarify an Addendum in Proof (4) which questioned the dose responsiveness to eflornithine.

**Statistical analysis.** A one-way Kruskal-Wallis analysis of
TABLE 1. Cyst counts for rats with *P. carinii* pneumonia given different treatments*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>No. of cysts (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>9</td>
<td>1,451 ± 762</td>
</tr>
<tr>
<td>TMP/SMZ</td>
<td>32.2/160.8</td>
<td>9</td>
<td>5 ± 16</td>
</tr>
<tr>
<td>Eflornithine</td>
<td>2%</td>
<td>3,390</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3%</td>
<td>4,440</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>5,220</td>
<td>9</td>
</tr>
<tr>
<td>Deferoxamine</td>
<td>250</td>
<td>7</td>
<td>106 ± 12</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>8</td>
<td>27 ± 10</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>8</td>
<td>9 ± 3</td>
</tr>
</tbody>
</table>

*All treatments were for 3 weeks after 5 weeks of conditioning to produce *P. carinii* pneumonia. The doses of eflornithine and TMP/SMZ were calculated from the observed intake of water which was available ad libitum. Desferrioxamine was administered as a single daily intraperitoneal bolus.

variance showed that the mean rank score of at least one of the six treatment groups was significantly different from that of the control (*P < 0.05*). A multiple comparison procedure (Dunnett analog) revealed that the TMP/SMZ, 3% eflornithine, 4% eflornithine, 500-mg/kg desferrioxamine, and 1,000-mg/kg desferrioxamine groups had a Z statistic value exceeding the critical value of Z0.05/2p, the recommended cutoff (5). The TMP/SMZ, 3% eflornithine, and 1,000-mg/kg desferrioxamine groups were significantly different from the control even when a much more stringent critical value (Z0.05/2p) was used. To confirm an apparent dose-response relationship for desferrioxamine, linear regression analysis was performed which produced an *r* value of −0.47 at *P < 0.05.

Mechanism. Eflornithine is a suicide inhibitor of ornithine decarboxylase. It is innocuous until activated by the target enzyme. Upon activation, a covalent bond forms between the inhibitor and the active site of the enzyme; thus, enzyme action leads to irreversible enzyme inhibition (25). Although the target enzyme, ornithine decarboxylase, has been reported to be absent in *P. carinii* (18), unpublished data from this laboratory indicate that this enzyme is present in *P. carinii*. These data were collected with the correct coenzyme, pyridoxal phosphate (2), rather than pyridoxine as used previously (18), and after removal of interfering substances.

While eflornithine is known to block polyamine biosynthesis, there are no data on the mechanism of action of desferrioxamine against *P. carinii*. Possibilities include deprivation of iron, a nutrient required by all cells, and interference with the function of iron-containing enzymes. A similar situation existed for *Plasmodium falciparum*. High concentrations of desferrioxamine rapidly kill this organism in vitro, as trophozoites become vacuolated and disrupted (22). That nutritional deprivation of iron was not responsible is suggested by the fact that enhancing the exchange of iron from transferrin to desferrioxamine decreased the inhibitory effect of desferrioxamine (19). Moreover, a desferrioxamine-dextran complex, which retained a high affinity for iron but was unable to cross cell membranes due to its size, was inactive against *P. falciparum* infection whether it was external to the erythrocyte or preloaded into the erythrocyte before infection. These data suggest that desferrioxamine acts directly on iron-containing enzymes of *P. falciparum*, a situation which may also be true for *P. carinii*.

These data are the first indication that the iron chelation is therapeutic for *P. carinii* pneumonia, and they confirm the activity of eflornithine against *P. carinii* pneumonia. Because of the design of the experiment, high doses of desferrioxamine were used to obtain a response. However, desferrioxamine may be active against *P. carinii* pneumonia in patients given a clinically relevant dose of this drug by subcutaneous or intravenous infusion. A recent case report of a single patient concluded there was a causal relationship between a high desferrioxamine dosage and the subsequent onset of *P. carinii* pneumonia in this patient (13), but it is difficult to balance this conclusion against the controlled dose-response data presented here. Recent developments suggest that orally effective iron chelators with antiparasitic activity may soon be available (10, 29). Thus, iron chelation may play a role in the treatment of *P. carinii* pneumonia.

LITERATURE CITED


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