Anti-Pneumocystis carinii Activity of PS-15, a New Biguanide Folate Antagonist

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A newly synthesized biguanide inhibitor of dihydrofolate reductase in Plasmodium species was evaluated for its anti-Pneumocystis carinii activity. The compound N-3-(2,4,5-trichlorophenoxypropoxy)-N'-(1-methylethyldiimidocarbonimidic diamide hydrochloride, designated PS-15, was administered prophylactically and therapeutically to immunosuppressed rats latently infected with P. carinii. Doses of 5 and 25 mg of PS-15 per kg of body weight per day given orally during 7 weeks of dexamethasone immunosuppression prevented P. carinii infection in all (100%) 19 rats given the drug, while 6 of 9 (67%) untreated control rats developed P. carinii pneumonitis. A single weekly dose of 50 mg of PS-15 per kg also prevented the infection in all 10 rats. P. carinii pneumonitis was established after 4 weeks of immunosuppression and was then treated orally for 3 weeks with 25, 5, and 1 mg of PS-15 per kg/day. Complete resolution of the infection occurred in all (100%) 10 rats given 25 mg of PS-15, 6 of 9 (67%) rats given 5 mg of PS-15, and 6 of 8 (75%) rats given 1.0 mg of PS-15 per kg per day and in all (100%) 9 rats treated with trimethoprim-sulfamethoxazole. PS-15 was well tolerated at all doses. Because drug studies in the P. carinii rat model have been highly predictable of the effects of drugs on the disease in humans, these experiments suggest that PS-15 may have promise as a drug for the treatment of P. carinii pneumonitis in humans.

Because Pneumocystis carinii may cause a life-threatening pneumonitis in up to 70% of patients with AIDS, it has come to prominence over the past decade as a major opportunistic pathogen of the immunocompromised host. Only three drugs have been approved by the U.S. Food and Drug Administration for the treatment of this disease. They are trimethoprim-sulfamethoxazole, pentamidine isethionate, and atovaquone. None is ideal; more effective and less toxic drugs are needed.

The immunosuppressed rat provides an excellent animal model for studies of P. carinii pneumonia. We have used this system to identify initially the anti-P. carinii activities of trimethoprim-sulfamethoxazole (7), dapsone (8), and atovaquone (5), drugs later proven to be effective in the treatment of human P. carinii pneumonia (4, 10).

It has become apparent that some drugs with antimarial activity may also have anti-P. carinii activity through interference with the folate metabolism of the microbe. Therefore, we have sought new compounds with these features for the treatment of P. carinii pneumonia using the corticosteroid-treated rat model.

The compound N-3-(2,4,5-trichlorophenoxypropoxy)-N'-(1-methylethyldiimidocarbonimidic diamide hydrochloride (PS-15) is a newly synthesized inhibitor of dihydrofolate reductase with in vitro and in vivo activities against drug-resistant Plasmodium falciparum (1). While PS-15 has intrinsic antimarial activity, some evidence suggests that it is also metabolized in vivo to 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(2,4,5-trichlorophenoxypropoxy)-1,3,5-triazine hydrochloride (WR99210), an active triazine inhibitor of dihydrofolate reductase (Fig. 1).

The experiments described herein provide evidence that PS-15 has anti-P. carinii activity similar to that of trimethoprim-sulfamethoxazole and that the prophylactic and therapeutic responses are dose dependent.

MATERIALS AND METHODS

Experimental plan. The experiments were based on the premise that rats latently infected with P. carinii and immunosuppressed for 6 or more weeks with dexamethasone develop overt pneumonitis caused by P. carinii, which was clearly evident by histological examination. The prophylactic efficacies of compounds can be tested by administration throughout the period of immunosuppression. Therapeutic efficacy can be evaluated by allowing the animals to progress to active P. carinii pneumonitis after 4 weeks of immunosuppression and then treatment with test drugs. The end point for assessment is the extent of P. carinii pneumonitis demonstrated by histological examination of the lungs at the completion of the study. Comparison was made with untreated immunosuppressed control animals.

Animals. Male Sprague-Dawley non-virus-free rats weighing 125 to 150 g were obtained from Hilltop Lab Animals, Scottsdale, Pa. After a 3-week quarantine, the rats were allocated to groups of 10 each and were begun on immunosuppression with dexamethasone. Five animals each were housed in wire-topped cages, and the animals were fed standard laboratory rat rations. Upon completion of the experiment, the surviving animals were sacrificed in a CO2 chamber.

Drugs. The compounds PS-15 and WR99210 (Jacobus Pharmaceutical Co. Inc., Princeton, N.J.) and trimethoprim-sulfamethoxazole (Hoffman-La Roche Inc., Nutley, N.J.) were used. All drugs were administered orally as components of food pellets.

The medicated food pellets were prepared by incorporat-
ing the daily dose into highly pulverized rat rations (Ralston Purina rat chow) at the amounts calculated to be consumed daily, mixed with water, and formulated into pellets dispensed to provide the daily calculated dosage. A 2-day supply of medicated pellets was made at one time.

Dexamethasone sodium phosphate, injection USP (Elkins-Sinn, Inc., Cherry Hill, N.J.), 2.0 mg, and tetracycline hydrochloride (Sumycin; E. R. Squibb & Sons, Princeton, N.J.), 500 mg, were added to 1 liter of drinking water.

**Dosage and administration of drugs.** All animals received immunosuppressive doses of dexamethasone throughout the experiment. Dexamethasone was provided in the sole drinking water source ad libitum. The daily consumption of dexamethasone was approximately 0.3 to 0.5 mg/kg of body weight per day.

Initially, PS-15 and WR99210 were tested along with other compounds for their anti-*P. carinii* activities at a dosage of 25.0 mg/kg/day.

The screening experiment showed evidence that PS-15 had activity against *P. carinii*. Another study was undertaken to determine the dose-dependent responses of PS-15 administered either prophylactically or therapeutically at dosages of 25, 5.0, and 1.0 mg/kg/day. The prophylactic doses were given throughout 6 weeks of the 7-week period of immunosuppression. The animals were started on dexamethasone 1 week before they were started on the study drugs, and drugs at the therapeutic dosages were initiated after 4 weeks of immunosuppression and were continued for a period of 3 weeks.

PS-15 was also administered prophylactically as a single weekly dose of 50 mg/kg.

Trimethoprim-sulfamethoxazole was administered both prophylactically and therapeutically at a dosage of 50 mg of trimethoprim and 250 mg of sulfamethoxazole per kg/day.

The control group received no drug other than the immunosuppressive regimen.

Groups of 10 rats each were allocated to each dosage level and to the control category.

**Histology.** The right lung was removed and placed in formalin. The fixed tissue was processed in paraffin block sections. Sectioned specimens were stained by Gomori’s methenamine silver nitrate method. Slides were coded and read separately by two investigators (W.T.H. and J.K.). The histological interpretations of the lung sections were scored as 0 if no cysts were seen in any portion of the lungs, 1+ if cysts were found to be sparsely distributed with less than one organism per 25 high-power fields; 2+ if focal areas of *P. carinii* pneumonitis were found surrounded by 10 to 25 high-power fields of normal lung parenchyma, and 3+ if the lung was diffusely and extensively infiltrated by cysts in almost all high-power fields. Differences in interpretation were resolved to the agreement of both readers before the code was broken.

**RESULTS**

In the initial screening experiment, all 10 untreated control animals had *P. carinii* pneumonitis after 7 weeks of immunosuppression, while none of the 10 rats given PS-15 had any evidence of *P. carinii* infection. All nine rats given the metabolite WR99210 progressed to *P. carinii* pneumonia while receiving the compound.

The results of the comparative experiment are summarized in Table 1. *P. carinii* pneumonia occurred in six of the nine (67%) evaluable control animals. Daily administration of PS-15 was totally effective in preventing *P. carinii* pneumonia at dosages of 25 and 5.0 mg/kg, as was trimethoprim-sulfamethoxazole. The dosage of 1.0 mg of PS-15 per kg/day was ineffective when compared with the control treatment (no treatment). The single weekly dose of 50 mg of PS-15 per kg prevented the infection in all nine evaluable rats.

Therapeutically, PS-15 at a dosage of 25 mg/kg and trimethoprim-sulfamethoxazole achieved complete clearing of *P. carinii* pneumonitis in all of the animals tested. However, residual *P. carinii* infection was found in 33 and 25% of the groups receiving PS-15 at dosages of 5.0 and 1.0 mg/kg, respectively.

![Chemical structures of PS-15 and WR99210](https://example.com/image)

**FIG. 1.** Chemical structures of PS-15 and WR99210.

### Table 1. Prophylactic and therapeutic effects of PS-15 compared with those of trimethoprim-sulfamethoxazole

<table>
<thead>
<tr>
<th>Category and drug (dose [mg/kg/day])</th>
<th>No. of evaluable rats</th>
<th>No. of rats with the following extent of <em>P. carinii</em> infection:</th>
<th>% of total with PCF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>None 1+ 2+ 3+</td>
<td>67</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>PS-15 (25 mg)</td>
<td>9 9 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>PS-15 (5 mg)</td>
<td>10 10 0 0 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PS-15 (1 mg)</td>
<td>10 4 0 2 4 60</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>PS-15 (50 mg/wk)*</td>
<td>9 9 0 0 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TMP-SMZ (50/250 mg)</td>
<td>7 7 0 0 0 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>PS-15 (25 mg)</td>
<td>8 8 0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>PS-15 (5 mg)</td>
<td>9 6 0 1 2 33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>PS-15 (1 mg)</td>
<td>8 6 0 1 1 25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>TMP-SMZ (50/250 mg)</td>
<td>9 9 0 0 0 0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*All animals received dexamethasone and tetracycline throughout the experiment. TMP-SMZ, trimethoprim-sulfamethoxazole.

*Each group had 10 rats initially; losses were due to cannibalism at death.

*PCF, *P. carinii* pneumonia.

*This group received only one dose per week.
observed. Thirty-eight of 40 rats given PS-15 over a period of 6 weeks survived the experiment, whereas 8 of 10 untreated control animals survived. Twenty-five of the 30 animals treated for 3 weeks with PS-15 for established P. carinii pneumonia survived the experiment. Weight losses and gross autopsy findings of the control and drug-treated groups were similar.

DISCUSSION

The present study showed that PS-15 is effective in both the prevention and treatment of murine P. carinii pneumonia. The effective therapeutic dose for 100% of animals (ED\textsubscript{100}) was 25.0 mg/kg/day or less, but it was greater than 5.0 mg/kg/day. The ED\textsubscript{100} for prophylaxis was 5.0 mg/kg/day or less, but it was greater than 1.0 mg/kg/day. The comparison of PS-15 with trimethoprim-sulfamethoxazole included only disproportionately higher doses of the latter drug combination, but equal anti-P. carinii effects were achieved. The clinical advantages or disadvantages that PS-15 might have over currently available drugs for use in the treatment of P. carinii pneumonia are unknown.

PS-15 is believed to represent a new class of antifolate drugs that Canfield et al. (1) have termed "oxyguanides," referring to hydroxylamine-derived biguanides.

Because PS-15 is metabolized to WR99210 in vivo, information about the latter compound is relevant to the expectations of PS-15. Rieckmann (11) first described the antimalarial effects of WR99210 in 1973. Subsequent studies showed that the antimalarial activity of WR99210 administered orally (ED\textsubscript{90}, 20 mg kg\textsuperscript{-1}) was significantly less than that when it was administered by the subcutaneous route (ED\textsubscript{90}, 0.34 mg kg\textsuperscript{-1}) (9). An initial clinical trial in humans was associated with severe gastrointestinal symptoms of sufficient magnitude that the study had to be abandoned (2, 3).

Based on the concept that some of the antimalarial activity of proguanil results from the metabolism of the biguanide proguanil to the triazine cycloguanil, a biguanide precursor for the triazine WR99210 was synthesized, which would lead us to expect a greater oral bioavailability and safety of the PS-15 compound.

The failure of WR99210 to elicit any discernible anti-P. carinii activity in the present study may be due to the limited absorption of this compound, while adequate bioavailability of PS-15 is evidenced by a dose-related effect on the prevention and treatment of P. carinii pneumonia.

The activity of PS-15 against bacteria, fungi, and other protozoa has not been determined.

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REFERENCES


