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Combination of PS-15, Epiroprim, or Pyrimethamine with Dapsone in Prophylaxis of Toxoplasma gondii and Pneumocystis carinii Dual Infection in a Rat Model

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In a rat model of dual infection, we studied such dihydrofolate reductase (DHFR) inhibitors as PS-15 (25 mg/kg of body weight), epiroprim (100 mg/kg), and pyrimethamine (3 mg/kg) alone or in combination with various doses of dapsone (50, 25, or 5 mg/kg) for the prevention of pneumocystosis and toxoplasmosis. Rats latently infected with Pneumocystis carinii were immunosuppressed by corticosteroids for 7 weeks, and the drugs were administered from the initiation of the corticosteroid treatment. At week 5, the rats were inoculated intraperitoneally with the RH strain of Toxoplasma gondii. Infections were monitored by the counting of P. carinii cysts in lung homogenates and the titration of T. gondii in organs by quantitative culture and an indirect immunofluorescence assay. Fourteen of the 15 untreated rats died after T. gondii challenge, with P. carinii infection in the lungs and T. gondii infection in the lungs, liver, spleen, and brain. Of the three tested DHFR inhibitors, only PS-15 exhibited anti-P. carinii activity; none prevented toxoplasmosis in 100% of the rats. After the DHFR inhibitors were combined with dapsone (50 or 25 mg/kg), both pneumocystosis and toxoplasmosis were completely prevented. On the basis of these results, PS-15 and epiroprim combined with dapsone are candidates for use for the prevention of both pneumocystosis and toxoplasmosis.

Despite the marked reductions in the incidence of pneumocystosis and toxoplasmosis in human immunodeficiency virus (HIV)-infected patients following primary prophylaxis and maintenance therapy (14), both infections are still highly prevalent, especially in Europe, where Toxoplasma gondii seroprevalence is high (65 to 70% of the general population); the arsenal of drugs remains limited. Case control studies (5) and controlled clinical trials (10) have demonstrated the high incidence of toxoplasmosis in HIV-infected patients latently infected with T. gondii who receive an anti-Pneumocystis carinii selective prophylaxis. Trimethoprim-sulfamethoxazole (TMP-SMX) is the first-choice drug, but side effects lead to a high frequency of discontinuation and so hamper its prescription (25). A substantial number of HIV-infected patients cannot tolerate long-term use of this combination, although the mechanisms of drug intolerance are poorly understood in this population (1, 4, 12, 23). The respective responsibilities of TMP and SMX in the occurrence of side effects are unclear, although SMX seems to predominate as a cause of side effects. There is clearly a need to develop new, effective combinations against both infections. Several experimental studies performed in vitro and in vivo have shown that PS-15, a new biguanide, and epiroprim (EPI), an analog of TMP, were effective against both P. carinii (7, 8, 15, 26) and T. gondii (6, 20) and that they might be good candidates for alternative therapy or prophylaxis. However, these results were obtained in experimental models of single infection and did not take account of the frequent association of these infections in AIDS patients. We have previously developed a rat model of dual infection with P. carinii and T. gondii (2) in which TMP combined with SMX and pyrimethamine (PYR) combined with dapsone (DAP) were shown to be effective prophylaxis against both pneumocystosis and toxoplasmosis. The aim of the present study was to determine whether other dihydrofolate reductase (DHFR) inhibitors, such as PS-15, EPI, and PYR, might also act against pneumocystosis and toxoplasmosis in this model of dual infection. We decided to evaluate these drugs both alone and in combination with DAP, because clinical evaluation of DAP has demonstrated its efficacy against pneumocystosis and improved tolerance of DAP by HIV-infected patients compared with that of TMP-SMX (17).

(Microorganisms and Their Environment) 1

This study was carried out in accordance with prevailing regulations regarding the use of laboratory animals in the European Communities (Journal Officiel des Communautés Européennes, 18 Décembre 1986, L358) and the care and use of laboratory animals in the European Communities (Journal Officiel des Communautés Européennes, 18 Décembre 1986, L358).

Experimental design. The animal protocol that we used has been described in detail elsewhere (2). P. carinii infection was induced in Wistar rats weighing about 200 g (Janvier Breeding Laboratories, Le Genest St Isle, France) by subjecting them to an immunosuppressive regimen (25 mg of cortisone acetate [Hydrocortisone] [Hoechst-Roussel, Paris, France] injected subcutaneously twice weekly) and a low-protein-level (8%) diet (Usine Alimentation Rationelle, Villebon-sur-Yvette, France). In each set of experiments, a group of rats was used to assess the development of P. carinii infection. Five rats were examined at the beginning of the study and after 5 and 7 weeks of immunosuppression.

Rats, for purposes of prophylaxis, were treated with DHFR inhibitors and DAP from the initiation of the corticosteroid treatment. Other rats received no therapy and were the control group; they were inoculated intraperitoneally with 105 tachyzoites of the virulent RH strain of T. gondii after 5 weeks of immunosuppression. All rats were sacrificed after 7 weeks of corticosteroid treatment.

Assessment of P. carinii and T. gondii infections. The procedures have been reported in detail in our earlier study (2). Briefly, P. carinii cysts were counted in lung tissue after enzymatic digestion and toluidine blue O staining (11). The number of cysts per gram of lung was expressed as a mean log value ± 1 standard deviation. T. gondii infection was assessed by determining parasite burdens in the brain, liver, lungs, spleen, and pleural fluid, with a tissue culture method and an indirect immunofluorescence assay being used as previously described (22).
Fourteen of the 15 rats that were challenged with T. gondii were evaluated. Since P. carinii indicated as zero. The parasitic burden of T. gondii per g of lung, and the mean values were, respectively, 6.6 log10 in lungs. They were 5.0 days after inoculation. One rat survived and was sacrificed at day 14 post-T. gondii infection. The P. carinii cyst level was log 7.2 per g of lung, and the T. gondii burden was log 1.7 per g of lung. The mean pleural fluid effusion was 5.0 ± 1.5 ml, with a T. gondii count of log 3.4 ± 1.4. T. gondii was found in the organs of all of the rats, with a mean parasitic burden of log 5.0 ± 0.9 in the lungs, 2.7 ± 1.4 in the brain, 4.9 ± 0.9 in the spleen, and 5.8 ± 1.4 in the liver being found. The mean numbers of P. carinii were not significantly different from cyst counts in immunosuppressed rats which had not been challenged with T. gondii (log 6.5 ± 0.5 in the lungs).

**TABLE 1. Prophylactic activity of PYR alone or combined with DAP against toxoplasmosis and pneumocystosis**

<table>
<thead>
<tr>
<th>Drug(s) and amt(s)</th>
<th>Outcome after T. gondii inoculation</th>
<th>Day</th>
<th>No. of rats</th>
<th>Pleural fluid vol (ml)</th>
<th>T. gondii count (log10)</th>
<th>P. carinii count (log10) in lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PYR (3 mg/kg)</td>
<td>Sacrifice</td>
<td>14</td>
<td>5</td>
<td>0</td>
<td>1.5 ± 1.2</td>
<td>0.5 ± 0.7</td>
</tr>
<tr>
<td>DAP (50 mg/kg)</td>
<td>Sacrifice</td>
<td>14</td>
<td>5</td>
<td>0.1 ± 0.2</td>
<td>0.8 ± 1.6*</td>
<td>1.2 ± 1.6</td>
</tr>
<tr>
<td>PYR (3 mg/kg) and DAP (50 mg/kg)</td>
<td>Sacrifice</td>
<td>14</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PYR (3 mg/kg) and DAP (25 mg/kg)</td>
<td>Death</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>1.5 ± 2.1</td>
<td>0.7 ± 1.0</td>
</tr>
<tr>
<td>PYR (3 mg/kg) and DAP (5 mg/kg)</td>
<td>Death</td>
<td>4.3 ± 2.3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sacrifice</td>
<td>14</td>
<td>12</td>
<td>0.6 ± 2.3</td>
<td>0</td>
<td>0.6 ± 1.4</td>
<td>4.0 ± 1.1*</td>
</tr>
</tbody>
</table>

* Drugs were given per os 5 days per week for 5 weeks and daily after T. gondii inoculation (day 0 began at week 5) until death or sacrifice.

† One rat with high levels of T. gondii and P. carinii died at day 4.

‡ One rat died at day 12. The P. carinii cyst level was log 2.7/g of lung, and the T. gondii burden was log 5.3/g of liver.

§ P < 0.001 versus values for the PYR-treated group. (For statistical analysis by Bonferroni’s adjusted t test, the values for the dead and sacrificed rats were pooled.)

DISCUSSION

Both P. carinii and T. gondii possess the necessary enzymes for de novo folate synthesis (18). Inhibitors of folic acid synthesis are the main drugs to have been evaluated for P. carinii and T. gondii infection, especially DHFR and dihydropteroate synthetase inhibitors. This model of dual infection was used because of several potential advantages. First, the development of both infections in immunosuppressed animals better
mimics the clinical setting in which the development of the two opportunistic infections is often found. Second, it avoids the variations in the pharmacokinetic behaviors of the drugs after they are administered to different animal species (rats for P. carinii and mice for T. gondii). Third, it takes account of the possible mutual interference in the development of the two infections. In the present study, we found evidence that the activities of two new folate inhibitors, PS-15 and EPI, were markedly reinforced when these drugs were given in combination with DAP.

Indeed, we have confirmed in the rat model of dual infection that the combination of PYR and DAP could be considered as a reference therapy. This combination has been shown previously to be effective for the treatment of either pneumocystosis (26) or toxoplasmosis (9) or both types of infection in the rat (2). The complementary experiments described here also demonstrated that the minimum effective dose of DAP could be reduced to 25 mg/kg without a loss of activity against either pathogen.

PS-15 is a synthesized inhibitor of DHFR and has previously been shown to be active against Plasmodium falciparum and P. carinii both in vitro (3, 7) and in vivo (8, 15, 16). In the latter studies, two experimental models of pneumocystosis were used: the SCID mouse model involving intratracheal infection and the rat model involving latent infection with P. carinii. PS-15 was found to be effective alone and had an additive effect when low doses were combined with DAP. The high efficacy of PS-15 against P. carinii has been confirmed here. However, we found that PS-15 alone was not effective against T. gondii, whereas PS-15 combined with DAP appeared to be as effective as the reference regimen (DAP and PYR) for preventing toxoplasmosis.

EPI (Ro 11-8958) is an analog of TMP and is substituted at position 4 of the benzyl moiety (24). Its main characteristics are a marked activity against gram-positive cocci, Nocardia species, and anaerobes and a larger volume of distribution and longer half-life than TMP. Although the anti-P. carinii and anti-T. gondii activities of EPI were demonstrated in vitro and in vivo individually in different models (6, 7, 13, 19, 20, 26), the rat model of dual infection is the first model in which T. gondii burdens were quantified in parallel with the number of P. carinii cysts. We have shown that EPI administered alone exhibited only mild anti-T. gondii activity (delayed death) and did not prevent pneumocystosis. On the other hand, EPI plus DAP acted in synergy against both P. carinii and T. gondii (even at a low dose of DAP), preventing pneumocystosis and toxoplasmosis.

The combinations of PS-15 or EPI and DAP (50 or 25 mg/kg) were more effective than PS-15 or EPI alone against toxoplasmosis. These results were similar to those obtained with the reference therapy of PYR combined with DAP (same doses). EPI seems poorly effective against both P. carinii and T. gondii after being administered alone, but a synergism was observed after EPI was combined with DAP against T. gondii. The anti-P. carinii activity was similar to that of DAP alone, preventing any detection of a synergistic effect. Of the drugs tested here, EPI plus a low dose of DAP (5 mg/kg) was significantly better than the reference treatment of PYR plus DAP (5 mg/kg).

PS-15 has a unique activity against P. carinii, which contrasts

<table>
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<th>Pleural fluid vol (ml)</th>
<th>T. gondi count (log_{10})</th>
<th>P. carinii count (log_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS-15 (25 mg/kg)</td>
<td>Death</td>
<td>5.2 ± 1.0</td>
<td>5†</td>
<td>3.4 ± 1.7</td>
<td>1.4 ± 2.0</td>
<td>1.4 ± 2.7</td>
</tr>
<tr>
<td>PS-15 (50 mg/kg)</td>
<td>Death</td>
<td>4.4 ± 1.5</td>
<td>5</td>
<td>2.6 ± 1.5</td>
<td>1.7 ± 2.1</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>PS-15 (25 mg/kg) + DAP (50 mg/kg)</td>
<td>Sacrifice</td>
<td>14</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PS-15 (25 mg/kg) + DAP (25 mg/kg)</td>
<td>Sacrifice</td>
<td>14</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PS-15 (5 mg/kg)</td>
<td>Death</td>
<td>8.5 ± 3.3</td>
<td>4</td>
<td>2.6 ± 2.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PS-15 (5 mg/kg)</td>
<td>Sacrifice</td>
<td>16</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0.7 ± 1.2</td>
</tr>
</tbody>
</table>

* Drugs were given per os 5 days per week for 5 weeks and daily after T. gondii inoculation (at week 5) until death or sacrifice.
† One rat survived and was sacrificed at day 14. The T. gondii burden in the liver was found to be log 3.6/g, and the P. carinii cyst level was log 3.1/g of lung.
‡ P < 0.001 versus values for PS-15-treated groups. (For statistical analysis by Bonferroni’s adjusted t test, the values for dead and sacrificed rats were pooled.)
with the poor activity of other DHFR inhibitors against this parasite. This drug could also be better tolerated, as it is not structurally related to other DHFR inhibitors, such as TMP, PYR, or trimetrexate and piritrexim. Its potential value for preventing opportunistic infections in AIDS patients is also supported by its activity against mycobacteria (21).

We conclude that both PS-15 and EPI are attractive candidates for mixed prevention of toxoplasmosis and pneumocystosis only after being given in combination with DAP.

ACKNOWLEDGMENTS

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REFERENCES


