Therapeutic Efficacies of GW471552 and GW471558, Two New Azasordarin Derivatives, against Pneumocystosis in Two Immunosuppressed-Rat Models

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Two new azasordarins, GW471552 and GW471558, were studied in vivo for treatment of Pneumocystis carinii pneumonia. In the Wistar rat spontaneous pneumonia model, both azasordarins significantly reduced the number of P. carinii cysts per gram of lung homogenate when administered at 1 mg/kg of body weight twice a day for 10 days. In a nude rat inoculation model, both compounds showed therapeutic efficacy at 0.25 mg/kg twice a day for 10 days.

Pneumocystis carinii, remains an important pathogen for the broad spectrum of immunocompromised individuals, despite significant advances in antimicrobial therapy (15). Deep impairments in cell immunity, probably associated with pulmonary surfactant changes, are critical conditions for proliferation (8). This agent is an important cause of community-acquired pneumonia in individuals with a wide variety of underlying immune deficiencies and remains the most common life-threatening opportunistic infection diagnosed in human immunodeficiency virus patients, despite the dramatic decline in incidence due to widespread use of highly active antiretroviral therapy (19). In most non-AIDS immunocompromised patients, such as organ transplant recipients, the risk of disease is in the range of 5 to 15% (depending on the nature and duration of the immunosuppression). Among patients who are not receiving prophylactic therapy, P. carinii causes pneumonia in 10% of heart, liver, and kidney transplant recipients in the first 6 months posttransplantation (11). In addition, the incidence of this infection appears to be higher in children than in adults (6).

The inability of many patients to tolerate prophylaxis or treatment with traditional therapeutic standards, such as trimethoprim-sulfamethoxazole, atovaquone, or pentamidine (19), originated a search for new agents for prevention and treatment of Pneumocystis infection in immunocompromised hosts.

Sordarins are a new class of antifungal agents that act by inhibiting the protein synthesis elongation cycle (5, 10). Sordarin derivatives have demonstrated a potent and relatively broad-spectrum antifungal activity in vitro (14) and in vivo studies (4, 20, 22). A further evolution of this class of compounds has led to a new family of substances, azasordarins, that have a similar biological profile but easier chemical synthesis. Azasordarins have demonstrated excellent in vitro activity against key fungal pathogens, including P. carinii (13), and therapeutic efficacy in experimental rodents of oral and vulvovaginal candidiasis (21).

In order to determine the potential in vivo profile of azasordarins, two compounds have been selected for the treatment of P. carinii pneumonia (PCP) as representatives of this new family of antifungal agents and have been evaluated in two experimental infection models of pneumonia in immunosuppressed rats.

(Antifungal agents. GW471552 and GW471558 were synthesized at GlaxoSmithKline (Tres Cantos, Madrid, Spain). The compounds, as potassium salts, were initially dissolved in sterile distilled water at a starting concentration of 2 mg/ml and diluted in sterile distilled water to reach the desired concentrations. Solutions were prepared just before use and protected from light. Wellcome Laboratory graciously provided trimethoprim-sulfamethoxazole as Septprim.

Experimental PCP. The therapeutic efficacy of GW471552 and GW471558 was evaluated with two experimental models in immunosuppressed rats: (i) Wistar rats, which develop spontaneous infection after immunosuppressive treatment; and (ii) nude rats intratracheally infected with P. carinii organisms. Seven-week-old female Wistar rats (Iffa Credo, Lyon, France) and 10-week-old female Fischer-344 RNU/nu rats from a Pneumocystis-free colony (Lille Pasteur Institute) were used in this study.

The research complied with national legislation and with company policy on the care and use of animals according to the related code of practice.

(i) Wistar rat model. Pneumonia developed spontaneously after 9 weeks of immunosuppressive treatment with dexameth-
After 2 weeks of dexamethasone treatment, rats were anesthe-
in the drinking water (1 mg/liter) throughout the study. The drug treatment was started after 9 weeks of immuno-suppression.

(ii) Nude rat model. The animals were given dexamethasone (2) in the drinking water (1 mg/liter) throughout the study. After 2 weeks of dexamethasone treatment, rats were anesthetized and infected by nonsurgical intratracheal inoculation with $9 \times 10^7$ Pneumocystis organisms per rat (E. M. Aliouat, S. Ferrar, J. C. Cailliez, A. E. Wakefield, J. Sparrowe, C. Recourt, D. Camus, and E. Dei-Cas, submitted for publication).

Antifungal treatment. Antifungal therapy was started 5 or 9 weeks after corticosteroid treatment in the nude or Wistar rat model, respectively. Groups of five rats each were treated subcutaneously with GW471552 or GW471558 twice a day for 10 consecutive days. Doses of 1 and 5 mg/kg of body weight were administered to the rats. Nude rats were treated with doses of 0.25 and 0.5 mg/kg of body weight. Septrim, used as reference compound in both models, was administered at 50 (trimethoprim)/250 (sulfamethoxazole) mg/kg of body weight orally (by gavage) once a day for 10 consecutive days.

Assessment of therapeutic efficacy. Therapeutic efficacy was assessed by counting $P$. carinii cysts in lung homogenates and comparing them with those of the untreated controls at the end of the experiment. Twenty-four hours after the end of the treatment, animals were sacrificed, and the lungs were aseptically removed and processed for parasite quantitation with toluidine blue O stain (Sigma Aldrich, Alcobendas, Madrid, Spain) as previously described (1, 24).

The total numbers of $P$. carinii cysts ($T_c$) were calculated according to the equation $T_c = (n \times S_o \times R)/F_a$, where $n$ is the average number of microorganisms per oil immersion field (20 fields counted for each smear), $S_o$ is the 2-μm smear area, $R$ is the ratio of the total volume of the microorganisms in suspension to the calibrate smear volume (2 μl), and $F_a$ is the oil immersion field area (2). The limit of detection of this procedure was $10^5$ cysts per g of lung.

One day before starting antifungal treatment, three animals were sacrificed, and lungs were processed to verify the level of infection and quantify the number of $P$. carinii cysts per gram of lung. The results indicate that all animals studied developed $P$. carinii pneumonia.

Statistical analysis. The Kruskal-Wallis nonparametric test was used to statistically compare the number of cysts of $P$. carinii recovered from the lungs of the experimental groups. Multiple comparisons of treated groups versus the control group were performed by Dunn’s method. All statistical evaluations were performed with the SigmaStat statistical package (Jandel Scientific, Erkrath, Germany). $P$ values of $\leq 0.05$ were considered statistically significant.

Two experimental models of pneumocystosis were used to evaluate the therapeutic efficacy of GW471552 and GW471558.

Therapeutic efficacy in Wistar rats. Before starting antifungal treatment, animals developed PCP with 7.0 ± 0.3 (n = 3) log cysts/g of lung. The number of organisms quantified 24 h after the end of the treatment from untreated animals was 6.9 ± 0.4 log cysts/g of lung. The experimental results obtained from rats with PCP, either untreated or treated with GW471552 or GW471558, are summarized in Table 1. After the antifungal chemotherapy, rats treated with 1.0 mg of GW471552 or GW471558 per kg showed a statistically significant reduction in the number of cysts recovered from lungs, with 5.0 ± 0.6 log cysts/g of lung in both cases, which represents reductions of 98.21 and 97.90% (P < 0.05), respectively. GW471552 and GW471558 administered at 5 mg/kg also reduced the log number of cysts per gram of lung: 98.88 and 98.96% (P < 0.05), respectively. Septrim significantly reduced (P < 0.05) the number of cysts per gram of lung compared to the level in controls (98.96%).

Therapeutic efficacy in nude rats. Before the start of antifungal treatment, the $P$. carinii burden in infected rats was 7.1 ± 0.2 log cysts/g of lung (n = 3). Saline-treated control animals showed 7.3 ± 0.2 log cysts per g of lung at the end of the treatment. The therapeutic effects of treatment with GW471552 and GW471558 on the $P$. carinii pulmonary burden in infected rats are summarized in Table 2. GW471558 was able to reduce the lung burden to below the limit of detection in all of the animals treated at 0.5 mg/kg. At this dose, GW471552 exhibited a 99.99% reduction compared with untreated controls. In addition, the two azasordarins were effective when administered at 0.25 mg/kg. Septrim reduced the

<table>
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<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Mean log cysts/g of lung ± SD</th>
<th>% Reduction of cysts&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>Control</td>
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<tr>
<td>GW471552</td>
<td>1.0</td>
<td>6.9 ± 0.4</td>
<td>98.21</td>
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<td>5.0</td>
<td>5.1 ± 0.2</td>
<td>98.88</td>
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<tr>
<td>GW471558</td>
<td>1.0</td>
<td>5.0 ± 0.6</td>
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<td></td>
<td>5.0</td>
<td>4.9 ± 0.4</td>
<td>98.96</td>
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<tr>
<td>Septrim</td>
<td>50/250</td>
<td>4.9 ± 0.4</td>
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<sup>a</sup> Rats were immunosuppressed with dexamethasone for 9 weeks. Compounds were administered subcutaneously every 12 h for 10 consecutive days. Septrim was administered once a day for 10 consecutive days.

<sup>b</sup> Therapeutic efficacy was assessed by counting $P$. carinii cysts in lung homogenates of treated rats and comparing them with those of the untreated control animals. For each entry, $P \leq 0.05$ versus the control treatment. The limit of detection was 3 log units/g of lung.

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<tr>
<td>GW471552</td>
<td>0.25</td>
<td>7.3 ± 0.2</td>
<td>99.49</td>
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<td></td>
<td>0.5</td>
<td>3.2 ± 0.2</td>
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<tr>
<td>GW471558</td>
<td>0.25</td>
<td>6.6 ± 0.4</td>
<td>74.88</td>
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<td></td>
<td>0.5</td>
<td>&lt;3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;99.99&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Septrim</td>
<td>50/250</td>
<td>6.7 ± 0.2</td>
<td>80.04</td>
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<sup>a</sup> Rats were intratracheally infected after 2 weeks of immunosuppression with $9 \times 10^7$ $P$. carinii organisms. Compounds were administered subcutaneously every 12 h for 10 consecutive days. Septrim was administered once a day for 10 consecutive days.

<sup>b</sup> Therapeutic efficacy was assessed by counting $P$. carinii cysts in lung homogenates of treated rats and comparing them with those of the untreated control animals. *, $P \leq 0.05$ versus the control treatment.

<sup>c</sup> No organisms detected. The limit of detection was 3 log units/g of lung.
number of cysts per gram of lung compared to controls, showing a reduction of 80.04%.

*P. carinii* remains an important pathogen in AIDS patients and other immunocompromised individuals (16). Pneumonia caused by *P. carinii* is usually treated with trimethoprim-sulfamethoxazole, atovaquone, or pentamidine, which are also used in primary and secondary prevention (12, 19). The relatively high frequency of adverse reactions (18) and appearance of resistance (17) to these drugs reflect the need for new therapeutic approaches.

Sordarins are a new kind of antifungal agents different from other antifungal compounds because of their novel mechanism of action. They are highly selective fungal protein synthesis inhibitors that interact with the translation elongation factor EF-2 and the large ribosomal subunit stalk, rpP0, thus inhibiting translation and elongation in fungal cells (5, 10). Azasordarins have demonstrated excellent in vitro and in vivo activity against key fungal pathogens. The efficacy of azasordarins against *P. carinii* has been demonstrated in vitro: 50% inhibitory concentration values of GW471552 and GW471558 against *P. carinii* used for experimental infections were 0.001 and ≤0.001 μg/ml, respectively. However, confirmation of therapeutic efficacy in rodent models is still necessary as a basis for clinical trials.

The aim of this study was to evaluate the therapeutic efficacy of two new azasordarins against experimental PCP. We have shown that azasordarins are highly potent, reducing the number of cysts from lungs with a good correlation between dose and response in the two immunosuppressed rat models evaluated. Azasordarins showed better therapeutic efficacies than those exhibited under our experimental conditions by antifungal derivatives against pathogenic yeasts, such as Septiplus and response in the two immunosuppressed rat models evaluated. Azasordarins showed better therapeutic efficacies than those exhibited under our experimental conditions by antifungal derivatives against pathogenic yeasts, such as Septiplus and aliyamines.

In addition, the low toxicity of GW471552 and GW471558 has been demonstrated in vitro (13) and confirmed by preliminary mouse toxicity tests [E. Herreros, A. Martinez, M. J. Almela, E. Jimenez, S. Lozano, M. J. Perez, and D. Gargallo-Viola, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1691, 2000]. In conclusion, the results of the present study are encouraging, although further investigations to confirm the potential of azasordarins for effective antifungal treatment of PCP in humans are needed.

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REFERENCES