NOTES

Low-Dose Trimethoprim-Sulfamethoxazole Alone and in Association with Zidovudine for Prevention and Treatment of Murine Pneumocystis carinii Pneumonia

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Low-dose trimethoprim-sulfamethoxazole (TMP-SMX) alone was found to be as effective as low-dose TMP-SMX plus zidovudine and standard-dose TMP-SMX alone in preventing and treating Pneumocystis carinii pneumonia (PCP) in an immunosuppressed-rat model. Zidovudine alone had no preventive or curative effect on PCP. We conclude that the initially reported reduced incidence of PCP in human immunodeficiency virus-infected patients treated with zidovudine alone is not due to anti-P. carinii activity of zidovudine. Furthermore, the clinical efficacy of low-dose TMP-SMX for the prevention and treatment of PCP should be further investigated.

Major efforts have been directed towards reducing the incidence of Pneumocystis carinii pneumonia (PCP) in human immunodeficiency virus (HIV)-infected patients; until recently, PCP was the most frequent AIDS-related opportunistic infection. Zidovudine, the first antiretroviral drug to be licensed, can reduce the frequency of PCP and other opportunistic infections in HIV-infected patients and can improve survival (4). Zidovudine shows anti-HIV activity in vitro and reduces p24 antigenemia in vivo, but it has also recently been shown to be active against nonviral microorganisms, including gram-negative bacteria (12, 15).

Effective anti-Pneumocystis treatment often involves drug synergy (5, 9, 11, 13). For example, Hughes and Killmar (8) recently found that erythromycin plus sulfisoxazole has a pronounced effect on the prevention and treatment of murine PCP, while erythromycin alone has no effect.

We therefore investigated the anti-Pneumocystis activity of zidovudine in vivo and its potential synergy with trimethoprim-sulfamethoxazole (TMP-SMX).

Fifty-six male Wistar rats (Janvier Breeding Laboratories, Le Genest St. Isle, France) weighing 200 to 210 g were used. Initially, before immunosuppression, three healthy rats were sacrificed to assess latent P. carinii infection. Then 50 rats were randomly assigned at the start of the immunosuppressive regimen to receive either prophylaxis or therapy for PCP (5 rats per group). The remaining three rats were sacrificed after 4 weeks of immunosuppression to monitor the level of the infection.

Immunodeficiency was induced by subcutaneous (s.c.) injections of 25 mg of cortisone acetate (hydrocortisone; Hoechst-Roussel, Paris, France) twice per week until sacrifice, as described by Frenkel et al. (5). The immunosuppression was enhanced by a low-protein (8%) diet (Usine Alimentation Rationelle, Villemoisson, France) (9). This model yields extensive PCP after 7 weeks (6).

Drugs. Zidovudine (3'-azido-3'-deoxythymidine) (Retrovir; Wellcome, Paris, France) was administered at 20 mg/kg of body weight per day in the drinking water and 20 mg/kg s.c. five times per week. The same dosing schedule was used for prophylaxis and treatment. TMP-SMX (Roche, Neuilly-sur-Seine, France) at the standard dose of 40 mg of TMP per kg and 200 mg of SMX per kg and at the low dose of 8 mg of TMP per kg and 40 mg of SMX per kg was injected s.c. twice per week for the prophylaxis group and five times per week for the treatment group. Zidovudine associated with low-dose TMP-SMX was administered at the same dosing schedule used for zidovudine and low-dose TMP-SMX alone. The doses were based on the average weights of the rats at the beginning of the treatment period. Tetracycline hydrochloride powder (Sigma, Paris, France) was added to the drinking water (1 mg/ml) to prevent bacterial infections in the untreated controls.

The drugs were given during the full 7-week period of immunosuppression to determine prophylactic efficacy or for the last 3 weeks of immunosuppression to determine therapeutic efficacy.

At the end of the study period, the surviving rats were anesthetized with pentobarbital (Pentothal; Abbott, Orsay, France) and exsanguinated via the abdominal aorta with a Vacutainer (Becton Dickinson, Meylan, France). The lungs were removed aseptically in order to detect bacterial infections and were weighed. P. carinii cysts were quantified as described elsewhere (1) using the technique of Yoshida and Ikai (20) with the modifications of Walzer et al. (18).

P. carinii cyst counts were processed by one-way analysis of variance (with Bartlett’s test for equal variance), and each
TABLE 1. Effects of zidovudine and low-dose TMP-SMX in prevention of PCP in immunosuppressed rats*

| Drug treatment       | No. of rats evaluated | Body wt (g) | Lung wet wt (g) | Geometric mean (CI) f P. carinii cysts/g of lung tissue (wet wt) |
|----------------------|-----------------------|-------------|-----------------|---------------------------------------------------------------|
| None (controls)      | 5/5                   | 198 ± 11    | 0.93 ± 0.04     | 8 × 10⁶ (1.3 × 10⁶–5 × 10⁶)††                                  |
| Zidovudine           | 4/5                   | 171 ± 22    | 1.33 ± 0.22     | 2 × 10⁷ (1 × 10⁷–4 × 10⁷)††                                  |
| Zidovudine + low-dose TMP-SMX | 3/5               | 180 ± 19    | 1.00 ± 0.06     | 5.3 × 10³ (9 × 10³–3 × 10⁴)††                                 |
| Standard-dose TMP-SMX| 5/5                   | 184 ± 19    | 0.96 ± 0.09     | 2 × 10³ (1.4 × 10³–3.3 × 10⁴)††                               |
| Low-dose TMP-SMX     | 5/5                   | 193 ± 126   | 0.95 ± 0.08     | 4.9 × 10³ (2.2 × 10³–1 × 10⁴)††                               |

* Rats were evaluated after 7 weeks of immunosuppression and prophylaxis.
† Zidovudine, 20 mg/kg/day in the drinking water and 20 mg/kg s.c. five times a week; standard-dose TMP-SMX, 40/200 mg/kg; low-dose TMP-SMX, 8/40 mg/kg. TMP-SMX was administered s.c. twice a week.
‡ Values are means ± standard deviations.
§ CI, 95% confidence interval.
** P < 0.001 versus value for Zidovudine (40/200 mg/kg).
†† One unevaluable rat died before the end of the study period.

A pair of groups that was of interest was compared by using Bonferroni’s adjusted t tests. The numbers of cysts are given as geometric means with 95% confidence intervals. Other data are presented as means ± 1 standard deviation.

The three untreated healthy control rats had 2 × 10³ P. carinii cysts per g of lung tissue.

**Prophylaxis regimen.** At the end of the study period, P. carinii cyst counts were 2 × 10⁷ and 8 × 10⁷ per g of lung tissue (wet weight) in the zidovudine-treated and untreated immunosuppressed rats, respectively (Table 1). Median cyst counts in the prophylaxis group treated with zidovudine plus low-dose TMP-SMX were similar to those obtained with the regular and low-dose TMP-SMX regimens alone (Table 1). Streptococcus pyogenes and Staphylococcus aureus were found in the lungs of rats treated with zidovudine alone.

**Therapeutic regimen.** After 4 weeks of immunosuppression, three untreated rats were sacrificed to evaluate pre-treatment infection. They had 2 × 10⁸ cysts per g of lung tissue (Table 2). At the end of the study period, pulmonary cyst counts in the rats treated with zidovudine alone were similar to those in the immunosuppressed control rats (1.7 × 10⁷ and 1.2 × 10⁷ cysts per g of lung tissue, respectively). Treatment with zidovudine plus low-dose TMP-SMX was no more effective than low-dose TMP-SMX alone. The lungs of both zidovudine-treated and control rats showed S. pyogenes infection.

We found that zidovudine alone does not act directly against P. carinii in severely immunosuppressed rats when a dose and mode of administration similar to those employed by Sinet et al. (16) to assess antiretroviral activity in mice are used. In addition, the combination of zidovudine and low-dose TMP-SMX was no more effective in preventing and treating PCP than low-dose TMP-SMX alone.

Our data also suggest that TMP-SMX may be equally effective at doses lower than those usually given. Low-dose TMP-SMX, i.e., 8/40 mg/kg (twice per week (16/80 mg/kg/week) for prophylaxis or five times per week (40/200 mg/kg/week) for therapy, was highly effective against P. carinii infection in our experimental model. This is inconsistent with a previous report that intermittent low doses of TMP-SMX in drinking water (100/500 mg/kg/week) prevented PCP in only 60% of rats (7) . Possibly because variable amounts of drug were ingested in the latter study.

In an uncontrolled study of patients treated with zidovudine, Ruskin and Lariviére (14) showed that intermittent low doses of TMP-SMX (160/800 mg every other day) were as effective as the previously evaluated dosage of 160/800 mg twice a day (2). Moreover, these reduced dosages (equivalent to 480/2,400 mg/week) were lower than those initially shown by Hughes et al. (10) to be effective in non-HIV-immunosuppressed children (150/750 mg/m² of body surface area 3 days a week, equivalent to 760/3,800 mg/week).

Our results thus indicate that the reduced incidence of PCP initially reported for patients treated with zidovudine (4) is due to antiretroviral activity leading to relative immune restoration as reflected by the increase in CD4 lymphocyte counts and is not due to a direct action on P. carinii. Actually, our results are consistent with the high incidence

TABLE 2. Effects of zidovudine and low-dose TMP-SMX in treatment of PCP in immunosuppressed rats*

| Drug treatment     | No. of rats evaluated | Body wt (g) | Lung wet wt (g) | Geometric mean (CI) f P. carinii cysts/g of lung tissue (wet wt) |
|--------------------|-----------------------|-------------|-----------------|---------------------------------------------------------------|
| None (controls)    | 3/5††                 | 161 ± 10    | 1.29 ± 0.23     | 1.2 × 10⁷ (2 × 10⁶–8 × 10⁷)††                                  |
| Zidovudine         | 5/5                   | 160 ± 18    | 1.50 ± 0.38*    | 1.7 × 10⁷ (5 × 10⁶–5 × 10⁷)††                                  |
| Zidovudine + low-dose TMP-SMX | 5/5               | 164 ± 10    | 1.00 ± 0.10     | 3.1 × 10³ (1.4 × 10³–6.8 × 10³)††                              |
| Standard-dose TMP-SMX | 5/5              | 151 ± 16    | 0.98 ± 0.08     | 3.7 × 10³ (3 × 10³–5 × 10³)††                                  |
| Low-dose TMP-SMX   | 5/5                   | 170 ± 15    | 0.97 ± 0.05     | 3.0 × 10³ (1 × 10³–1 × 10⁴)††                                  |

* Rats were evaluated after 7 weeks of immunosuppression and 3 weeks of treatment.
† Zidovudine, 20 mg/kg/day in the drinking water and 20 mg/kg s.c. five times a week; standard-dose TMP-SMX, 40/200 mg/kg; low-dose TMP-SMX, 8/40 mg/kg. TMP-SMX was administered s.c. five times a week.
‡ Values are means ± standard deviations.
§ CI, 95% confidence interval.
** P < 0.01 versus value for Zidovudine (40/200 mg/kg).
†† Two rats died and could not be evaluated.
of PCP in patients treated with zidovudine alone as reported recently in controlled trials with longer follow-up (3).

By extension, the efficacy of low-dose TMP-SMX in HIV-infected patients treated concomitantly with zidovudine appears to be due to TMP-SMX alone (14, 17, 19). Our data further support the clinical evaluation of lower dosages of TMP-SMX in the prevention and treatment of PCP, as previously reported (17, 19).

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