

## Use of Fluoroquinolones for Prophylaxis of Murine *Pneumocystis carinii* Pneumonia

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**We compared the prophylactic activities of six fluoroquinolones against *Pneumocystis carinii* pneumonia in immunosuppressed rats. Pefloxacin was the only agent which was as effective as the reference drug trimethoprim-sulfamethoxazole. Clinical trials with pefloxacin in patients at risk for *P. carinii* pneumonia appear to be justified.**

*Pneumocystis carinii* is one of the major opportunistic pulmonary pathogens in patients with AIDS (6, 11). Because of the side effects of oral trimethoprim-sulfamethoxazole (TMP-SMX) and intravenous pentamidine classically used for the treatment and prophylaxis of *P. carinii* pneumonia (PCP), newer agents and novel routes of administration are being sought. Such approaches have been initially tested in a rat model of PCP and include sulfones (14, 23), clindamycin-primaquine (21), aerosolized pentamidine (5, 8), and hydroxynaphthoquinone (12).

As we have previously shown the efficacy of pefloxacin (PEF) against PCP in rats with curative and prophylactic treatments (2), we compared the efficacy of PEF with those of five other fluoroquinolones: ciprofloxacin (CIP), norfloxacin (NOR), ofloxacin (OFL), sparfloxacin (SPFX), temafloxacin (TEM), and TMP-SMX for the prophylaxis of PCP in immunosuppressed rats.

Male Sprague-Dawley rats weighing 200 to 220 g were randomly assigned to the different treatment groups as discussed below, and drugs were administered throughout the period of immunosuppression. Immunodeficiency was induced by the method of Frenkel et al. (7) by subcutaneous (s.c.) injections of 25 mg of cortisone acetate twice weekly. Doxycycline was injected s.c. at 10 mg twice weekly to prevent bacterial infection. The immunosuppression was enhanced (13) with a low-protein (8%) diet. This protocol induces PCP after 4 weeks.

The following forms of quinolones were administered orally (by gavage), 100 mg/kg of body weight thrice weekly: (i) CIP (Bayer, Pharma. Puteaux, France) in powder form, suspended in a balanced salt solution; (ii) NOR (Merck Sharp and Dohme, La Celle St. Cloud, France) in powder form, suspended in 0.2 g% carboxymethyl cellulose; (iii) OFL (Roussel Uclaf, Romainville, France) in powder form, suspended in a balanced salt solution; (iv) PEF (Rhône-Poulenc Santé, Antony, France) as an injectable solution; (v) SPFX (Rhône-Poulenc Santé) in powder form, suspended in 0.2 g% carboxymethyl cellulose; and (vi) TEM (Abbott, Rungis, France) in powder form, suspended in a balanced salt solution. TMP-SMX (Roche, Neuilly-sur-Seine, France) at 40 and 200 mg/kg, respectively, was injected s.c. twice weekly. The drug efficacies were evaluated in different

experiments which included untreated control and TMP-SMX-treated groups in each experiment.

After 4 weeks of prophylaxis and 24 h after the last drug administration, the anesthetized rats were exsanguinated via the abdominal aorta. The lungs were weighed, and one portion was used for quantification of *P. carinii* cysts and the other for antibiotic assay. *P. carinii* cysts were quantified by the technique of Walzer et al. (24). Two 5- $\mu$ l drops of the enzymatically digested lungs were placed on a slide, dried, and stained with toluidine blue O (9). Cysts were either blindly counted in duplicate in the whole drop or, when too many, calculated by the technique of Yoshida and Ikai (26). Concentrations of CIP, SPFX, TEM, and PEF in plasma and lungs were measured by high-pressure liquid chromatography (3, 3a). The lung contamination by the residual blood determined by the technique of Cross et al. (4) was found to be negligible (6.5% of serum). *P. carinii* cyst counts were analyzed by one-way analysis of variance and Student's *t* test.

After the 4-week period of immunosuppression, as no differences between untreated control groups and TMP-SMX-treated groups for each experiment were found, the results were combined to facilitate presentation of the data. All the control rats were heavily infected with *P. carinii* cysts ( $5.7 \times 10^6$  cysts per g). TMP-SMX and PEF were equally effective in preventing PCP, with median counts of  $9 \times 10^2$  and  $2.7 \times 10^3$  cysts per g of lung tissue, respectively (Table 1). TEM and OFL provided little coverage, while

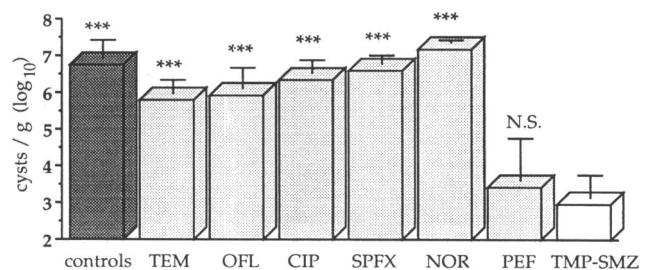


FIG. 1. Intensity of PCP in control and quinolone-treated rats versus that in rats treated with TMP-SMX after 4 weeks of prophylaxis. The bars are means  $\pm$  standard deviations. \*\*\*,  $P < 0.001$ ; NS,  $P > 0.05$ .

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TABLE 1. Prophylaxis against PCP in rats<sup>a</sup>

Drug treatment <sup>b</sup>	No. of rats	Geometric mean (CI) <sup>c</sup> <i>P. carinii</i> cysts/g of lung tissue	g of lung tissue (mean ± SD)
None (controls)	18 <sup>d</sup>	5.7 × 10 <sup>6</sup> (3.2 × 10 <sup>6</sup> –1.1 × 10 <sup>7</sup> )	1.02 ± 0.40
TMP-SMX	18 <sup>d</sup>	9 × 10 <sup>2</sup> (4.5 × 10 <sup>2</sup> –1.8 × 10 <sup>3</sup> )	0.89 ± 0.07
<b>Quinolones</b>			
PEF	10	2.7 × 10 <sup>3</sup> (4.1 × 10 <sup>2</sup> –1.7 × 10 <sup>4</sup> )	0.86 ± 0.06
TEM	5	6 × 10 <sup>5</sup> (2 × 10 <sup>5</sup> –2 × 10 <sup>6</sup> )	0.86 ± 0.05
OFL	5	7.9 × 10 <sup>5</sup> (1.4 × 10 <sup>5</sup> –4.4 × 10 <sup>6</sup> )	0.92 ± 0.04
CIP	5	2.2 × 10 <sup>6</sup> (8 × 10 <sup>5</sup> –6 × 10 <sup>6</sup> )	0.91 ± 0.06
SPFX	5	3.8 × 10 <sup>6</sup> (1.9 × 10 <sup>6</sup> –7.9 × 10 <sup>6</sup> )	1.00 ± 0.11
NOR	5	1.5 × 10 <sup>7</sup> (1.2 × 10 <sup>7</sup> –1.8 × 10 <sup>7</sup> )	1.17 ± 0.27

<sup>a</sup> Rats were evaluated after 4 weeks of immunosuppression.

<sup>b</sup> TMP-SMX, 40 and 200 mg/kg, respectively, administered s.c. twice weekly; quinolones, 100 mg/kg administered orally thrice weekly.

<sup>c</sup> CI, confidence interval.

<sup>d</sup> Two rats died and could not be evaluated.

CIP, SPFX, and NOR were ineffective (Fig. 1). The mean concentrations of the quinolones are shown in Table 2. Pulmonary concentrations of PEF and SPFX were similar and were approximately seven times higher than those of TEM and CIP.

Our results show that PEF, when administered orally three times a week at 100 mg/kg for 4 weeks, is as effective as TMP-SMX (40 and 200 mg/kg, respectively, s.c. twice weekly) in preventing PCP in rats. The other fluoroquinolones tested were either poorly effective (TEM and OFL) or ineffective (CIP, SPFX, and NOR). There are few studies of quinolone activity against parasitic and fungal infections: Salmon et al. (22) recently showed that PEF was more effective than CIP against *Plasmodium falciparum* in mice, although it is ineffective against *Toxoplasma gondii* (5a).

Twenty-four hours after the last quinolone administration, higher concentrations in plasma and lungs were obtained with SPFX and PEF than with TEM and CIP. The differences could be explained by the longer plasma half-lives of PEF (18) and SPFX (20) (3.3 and 3.8 h, respectively) than of the other quinolones (1 to 2 h) (10, 16, 19, 20). Higher concentrations in lungs may be obtained with larger doses. A lower level of lung diffusion cannot explain the difference in efficacy between PEF and SPFX, since concentrations in lungs were the same, whereas only PEF was effective in preventing PCP. Although OFL and NOR were not tested, since Azoulay-Dupuis et al. (1) have shown that similar concentrations of OFL and CIP are achieved in the lungs of mice, it seems reasonable to think that similar concentrations in lungs were achieved with CIP and OFL.

TABLE 2. Levels of quinolones in plasma and lungs after prophylaxis

Drug treatment <sup>a</sup>	Mean ± SD quinolone concn <sup>b</sup>		
	µg/g of lung tissue	µg/ml of plasma	Lung/plasma ratio
PEF	7.29 ± 7.09	1.76 ± 0.26	4.05 ± 3.44
TEM	1.08 ± 0.66	1.40 ± 0.60	0.66 ± 0.22
CIP	0.72 ± 0.22	0.25 ± 0.10	3.30 ± 1.62
SPFX	7.63 ± 6.79	2.88 ± 1.26	2.37 ± 1.23

<sup>a</sup> For each quinolone, 100 mg/kg was administered orally thrice weekly for 4 weeks.

<sup>b</sup> Values are means for five rats. All determinations were done 24 h after the last administration.

The primary target of fluoroquinolones is DNA-gyrase (25). PEF could possibly act on this enzyme system in *P. carinii*, but this would not explain the discrepancy among the quinolones tested. Kovacs et al. (17) demonstrated that agents clinically effective in PCP (TMP-SMX, pentamidine, and dapsone) inhibit de novo folate synthesis by *P. carinii* in vitro. Such a mechanism of action has not been described for fluoroquinolones. PEF does not appear to act on this metabolic pathway in *P. carinii*, since it is ineffective against *T. gondii*, a pathogen which possesses the same enzyme necessary for de novo folate synthesis (17).

As has been demonstrated for two sulfonyleurea compounds (15), the specific activity of PEF may be due to a particularity of its structure, given the inactivity of NOR, its major metabolite. On the basis of this study, clinical trials of PEF for the prophylaxis of PCP in patients with AIDS and in patients who present adverse reactions to traditional drugs appear to be justified.

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