

## Efficacy of Ciprofloxacin and Moxifloxacin against *Nocardia brasiliensis* In Vitro and in an Experimental Model of Actinomycetoma in BALB/c Mice<sup>∇</sup>

Brenda Edith Chacon-Moreno,<sup>1</sup> Oliverio Welsh,<sup>1</sup> Norma Cavazos-Rocha,<sup>2</sup> Maria de la Luz Salazar-Cavazos,<sup>2</sup> Hector Gerardo Garza-Lozano,<sup>3</sup> Salvador Said-Fernandez,<sup>3</sup> Jorge Ocampo-Candiani,<sup>1</sup> and Lucio Vera-Cabrera<sup>1\*</sup>

Servicio de Dermatología, Hospital Universitario José E. González, Monterrey, NL,<sup>1</sup> Departamento de Química Analítica, Facultad de Medicina, UANL, Monterrey, NL,<sup>2</sup> and Centro de Investigación Biomédica del Noreste, IMSS, Monterrey, NL,<sup>3</sup> México

Received 31 July 2008/Returned for modification 2 September 2008/Accepted 7 October 2008

**The efficacy of ciprofloxacin and moxifloxacin against *Nocardia brasiliensis* was evaluated by applying 25 mg of each drug/kg subcutaneously every 8 h in BALB/c mice infected with *N. brasiliensis*. A statistically significant difference was observed only with moxifloxacin. A moxifloxacin–trimethoprim-sulfamethoxazole combination was as active as when each compound was used alone.**

Mycetoma in Mexico is mainly produced by actinomycetes, with *Nocardia brasiliensis* being the most predominantly isolated agent in 86.6% of cases and *Actinomadura madurae* being the most predominantly isolated agent in 9.6% of cases. Therapy of this infectious disease is not easy. Drugs must be taken for several months, and in some cases resistance may appear (10). We present here the results of an experimental model of infection of *N. brasiliensis* in the BALB/c mouse using ciprofloxacin (CIP) and moxifloxacin (MXF) alone or in combination with trimethoprim-sulfamethoxazole (SXT).

(The work presented here was performed in part to fulfill the requirements for the Doctor of Medicine degree [Facultad de Medicina, Universidad Autónoma de Nuevo León] of B.E.C.-M.)

For in vitro susceptibility assays with CIP, we used a previously described broth microdilution method (9) that is based on Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) document A-4 to evaluate 30 isolates of *N. brasiliensis*.

In order to determine how many bacteria are naturally resistant to the quinolones, we calculated the mutation rate of *N. brasiliensis* HUJEG-1 with the *p0* method (5, 7) using Mueller-Hinton medium containing 1×, 4×, and 8× the MIC for each antimicrobial adjusted to contain 10<sup>9</sup> CFU per plate.

To quantitate the quinolone plasma levels in mice, we injected 8- to 12-week-old female BALB/c mice subcutaneously (s.c.) with CIP at 12.5 and 25 mg/kg and with MXF at 12.5 and 25 mg/kg as previously described (3). SXT was administered by gavage at 50 mg/kg, and blood samples were taken at 0, 20, 40, 60, 120, 240, 360, 480, and 600 min. SXT was also given to mice after the compound was suspended in the drinking water, and blood samples were obtained at 0, 3, 6, 9, and 12 h. The concentrations of MXF, CIP, and SXT were analyzed by using

a high-pressure liquid chromatography method developed in our laboratory.

Experimental mycetomas were produced in 8- to 12-week-old female BALB/c mice using *N. brasiliensis* HUJEG-1 (3, 4). One week later, therapy was started. Groups of 15 animals were tested. One group of animals was injected with saline solution as a negative control. The rest were treated with MXF at 25 mg/kg s.c. three times a day; with CIP at 25 mg/kg s.c. three times a day; with MXF at 25 mg/kg s.c. three times a day plus SXT at 50 mg/kg in the drinking water twice a day; or with SXT alone at 50 mg/kg in the drinking water twice a day. The degree of infection was determined after 9 weeks (three cycles of 3 weeks of treatment and 1 week of rest) as previously described (3). Two independent evaluators scored the development of lesions, and potential differences among the groups against a control inoculated with saline solution were established by using an analysis of variance test.

The MIC range for CIP was 4 to 64 μg/ml, and the MIC<sub>50</sub> and MIC<sub>90</sub> were 4 and 64 μg/ml, respectively. The MICs of MXF, CIP, and SXT for *N. brasiliensis* HUJEG-1 were 0.25, 4, and 2.3/0.12 μg/ml, respectively.

The frequency of the in vitro appearance of antimicrobial-resistant mutants is presented in Table 1. The mutation rate among quinolones was about the same (10<sup>-9</sup>) for all quinolones at high concentrations of the antimicrobials. The addi-

TABLE 1. Mutation rate of *N. brasiliensis* HUJEG-1 in response to several quinolones and SXT

Quinolone(s) <sup>a</sup>	Concn in agar		
	1× the MIC	4× the MIC	16× the MIC
CIP	5.3 × 10 <sup>-7</sup>	6 × 10 <sup>-9</sup>	1 × 10 <sup>-9</sup>
SPF	>1 × 10 <sup>-9</sup>	>1 × 10 <sup>-9</sup>	8 × 10 <sup>-8</sup>
GAT	7.3 × 10 <sup>-7</sup>	<1 × 10 <sup>-9</sup>	<1 × 10 <sup>-9</sup>
MXF	4.8 × 10 <sup>-8</sup>	<1 × 10 <sup>-9</sup>	<1 × 10 <sup>-9</sup>
SXT	>1 × 10 <sup>-9</sup>	6.9 × 10 <sup>-8</sup>	1 × 10 <sup>-9</sup>
MXF + SXT	3.4 × 10 <sup>-8</sup>	<1 × 10 <sup>-9</sup>	<1 × 10 <sup>-9</sup>
GAT + SXT	2 × 10 <sup>-9</sup>	<1 × 10 <sup>-9</sup>	<1 × 10 <sup>-9</sup>

<sup>a</sup> SPF, sparfloxacin; GAT, gatifloxacin.

\* Corresponding author. Mailing address: Servicio de Dermatología, Hospital Universitario José E. González, Madero y Gonzalitos, Col. Mitras Centro, Monterrey, NL, CP 64460, Mexico. Phone: (5281) 83480383. Fax: (5281) 83484407. E-mail: luvera\_99@yahoo.com.

<sup>∇</sup> Published ahead of print on 13 October 2008.

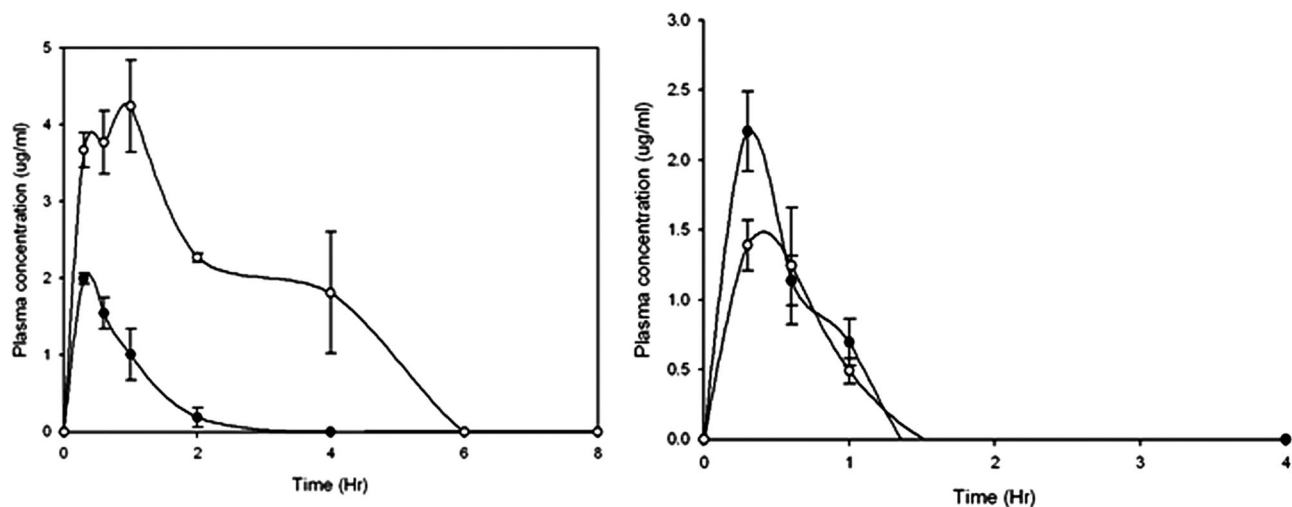


FIG. 1. Plasma levels produced in BALB/c mice after s.c. injection of MXF (left) at 50 and 12.5 mg/kg and of CIP (right) at the same doses. Three mice were bled and sacrificed at each point. Bars represent the standard deviation.

tion of SXT did not modify this value significantly. Some colonies growing in the plates with a high concentration of quinolones (8 $\times$ ) were subcultured, and the MICs were retested. In the case of CIP, a 16-fold increase in the MIC was observed (from 4 to 64  $\mu$ g/ml); the MIC of MXF increased 64-fold (from 0.25 to 16  $\mu$ g/ml).

MXF plasma levels were determined at 25 and 12.5 mg/kg. At 25 mg/kg, the levels were maintained over the MXF MIC of *N. brasiliensis* HUJEG-1 (0.25  $\mu$ g/ml) for about 5 h (Fig. 1), with a  $C_{max}$  of 4.3  $\mu$ g/ml. At a dose of 12.5 mg/kg the concentrations were very poor. CIP administered s.c. did not reach plasma levels greater than the MIC for *N. brasiliensis* HUJEG-1 (4  $\mu$ g/ml), not even at the highest dose tested (25 mg/kg) (Fig. 1, right). The  $C_{max}$  at 25 mg/kg was about 2.25  $\mu$ g/ml. In contrast, the plasma levels of SXT administered by gavage at 50 mg/kg were sustained over the SXT MIC for *N. brasiliensis* HUJEG-1 (2.3/0.12  $\mu$ g/ml) for about 8 h (Fig. 2). The animals taking the drug in drinking water could sustain a plasma concentration greater than the MIC for about 8 h, even

though the values were lower than those observed with the gavage method.

When CIP or MXF was applied s.c. (Fig. 3), only MXF had a statistically significant effect compared to the control ( $P = 0.017$ ). No statistical difference was found in the CIP-treated group ( $P = 1.0$ ). As shown in Fig. 3, SXT also exerted a significant effect on the development of experimental mycetoma lesions in the mouse model ( $P = 0.006$ ). When SXT with MXF were combined (25 mg/kg s.c., three times a day), the effect was similar to that observed when SXT was applied alone ( $P = 0.007$ ).

Quinolones have been widely used to treat infections by aerobic actinomycetes. CIP has been observed to be active against other species of *Nocardia* and mycobacteria other than tuberculosis (1, 2, 8, 12). In contrast, we observed here higher CIP MICs for *N. brasiliensis* than we observed with MXF and gatifloxacin. This poor in vitro susceptibility level, together with low levels of the drugs reached in mouse plasma, had no effect on the development of experimental mycetoma by *N. brasiliensis*.

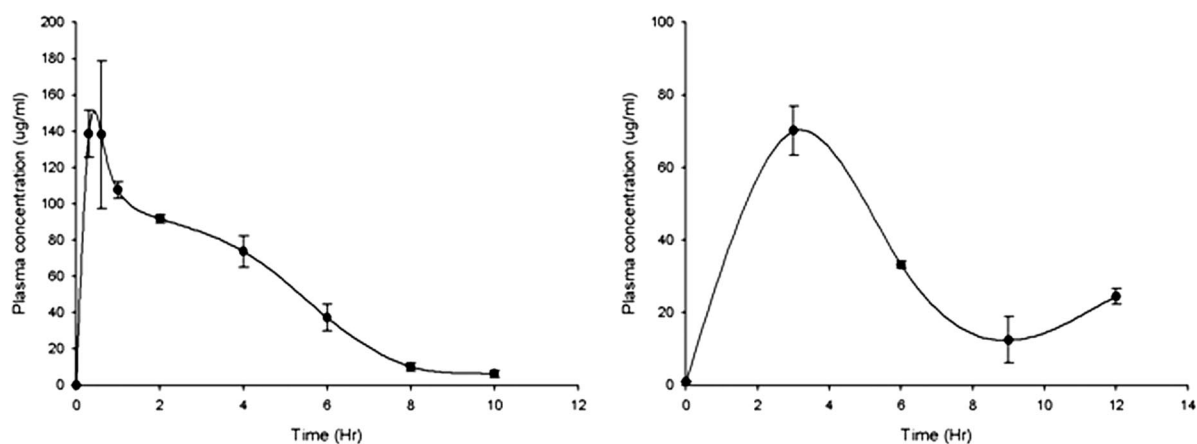


FIG. 2. Sulfamethoxazole plasma levels produced in BALB/c mice after the application of SXT by gavage (left) and in drinking water (right) in both cases at 50 mg/kg. Three mice were bled and sacrificed at each point. Bars represent the standard deviation.

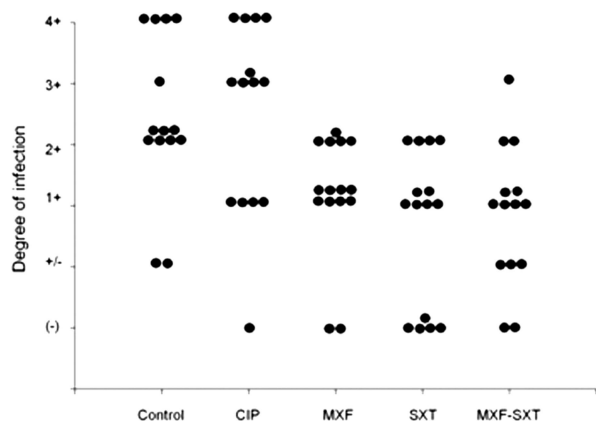


FIG. 3. Effect of CIP, MXF, SXT, and the combination MXF-SXT in the development of mycetoma lesions in BALB/c mice infected with *N. brasiliensis* HUJEG-1. According to the analysis of variance test, significant differences were found for treatment with MXF, SXT, and MXF-SXT, with *P* values of 0.017, 0.006, and 0.007, respectively.

Gatifloxacin has been shown to be active *in vivo* against *N. brasiliensis* (3). However, since there have been reports of dislycemia with this fluoroquinolone (11), it will be important to properly select young patients without diabetes before using it is used to treat actinomycetoma cases.

MXF has excellent plasma levels in humans, with a maximum plasma concentration of 6.13 at a dose of 400 mg daily and plasma levels greater than 1  $\mu\text{g}/\text{ml}$  for about 8 h (6), and it has low toxicity in long-term applications. According to the results obtained here we feel MXF may be useful in the treatment of actinomycetoma using 400 mg every 12 h to keep the

plasma levels greater than the MIC and to avoid the selection of naturally resistant bacteria.

This research was supported by CONACYT grant 52219.

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