

## Accumulation of $^{99m}\text{Tc}$ -Ciprofloxacin in *Staphylococcus aureus* and *Pseudomonas aeruginosa*<sup>∇</sup>

J. M. Sierra,<sup>1\*</sup> D. Rodriguez-Puig,<sup>2</sup> A. Soriano,<sup>1</sup> J. Mensa,<sup>1</sup> C. Piera,<sup>2</sup> and J. Vila<sup>3</sup>

Department of Infectious Diseases,<sup>1</sup> CDB, Department of Nuclear Medicine,<sup>2</sup> and Department of Microbiology,<sup>3</sup> Hospital Clinic, IDIBAPS, School of Medicine, University of Barcelona, Barcelona, Spain

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**The use of radiopharmaceuticals for the diagnosis of infection is increasing due to their ability to distinguish between septic and aseptic inflammation. The aim of this study was to analyze the intracellular accumulation of technetium-99m-ciprofloxacin in strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* harboring an overexpression of NorA and MexAB-OprM, respectively.**

The use of radiolabeled antibiotics is an emergent technique for the diagnosis of infection due to the agents' abilities to differentiate between septic and aseptic inflammation (3, 11). The usefulness of technetium-99m ( $^{99m}\text{Tc}$ )-ciprofloxacin to diagnose infections with orthopedic devices or to identify the source of an infectious fever of unknown origin has been described previously. However, the influence that the susceptibility of the bacterium to the radiolabeled antibiotic has on the efficacy of this tracer for the diagnosis of infection is not well established (8).

Ciprofloxacin is a broad-spectrum fluoroquinolone that inhibits DNA gyrase and/or topoisomerase IV of bacteria (7). Resistance to ciprofloxacin is mediated by the alteration of these targets and/or the overexpression of efflux pumps (1, 7, 9, 10). The second mechanism of resistance decreases intracellular concentration of ciprofloxacin. Therefore, the expression of efflux pumps could reduce the ability of  $^{99m}\text{Tc}$ -ciprofloxacin to detect the presence of resistant microorganisms. The aim of this study was to analyze the intracellular accumulation of  $^{99m}\text{Tc}$ -ciprofloxacin in *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains with and without the presence of an efflux pump as the mechanism of resistance.

The accumulation of free ciprofloxacin,  $^{99m}\text{Tc}$ -ciprofloxacin, and  $^{99m}\text{TcO}_4^-$  (pertechnetate) was evaluated with two strains of *S. aureus*, 1199B and 1199 (with and without the overexpression of the NorA efflux system) (2), and two strains of *P. aeruginosa*, PAOLC1-6 and KG2239 (with and without the overexpression of the MexAB-OprM efflux system) (1). Evaluation of the accumulation of  $^{99m}\text{TcO}_4^-$  was performed to ensure that all of the radioactivity signal was due to the  $^{99m}\text{Tc}$ -ciprofloxacin. Also, the accumulation of nonlabeled ciprofloxacin was done to ensure that *S. aureus* and *P. aeruginosa* strains were able to pump out this antibiotic.

The labeling of ciprofloxacin with  $^{99m}\text{Tc}$  was performed by following a previously described method (6). Briefly, a solution of tin(II) chloride dihydrate (0.1 mg in 0.1 ml of hydrochloric

acid, 0.01 N) was added to a solution of ciprofloxacin hydrochloride (2 mg) in 1 ml of saline. Next, 0.1 ml of an aqueous solution of L-tartaric acid was added to the resulting solution, which was vigorously stirred. Finally, freshly eluted pertechnetate (0.2 to 1.1 GBq) was added, and the resulting solution was vigorously stirred and kept at room temperature for 15 min.

Accumulation was performed as described previously and measured at two different end points, 5 and 30 min (4, 5, 9). Briefly, strains were cultured in Luria-Bertani broth overnight at 37°C. Cells were washed and resuspended in phosphate-buffered saline to an optical density at 600 nm of 1.5. After that, 490  $\mu\text{l}$  was taken, and 10  $\mu\text{l}$  of ciprofloxacin was added to obtain an extracellular concentration of 10  $\mu\text{g/ml}$  ( $^{99m}\text{Tc}$ -ciprofloxacin presented an activity of about 555 kBq/sample, and free  $^{99m}\text{TcO}_4^-$  presented an activity of about 555 kBq/sample), and samples were incubated at 37°C for 5 or 30 min. Finally, samples were washed three times with phosphate-buffered saline. The accumulation of ciprofloxacin (unlabeled) was measured by fluorimetry, the wavelength for excitation/emission used was 278/447 nm, respectively (previously, samples were lysed with glycine-HCl buffer), and accumulations of  $^{99m}\text{Tc}$ -ciprofloxacin and  $^{99m}\text{TcO}_4^-$  were measured by a gamma counter. The percentage of each accumulation of  $^{99m}\text{Tc}$ -ciprofloxacin and  $^{99m}\text{TcO}_4^-$  was calculated as the ratio of the radioactivity in the pellet (cells) and that of the total radioactivity used per sample. All the samples were processed in duplicate, and three independent experiments were performed.

Results of the accumulation of free ciprofloxacin,  $^{99m}\text{Tc}$ -ciprofloxacin, and  $^{99m}\text{TcO}_4^-$  are summarized in Table 1. The accumulation of unlabeled ciprofloxacin in the *S. aureus* and *P. aeruginosa* strains showing overexpression of an efflux pump was lower than that for strains without an efflux pump overexpressed.  $^{99m}\text{Tc}$ -ciprofloxacin accumulated equally intracellularly in all the strains tested, disregarding the presence or absence of an efflux system, while  $^{99m}\text{TcO}_4^-$  did not show accumulation in any of the strains. The absence of intracellular  $^{99m}\text{TcO}_4^-$  demonstrated that the entire radioactivity detected in the  $^{99m}\text{Tc}$ -ciprofloxacin assay was due to the accumulation of the radiopharmaceutical compound and not due to free  $^{99m}\text{TcO}_4^-$ . The reason why  $^{99m}\text{Tc}$ -ciprofloxacin was not pumped out remains unknown, but two different explanations are possible: first, this complex may not be recognized by the

\* Corresponding author. Mailing address: Dept. of Infectious Diseases, Hospital Clinic of Barcelona, Villarroel 170, Esc 11-5 planta, 08036 Barcelona, Spain. Phone: 34 93 2275708. Fax: 34 93 4514438. E-mail: jmsierra@clinic.ub.es.

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TABLE 1. Accumulation of free ciprofloxacin,  $^{99m}\text{Tc}$ -ciprofloxacin, and  $^{99m}\text{TcO}_4^-$  in *S. aureus* and *P. aeruginosa*

Strain	Accumulated unlabeled ciprofloxacin at 30 min (ng ciprofloxacin/mg [dry wt] $\pm$ SD)	Accumulated radioactivity (% $\pm$ SD) <sup>a</sup>			
		Tc $^{99m}$ -ciprofloxacin at 5 min	Tc $^{99m}$ -ciprofloxacin at 30 min	$^{99m}\text{TcO}_4^-$ at 5 min	$^{99m}\text{TcO}_4^-$ at 30 min
<i>S. aureus</i> wild type 1199	47.72 $\pm$ 5.37	6.5 $\pm$ 1.5	22 $\pm$ 3.2	0.02 $\pm$ 0.01	0.02 $\pm$ 0.005
<i>S. aureus</i> efflux 1199B	27.24 $\pm$ 2.89	7.1 $\pm$ 2.1	23 $\pm$ 2.9	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01
<i>P. aeruginosa</i> wild type KG2239	68.74 $\pm$ 4.93	8.7 $\pm$ 2.3	16.3 $\pm$ 1.7	0.01 $\pm$ 0.005	0.01 $\pm$ 0.01
<i>P. aeruginosa</i> efflux PaoLC1-6	21.65 $\pm$ 6.04	9.7 $\pm$ 1.5	18.7 $\pm$ 2.0	0.02 $\pm$ 0.01	0.01 $\pm$ 0.005

<sup>a</sup> Percentages of radioactivity were derived by comparing radioactivity inside the bacterial cells with the total radioactivity used in the assay. All the results are the means  $\pm$  standard deviations (SD) of at least three independent experiments.

efflux systems, or second, the  $^{99m}\text{Tc}$ -ciprofloxacin complex inside the cell could be disassociated, and free pertechnetate did not cross the membrane, as the result of the accumulation of free pertechnetate showed.

Finally, our results suggest that (i) radiolabeled ciprofloxacin accumulates in the bacteria and (ii) this compound is not recognized by the efflux pumps, resulting in a high intracellular concentration even in the case of overexpression of efflux pumps. In the future, it will be necessary to confirm these results in a clinical setting.

#### REFERENCES

1. Conejo, M. C., L. Martinez-Martinez, I. Garcia, L. Picabea, and A. Pascual. 2003. Effect of siliconized latex urinary catheters on the activity of carbapenems against *P. aeruginosa* strains with defined mutations in *ampC*, *oprD*, and genes coding for efflux systems. *Int. J. Antimicrob. Agents* **22**:122–127.
2. Kaatz, G. W., and S. M. Seo. 1997. Mechanisms of fluoroquinolone resistance in genetically related strains of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **41**:2733–2737.
3. Malamitsi, J., H. Giamarellou, K. Kanellakopoulou, E. Dounis, V. Grecka, J. Christakopoulos, G. Koratzanis, A. Antoniadou, G. Panoutsopoulos, C. Batsakis, and C. Proukakis. 2003. Infection: a  $^{99m}\text{Tc}$ -ciprofloxacin radiopharmaceutical for the detection of bone infection. *Clin. Microbiol. Infect.* **9**:101–109.
4. Martinez-Martinez, L., I. Garcia, M. C. Conejo, P. Joyanes, and A. Pascual. 2000. Accumulation of b-lactams and quinolones into bacteria cells, p. 63–72. *In* V. J. Benedi, R. Canton, M. A. Dominguez, L. Martinez, and J. Vila (ed.), *Antimicrobial resistance: a laboratory approach*. Biomerieux, Madrid, Spain.
5. Piddock, L. V. J. 1997. Mechanism of quinolone uptake into bacterial cells. *J. Antimicrob. Chemother.* **27**:399–403.
6. Rodriguez-Puig, D., C. Piera, D. Fuster, A. Soriano, J. M. Sierra, S. Rubi, and J. Suades. 2006. A new method of [ $^{99m}\text{Tc}$ ]-ciprofloxacin preparation and quality control. *J. Labelled Comp. Radiopharm.* **49**:1171–1176.
7. Ruiz, J. 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J. Antimicrob. Chemother.* **51**:1109–1117.
8. Sarda, L., A. C. Cremieux, Y. Lebellec, A. Meulemans, R. Lebtahi, G. Hayem, R. Genin, N. Delahaye, D. Hutten, and D. Le Guludec. 2003. Inability of  $^{99m}\text{Tc}$ -ciprofloxacin scintigraphy to discriminate between septic and sterile osteoarticular diseases. *J. Nucl. Med.* **44**:920–926.
9. Sierra, J. M., J. G. Cabeza, M. Ruiz Chaler, T. Montero, J. Hernandez, J. Mensa, M. Llagostera, and J. Vila. 2005. The selection of resistance to and the mutagenicity of different fluoroquinolones in *Staphylococcus aureus* and *Streptococcus pneumoniae*. *Clin. Microbiol. Infect.* **8**:750–758.
10. Sierra, J. M., F. Marco, J. Ruiz, M. T. Jimenez de Anta, and J. Vila. 2002. Correlation between the activity of different fluoroquinolones and the presence of mechanisms of quinolone resistance in epidemiologically related and unrelated strains of methicillin-susceptible and resistant *Staphylococcus aureus*. *Clin. Microbiol. Infect.* **8**:781–790.
11. Welling, M. M., A. Paulusma-Annema, H. S. Balter, E. K. Pauwels, and P. H. Nibbering. 2000. Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. *Eur. J. Nucl. Med.* **27**:292–301.