

# Uptake of ciprofloxacin by macrophages

CSF EASMON, JP CRANE

From the Department of Medical Microbiology, Wright-Fleming Institute, St Mary's Hospital Medical School, London W2

**SUMMARY** Ciprofloxacin was concentrated within mouse peritoneal macrophages to between two and three times extracellular values. Uptake was rapid, occurred equally well with dead cells, and was not affected by lowering the pH or by prior ingestion of *Staphylococcus aureus*. Intracellular staphylococci were killed by extracellular concentrations of ciprofloxacin as low as 0.5 mg/l.

Ciprofloxacin (1-cyclopropyl-6-fluoro-1, 4 dihydro-4-oxo-7)-(1-piperazinyl)-3-quinolone carboxylic acid hydrochloride) is a new quinolone derivative with potential for use in systemic infection. It is active against a number of intracellular pathogens (mycobacteria, legionella,<sup>1</sup> chlamydia) and we have shown that it is taken up by neutrophils.<sup>2</sup> The other main phagocytic cells are macrophages and we present data to show that they too take up ciprofloxacin.

## Material and methods

### BACTERIA AND SENSITIVITY TESTING

*Staphylococcus aureus* NCTC 6571 was used and minimum inhibitory and bactericidal concentrations were measured by the broth dilution method using medium 199 (Gibco) buffered with 25 mM HEPES (Gibco). The inoculum was 10<sup>5</sup> colony forming units (cfu)/ml.

### MACROPHAGES

Mice were injected intraperitoneally with 1 ml of thioglycollate broth or 5% sodium caseinate. Four to five days later they were killed and the peritoneal cavity washed with medium 199 supplemented with 0.1% gelatin and 1% fetal calf serum to remove the cells. The cells were washed and macrophages purified on a Ficoll-metrizoate gradient (Lymphoprep, Nyegaard). The intracellular volume of the macrophages was measured using tritiated water and inulin as previously described.<sup>3</sup> For experiments with dead cells macrophages were killed by exposure to 10% formalin for 30 min followed by extensive washing.

### BACTERICIDAL ACTIVITY OF MACROPHAGE SONICATES

Macrophages were incubated with ciprofloxacin and then rapidly centrifuged through silicon fluid (a 4:7 mixture of silicon fluid, specific gravity 0.94 g/ml and 1.07 g/ml (Dow Corning)). The cell pellet was lysed by sonication and the ciprofloxacin content was measured by a plate diffusion assay. The test organism was *Klebsiella edwardsii* NCTC 10896.

### EFFECT OF CIPROFLOXACIN AND RIFAMPICIN ON INTRACELLULAR SURVIVAL OF STAPH AUREUS

*Staph aureus* was opsonised with 10% pooled normal human serum for 15 min at 37°C, washed, and resuspended at a concentration of about 5 × 10<sup>6</sup> cfu/ml. Staphylococci and macrophages were mixed for 30 min to allow ingestion of the former. Extracellular and cell adherent staphylococci were removed by treatment with 5 mg/l lysostaphin (Becton Dickinson). Macrophages can take up lysostaphin, depending on concentration and exposure time, and so these were kept to a minimum. A viable count of intracellular staphylococci was made at the start of the experiment by adding a 0.1 ml aliquot of the cell suspension to 0.9 ml of distilled water, sonicating, and making a series of 10-fold dilutions. Macrophages with intracellular *Staph aureus* were incubated in the presence of varying extracellular concentrations of ciprofloxacin (Bayer) and rifampicin (Lepetit). At 2 h and 20 h repeat intracellular viable counts were performed and the percentage of surviving intracellular *Staph aureus* calculated. In each case results shown are taken from typical experiments repeated at least four times.

## Results

The minimum inhibitory concentration of ciprofloxacin for *Staph aureus* NCTC 6571 was 0.3

Table 1 Uptake of ciprofloxacin by mouse peritoneal macrophages

Time (min)	Intracellular: extracellular ciprofloxacin ratio		
	Live cells	Live cells + <i>Staph aureus</i>	Dead cells
0.5	0.18	0.22	0.22
5.0	2.3	1.9	2.3
30.0	2.7	2.7	3.0

Table 2 Killing of intracellular *Staph aureus* in the presence of ciprofloxacin and rifampicin

Antibiotic concentration (mg/l)	Viable count ( $\times 10^6$ )	<i>Staph aureus</i> survival	
		2 h	20 h
Control	4.0	11.0	overgrowth
Ciprofloxacin	2.0	1.7	0.05
Ciprofloxacin	0.5	3.0	0.1
Ciprofloxacin	0.1	13.0	11.0
Rifampicin	0.1	0.55	<0.01

mg/l and the bactericidal concentration 1.25 mg/l. Table 1 shows the uptake of ciprofloxacin by macrophages. This was rapid, and neither the killing of the cells nor allowing them to ingest *Staph aureus* before exposure to ciprofloxacin affected uptake. A reduction of extracellular pH was also without effect.

Table 2 shows the effect of extracellular ciprofloxacin on intracellular staphylococcal survival. Rifampicin, an antibiotic known to kill intracellular staphylococci,<sup>4</sup> was included as a positive control. Ciprofloxacin at 2.0 or 0.5 mg/l produced a considerable reduction in intracellular staphylococcal survival at 2 or 4 h, whereas the lower concentration of 0.1 mg/l did not. At 20 h there was always overgrowth of *Staph aureus* in the control tubes. At that time the viable count of *Staph aureus* was further reduced with 2.0 and 0.5 mg/l ciprofloxacin but stayed the same with 0.1 mg/l.

## Discussion

Biologically active ciprofloxacin is taken up rapidly by peritoneal macrophages. The results with dead cells suggest that this is a passive rather than an active process. The membrane stimulation produced by prior ingestion of *Staph aureus* did not enhance ciprofloxacin uptake in the way recently described for the active transport of clindamycin.<sup>5</sup> These findings are similar to those for human neutrophils, although the level of ciprofloxacin uptake for macrophages was less than half that of the neutrophils.<sup>2</sup> We cannot be certain how macrophages from other sites or species will behave, and the peritoneal macrophages used here were stimulated by the injection of caseinate or thioglycollate. Work on human alveolar macrophages is planned.

Although *Staph aureus* is not primarily an

intracellular pathogen, it can act in this way where there are defects of phagocytic killing—for example, chronic granulomatous disease. This is a special area in which ciprofloxacin should be tried clinically. Its broad spectrum of activity, covering Gram negative bacilli that sometimes cause problems in chronic granulomatous disease, could be useful.<sup>6</sup> Typhoid is another clinical area. Salmonellae are far more sensitive to ciprofloxacin than *Staph aureus* and are, of course, intracellular pathogens. We have had some success in treating systemic murine salmonella infections with ciprofloxacin. Legionnaires' disease is a third condition where survival within macrophages appears to be important, and *Legionella pneumophila* too is sensitive to ciprofloxacin.<sup>1</sup> In fact, the relative lack of sensitivity of *Staph aureus* to ciprofloxacin makes it a good test organism for this type of in vitro experiment.

Mycobacteria are perhaps the most interesting prospect for ciprofloxacin. These bacteria above all are intracellular pathogens, surviving within macrophages. The advent of rifampicin, a drug that kills intracellular bacteria, revolutionised antituberculous chemotherapy. If the activity of ciprofloxacin against mycobacteria is confirmed, this, coupled with its uptake by phagocytic cells, could make it an effective antituberculous agent.

Professional phagocytes clearly take up appreciable amounts of ciprofloxacin. We plan to examine non-professional phagocytes such as epithelial cells. This is the key cell involved with another common intracellular pathogen *Chlamydia trachomatis*.

We thank Bayer UK for financial support.

## References

- Greenwood D, Laverick A. Activities of newer quinolones against legionella group organisms. *Lancet* 1983;ii:279-80.

- <sup>2</sup> Easmon CSF, Crane PJ. Uptake of ciprofloxacin by human neutrophils. *J Antimicrob Chemother* (in press).
- <sup>3</sup> Easmon CSF, Crane PJ. Cellular uptake of clindamycin and lincomycin. *Br J Exp Pathol* 1984; **65**:725-30.
- <sup>4</sup> Mandell GL, Vest TK. Killing of intraleucocytic *Staphylococcus aureus* by rifampicin *in vitro* and *in vivo* studies. *J Infect Dis* 1972; **125**:486-90.
- <sup>5</sup> Steinberg TH, Hand WL. Effects of phagocytosis on antibiotic and neoside uptake by human polymorphonuclear leucocytes. *J Infect Dis* 1984; **149**:397-403.

- <sup>6</sup> Wise R, Andrews JM, Edwards LJ. *In vitro* activity of BAY 09867 a new quinolone derivative compared with those of other antimicrobial agents. *Antimicrob Agents Chemother* 1983; **23**:559-64.

Requests for reprints to: Professor CSF Easmon, Department of Medical Microbiology, Wright-Fleming Institute, St Mary's Hospital Medical School, London W2 1PG, England.

## The March 1985 issue

THE MARCH 1985 ISSUE CONTAINS THE FOLLOWING PAPERS

The pathobiology of the osteoclast TJ CHAMBERS  
Glomerular thrombi in renal allografts associated with cyclosporin treatment GH NEILD, ROWENA REUBEN, RB HARTLEY, JS CAMERSON

Incidence of intraglomerular platelets in steroid sensitive nephrotic syndrome JMC UTTLEY, RA ELTON, CM HILL, MK MACDONALD

Functional and structural changes of the human proximal small intestine after cytotoxic therapy D CUNNINGHAM, RJ MORGAN, PR MILLS, LM NELSON, PG TONER, M SOUKOP, CS McARDLE, RI RUSSELL

The Cronkhite-Canada syndrome: an ultrastructural study of pathogenesis D JENKINS, PM STEPHENSON, BB SCOTT

Mucin producing microfollicular adenoma of the thyroid C RIGAUD, F PELTIER, WV BOGOMOLETZ

Morphometric studies of age related changes in normal human breast and their significance of evolution of mammary cancer SW HUTSON, PN COWEN, CC BIRD

Subcutaneous phaeohyphomycosis: a histopathological study of nine cases from Malawi PJ O'DONNELL, MSR HUTT

Gastro-oesophageal candidiasis: diagnosis by brush cytology JENNIFER A YOUNG, E ELIAS

Refractory anaemia terminating in a combined lymphoproliferative and myeloproliferative disorder L CAMBA, MV JOYNER

Granulocyte and monocyte surface membrane markers in the myelodysplastic syndromes RE CLARK, TG HOY, A JACOBS

Evaluation of the common conditions associated with eosinophilia CG TEO, M SINGH, WC TING, LC HO, YW ONG, LC SEET

Postsplenectomy sepsis 10 years or more after operation DIK EVANS

Acute phase response to serum amyloid A protein and C reactive protein to the common cold and influenza JT WHICHER, RE CHAMBERS, J HIGGINSON, L NASHEF, PG HIGGINS

Enhanced luminescence enzyme immunoassay for factor VIII related antigen HX WANG, J GEORGE, CHG THORPE, RA STOTT, LJ KRICKA, TP WHITBREAD

Blood group antigens A, B, H, Le<sup>a</sup>, Le<sup>b</sup>, and I(Ma) in resting and tetragastrin stimulated gastric juice of patients with non-neoplastic diseases of the stomach JEAN K PICARD, KYOKO HOTTA, TEN FEIZI

Familial abnormalities of thyroxine binding proteins: some problems of recognition and interpretation JULIA E NEILD, PGH BYFIELD, MRA LALLOZ, DIANA TAIT, JH MARIGOLD, DN CROFT, BRENDA M SLAVIN

Simplified restriction endonuclease method for typing and subtyping adenoviruses JM DARVILLE

Effects of media, working practice, and automation on the rapid detection of bacteraemia JE CORKILL

Assessment of rapid methods of pneumococcal antigen detection in routine sputum bacteriology M WHITBY, KG KRISTINSSON, M BROWN

Comparison of enzyme linked immunosorbent assay and enzyme linked fluorescence immunoassay for detection of antibodies against *Chlamydia trachomatis* K NUMAZAKI, S CHIBA, T MOROBOSHI, T KUDOH, T YAMANAKA, T NAKAO

Incidence of anti-intermediate filament antibody in serum samples of students with suspected glandular fever PK KATAAHA, EJ HOLBOROW, JMB EDWARDS

### Letters to the Editor

### Book reviews

### Notice