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Uptake of ciprofloxacin by macrophages

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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-fluro-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,[1] chlamydia) and we have shown that it is taken up by neutrophils.[2] 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 10^5 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.[3] 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 5×10^6 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

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Table 1 *Uptake of ciprofloxacin by mouse peritoneal macrophages*

Time (min)	*Intracellular: extracellular ciprofloxacin ratio*		
	Live cells	*Live cells + Staph aureus*	*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 (× 10⁶)*	*Staph aureus survival*	
			2 h	*20 h*
Control		4·0	11·0	overgrowth
Ciprofloxacin	2·0	3·7	1·7	0·05
Ciprofloxacin	0·5	5·0	3·0	0·1
Ciprofloxacin	0·1	3·8	13·0	11·0
Rifampicin	0·1	3·6	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,[4] 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.[5] 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.[2] 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.[6] 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.[1] 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*.

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