In vitro effects of cefotaxime and ceftriaxone on *Salmonella typhi* within human monocyte-derived macrophages

B. Ekinci, A. Y. Coban, A. Birinci, B. Durupinar and M. Erturk*

Ondokuz Mayis University, Medical School, Dept. Microbiology and Clin. Microbiology, Samsun, Turkey

*Tel: +90 362 4576000/2539 Fax: +90 362 4576041 E-mail: microbora@hotmail.com

The main objective of this in vitro study was to assess the effects of cefotaxime and ceftriaxone in killing *Salmonella typhi* in infected human macrophages. Human monocyte-derived macrophages isolated from peripheral blood of human volunteers were cultured in vitro for macrophage differentiation, and subsequently infected with *S. typhi* strains (a clinical isolate and a standard strain TA-42) at a cell ratio of 10 : 1. MICs of cefotaxime and ceftriaxone were determined by broth microdilution, and the antibiotics were included in the culture medium at one and five times their MIC values. Samples of cell culture medium taken at 0, 3, 6 and 24 h of incubation were cultured for growth of *S. typhi* on nutrient agar. Gentamicin (10 mg/L) was included in each well except for the control wells, in order to prevent growth of extracellular *S. typhi*. Both antibiotics showed good in vitro antibacterial effects against *S. typhi* strains. There were no statistically significant differences between the extracellular and intracellular effects of antibiotics with regard to elimination of the bacteria. Cefotaxime and ceftriaxone are highly effective against extracellular bacterial growth. The results of our in vitro experiments suggest that cefotaxime and ceftriaxone might also be used clinically against susceptible intracellular pathogens such as *S. typhi*.

Keywords *Salmonella typhi*, macrophage, cefotaxime, ceftriaxone

Accepted 15 January 2002

Clin Microbiol Infect 2002; 8: 810–813

INTRODUCTION

Typhoid fever is still a major public health problem associated with significant morbidity and mortality in many countries [1]. Clinically, the cardinal features of the infection arise as a result of gastrointestinal disturbance, which follows infiltration of the microorganism into the intestinal mucosa. *Salmonella* species penetrate the intestinal mucus layer through bacteria-induced endocytosis. Thereafter, a similar process also takes place in the infection of mucosa-associated tissue cells, in particular the macrophages, a step which is clearly important in the pathogenesis of typhoid fever [2]. Salmonellae survive and may also replicate within the phagolysosome [3,4].

In the treatment of typhoid fever, the choice of an antimicrobial agent requires consideration of the current understanding of the behavior of intracellular salmonellae. Thus, both effective penetration into cells and resistance to environmental conditions are important prerequisites for the antibiotics to be effective. It has been shown that the treatment of typhoid fever and, most importantly, the chronic carriers of *Salmonella typhi* with certain antibiotics is ineffective despite good in vitro activity, since salmonellae within phagolysosomes create an acidic and viscous environment which causes antimicrobials to be less effective and even inactive [1,5].

Here, we studied the efficacy of cefotaxime and ceftriaxone in killing *S. typhi* in human monocyte-derived macrophages infected in vitro with a standard and a clinical *Salmonella* isolate.

MATERIALS AND METHODS

Bacteria

A standard strain of *S. typhi*—42 TA(2-1)—was obtained from Refik Saydam Public Health

© 2002 Copyright by the European Society of Clinical Microbiology and Infectious Diseases

Institute, Ankara, and a clinical strain isolated from a fecal specimen was obtained from the Bacteriology Section of the Central Laboratory at the Ondokuz Mayis University Hospital, Samsun. Strains were stored in brain–heart infusion (BHI) (Difco, Detroit, MI, USA) broth containing 20% glycerol at −70 °C, until used.

Human monocyte-derived macrophages

Human monocyte-derived macrophages were obtained by the Ficoll–Hypaque density gradient method and cultured as described elsewhere [6,7]. Monocytes were obtained from the blood of healthy human volunteers, serologically negative for hepatitis, syphilis, HIV and typhoid fever, and without a history of vaccination against typhoid fever [1]. Isolated monocytes were seeded into T25 tissue-culture flasks and incubated with RPMI 1640 (Sigma, St Louis, MO, USA) with 10% fetal calf serum (FCS) (Sigma) to form monolayers at 37 °C in a 5% CO_2 humidified incubator for 7–12 days. The medium was replaced every three days.

Determination of minimal inhibitory concentration (MIC)

The antibiotics used in this study were cefotaxime and ceftriaxone, obtained from Eczacibasi, Istanbul, and gentamicin, from Bilim Co., Istanbul. They were dissolved in solvents as recommended by the manufacturers. MIC testing was performed by the macrodilution method in Mueller–Hinton broth (MHB) (Difco) supplied with Ca^{2+} and Mg^{2+} cations [8,9].

Infection of macrophages

Macrophage monolayers were removed with a cell scraper from the surface of culture flasks by treating with ice-cold 0.02% EDTA/PBS for 10 min. Cell viability was >90% as determined by the trypan blue exclusion test [6].

Macrophages were seeded in 96-well plates at 10^5 cells/mL one night before infection with *S. typhi* strains. They were incubated for one night at 37 °C in a humidified 5% CO_2 incubator. After replacement of the medium, the macrophages in the 96-well plates were infected with 10^6 CFU/mL of the *S. typhi* strains (opsonized with 10% normal human serum for 30 min at 37 °C) at a ratio of 10 bacteria/1 macrophage. Plates were rotated at 200 rev/min for 5 min at room temperature, prior to incubation for 30 min at 37 °C for phagocytosis to occur. At the end of phagocytosis, the cells were washed with PBS to remove non-phagocytosed bacteria [1,7,10–12]. The cells were then incubated with fresh maintenance medium (RPMI with 5% FCS) containing gentamicin (10 mg/L) to prevent extracellular bacterial growth [1,11]. Antibiotics were added at one and five times the MIC to each well except for control wells. Macrophages were not included in the wells when the extracellular activities of the antibiotics were tested.

Samples

Wells were sampled at 0, 3, 6 and 24 h after infection. After washing wells twice with PBS to remove all the gentamicin, the macrophages were lyzed with 0.5% sodium deoxycholate, and the samples were pooled [4,7,10]. Of these lysates, 100 μL was plated on an agar base to determine the number of CFUs [13].

Statistics

The results were tested for significance using Student's *t*-test and the chi-square test.

RESULTS

The MIC values of cefotaxime and ceftriaxone are summarized in Table 1; the MICs of ceftriaxone against the standard strain and the clinical isolate differed by one dilution.

The effects of cefotaxime and ceftriaxone at MIC and 5 × MIC on *S. typhi*, both inside and outside macrophages, are summarized in Figures 1–4. In a control experiment, gentamicin did not exhibit any significant inhibitory effect on intracellular *Salmonella typhi* (data not shown). On the other hand, both cephalosporins at MIC and 5 × MIC showed similar effects in the macrophage monolayers

Table 1 MIC values of cefotaxime and ceftriaxone against *Salmonella typhi*

	MIC (mg/L)	
Antibiotics	**Clinical strain**	**Standard strain**
Cefotaxime	8.0	8.0
Ceftriaxone	4.0	8.0

© 2002 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **8**, 810–813

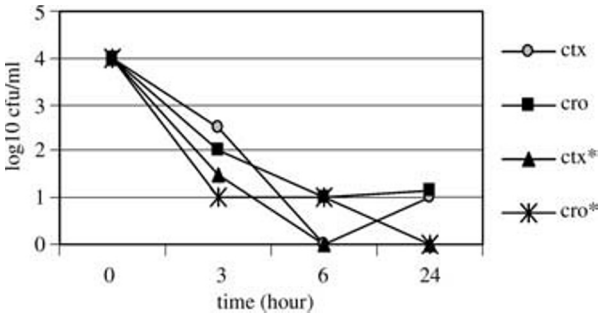

Figure 1 Effects of cefotaxime (ctx) and ceftriaxone (cro) on the standard strain within macrophages. *5 × MIC.

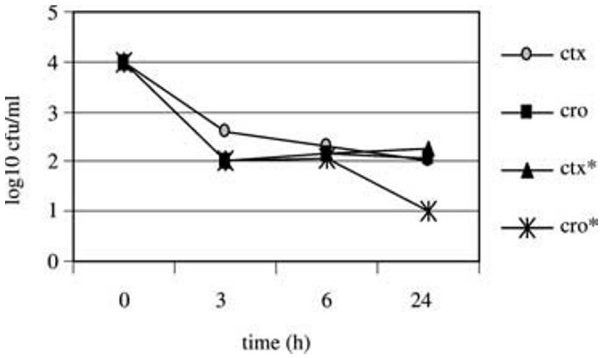

Figure 2 Effects of cefotaxime (ctx) and ceftriaxone (cro) on the clinical strain within macrophages. *5 × MIC.

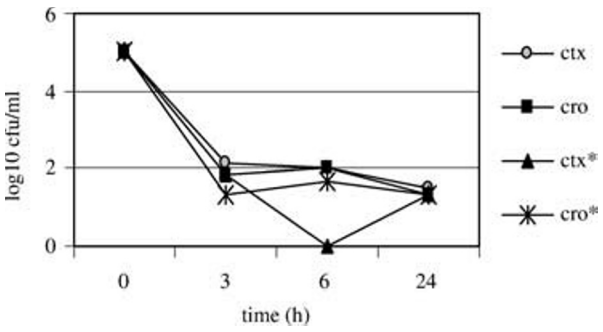

Figure 3 Effects of cefotaxime (ctx) and ceftriaxone (cro) on the standard strain without macrophages. *5 × MIC.

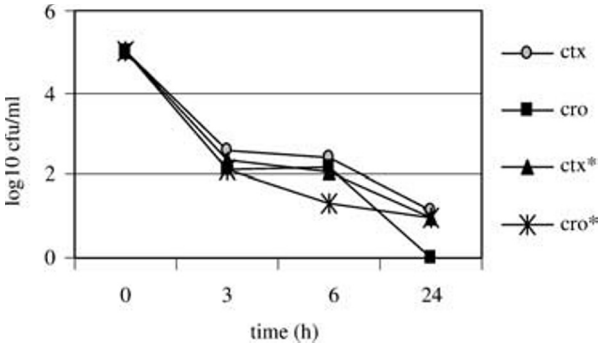

Figure 4 Effects of cefotaxime (ctx) and ceftriaxone (cro) on the clinical strain without macrophages. *5 × MIC.

infected with both strains. Some killing took place in the first 3 h, whereas their maximum efficiencies were attained at 6–24 h. On the other hand, as shown in Figures 1 and 2, the clinical strain appeared to be more resistant than the standard strain, especially at 5 × MIC of cefotaxime ($P < 0.05$). The effects of antibiotics on both strains in wells without macrophages were similar ($P > 0.05$) (Figures 3 and 4).

DISCUSSION

To spread and cause infection, *S. typhi* needs to invade a macrophage, survive within it and be resistant to the bactericidal effects of macrophages [14].

For treating a disease caused by an intracellular pathogen, the antibiotics must have good penetration ability and effective killing capacity, in order to treat intracellularly infected cells. In countries like India, Pakistan and Vietnam, drug resistance is a common problem [15,16]. Treatments of *S. typhi* infections with ampicillin, chloramphenicol and trimethoprim-sulfamethoxasole, which penetrate cells well, have been used for years. However, increasing resistance to these drugs has necessitated the search for alternative drugs. Now new quinolones and third-generation cephalosporins, including cefotaxime and ceftriaxone, are successfully used in clinical practice [15–17].

In the present study, we compared the intracellular and extracellular effects of cefotaxime and ceftriaxone on *S. typhi*, both inside and outside macrophages.

Both antibiotics had good effects on the standard strain, especially at 5 × MIC within macrophages ($P < 0.05$), but outside macrophages they had similar effects at both concentrations (Figures 2 and 3). Maximum efficiencies of antibiotics were observed at 6 and 24 h at MIC.

We found that antibiotics had different efficiencies in intracellular and extracellular environments, especially with regard to the clinical strain. For example, at the end of 24 h, the extracellular effects of antibiotics on the clinical strain were better than the intracellular effects ($P < 0.05$), except for ceftriaxone at 5 × MIC. Ceftriaxone had similar effects on the clinical isolates inside and outside macrophages. The extracellular and intracellular activities of both antibiotics did not differ when tested against the standard strain ($P > 0.05$).

Chang et al. [1] reported that cefotaxime and ceftriaxone exhibit good intracellular effects,

© 2002 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **8**, 810–813

despite the well known poor concentrating ability of penicillins and cephalosporins inside polymorphonuclear leukocytes [1]. Our results are in agreement with their results, and further suggest that these antibiotics are able to enter macrophages to act on *S. typhi* intracellularly. Furthermore, it has been shown that cephalosporins are able to kill phagocytosed *Staphylococcus aureus* when the killing mechanisms of monocytes have been blocked [18].

Finally, our in vitro study suggests that third-generation cephalosporins (cefotaxime and ceftriaxone) have good intracellular effects against *S. typhi*, which suggests that they might be used when resistance to chloramphenicol or ampicillin is experienced.

ACKNOWLEDGMENTS

This work was presented in part at the 11th European Congress of Clinical Microbiology and Infectious Diseases (Istanbul, Turkey), 1–4 April 2001 (Abstract P1443).

REFERENCES

1. Chang HR, Vladoianu IR, Pechere JC. Effects of ampicillin, ceftriaxone, pefloxacin, and trimethoprim–sulphamethoxazole on *Salmonella typhi* within human monocyte-derived macrophages. *J Antimicrob Chemother* 1990; 26: 689–94.
2. Sizemore DR, Elsinghorst EA, Eck LC *et al.* Interaction of *Salmonella typhi* strains with cultured human monocyte-derived macrophages. *Infect Immun* 1997; 65: 309–12.
3. Arias M, Rojas M, Zabaleta J *et al.* Inhibition of virulent *Mycobacterium tuberculosis* by Bcg^r and Bcg^s macrophages correlates with nitric oxide production. *J Infect Dis* 1997; 176: 1552–8.
4. Fields PI, Swanson RV, Haidaris CG, Heffron F. Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. *Proc Natl Acad Sci USA* 1986; 83: 5189–93.
5. Balland O, Pinto-Alphandry H, Viron A, Puvion E, Andremont A, Couvreur P. Intracellular distribution of ampicillin in murine macrophages infected with *Salmonella typhimurium* and treated with (^{3}H) ampicillin-loaded nanoparticles. *J Antimicrob Chemother* 1996; 37: 105–15.
6. Coligan JE, Kruisbeek AM, Morgulies DH, Schevach EM, Strober W. In: Coligan *et al*, eds. *Current protocols in immunology*. Indianapolis, IN, USA: Wiley, 1994: Section 7.1–7.6.
7. Buchmeier NA, Heffron F. Intracellular survival of wild-type *Salmonella typhimurium and* macrophage-sensitive mutants in diverse populations of macrophages. *Infect Immun* 1989; 57: 1–7.
8. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn Jr WC. *Color atlas and textbook of diagnostic microbiology*, 5th edn. Philadelphia: Lippincott, 1997: 171–251.
9. Isenberg HD. *Clinical microbiology procedures handbook*. Washington, DC: American Society for Microbiology, 1992: Section 5.2–5.3.
10. Buchmeier NA, Libby SJ. Dynamics of growth and death within a *Salmonella typhimurium* population during infection of macrophages. *Can J Microbiol* 1997; 43: 29–34.
11. Oh YK, Aranda CA, Berthiaume E, Jinks T, Miller SI, Swanson JA. Rapid and complete fusion of macrophage lysosomes with phagosomes containing *Salmonella typhimurium*. *Infect Immun* 1996; 64: 3877–83.
12. Shiloh MU, Ruan J, Nathan C. Evaluation of bacterial survival and phagocyte function with a fluorescence-based microplate assay. *Infect Immun* 1997; 65: 3193–8.
13. Bilgehan H. *Klinik mikrobiyolojik taný*, 2nd edn. Ýzmir: Barýþ Yayýnlarý, 1995: 117–46.
14. Buchmeier NA, Heffron F. Induction of Salmonella stress proteins upon infection of macrophages. *Science* 1990; 248: 730–2.
15. Bhat KG, Andrade AT, Kardesai SG, Hemashethar BM, Patil CS. Antimicrobial susceptibility of *Salmonella typhi* to quinolones and cephalosporins. *Ind J Med Res* 1998; 107: 247–51.
16. Topçu AW, Söyletir G, Doðanay M. *Ýnfeksiyon hastalýklarý*. Istanbul: Nobel Týp Kitabevleri, 1996: 491–505.
17. Ünal S, Hayran M, Tuncer S *et al.* Treatment of enteric fever with pefloxacin for 7 days versus 5 days. Randomized clinical trial. *Antimicrob Agents Chemother* 1996; 40: 2898–900.
18. Van Den Broek PJ, Buys LFM, Van Den Barselaar MT, Leijh PCJ, Van Furth R. Influence of human monocytes on the antibacterial activity of kanamycin and gentamicin for *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1986; 29: 1032–9.

© 2002 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, *CMI*, **8**, 810–813