Bactericidal activities and post-antibiotic effects of ofloxacin and ceftriaxone against drug-resistant *Salmonella enterica* serovar Typhi

John Wain1,2, Julie A. Simpson3,4, Luong Thi Diem Nga1, To Song Diep5, Pham Thanh Duy1, Stephen Baker1,6,7, Nicholas P. J. Day6,6, Nicholas J. White4,6 and Christopher M. Parry1,6*

1Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam; 2Quadram Institute Bioscience, Norwich Research Park, Norwich, UK; 3Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Australia; 4Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; 5Hospital for Tropical Diseases, Vo Van Kiet, District 5, Ho Chi Minh City, Vietnam; 6Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK; 7Department of Medicine, University of Cambridge, Cambridge, UK

*Corresponding author. E-mail: christopher.parry@ndm.ox.ac.uk

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**Background:** The clinical response to ceftriaxone in patients with typhoid fever is significantly slower than with ofloxacin, despite infection with *Salmonella enterica* serovar Typhi (*S. Typhi*) isolates with similar susceptibilities (MIC 0.03–0.12 mg/L). The response to ofloxacin is slower if the isolate has intermediate susceptibility (MIC 0.25–1.0 mg/L).

**Objectives:** To determine the bactericidal activity and post-antibiotic effect (PAE) of ceftriaxone and ofloxacin against *S. Typhi*.

**Methods:** The mean time to reach a 99.9% reduction in log10 count (bactericidal activity) and PAE of ceftriaxone and ofloxacin were determined for 18 clinical isolates of *S. Typhi* in time–kill experiments (MIC range for ofloxacin 0.06–1.0 mg/L and for ceftriaxone 0.03–0.12 mg/L).

**Results:** The mean (SD) bactericidal activity of ofloxacin was 33.1 (15.2) min and 384.4 (60) min for ceftriaxone. After a 30 min exposure to ofloxacin, the mean (SD) duration of PAE was 154.7 (52.6) min. There was no detectable PAE after 1 h of exposure to ceftriaxone. For ofloxacin, bactericidal activity and PAE did not significantly differ between isolates with full or intermediate susceptibility provided ofloxacin concentrations were maintained at 4% MIC.

**Conclusions:** Infections with *S. Typhi* with intermediate ofloxacin susceptibility may respond to doses that maintain ofloxacin concentrations at 4% MIC at the site of infection. The slow bactericidal activity of ceftriaxone and absent PAE may explain the slow clinical response in typhoid.

**Introduction**

Typhoid (enteric) fever, caused by *Salmonella enterica* serovar Typhi (*S. Typhi*) and serovar Paratyphi A (*S. Paratyphi A*), causes morbidity and mortality in children and young adults in low- and middle-income countries where adequate sanitation and clean water are lacking. Effective antimicrobial therapy shortens illness duration and reduces complications and mortality, but resistance to commonly used agents is widespread. A recent systematic review reports a pooled prevalence (95% CI) of fluoroquinolone-non-susceptible *S. Typhi* in South Asia between 2015 and 2018 of 70% (38%–94%). A large outbreak of ciprofloxacin- and ceftriaxone-resistant typhoid has affected the Sindh province in Pakistan since 2016. The *S. Typhi* clade H58 has been particularly dominant in the spread of these resistant strains across Asia and some parts of Africa.

Typhoid fever treatment with ofloxacin results in rapid recovery times and high cure rates even with short treatment courses, provided the infecting isolates have an MIC ≤0.1 mg/L. Infections with isolates with intermediate susceptibility to ofloxacin, defined by an ofloxacin MIC of 0.25–1.0 mg/L, or resistance to nalidixic acid or pefloxacin, have prolonged recovery times and increased clinical failure rates. Ceftriaxone has an MIC of ≤0.1 mg/L, in a similar range to susceptible fluoroquinolones, but, when used for treatment, the fever recovery times are slow.
We compared the in vitro bactericidal activities and the post-antibiotic effects (PAEs) of ofloxacin and ceftriaxone against clinical isolates of S. Typhi.

Materials and methods

Bacterial strains and patients

The study used 18 unique blood culture isolates of S. Typhi from Vietnamese patients with uncomplicated typhoid fever before entry into randomized controlled trials of treatment with short courses of ofloxacin (Oflocet©; Roussel-UCLAF, France) and ceftriaxone (Rocephin®; Roche, Hong Kong, China) at a concentration of 8×MIC for the strain used in that experiment. The response to treatment was assessed by fever clearance time, defined as the time since treatment began for the temperature to fall below 37.5°C and remain at or below 37.5°C for 48 h.

Strains were identified by standard biochemical tests and by agglutination with specific antisera (Murex, Dartford, UK). Antimicrobial susceptibility testing against chloramphenicol (30 μg), ampicillin (10 μg), co-trimoxazole (1.25/23.75 μg), ceftriaxone (30 μg) and nalidixic acid (30 μg) discs was performed and interpreted using CLSI guidelines. The MIC and MBC of ofloxacin (Roussel-UCLAF, France) and ceftriaxone (Rocephin®; Roche, Hong Kong, China) were determined by the microdilution method with CAMHB (Difco, MI, USA).

Molecular typing

We determined by modified pyrosequencing if the serovar Typhi strains were the H58 clade, by inferring genotype though the detection of the H58-specific SNP and the common SNPs located at positions 83 and 87 in the gyrA gene and position 80 in the parC that determine intermediate susceptibility to ofloxacin in the isolates resistant to nalidixic acid.

Table 1. Pharmacodynamic parameters for 18 isolates of S. Typhi versus ofloxacin and ceftriaxone [values are the median (range) of three separate experiments and the mean (SD) for all patient data combined]

| Patient code | Susceptibility pattern | Ofloxacin | Ceftriaxone |<br>time–kill (min) | PAE (min) |<br>time–kill (min) |
|--------------|------------------------|-----------|-------------|------------------|-----------|------------------|
| TY65         | FS                     | 35 (25–45)| 125 (125–215)| 320 (155–450)    |
| TY73         | FS                     | 25 (25–25)| 190 (185–245)| 315 (305–450)    |
| TY86         | FS                     | 20 (20–20)| 240 (85–255) | 280 (265–330)    |
| TY98         | FS                     | 25 (25–25)| 60 (165–165) | 475 (192–475)    |
| CT4          | FS                     | 35 (35–70)| 155 (145–165)| 425 (350–435)    |
| CT66         | FS                     | 50 (50–50)| 110 (30–125) | 455 (455–455)    |
| All FS [mean (SD)] |             | 31.7 (10.8)| 146.7 (63.2)| 378.3 (83.0)     |
| TY77         | MDR NA<sup>a</sup>     | 25 (25–25)| 215 (210–285)| 385 (255–475)    |
| TY84         | MDR NA<sup>a</sup>     | 40 (40–40)| 160 (135–185)| 440 (415–490)    |
| TY90         | MDR NA<sup>a</sup>     | 16 (16–16)| 200 (135–300)| 405 (385–415)    |
| TY97         | MDR NA<sup>a</sup>     | 35 (21–66)| 180 (125–280)| 315 (280–330)    |
| CT55         | MDR NA<sup>a</sup>     | 42 (30–50)| 100 (65–190) | 415 (395–495)    |
| CT65         | MDR NA<sup>a</sup>     | 70 (30–100)| 100 (50–160) | 330 (330–345)    |
| All MDR NA<sup>a</sup> [mean (SD)] |             | 38.0 (18.5)| 159.2 (49.4)| 381.7 (49.4)     |
| TY62         | MDR NA<sup>b</sup>     | 55 (49–55)| 110 (85–115) | 435 (420–450)    |
| TY169        | MDR NA<sup>b</sup>     | 45 (20–75)| 125 (105–180)| 420 (355–445)    |
| CT30         | MDR NA<sup>b</sup>     | 23 (18–25)| 175 (60–190) | 425 (420–605)    |
| CT31         | MDR NA<sup>b</sup>     | 25 (25–25)| 105 (25–130) | 425 (325–425)    |
| CT75         | MDR NA<sup>b</sup>     | 10 (10–40)| 200 (105–270)| 345 (320–450)    |
| CT76         | MDR NA<sup>b</sup>     | 20 (20–95)| 235 (205–235)| 310 (300–325)    |
| All MDR NA<sup>b</sup> [mean (SD)] |             | 29.7 (16.9)| 158.3 (53.3)| 393.3 (52.4)     |

<sup>a</sup>FS, susceptible to chloramphenicol, ampicillin, co-trimoxazole, ceftriaxone and nalidixic acid, and with an ofloxacin MIC <0.12 mg/L; MDR NA<sup>a</sup>, resistant to chloramphenicol, ampicillin and co-trimoxazole, but susceptible to ceftriaxone and nalidixic acid, and with an ofloxacin MIC <0.12 mg/L. MDR NA<sup>b</sup>, resistant to chloramphenicol, ampicillin, co-trimoxazole and nalidixic acid, but susceptible to ceftriaxone, and with an ofloxacin MIC of 0.25–1.0 mg/L.
**Determination of bactericidal activity and PAE**

Log viable counts were plotted versus time. The bactericidal activity was the time to reach a 99.9% reduction in log_{10} count and was calculated by two people with differences reconciled. The PAE was calculated as the PAE = T – C, where C is the time for a 1 log_{10} increase in the viable count from the moment of dilution of the PAE control and T is the time for a 1 log_{10} increase from the time of dilution for the test. The mean (SD) for the bactericidal activity and PAE for each resistance group was compared using one-way analysis of variance.

**Results**

**MICs and MBCs, haplotype and gyrA mutations**

Six isolates were fully susceptible to the drugs tested (FS: susceptible to chloramphenicol, ampicillin, co-trimoxazole, ceftriaxone and nalidixic acid, and with an ofloxacin MIC ≤0.12 mg/L), six were MDR NA^S (resistant to chloramphenicol, ampicillin and co-trimoxazole, but susceptible to ceftriaxone and nalidixic acid, and with an ofloxacin MIC ≤0.12 mg/L) and six were MDR NA^R (resistant to chloramphenicol, ampicillin, co-trimoxazole and nalidixic acid, but susceptible to ceftriaxone, and with an ofloxacin MIC of 0.25–1.0 mg/L). One MDR NA^R strain had an ofloxacin MIC of 0.12 mg/L. The response of patients infected with isolates in each resistance group to ofloxacin treatment is shown in Table S1 and Figure S1 (both available as Supplementary data at JAC Online).

**Time–kill studies and PAE**

The mean (SD) bactericidal activity of ofloxacin in the 18 experiments was 33.1 (15.2) min, compared with 384.4 (60) min for ceftriaxone (Table 1). Typical curves are shown in Figure 1. After 30 min of exposure to ofloxacin, all isolates gave a PAE; mean (SD) PAE of 157.7 (11.2) min. There were no significant differences for the time–kill and PAE when comparing the FS, MDR NA^S and MDR NA^R isolates exposed to ofloxacin at 4×MIC. There was no detectable PAE after 1 h of exposure to ceftriaxone.

**Discussion**

Ofloxacin at a concentration of 4×MIC was rapidly bactericidal against susceptible and MDR strains of S. Typhi that had ofloxacin MICs ≤0.12 mg/L and those with an MIC of 0.25–1.0 mg/L. Ofloxacin also provided an average PAE of 158 min against these organisms. In contrast, ceftriaxone at four times the MIC of ≤0.12 mg/L demonstrated a slow bactericidal action against all strains and no PAE.

A pharmacokinetic-pharmacodynamic measure that correlates with in vivo efficacy of ofloxacin in typhoid is thought to be f_{C_max}/MIC. In Vietnamese children with uncomplicated typhoid fever, treated with 15 mg/kg/day of oral ofloxacin in two divided doses, the mean (95% CI) peak serum level was 5.5 (4.7–6.3) mg/L. Ofloxacin is approximately 35% bound to plasma proteins, so this corresponds with an unbound peak level of approximately 3.6 mg/L. In adult healthy volunteers given 200 mg of ofloxacin, a dose used in a number of the clinical trials, the serum level at 2 h was 2.07 mg/L with an estimated unbound concentration of
1.3 mg/L.\textsuperscript{19} For isolates of S. Typhi with an MIC of $\leq 0.06$ mg/L the $f_{C_{\text{max}}}/\text{MIC}$ ratio will therefore vary between 60:1 and 22:1, well above the 4$\times$MIC. For isolates with intermediate ofloxacin susceptibility the $f_{C_{\text{max}}}/\text{MIC}$ ratio will range between 14.4:1 and 3.6:1 in children and between 5.2:1 and 1.3:1 in adults. Although isolates with intermediate susceptibility also demonstrated a PAE, the unbound serum level will fall below a value of 4$\times$MIC during the dosing cycle.

Ceftriaxone had a slow bactericidal action against all strains and no PAE against all the S. Typhi. A pharmacokinetic-pharmacodynamic target associated with treatment success of ceftriaxone against other Gram-negative infections is a target attainment of drug levels above the MIC for 100% of the dosing cycle. The mean (SD) peak serum ceftriaxone level in adolescents and adults with typhoid in Nepal, treated with 3 g of IV ceftriaxone once daily, was 291 (92) mg/L and the trough was 21.7 (25.4) mg/L.\textsuperscript{20} Even considering that ceftriaxone is 85%–95% protein bound, the ceftriaxone level is still above the MIC for 100% of the dosing cycle.

This study has shown that an ofloxacin concentration of 4$\times$MIC is rapidly bactericidal and has a prolonged PAE in time–kill experiments against S. Typhi isolates with intermediate ofloxacin susceptibility. In principle, higher doses of ofloxacin than used in these trials (>$15$ mg/kg/day) could be clinically effective against such infections, but may be limited by adverse effects, such as QT interval prolongation, tendinopathy and cardiac valve disorders. Low-dose regimens of ofloxacin (such as 200 mg twice daily in adults and 10–15 mg/kg/day in children used in these trials) risk selecting resistant S. Typhi and are best avoided. In contrast, ceftriaxone demonstrated slow bactericidal activity and an absent PAE against S. Typhi and this may contribute to the slow clinical responses seen with typhoid fever patients treated with ceftriaxone. With the emergence of isolates resistant to both ceftriaxone and fluoroquinolones the need to understand the activity of older antimicrobials and to study new agents against S. Typhi is critical.\textsuperscript{3}

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Transparency declarations
None to declare.

Supplementary data
Supplementary Methods, Table S1 and Figure S1 are available as Supplementary data at JAC Online.

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