The antibacterial efficacy of trovafloxacin against an experimental infection with *Listeria monocytogenes* in hydrocortisone-treated mice

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The efficacy of trovafloxacin in treating *Listeria monocytogenes* infections in glucocorticosteroid-treated mice was compared with the efficacy of amoxycillin. Swiss mice were treated with daily injections of 2.5 mg hydrocortisone sc and then infected iv with $1 \times 10^7$ cfu of *L. monocytogenes*. Untreated, this level of infection resulted in 100% mortality between day 3 and day 5 after infection. Both sc trovafloxacin and amoxycillin were effective in reducing the number of viable *L. monocytogenes* in the liver and spleen. Although the MIC of amoxycillin for this isolate of *L. monocytogenes* was lower than that of trovafloxacin (0.063 mg/L versus 0.5 mg/L, respectively), trovafloxacin was more efficacious *in vivo* after a single dose in the dose range between 12.5 and 100 mg/kg than was amoxycillin. After treatment with trovafloxacin at 100 mg/kg bodyweight od for 3 days, a mean $\log_{10}$ cfu of 1.58 and 2.52 *L. monocytogenes* could be recovered from the spleens and livers, respectively, whereas after treatment with amoxycillin at 100 mg/kg bodyweight every 8 h for 3 days, the mean $\log_{10}$ cfu values were 2.36 and 2.02, respectively. These differences were statistically not significant. Results of the present study show that the antibacterial efficacy of trovafloxacin against *L. monocytogenes* in our animal model is equivalent to that of amoxycillin.

Introduction

*Listeria monocytogenes* is a facultative intracellular bacterial pathogen that causes severe infections, such as septicemia and meningitis, which can be life-threatening in immunocompromised patients. The patients most often affected by severe listeriosis are neonates, transplant recipients, patients on glucocorticosteroids and patients with lymphomas.1 *L. monocytogenes* is widespread in nature, but humans are only sporadically infected. It is estimated that approximately 1850 cases occur yearly in the USA with an overall mortality of 25%.2,3 Standard therapy for listeriosis consists of ampicillin, amoxycillin or penicillin, sometimes combined with an aminoglycoside. There are few well-documented alternatives for the treatment of listeriosis. Co-trimoxazole, vancomycin, tetracycline, chloramphenicol and cephalothin have all been suggested as alternatives for amoxycillin, but controlled studies have not been performed. Trovafloxacin is a fluorinated quinolone with in-vitro bactericidal activity against *L. monocytogenes;*4 moreover, it easily penetrates phagocytic cells.5 On theoretical grounds these characteristics make trovafloxacin an interesting alternative therapy for listeriosis.

The aim of the present study was to compare the antimicrobial efficacy of trovafloxacin with that of amoxycillin for the treatment of an experimental infection with *L. monocytogenes* in mice immunocompromised by treatment with glucocorticosteroids. The design of the study was first to determine the dosage interval for the treatment of this experimental infection using a maximally effective dose of amoxycillin or trovafloxacin, then to determine the maximally effective dose of either drug after a single dose, and finally to determine the antibacterial efficacy of both antimicrobial drugs by giving the maximally effective doses at the determined dosage intervals for a period of 3 days.

Materials and methods

**Antibiotics**

Trovafloxacin powder (79.8% potency) was obtained from Pfizer (Groton, CT, USA). Stock solutions were prepared...
in distilled water and used within 1 h. Amoxycillin (85%) was obtained from SmithKline Beecham (Brockham Park, UK). Stock solutions were prepared in distilled water and used within 1 h.

**Animals**

Female specific pathogen free Swiss mice (IFFA Credo, l’Arbresle, France) were used throughout the study. The animals were housed in groups of 10 in polycarbonate cages on sterile sawdust; they received acidified tap water and non-sterilized food pellets (type AM-II, Hope Farms, Woerden, The Netherlands) ad libitum. This study was approved by the Committee on Animal Experiments of the State University of Leiden.

**Bacteria**

An overnight culture of *L. monocytogenes* (isolate EGD) in tryptone soya broth (TSB) (Oxoid Ltd, Basingstoke, UK) was stored in small aliquots at −70°C. The virulence of this isolate was maintained by repeated passage through mice. Before each experiment, aliquots were rapidly thawed in a water bath at 37°C. The MIC of amoxycillin for this isolate was 0.063 mg/L, and that of trovafloxacin was 0.5 mg/L. The MICs were determined by the agar dilution method on Mueller–Hinton agar (Oxoid), using an inoculum of 10^3–10^4 cfu/spot.

**In-vitro growth experiments**

A 1:2000 dilution of an overnight culture of *L. monocytogenes* in TSB was incubated in a shaking water bath at 37°C for 1 h and then distributed in 20 mL aliquots over 50 mL flasks that contained various concentrations of the antimicrobial drugs. Samples were taken at 1 h intervals over a period of 6 h. After appropriate dilutions in phosphate-buffered saline (PBS) plating on blood agar and incubation overnight at 37°C, the cfu were counted. To prevent carry-over of the antimicrobial agents, samples expected to have low counts of viable *L. monocytogenes* were washed once with ice-cold PBS. The washing procedure was performed as follows: a 200 μL sample was diluted with 1800 μL PBS and centrifuged at 2000g for 10 min at 4°C; the upper ninetenths of the volume were then removed with a pipette. Recovery of the bacteria with this procedure was 99.8% (s.d. 17.7%).

**Effect of amoxycillin and trovafloxacin on the growth of *L. monocytogenes* in vivo**

The experimental infection model has been validated previously. In short, mice were treated od with 2.5 mg hydrocortisone sodium succinate (Upjohn, Ede, The Netherlands) subcutaneously in the nuchal region. One day after the start of hydrocortisone treatment the mice received an iv injection of 1 × 10^7 cfu *L. monocytogenes*. Twenty-four hours after the injection of *L. monocytogenes* the antimicrobial treatment was started. The dose range studied for amoxycillin as well as trovafloxacin was 12.5–100 mg/kg. The dosage interval in these studies was either 8 or 24 h. The outgrowth of *L. monocytogenes* in the livers and spleens of hydrocortisone-treated mice was determined by quantitative culturing.

At specific intervals after the start of infection the mice were killed by cervical dislocation and the livers and spleens were excised, weighed and homogenized in a tissue homogenizer (Ystral, type X-1020, International Laboratorium Apparate GmbH, Dottingen, Germany). For counting of the cfu in the homogenate, samples were processed as described for the in-vitro experiments. The lower limit of detection for this assay was approximately 5 cfu/organ; when no bacteria were recovered from the organs, the number of cfu was arbitrarily set at unity for further calculations.

**Pharmacokinetics**

After a single dose of trovafloxacin 100 mg/kg the pharmacokinetics in plasma were determined in a group of 20 uninfected mice. At consecutive time points between 15 min and 24 h after administration of the antimicrobial agent some of the mice were killed by exposure to 100% CO₂. Blood samples were taken by cardiac puncture with heparinized syringes and centrifuged at 1500g for 10 min at room temperature and the plasma was removed; the drug concentration was measured as described below.

Pharmacokinetic analysis was performed by the Scientist program (MicroMath, Salt Lake City, UT, USA). The data were fitted to a one-compartment model.

Pharmacokinetic experiments with amoxycillin were not performed, because there were previous published data from our institute.

**Drug assays**

Trovafloxacin concentrations were determined by means of an agar diffusion method in a microbiological assay with an *Escherichia coli* isolate as test organism and Nutrient agar (pH 7.4) (Oxoid) as medium. Appropriate two-fold dilutions of the samples were prepared with pooled murine plasma. Standards were prepared in the same way. The detection limit of the assay was 0.1 mg/L.

**Statistical analysis**

The results of assessment of the quantitative cultures are given as the mean log cfu per organ. Sigmastat software (Jandel, San Rafael, CA, USA) was used for making statistical calculations. One-way analysis of variance was used.

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for statistical comparisons between multiple groups, with the Tukey test used as a follow-up test. A $P$-level of 0.05 was considered significant.

**Results**

*In-vitro growth experiments*

The exponential growth rate for *L. monocytogenes* in TSB, as determined by linear regression analysis, was 0.28/h, which corresponds to a doubling time of 1.07 h. Amoxicillin showed antibacterial activity at concentrations above 0.032 mg/L. The maximum antibacterial effect of amoxicillin on *L. monocytogenes* was demonstrated at concentrations of 0.063 mg/L and higher, apparently without concentration-dependent activity. At concentrations above 0.063 mg/L the effect was bacteriostatic in the first 6 h of the experiment (Figure 1). After this period some extent of bactericidal activity occurred in the first 24 h of exposure to amoxicillin (results not shown).

Trovafloxacin showed antibacterial activity at concentrations above 0.125 mg/L. Concentrations of trovafloxacin above 0.5 mg/L were bactericidal, apparently with concentration-dependent killing (Figure 1).

**Effect of a single dose of amoxicillin or trovafloxacin on the growth of L. monocytogenes in vivo**

For both amoxicillin and trovafloxacin, initial experiments were carried out using a dosage of 100 mg/kg. This dosage was chosen, because for trovafloxacin the limit of solubility in water was approximately 10 mg/mL. After administration of a single sc dose of amoxicillin 100 mg/kg the number of *L. monocytogenes* in the liver and spleen initially declined, but regrowth of *L. monocytogenes* occurred after more than 8 h (Figure 2). For a single sc dose of trovafloxacin 100 mg/kg bactericidal activity was shown for a period of 24 h (Figure 2). Therefore an 8 h dosage interval was considered to be the optimum interval for amoxicillin, whereas this period was 24 h for trovafloxacin.

To determine the dose–effect relationship for amoxicillin and trovafloxacin, the drugs were administered sc to hydrocortisone-treated mice 24 h after an iv injection of $1 \times 10^7$ cfu of *L. monocytogenes*. The mice were killed 24 h after administration of the antimicrobial agent. Treatment with amoxicillin caused a significant decrease in the number of *L. monocytogenes* in the livers and spleens, with an apparent dose-dependent effect in the dose range between 12.5 and 100 mg/kg (Figure 3). The mean log number of *L. monocytogenes* in the livers of mice treated with amoxicillin 100 mg/kg 24 h after administration of the antimicrobial agent was 5.18 (s.d. 0.57) compared with 6.97 (s.d. 0.57) for control mice; for the spleen it was 6.41 (s.d. 0.35) compared with 7.66 (s.d. 0.48). For amoxicillin-treated mice, the liver and spleen counts of mice treated with doses of 25 mg/kg or higher were significantly different from those of control mice ($P < 0.05$). The same was true for the difference in liver counts between mice treated with amoxicillin 12.5 mg/kg and 100 mg/kg, and for the spleen counts between mice treated with amoxicillin 12.5 mg/kg and 50 mg/kg.

Treatment with trovafloxacin 12.5–100 mg/kg also had an apparent dose-dependent effect (Figure 3). Twenty-four
hours after the administration of trovafloxacin 100 mg/kg
the mean log number of *L. monocytogenes* in the livers was
4.54 (S.D. 0.61) compared with 6.97 (S.D. 0.57) for the
control mice; for the spleens it was 5.05 (S.D. 0.87) compared
with 7.66 (S.D. 0.48).

For trovafloxacin-treated mice, the liver and spleen
counts of mice treated with doses of 25 mg/kg or higher
were significantly different from those of control mice
($P < 0.05$). The same was true for the difference in liver and
spleen counts between mice treated with trovafloxacin
12.5 mg/kg and 50 or 100 mg/kg.

Comparing equivalent doses between amoxycillin- and
trovafloxacin-treated mice, there was no statistically sig-
nificant difference for the liver counts, but the difference
was statistically significant for the spleen counts at doses of
25, 50 and 100 mg/kg ($P < 0.05$).

**Effect of 3 days of amoxycillin or trovafloxacin
therapy on the growth of *L. monocytogenes* in vivo**

Treatment with the antibiotics for a prolonged period led
to a large reduction in the number of *L. monocytogenes*
in the livers and spleens. Treatment with amoxycillin
100 mg/kg tds resulted in a significant decrease ($P < 0.001$)
in the number of *L. monocytogenes* after 1 day of treatment
(Figure 4). Thereafter the numbers of *L. monocytogenes*
decayed to a mean log cfu of 2.02 (S.D. 0.70) in the livers
and 2.36 (S.D. 1.18) in the spleens after 3 days. Treatment
with trovafloxacin 100 mg/kg od also led to a significant
decrease ($P < 0.001$) in the number of *L. monocytogenes*
in the livers and spleens. After 3 days of treatment the mean
log number of *L. monocytogenes* from the livers was 2.52
(S.D. 0.74), and that from the spleens was 1.58 (S.D. 1.09).
The difference in numbers between amoxycillin- and trova-
fl oxacin-treated mice at day 3 was not statistically signifi-
cant for the spleens ($P = 0.22$) or for the livers ($P = 0.26$).

**Pharmacokinetic studies**

The pharmacokinetic data on an sc dose of trovafloxacin
100 mg/kg in the plasma of mice are shown in Figure 5.
Trovafloxacin reached a peak concentration of 4.5 mg/L in
plasma at 4 h after administration. The apparent elimina-
tion half-life was 4.2 h. The AUC$_{0-24h}$ was 72 mg h/L.
Discussion

The present study shows that amoxycillin and trovafloxacin exhibit antibacterial activity in an experimental *Listeria* spp. infection in mice immunocompromised by treatment with glucocorticosteroids, and that the antibacterial efficacy of trovafloxacin did not differ significantly from that of amoxycillin. The antimicrobial efficacy of trovafloxacin can be explained by the finding that trovafloxacin was bactericidal against this isolate of *L. monocytogenes in vitro*, and that trovafloxacin has a long plasma half-life. Amoxycillin, on the other hand, has a half-life of approximately 0.40 h in Swiss mice, with a peak concentration of 11.9 mg/L after an sc dosage of 10 mg/kg. Other researchers have found a relatively lower $C_{\text{max}}$ of 67.0 mg/L at a dosage of 100 mg/kg, but with a comparable elimination half-life of 0.33 h. The pharmacokinetic data explain why for amoxycillin three times daily dosing was optimal, whereas for trovafloxacin od sufficed.

Using the pharmacokinetic data, we can calculate that a single dose of trovafloxacin 100 mg/kg reaches an AUC/MIC ratio of 142. This pharmacokinetic/pharmacodynamic parameter should be considered adequate for treatment of infections, as for quinolones an AUC/MIC ratio of >125 is generally correlated with clinically successful treatment of infections. It can also be calculated that amoxycillin 100 mg/kg tds leads to 54% of the time above the MIC. This is also more than the required 40% of the time above the MIC that is needed for clinical success. Therefore, both antibiotics were administered in doses that reached efficacious concentrations, provided that the data that have been generated for mainly extracellular bacteria also apply for *L. monocytogenes*. The value of quinolones in treating listeriosis is questionable. In a previous study comparing ciprofloxacin and ampicillin, ampicillin showed superior efficacy compared with ciprofloxacin, but other researchers found that newer quinolones showed some promise in treating experimental listeriosis.

The pharmacokinetics of trovafloxacin in mice are remarkably similar to those in humans. The plasma elimination half-life in humans is 7.8 h, and the maximum concentration after an oral dose of 200 mg is 2.9 mg/L. In humans, approximately 5% of unchanged trovafloxacin is excreted in the urine. The urinary clearance of trovafloxacin in mice is not known, but the relatively long elimination half-life in mice points to a lack of renal elimination. Considering the similarity of the pharmacokinetics of trovafloxacin in mice and humans, murine infection models are well suited to study the antimicrobial efficacy of this drug. This is in strong contrast to most $\beta$-lactam antibiotics, which have a much shorter half-life in mice.

Despite the different in-vitro behaviour of amoxycillin and trovafloxacin, the antibacterial efficacy in our model of listeriosis was similar. It is especially noteworthy that trovafloxacin was bactericidal, whereas amoxycillin exhibited only delayed bactericidal activity. Furthermore, trovafloxacin accumulates well intracellularly and should therefore theoretically be more efficacious against facultative intracellular bacteria than $\beta$-lactam antibiotics, which accumulate more slowly and to a lesser extent. The results of the present study do not lend support to the theory that rapid intracellular accumulation of antibiotics contributes significantly to the antibacterial efficacy against infections by facultative intracellular bacteria.

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