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# Comparative Efficacies of Antibiotics in a Rat Model of Meningoencephalitis Due to *Listeria monocytogenes*

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**The antibacterial activities of amoxicillin-gentamicin, trovafloxacin, trimethoprim-sulfamethoxazole (TMP-SMX) and the combination of trovafloxacin with TMP-SMX were compared in a model of meningoencephalitis due to *Listeria monocytogenes* in infant rats. At 22 h after intracisternal infection, the cerebrospinal fluid was cultured to document meningitis, and the treatment was started. Treatment was instituted for 48 h, and efficacy was evaluated 24 h after administration of the last dose. All tested treatment regimens exhibited significant activities in brain, liver, and blood compared to infected rats receiving saline ($P < 0.001$). In the brain, amoxicillin plus gentamicin was more active than all of the other regimens, and trovafloxacin was more active than TMP-SMX (bacterial titers of 4.1 ± 0.5 $\log_{10}$ CFU/ml for amoxicillin-gentamicin, 5.0 ± 0.4 $\log_{10}$ CFU/ml for trovafloxacin, and 5.8 ± 0.5 $\log_{10}$ CFU/ml for TMP-SMX; $P < 0.05$). In liver, amoxicillin-gentamicin and trovafloxacin were similarly active (2.8 ± 0.8 and 2.7 ± 0.8 $\log_{10}$ CFU/ml, respectively) but more active than TMP-SMX (4.4 ± 0.6 $\log_{10}$ CFU/ml; $P < 0.05$). The combination of trovafloxacin with TMP-SMX did not alter the antibacterial effect in the brain, but it did reduce the effect of trovafloxacin in the liver. Amoxicillin-gentamicin was the most active therapy in this study, but the activity of trovafloxacin suggests that further studies with this drug for the treatment of *Listeria* infections may be warranted.**

*Listeria monocytogenes*, a gram-positive bacillus, is a ubiquitous bacterium transmitted by food that causes infections in humans and animals (23, 24). Bacteremia (with liver and spleen involvement) and central nervous system (CNS) infections are the most common clinical presentations in humans (6). Listeriosis is associated with a high mortality rate of up to 30% in infants and in patients with underlying diseases (5, 8, 26). In a recent study, *L. monocytogenes* was the fourth most frequent cause of community-acquired bacterial meningitis overall and was the second most common pathogen in patients older than 60 years and in newborns younger than 1 month (25). Listeriosis of the central nervous system presents itself as a meningoencephalitis or rhomboencephalitis, with clinical signs of meningitis, cranial nerve deficits, sensorimotor impairments, seizures, and other signs of encephalitis.

To date, no controlled trials have been performed to delineate the most effective antibiotic regimen of listeriosis in humans, but therapy studies have been performed in animal models of the disease (7, 22). Among the β-lactam antibiotics, ampicillin (or amoxicillin) appears to be the most effective, even though its bactericidal activity is relatively slow and complete elimination of bacteria is only achieved in synergy with the host immune response (7, 22). We previously demonstrated that amoxicillin had the best activity against intracellular *L. monocytogenes* in infected HeLa cells, but it could not completely eradicate the cultures (17). Based on in vitro and animal data, the combination of ampicillin with gentamicin is generally recommended as first-line therapy for the treatment of listeriosis in humans (12, 15). Trimethoprim-sulfamethoxazole (TMP-SMX), which can penetrate cells well, is effective in penicillin-allergic patients with listeriosis, but its activity in cell cultures is lower than that of amoxicillin (17, 31). Several other antibiotics, including rifampin, have also been examined in animal models, but the available data are insufficient to propose their use in cases of human listeriosis. New fluoroquinolones with improved activity against gram-positive bacteria are rapidly bactericidal against sensitive organisms, penetrate well into cells, cross the blood-brain barrier, and have been shown to be effective in cell cultures infected with *L. monocytogenes* and in experimental pneumococcal meningitis (9, 14, 19, 20).

Evaluation of therapeutic regimens in experimental models of listeriosis must take into account the complex features of the disease, such as the intracellular location of the pathogen, systemic involvement, and the devastating involvement of both the meninges and the brain parenchyma in CNS listeriosis. We have developed a model of meningoencephalitis in infant rats that allows assessment of many features of the disease, including the determination of bacterial titers in various organs, the clinical parameters, and the brain histopathology. The model thus expands on the information obtained in the classic rabbit model of meningitis, where bacterial titers in the cerebrospinal fluid (CSF) are the primary endpoint (22). In the present study, we used the new rat model to compare the antibacterial activity and clinical efficacy of ampicillin plus gentamicin with that of trimethoprim-sulfamethoxazole and a new quinolone (trovafloxacin). Trovafloxacin was chosen because its MICs against *L. monocytogenes* range between 0.12 and 0.25 mg/liter (21), and most strains are killed by concentrations of <1 mg/liter (3). Furthermore, trovafloxacin showed good CSF penetration in rabbits with experimental meningitis and was effective against *Streptococcus pneumoniae* in these models (9, 20).

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## MATERIALS AND METHODS

**Strain.** The strain of *L. monocytogenes* (serotype 4b) used in the present study was isolated from the CSF of a patient with meningitis. Bacteria were grown on blood agar plates, and one colony was cultured overnight in brain heart infusion.

For infection, 50 μl was diluted in 5 ml of fresh medium and grown at 37°C for 3 h to logarithmic phase, pelleted, and resuspended in normal saline to be used as the inoculum. The accuracy of the inoculum size was confirmed by quantitative cultures for each experiment.

**Meningitis model in infant rats.** The present model is a modification of the model of group B streptococcal meningitis in infant rats described previously (10, 11). Briefly, nursing 11-day-old Sprague-Dawley infant rats weighing 25 ± 2 g were purchased (RCC Biotechnology and Animal Breeding, BL, Füllinsdorf, Switzerland) with their dam and were infected by direct intracisternal injection of 10 μl of a suspension of $5 \times 10^4$ to $1 \times 10^5$ CFU of *L. monocytogenes* in sterile saline by using a 32-gauge needle. After infection, pups were returned to their mother. At 22 h after infection, 5 to 10 μl of CSF was obtained by puncture of the cisterna magna and cultured quantitatively to document *L. monocytogenes* meningitis. Treatment was initiated 22 h after infection (H22), and animals received antibiotics for 2 consecutive days (i.e., receiving four or eight antibiotic injections). Animals were sacrificed by intraperitoneal injection of pentobarbital (200 mg/kg) 24 h after the last injection of antibiotics or when they became terminally ill (coma, protracted seizures, and/or cyanosis).

**In vitro studies.** MICs and minimal bactericidal concentrations (MBCs) were determined by standard tube macrodilution methods with inocula of $1 \times 10^6$ and $9 \times 10^7$ CFU/ml, respectively. MICs were defined as the lowest concentration inhibiting visible growth after 24 h of incubation at 37°C, and MBCs were defined as the lowest concentration killing more than 99.9% of the initial inoculum.

**Antibiotics and administration in vivo.** Amoxicillin was provided by Smith-Kline Beecham (Puteaux, France), and trovafloxacin was provided by Pfizer, Inc. (Groton, Conn.), as powder (Mesylate) for in vitro testing and as injectable prodrug (alatrofloxacin) for animal experiments. Gentamicin and TMP-SMX were obtained from commercial sources. Animals were randomized to receive either saline or active compounds. All antibiotics were dissolved or diluted in sterile water and were injected intraperitoneally with a volume of 100 or 150 μl. Alatrofloxacin was dosed at 20 mg/kg, either two or four times per day. The drug was also tested at a dose of 40 mg/kg per injection, but all of these rats developed seizures and died and so use of this concentration was discontinued. Amoxicillin was used at the dose of 50 mg/kg given four times a day, gentamicin at the dose of 5 mg/kg given twice a day, and TMP-SMX at the dose of 25 mg of SMX and 5 mg of TMP per kg four times a day.

**Bacterial culture.** Immediately after spontaneous death or sacrifice, animals were perfused via the left cardiac ventricle with 40 ml of ice-cold phosphate-buffered saline (PBS). The liver and brain were aseptically removed. The liver and cerebellum were washed in sterile PBS on ice, large vessels were removed, and the organs were weighed and homogenized in 1 ml of saline. Samples were kept on ice for approximately 1 h before 10-fold serial dilution (100 ml) in saline. Bacterial counts were obtained by quantitative cultures of the samples for 24 h at 37°C. In preliminary experiments, no major differences were found when bacterial titers in the cerebellum were compared to those in other regions of the brain. Bacterial counts in blood were determined by culture of undiluted (0.1 ml) and serially diluted blood, and bacterial counts in CSF were determined by plating serially diluted CSF samples. Results were expressed in $\log_{10}$ CFU per gram of tissue for brain and liver, with a detection limit of 20 CFU/g. For blood and CSF, bacterial counts were expressed as $\log_{10}$ CFU per milliliter, with detection limits of 10 and 100 CFU/ml, respectively.

**Histopathology.** Brains were harvested immediately after sacrifice and processed on ice. After the cerebellum was removed for bacterial cultures, the brains were immersion fixed in 4% paraformaldehyde in PBS for 48 h, placed in 30% phosphate-buffered sucrose for an additional 12 h, and cut at 30- to 50-μm intervals on a vibratome. Sections were mounted on gelatinized glass slides for staining. After dehydration, sections were Nissl stained with cresyl violet and quantitatively assessed for the presence of abscesses in the cortex, the periventricular spaces, and ependymal cell layers. Histopathological examinations were performed by an investigator blinded to the clinical, microbiological, and treatment data of the animals. Sections were also stained by the Brown and Brenn Gram stain modified for the microscopic detection of *Listeria* spp. in the meningeal spaces and inside the abscesses (13).

**Disease assessment.** The clinical severity of the disease was scored in every animal 22 h after infection and then twice daily until death. The activity scale was graded from 5 to 0 as follows: 5, normal activity and ambulation; 4, minimal disease (ability to right themselves within 5 s); 3, moderate disease (unable to right themselves within 5 s or evidence of paralysis); 2, severe disease (lethargic, no ambulation); 1, coma; and 0, death. Survival time was determined to be the time between infection and spontaneous death or the time from infection to sacrifice after completion of antibiotic therapy (i.e., 82 h of treatment with trovafloxacin twice a day and 88 h for all other regimens).

**Antibiotic concentrations.** Antibiotic concentrations were determined in blood and CSF samples obtained 1 and 6 h after intraperitoneal administration. Blood (250 μl) was removed by intracardiac puncture, and 10 μl of CSF was removed by puncture of the cisterna magna. CSF samples that were visibly contaminated with blood were excluded from analysis. Concentrations of trovafloxacin and amoxicillin were measured by an agar disk diffusion microbioassay with *Bacillus subtilis* ATCC 6633 by using antibiotic medium Number 11, pH 8 (Difco Laboratories, Detroit, Mich.), for trovafloxacin and antibiotic medium Number 5, pH 6, for amoxicillin. Standard curves for serum were generated in 100% rat serum, while standard curves for CSF were generated in saline containing 5% rat serum.

TABLE 1. In vitro activities of antibiotics against experimental *L. monocytogenes* strain (serotype 4b) after injection with $10^6$ CFU/ml

| Antibiotic | Antibiotic activities (mg/liter): | |
|---|---|---|
| | MIC | MBC |
| Trovafloxacin | 0.5 | 1 |
| Amoxicillin | 0.25 | 2 |
| Gentamicin | 0.12 | 0.25 |
| TMP | 0.25 | 2 |
| TMP-SMX | 0.25 | 2 |

To minimize variability, the concentrations of drugs in all samples containing the same drug were determined on a single day. Serum and CSF from untreated infected rats induced a mild inhibition of the assay organisms, and the limits of detection for trovafloxacin were 0.59 μg/ml for serum and 0.31 μg/ml for CSF and for amoxicillin were 0.44 μg/ml for serum and 0.22 μg/ml for CSF. Concentrations of gentamicin in plasma were measured by the enzyme multiplied immunoassay technique (EMIT; Behring, Inc., Cupertino, Calif.). Plasma and CSF concentrations of SMX and TMP were measured by high-performance liquid chromatography according to the methods described by Metz et al. and van der Steuijt et al. (16, 29).

**Statistical analysis.** All results were expressed as means ± the standard deviation. Comparisons between groups were performed by one-way analysis of variance. In case of significance ($P < 0.05$), this was followed by the Newman-Keuls test for pairwise comparisons. Proportions were compared by the Fisher exact test.

## RESULTS

**Experimental meningitis.** At 21 h after intracisternal infection with *L. monocytogenes*, rats were sick, with reduced activity scores (see Table 2), weight loss (not exceeding 2% of the baseline body weight), and documented meningitis on CSF examination with a mean CSF leukocyte count of 3,052 cells/$mm^3$ (range, 850 to 6,550 cells/$mm^3$) and mean bacterial titers of 5.1 ± 0.6 $\log_{10}$ CFU/ml. All animal sacrificed at H22 ($n$ = 10) were bacteremic (mean, 2.64 ± 0.17 $\log_{10}$ CFU/ml). At the same time, the brain and liver samples exhibited high bacterial titers (brain, 7.8 ± 0.7 $\log_{10}$ CFU/g; liver, 6.7 ± 0.6 $\log_{10}$ CFU/g).

By 22 h after infection, histological evidence of CNS involvement was observed, with an inflammatory reaction in the subarachnoidal spaces containing numerous *L. monocytogenes*. By the next day, cortical abscesses consisting of inflammatory cells and numerous intracellular organisms had developed in the vicinity of small blood vessels, especially in the ventral part of the cerebrum and along the interhemispheric fissure (Fig. 1a and c). *L. monocytogenes* was often observed in the cytoplasm of ependymal cells of the ventricles and in the adjacent brain parenchyma (Fig. 1d and f). In untreated, infected animals dying of the infection, the mean number of abscesses per section was 16 ± 10 and the ependymal layer, especially in the third ventricle, was uniformly infiltrated by listeriae, causing the formation of large abscesses in the subependymal area (Fig. 1f). These findings were not specific for the *Listeria* strain used in these experiments, since identical histopathological features of meningoencephalitis and similar bacterial counts in the CSF and cerebellum were obtained with the reference strain EGD (serotype 1/2a) (4) obtained from the Trudeau Institute (Sarnac Lake, N.Y.) (data not shown).

**In vitro susceptibility.** All of the antibiotics tested were bacteriostatic and bactericidal at relatively low concentrations against the strain of *L. monocytogenes* used in these experiments (Table 1). When the inoculum was increased from $1 \times 10^6$ to $9 \times 10^7$ CFU/ml, corresponding to the titers in the brains of rats 22 h after inoculation, an inoculum effect was

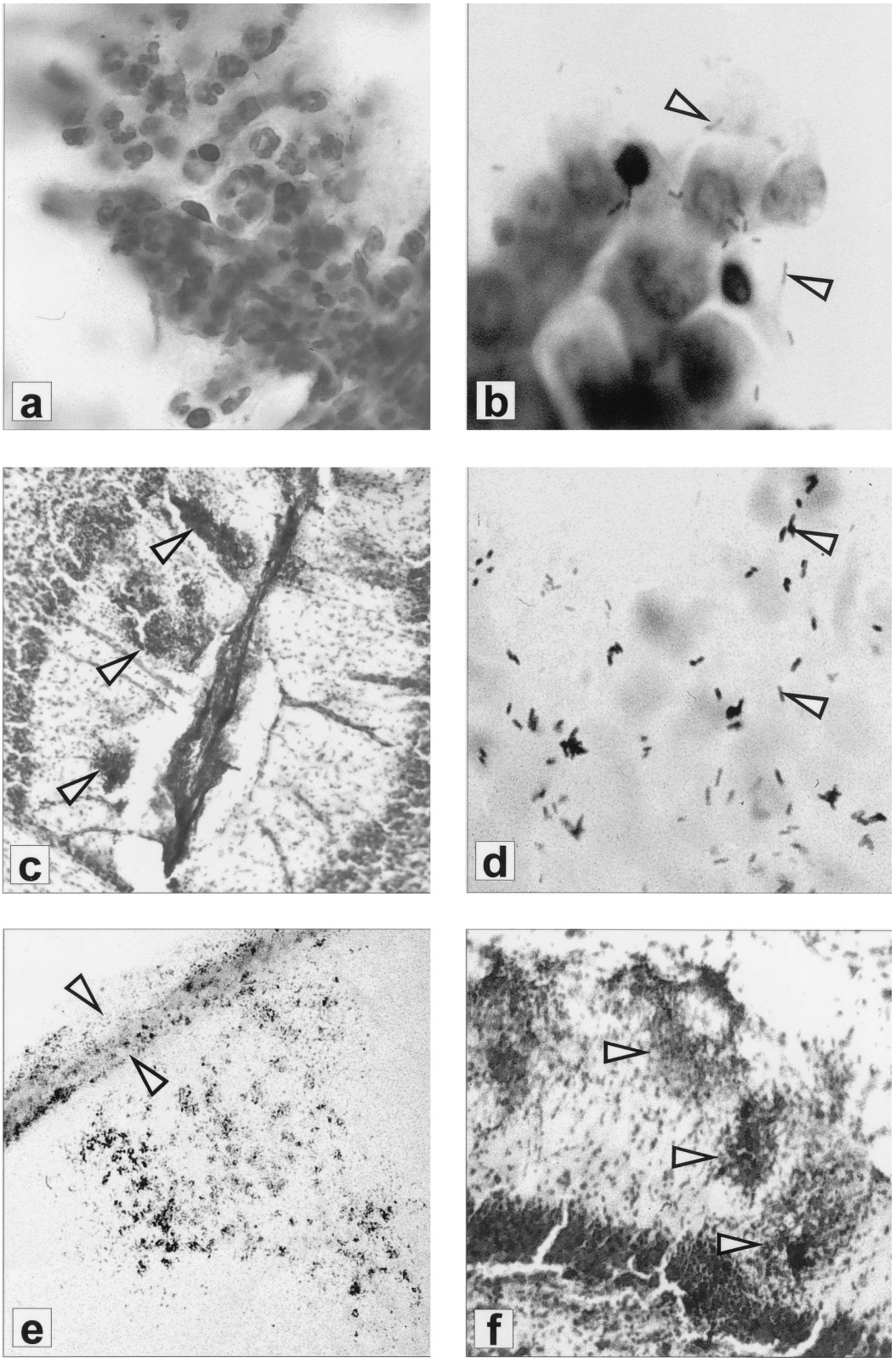


FIG. 1. Histopathological findings in infant rats with experimental meningoencephalitis caused by *L. monocytogenes*. (a) Cortical abscess in the amygdala developed 22 h after infection (Nissl stain; magnification, ×1,600). (b) *L. monocytogenes* (arrowheads) in the cytoplasm of ependymal cells of the lateral ventricular layer at 22 h after intracisternal infection (Nissl stain; ×1,600). (c) Interhemispheric abscesses (arrowheads), which are often associated with cerebral vasculature, in an untreated rat at 52 h after infection (Nissl stain; ×320). (d) Numerous *L. monocytogenes* (arrowheads); in the cytoplasm of ependymal cells 52 h after infection (Nissl stain; ×1,600). (e) *L. monocytogenes* (dark clusters) in the subarachnoidal space (arrowheads) and in the underlying cortical brain parenchyma (Brown-Brenn stain; ×800). (f) Paraventricular abscesses (arrowheads), with inflammatory cells penetrating into the dentate gyrus (dark cell band below) (Nissl stain; ×320).

TABLE 2. Clinical course of *L. monocytogenes* meningoencephalitis in infant rats

| Therapy regimen | Dose (mg/kg) | No. of injections per day | No. of animals | Illness score at: | | No. of survivors (%) | Survival time (h) |
|---|---|---|---|---|---|---|---|
| | | | | H22 | H82 | | |
| Amoxicillin plus gentamicin (AG) | 50<br>5 | 4<br>2 | 13 | 3.6 ± 0.6 | 4.5 ± 0.7 | 13 (100)[c] | 88 ± 0[d] |
| Trovafloxacin (Tq) | 20 | 4 | 13 | 3.7 ± 0.6 | 4.7 ± 0.5 | 8 (61) | 76 ± 18 |
| Trovafloxacin (Tb) | 20 | 2 | 18 | 3.6 ± 0.4 | 3.7 ± 0.8[b] | 10 (55) | 70 ± 17 |
| TMP-SMX (TS) | 5/25 | 4 | 8 | 4.6 ± 0.5[a] | 4.4 ± 0.5 | 6 (75) | 86 ± 4 |
| TMP-SMX plus trovafloxacin (TT) | 5/25<br>20 | 4<br>4 | 9 | 4.3 ± 0.9 | 4.1 ± 0.9 | 6 (66) | 79 ± 14 |
| Saline (C) | | 2 or 4 | 12 | 3.6 ± 0.8 | ND[f] | 2 (17) | 51 ± 20[e] |

[a] $P < 0.05$ versus Tb, Tq, AG, C.
[b] $P < 0.05$ versus Tq and AG.
[c] $P < 0.05$ versus Tb and C.
[d] $P < 0.05$ versus Tb and C.
[e] $P < 0.05$ versus all others.
[f] ND, not done.

observed (MIC/MBC ratios were 1/8, 1/8, and 4/>16 mg/liter for trovafloxacin, amoxicillin, and TMP-SMX, respectively). The MIC of trovafloxacin was also determined for five isolates recovered from the brains of animals treated with trovafloxacin for the full duration of the experiment. The MIC of trovafloxacin for these strains was identical to that of the original isolate.

**In vivo activity.** All animals treated with amoxicillin plus gentamicin survived the full duration of therapy ($P < 0.05$ versus untreated animals and versus animals treated with trovafloxacin twice a day [b.i.d.]). Mean survival times were also longest for amoxicillin plus gentamicin, though shorter in untreated animals than in treated animals ($P < 0.05$; Table 2). Clinical scoring was similar among groups at the beginning of therapy, with the exception of animals treated with TMP-SMX, which suffered less-severe disease (Table 2) as a reflection of a slightly lower inoculum (4.5 ± 0.2 $\log_{10}$ CFU/ml compared to 4.8 ± 0.1 $\log_{10}$ CFU/ml for trovafloxacin four times a day [q.i.d.] and amoxicillin plus gentamicin). At the end of therapy, animals treated with the suboptimal regimen of trovafloxacin b.i.d. had more-severe disease than animals treated with the more effective regimens ($P < 0.05$ versus trovafloxacin q.i.d. and amoxicillin plus gentamicin; Table 2).

Bacteriologic outcome was analyzed primarily for animals completing the entire treatment period. No significant difference was detectable between the experimental groups in terms of bacterial count in the CSF before the initiation of therapy (H22) ($P > 0.05$; Table 3). All antibiotic regimens showed significant activity compared to untreated controls (Table 3). While untreated rats were uniformly bacteremic at the time of death (3.3 ± 0.6 $\log_{10}$ CFU/ml), blood cultures of treated animals were below the limit of detectability (<1 $\log_{10}$ CFU/ml), with the exception of the group of rats treated with trovafloxacin b.i.d., where 5 of 10 rats (50%) had positive blood cultures (Table 3). In the brain, amoxicillin combined with gentamicin showed the best activity, with a mean bacterial titer per gram of tissue that was approximately 1 $\log_{10}$ CFU/ml lower than with any other therapy ($P < 0.05$ versus all other groups; Table 3). Trovafloxacin given q.i.d., alone or combined with TMP-SMX, was intermediately active in the brain, whereas TMP-SMX alone and trovafloxacin given b.i.d. were least active (Table 3). In the liver, the two most effective regimens, amoxicillin plus gentamicin and trovafloxacin given q.i.d. had similar activities and were significantly better than the comparison regimens ($P < 0.05$; Table 3). The addition of TMP-SMX to trovafloxacin significantly reduced the activity of trovafloxacin in the liver ($P < 0.05$; Table 3). For technical reasons, CSF samples could be obtained only in a small fraction of the animals prior to sacrifice, and the bacterial titers were below the limit of detection in all treated rats. Inclusion of animals dying prior to the completion of therapy did not significantly alter these results, even though animals dying prematurely had slightly higher titers than animals receiving full treatment (data not shown).

Histological evaluation showed that rats treated with antibi-

TABLE 3. Efficacy of different antibiotic treatment regimens in experimental meningoencephalitis caused by *L. monocytogenes*

| Therapy regimen | Dose (mg/kg) | No. of injections per day | No. of animals | CSF titers at H22 ($\log_{10}$ CFU/ml) | Brain titers ($\log_{10}$ CFU/g) | Liver titers ($\log_{10}$ CFU/g) | Blood titers ($\log_{10}$ CFU/ml) |
|---|---|---|---|---|---|---|---|
| Amoxicillin plus gentamicin (AG) | 50<br>5 | 4<br>2 | 13 | 5.1 ± 0.7 | 4.1 ± 0.5[b] | 2.8 ± 0.6[f] | <1 |
| Trovafloxacin (Tq) | 20 | 4 | 10 | 5.4 ± 0.8 | 5.0 ± 0.4 | 2.7 ± 0.8[e] | <1 |
| Trovafloxacin (Tb) | 20 | 2 | 10 | 5.4 ± 0.9 | 6.1 ± 1.0[a] | 4.2 ± 0.9[d] | 1.4 ± 0.7 |
| TMP-SMX (TS) | 5/25 | 4 | 8 | 4.8 ± 0.6 | 5.8 ± 0.5[c] | 4.4 ± 0.6 | <1 |
| TMP-SMX plus trovafloxacin (TT) | 5/25<br>20 | 4<br>4 | 8 | 4.6 ± 0.5 | 5.0 ± 0.6 | 3.8 ± 0.4 | <1 |
| Saline (C) | | 2 or 4 | 12 | 5.3 ± 0.9 | 7.9 ± 0.8 | 7.0 ± 0.8 | 3.3 ± 0.6 |

[a] $P < 0.05$ versus Tq.
[b] $P < 0.05$ versus all other groups.
[c] $P < 0.05$ versus Tb, Tq, TT, and C.
[d] $P < 0.05$ versus Tq, AG, and C.
[e] $P < 0.05$ versus TS, TT, and C.
[f] $P < 0.05$ versus TS and TT.

TABLE 4. Concentration of antibiotics in serum and CSF after a single injection

| Antibiotic | Dose (mg/kg) | 1 h postinjection (mean ± SD) | | 6 h postinjection (mean ± SD) | |
|---|---|---|---|---|---|
| | | CSF (μg/ml) | Serum (μg/ml) | CSF (μg/ml) | Serum (μg/ml) |
| Trovafloxacin | 20 | 2.3 ± 0.7 | 9.6 ± 3.0 | 0.5 ± 0.1 | 1.2 ± 0.8 |
| Amoxicillin | 50 | 13.7 ± 1.9 | 40 ± 10.8 | 4.5 ± 1.3 | 1.0 ± 0.3 |
| Gentamicin | 5 | ND[a] | 5 ± 0.5 | ND | 3.5 ± 0.4 |
| SMX | 25 | 32.3 ± 8.0 | 67.6 ± 12.0 | 18.6 ± 2.1 | 43.4 ± 11.8 |
| TMP | 5 | 1.1 ± 0.3 | 1.5 ± 0.4 | 0.7 ± 0.2 | 0.8 ± 0.1 |

[a] ND, not done.

otics, in contrast to saline-treated rats, had no visible *L. monocytogenes* organisms in the subarachnoidal space. Abscesses were rare, with a mean of 0.3 ± 1.1 abscesses per section in rats treated with amoxicillin plus gentamicin, 0.5 ± 1.0 in rats treated with trovafloxacin q.i.d., 0.7 ± 1.2 in rats treated with trovafloxacin b.i.d., and 1.5 ± 1.3 in rats treated with TMP-SMX (*P* values were not significant). Microscopic demonstration of *L. monocytogenes* in abscesses was infrequent, and ventricular cell layers were not disrupted in treated animals.

**Pharmacokinetics.** After a single injection of 20 mg of alatrofloxacin per kg, trovafloxacin penetrated well into the CSF of rats with meningitis, with a CSF-to-serum concentration ratio at 1 h of 0.24 (Table 4). The CSF concentrations at 1 h exceeded the MBCs for the experimental strain by 2.3-fold, while they were in the range of the MICs after 6 h. The half-life of trovafloxacin in serum was approximately 1 h in this model, thereby providing an explanation for the better results obtained with doses of trovafloxacin given every 6 h compared to every 12 h. The ratio of CSF to serum concentrations of amoxicillin at 1 h after injection (0.34) was similar to that of trovafloxacin (Table 4). CSF concentrations of amoxicillin exceeded the MBC by almost sevenfold at 1 h and by twofold at 6 h. CSF concentrations of trimethoprim exceeded the MIC by fourfold at 1 h and by threefold at 6 h, but the concentrations were always below the MBC. The mean ratios of CSF to plasma concentrations were 0.47 for SMX and 0.70 for TMP at 1 h after injection.

## DISCUSSION

In the present study, we have compared one of the newer quinolones, trovafloxacin, with two standard regimens for treating *Listeria* infection: (i) amoxicillin plus gentamicin and (ii) TMP-SMX. All treatment regimens exhibited antibacterial activities in the brains, livers, and blood of rats with CNS and systemic experimental listeriosis. However, amoxicillin plus gentamicin was more active in the brain than the comparison regimens. The inferior activity of trovafloxacin in the CNS may be related to the relatively low trovafloxacin concentrations achieved in CSF and perhaps in the brain. Fluoroquinolones exhibit concentration-dependent killing of *S. pneumoniae* and the area under the concentration-time curve/MBC ratio is likely to be an important predictive factor of bactericidal activity of these fluoroquinolones in CSF (14, 20). In our study, the peak concentration of trovafloxacin achieved in CSF exceeded the MBC by only 2.3-fold. Moreover, trovafloxacin concentrations in CSF remained above the MBC for only about half of the 6-h dosing interval. In rabbits with *Listeria* meningitis, we have found that the antibacterial activity of trovafloxacin increases with increased doses of the drug (28). Thus, it is likely that higher doses of trovafloxacin in the present study would have led to antibacterial activity comparable to that of amoxicillin plus gentamicin. However, the peak serum and CSF concentrations of trovafloxacin with the doses examined here were already twice those achieved in humans with standard doses and higher doses would be of questionable clinical relevance (1, 30). Serum and CSF concentrations of amoxicillin in the rat model were, on the other hand, very similar to those achieved in humans during therapy for severe infections by using high doses. It appears, therefore, that at clinically achievable concentrations trovafloxacin is inferior to amoxicillin plus gentamicin in eradicating *Listeria* organisms from the brain. In contrast, trovafloxacin is very active in cases of experimental meningitis caused by *S. pneumoniae*, including penicillin-resistant pneumococci (9), and is likely to be active against other meningeal pathogens for which MICs are even lower, such as *Neisseria meningitidis* ($MIC_{90}$ = 0.06 mg/liter).

TMP-SMX has been effective in humans and is currently considered the drug of choice in β-lactam-hypersensitive patients (27, 31). Somewhat surprisingly, TMP-SMX was the least active regimen tested in the present study, both in the brain and in the liver. Of the two drugs, only TMP had a relatively low MIC for *L. monocytogenes* (0.25 mg/liter for our experimental strain), and the in vitro activity of the combination was the same as that of TMP alone. TMP peak concentrations achieved in CSF exceeded the MIC by fourfold but were always below the MBC, and these low TMP concentrations achieved in the CSF were likely responsible for the limited efficacy of the drug in the present study.

We also tested the combination of trovafloxacin with TMP-SMX in search of an optimally active regimen that avoided β-lactams and so could be used in allergic patients. Unexpectedly, our data showed that the addition of TMP-SMX to trovafloxacin, while as effective in the brain as trovafloxacin used alone, reduced the activity of the quinolone in the liver. The reasons for this antagonistic effect in one but not another organ are not clear. We have previously shown that new fluoroquinolones and TMP-SMX in combination had an additive effect on intracellular *L. monocytogenes* in cell cultures (18). In vitro killing curves with concentrations corresponding to those achieved in plasma in our rat model (2 and 8 mg/liter for trovafloxacin and 4 and 8 mg/liter for TMP and 20 and 40 mg/liter for SMX in combination) demonstrated a synergistic bactericidal effect after 24 h (data not shown) and thus also failed to duplicate the findings in the liver.

The present model combines several important features of the disease in humans and allowed a detailed analysis of bacterial titers in the brain, blood, and liver (as a representative organ of the reticuloendothelial system). The histopathological changes observed in the brains of infected rats were similar to those observed in patients dying from the disease (6). Furthermore, bacteremia is an important feature in humans and was uniformly present in the infected rats despite the intracisternal route of infection. The ability of *L. monocytogenes* to invade cells, including endothelial cells, may have favored its spread from the CNS to the rest of the body, where high-titer organ involvement was established (2).

As the results of the present study document, the relative efficacy of an antibiotic regimen may differ from organ to organ. Supporting the current recommendations for the treatment of listeriosis in humans, the combination of an aminopenicillin with gentamicin was the most effective regimen tested here. Limited drug concentrations in the CNS relative to their in vitro activity may at least in part explain why both of the comparison regimens, trovafloxacin and TMP-SMX, were less effective. The moderate activity of TMP-SMX in this model is noteworthy in light of its generally accepted effective-

ness in humans. However, its potency in humans with listeriosis has not been rigorously compared to other treatment regimens, and differences to amoxicillin plus gentamicin cannot be ruled out. Further clinical studies will have to evaluate whether new fluoroquinolones offer an alternative to TMP-SMX in β-lactam-allergic patients.

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