Moxifloxacin versus ampicillin + gentamicin in the therapy of experimental *Listeria monocytogenes* meningitis

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Objectives: This study aimed to compare the antibacterial activity of moxifloxacin and ampicillin + gentamicin in the treatment of *Listeria monocytogenes* meningitis in a rabbit meningitis model.

Methods: Meningitis was induced by direct inoculation of a clinical strain isolated from an immunocompromised patient (10^7 cfu/mL) into the cisterna magna of New Zealand rabbits. After 16 h of incubation, rabbits were separated into four groups: moxifloxacin (M), ampicillin + gentamicin (A), ampicillin + gentamicin 2 (A2) and control (C). Group M received 20 mg/kg moxifloxacin at the end of the incubation time and 5 h later by intravenous (iv) route. Group A received ampicillin (30 mg/kg/h) and gentamicin (2.5 mg/kg/h) by iv route with continuous infusion for 8 h in 36 mL of 0.9% NaCl, group A2 received the same dosage of gentamicin and ampicillin in two different 36 mL 0.9% NaCl solutions and group C did not receive any treatment. Cerebrospinal fluid (CSF) samples (0.1–0.25 mL) were obtained 16 and 24 h after induction of meningitis.

Results: At the end of the 16 h of incubation, CSF bacterial counts were similar in all groups (P > 0.05). At the final stage of the study (24 h after induction of meningitis), bacterial counts in all treatment groups were significantly lower than the control group (P < 0.05). When the three treatment groups were compared, bacterial counts were found to be similar (P > 0.05).

Conclusions: These data suggest that antibacterial activity of moxifloxacin is similar to ampicillin + gentamicin in the treatment of experimental *L. monocytogenes* meningitis of rabbits.

Keywords: listeriosis, quinolones, rabbits, central nervous system infections

Introduction

*Listeria* spp. has become an important subject in medical research due to its significant status in medical microbiology, food microbiology and infectious diseases. This Gram-positive bacillus may be spread by contaminated food and may eventually cause outbreaks of listeriosis.1,2

Bacterial meningitis is a life-threatening disease. *Listeria monocytogenes* is an important pathogen causing meningitis and may cause disease in either immunocompromised or immunocompetent hosts.1,2

The main therapeutic choice in *Listeria* spp. meningitis is ampicillin + gentamicin, which has been shown to be synergic in an experimental meningitis model in rabbits.1,3 Moxifloxacin is effective against *L. monocytogenes in vitro* and in macrophages.4,5

There is no human or animal study comparing ampicillin + gentamicin versus moxifloxacin in *L. monocytogenes* meningitis. This study aimed to compare the antibacterial activity of moxifloxacin and ampicillin + gentamicin in the treatment of *L. monocytogenes* meningitis in an experimental rabbit meningitis model.

Methods

Test organism

The inoculum was a clinical strain isolated from the cerebrospinal fluid (CSF) of an immunocompromised patient. The strain was identified by conventional methods and automated systems (VITEK, bioMérieux, Marcy l’Etoile, France). MICs of ampicillin, gentamicin and moxifloxacin were 0.016, 1 and 0.5 mg/L, respectively (measured in duplicate using the Etest; AB BIODISK, Solna, Sweden).
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In vivo studies

Male white New Zealand rabbits weighing 2.75–3 kg were anaesthetized by intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) before each intraventricular intervention including induction of meningitis and CSF sampling. The duration of anaesthesia was 10–15 min.

Meningitis was induced by direct inoculation of $0.3\text{ mL}$ of physiological serum containing $10^7\text{ cfu/mL}$ L. monocytogenes into the cisterna magna of rabbits using a 22 G syringe (Hayat Ticaret, Istanbul, Turkey).

After 16 h of incubation, rabbits were separated into four groups: moxifloxacin (M), ampicillin + gentamicin (A), ampicillin + gentamicin (A2) and control (C). Group M received 20 mg/kg moxifloxacin (Bayer, Germany) at the end of the incubation time and 5 h later by intravenous (iv) infusion.$^{5,7}$ Group A received ampicillin (Mustafa Nevzat, Turkey; $30\text{ mg/kg/h}$) and gentamicin (IE Ulagay, Turkey; $2.5\text{ mg/kg/h}$) by iv route with continuous infusion (Abbott, Lifecare 5000 infusion pumps) for 8 h in $36\text{ mL}$ of $0.9\%\text{ NaCl}$. Group A2 received the same dosage of gentamicin and ampicillin in two different $36\text{ mL}$ $0.9\%\text{ NaCl}$ solutions with two different lines. Group C did not receive any treatment.

Meningitis criteria were as follows: fever (>$40\%\text{ C}$); CSF pleocytosis of $>1000\text{ cells}$ with $>96\%$ polymorphonuclear leucocytes; and a CSF bacterial count $>10^5\text{ cfu/mL}$.

CSF samples ($0.1–0.25\text{ mL}$) were obtained 16 and 24 h after induction of meningitis by puncture of the cisterna magna using a 22 G needle (Hayat Ticaret). At the end of the study period (24 h), animals were humanely killed by intravenous infusion of high dose nembutal.

The bacterial count in CSF was measured by standard serial dilutions of $10\text{ mL}$ of CSF in $0.9\%\text{ NaCl}$ and incorporation into sheep blood agar (Oxoid, Basingstoke, UK) pour plates. The limit of detection of bacterial counts was $2 \times 10^5\text{ cfu/mL}$.

The evaluation of bacteriological response was defined using three categories: full response, sterilization of CSF; partial response, any decrease in bacterial count; and bacteriological failure, a stable or increased bacterial count.

Statistical analysis

Data were evaluated by the SPSS 11.0 package program using Mann–Whitney U-test, Kruskal–Wallis test and Fisher’s exact test. A $P$ value <0.05 was considered significant.

Ethics

The study protocol was approved by the local Ethics Committee on animal studies (approval no. 2006-38).

Results

At the beginning of the study, 51 animals were inoculated with L. monocytogenes. Of these 51, 40 were alive at the end of the 16 h of incubation. These 40 animals were separated into four groups: group M, 11 animals; group A, 7 animals; group A2, 5 animals; group C, 17 animals. Due to the lack of adequate infusion pumps, groups A and A2 consisted of seven and five animals, respectively.

At 16 h, all rabbits had developed meningitis according to the above mentioned criteria. CSF bacterial counts were similar in all groups ($P > 0.05$) (Table 1). At 24 h (8 h after the end of the incubation time, i.e. end of the study), bacterial counts in groups M, A and A2 were significantly lower ($P < 0.05$) than group C, but there were no significant differences between the treatment groups (Table 1).

During the study, mortality among animals was similar in all three groups (group M, 2/11; group C, 2/12; group A, 0/7; and group A2, 0/5). When groups M, A and A2 were compared at 24 h, there was no full response in any of the treatment groups. The rate of partial bacteriological responses was similar (9/9 in group M, 6/7 in group A and 5/5 in group A2; $P > 0.05$). The decrease in bacterial counts in groups A, A2 and M was also similar ($-3.910 \pm 4.138$ versus $-4.312 \pm 0.896$ versus $-5.346 \pm 0.623$, $P > 0.05$).

Discussion

Despite developments in intensive care and antibiotherapeutic agents, meningitis is still associated with significant mortality and morbidity. L. monocytogenes is an important pathogen causing meningitis both in immunocompromised and immunocompetent hosts.

The primary treatment option recommended in L. monocytogenes meningitis is ampicillin + gentamicin. The most important evidence for this choice is the synergic effect of both drugs shown in the historical study of Scheld et al.$^5$ where they compared penicillin, ampicillin, gentamicin, rifampicin, penicillin + rifampicin, penicillin + gentamicin and ampicillin + gentamicin in a rabbit experimental meningitis model and proved that ampicillin + gentamicin was the most effective regimen. For patients allergic to β-lactams, co-trimoxazole has been recommended as a second choice for the treatment of intracranial as well as extracranial manifestations.$^1$

Despite available treatment modalities, prognosis of L. monocytogenes meningitis is still not favourable.$^{1,2}$ Levvidiotou et al.$^2$ reported three patients with L. monocytogenes meningitis who were treated with ampicillin + gentamicin but died a short time after the start of the treatment.

Moxifloxacin is a relatively new 8-methoxy quinolone antibacterial. Although its main indication is pneumonia of pneumococci, Haemophilus influenzae and Moraxella catarrhalis, it is also very effective in vitro against a variety of other Gram-negative and -positive bacteria including L. monocytogenes strains. Rolston et al.$^5$ compared the MIC$_{90}$ of moxifloxacin, ciprofloxacin and levofloxacin in 10 L. monocytogenes strains with broth dilution method; the MIC$_{90}$ for moxifloxacin was 0.12 mg/L whereas it was 0.5 mg/L for ciprofloxacin and 1 mg/L for levofloxacin. When compared with other quinolones,
moxifloxacin is effective against intracellular and extracellular *L. monocytogenes*. Carryn et al. compared the activities of ampicillin, meropenem, azithromycin, gentamicin, ciprofloxacin and moxifloxacin against intracellular haemolysin-positive *L. monocytogenes* in human THP-1 macrophages. They reported that in cells ampicillin, meropenem, azithromycin and ciprofloxacin were slightly bactericidal (0.3–0.8 log_{10} cfu/mL reductions), moxifloxacin was strongly bactericidal (2.1 log_{10} cfu/mL reduction) and gentamicin was virtually inactive.

To our knowledge, these are the first data comparing moxifloxacin and ampicillin + gentamicin in experimental *L. monocytogenes* meningitis. In previous *in vivo* studies, levofloxacin or moxifloxacin was shown to cure infected animals in mouse models. Schmidt et al. reported that moxifloxacin had similar antibacterial activity to ceftriaxone in a pneumococcal rabbit meningitis model. Moxifloxacin was also reported to have superior antibacterial activity to meropenem and similar antibacterial activity to ceftriaxone in an *Escherichia coli* rabbit meningitis model. Despite these data, to our knowledge, there is no reported case of meningitis treated with moxifloxacin but Viale et al. recently reported a case of *L. monocytogenes* meningitis treated with cefotaxime (switched to meropenem 2 days later) + levofloxacin.

Due to the problem of inactivation of gentamicin by ampicillin, the present study consisted of two ampicillin + gentamicin arms. Although both groups received the same dosage, group A received the antibiotics together in accordance with the study of Scheld et al. and group A2 received the antibiotics through separate lines. It was observed that different lines yielded a mean additional 0.4 log cfu/mL decrease in bacterial load.

Since antibacterial efficacies of β-lactams are rather time-dependent and quinolones are concentration-dependent, it would have been of major interest to have examined antibiotic efficacy over a longer period of time and to have tested different dosages but this was not possible because of economic reasons. Another criticism may be the continuous administration of gentamicin which is in contradiction with the pharmacodynamics of aminoglycosides. The reason we chose continuous infusion was to be in concordance with the study of Scheld *et al.*. In the present study, the decrease in the bacterial load in the moxifloxacin arm was ~1 log more than in the ampicillin + gentamicin arm (*P* > 0.05). Due to these reasons, if the gentamicin was given as multiple dosages or if the duration of treatment i.e. the duration of the experiment was extended, bacterial load decrease in the ampicillin + gentamicin arm would probably be more similar to the moxifloxacin arm. Other limitations of our study are the lack of pharmacokinetic data, which were not analysed since the main aim was to compare antibacterial efficacy of the drugs, the presence already of data related to the distribution of ampicillin, gentamicin and moxifloxacin in rabbits and economic reasons. Penetration of ampicillin and gentamicin into CSF of rabbits in *L. monocytogenes* meningitis (same methodology for the ampicillin + gentamicin arm of the present study) is 18.38 ± 2.69% and 26.82 ± 6.74%, respectively. AUC_{CSF}/AUC_{plasma} rate was reported to be 85% in *E. coli* experimental meningitis in rabbits with a similar moxifloxacin dosage and regimen used in the present study. Thus, it is possible that relatively higher penetration of moxifloxacin into CSF played a critical role in the 1 log additional decrease in the bacterial load of CSF in the moxifloxacin group.

In conclusion, our study suggests that the antibacterial activity of moxifloxacin is similar to ampicillin + gentamicin in the treatment of experimental *L. monocytogenes* meningitis of rabbits. The rabbit meningitis model has the disadvantage of not mimicking the normal pathophysiological process in humans. Thus, additional data should confirm our experiments in advance of clinical trials or experience to assess efficacy in humans. Such studies will help to see if our findings are translatable to clinical practice or not.

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