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# Correspondence

## Activity of β-lactams (ampicillin, meropenem), gentamicin, azithromycin and moxifloxacin against intracellular *Listeria monocytogenes* in a 24 h THP-1 human macrophage model

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Sir,

*Listeria monocytogenes* is a facultative intracellular organism causing persistent and life-threatening infections. Recurrence and dissemination of the infection is due to the lack of eradication of intracellular forms, particularly from phagocytes.[1] Currently recommended antibacterial therapy of listeriosis combines gentamicin and ampicillin (or meropenem).[2] Yet, gentamicin is inactive against the intracellular forms of *Listeria*, and ampicillin and meropenem are only bacteriostatic in a short-term (5 h) cell culture model.[3] This suggested to us that mechanisms other than intrinsic antibacterial properties could play a critical role *in vivo*, or that the model used did not sufficiently take into account the influence of time (a point of particular importance for β-lactams that are time-dependent antibiotics[4]). We have therefore examined the activity of ampicillin, meropenem and gentamicin against extracellular and intracellular *L. monocytogenes* in a 24 h model. For the sake of comparison, we also examined azithromycin (ineffective against intracellular *Listeria* in a short-term model in spite of a rapid and very large cellular accumulation) and moxifloxacin (markedly bactericidal at 5 h towards both extracellular and intracellular bacteria).[3]

Time–kill studies were carried out as described previously.[3] For determination of activity against extracellular bacteria, cultures were used at a density of $10^6$ cfu/mL in tryptic soy broth. Antibiotics were then added at a fixed concentration corresponding to the highest serum concentration ($C_{max}$) currently observed after conventional administration to patients.[3] Incubation was carried out for 5 and 24 h, and cfu were determined by plate assay.

As shown in Figure 1(a), ampicillin and meropenem acted very slowly and were only slightly bactericidal (0.8 and 0.7 log cfu decrease) at 24 h. In contrast, gentamicin and moxifloxacin were highly bactericidal (gentamicin achieving complete eradication within 5 h). Azithromycin slowed down bacterial growth at 5 h but was without effect at 24 h.

For measurement of activity against intracellular *L. monocytogenes*, macrophages ($5 \times 10^5$/mL) were exposed for 1 h to bacteria ($2.5 \times 10^6$ cfu/mL), washed extensively, and incubated for 5 and 24 h with or without antibiotics. Cells were collected, washed, lysed in distilled water and the number of viable bacteria determined by plate assay. Cell protein was determined in parallel.

As shown in Figure 1(b), ampicillin and meropenem again acted slowly but were definitely bactericidal at 24 h (2 log reduction of cfu). In sharp contrast with what was observed in broth, gentamicin caused only a modest slow down of the rate of growth of intracellular bacteria. Azithromycin was bacteriostatic both at 5 and 24 h. Moxifloxacin was bactericidal at 5 h (2 log cfu reduction) and its effect was increased at 24 h (3.2 log reduction).

The present data extend our previous studies on activities of antibiotics in *Listeria*-infected THP-1 macrophages,[3] and bring several new pieces of information of both pharmacological and clinical significance.

First we show that gentamicin does not control the growth of intracellular *L. monocytogenes* for at least 24 h, even though it is highly and very quickly bactericidal against extracellular forms. This could result from the inappropriate subcellular localization of gentamicin (intracellular aminoglycosides being largely if not exclusively sequestered in lysosomes,[5] whereas *L. monocytogenes* multiplies in the cytosol). The usefulness of gentamicin seems therefore limited to eradication of the extracellular forms of *Listeria*.

Secondly, the data indicate that azithromycin will be ineffective against *L. monocytogenes in vivo* not only against extracellular bacteria [mainly due to its unfavourable $C_{max}$/MIC ratio (<1)], but also against intracellular forms. This probably results from the fact that azithromycin is intrinsically bacteriostatic, making its cellular accumulation largely useless in this context.

Thirdly, both ampicillin and meropenem kill intracellular bacteria more rapidly and more extensively than extracellular bacteria, even though they do not accumulate in THP-1 cells.[3] Yet, β-lactams penetrate macrophages where they are distrib-

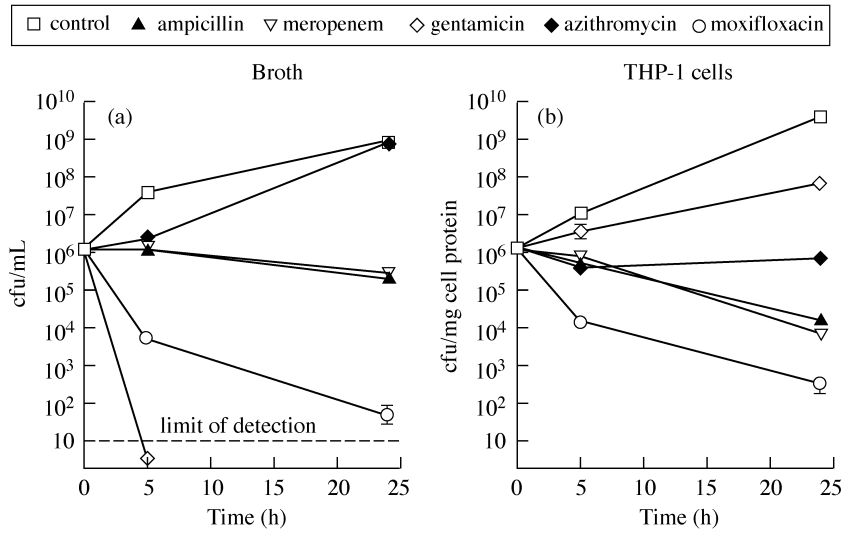


**Figure 1.** Activity of antibiotics towards *L. monocytogenes* [as determined by colony counting (cfu)] upon incubation at a fixed concentration corresponding to the maximal concentration ($C_{max}$) commonly observed in serum after administration of conventional doses to humans (ampicillin and meropenem 50 mg/L; gentamicin 18 mg/L; azithromycin 0.4 mg/L; moxifloxacin 4 mg/L[3]). Data are shown as arithmetic means ± S.D. ($n = 3$; when not visible, the error bars are smaller than the symbols).

uted into the cytosol.[5] This should bring them into direct contact with their target in the case of *Listeria*. The present data indicate therefore some sort of enhancement of the activity of β-lactams by the intracellular milieu compared with broth. Future studies will be needed to distinguish between a modification of the intrinsic pharmacological properties of β-lactams and a direct co-operation between host defences and β-lactams for bacterial killing. It nevertheless clearly appears that prolonged treatment with ampicillin or meropenem may contribute to the eradication of the intracellular forms of *L. monocytogenes* (as was also observed in another model[6]).

Finally, we confirm that moxifloxacin is, so far, the most active antibiotic towards both the extracellular and intracellular forms of *L. monocytogenes*. Therefore, animal trials with moxifloxacin for eradication of *L. monocytogenes* appear justified.

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