Increased susceptibility of intracellular *Listeria monocytogenes* to ampicillin: studies with Caco-2 cells and THP-1 macrophages

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ABSTRACT

Objectives : Invasion and intracellular survival of *Listeria monocytogenes* in enterocytes and macrophages are two critical determinants in both growth and further spreading of this bacterium in human. The use of antibiotics is essential because cell host defences are impaired in immunocompromised patients. We have compared the antibacterial activities of ampicillin towards extracellular and intracellular forms of *Listeria monocytogenes* using human cellular models (enterocytes Caco-2 cells; THP-1 macrophages).

Methods : Infection of Caco-2 cells and THP-1 macrophages was performed as previously described (JID 180:1195-204; JAC 51:1051-1052). Activities, after 24h in broth (Δlog (CFU/mL)) and cells (Δlog (CFU/mg of cell proteins)) were compared at a fixed ampicillin concentration of 50µg/mL (Cmax in human serum after conventional dosing).

Results:

	Broth	THP-1	THP-1
Control	3.06 ± 0.07	3.51 ± 0.07	4.09 ± 0.06
Ampicillin	-0.79 ± 0.03	-1.92 ± 0.06*	-1.81 ± 0.18*

All values are means SEM (n=3 independent experiments)
* p<0.05 (Student t test) ampicillin in cell line versus broth

Thus, ampicillin is more cidal towards intracellular than extracellular *Listeria monocytogenes*. Addition of inhibitors of cell defence mechanisms to THP-1 macrophages (L-NAME (400µM)/catalase (1500U/mL) (JID 180:1195-204), ambroxol (100µM) (JPET 300:629-637), dexamethasone (10-7M) (JCI 82:913-919) and leupeptine (100µM)/pepstatine (100µM) (Neuroscience 91:233-249)) did not decreased the antibacterial effect of ampicillin.

Conclusions: *Listeria monocytogenes* is more susceptible to ampicillin intracellularly than extracellularly. This observation justifies the use of ampicillin to control *Listeria* infection, but his mechanism remains unclear and needs to be investigated.

INTRODUCTION

L. monocytogenes is an intracellular bacteria responsible for life-threatening infections in immunocompromised patients and pregnant women. The current therapeutic treatment consists in the combination of ampicillin and an aminoglycoside.

The colonization and dissemination of the bacteria in vivo depend on its capacity to penetrate and to grow inside cells, to further spread in a adjacent cell. It is therefore important to use antibiotics able to control intracellular forms of the bacteria to avoid its rapid dissemination.

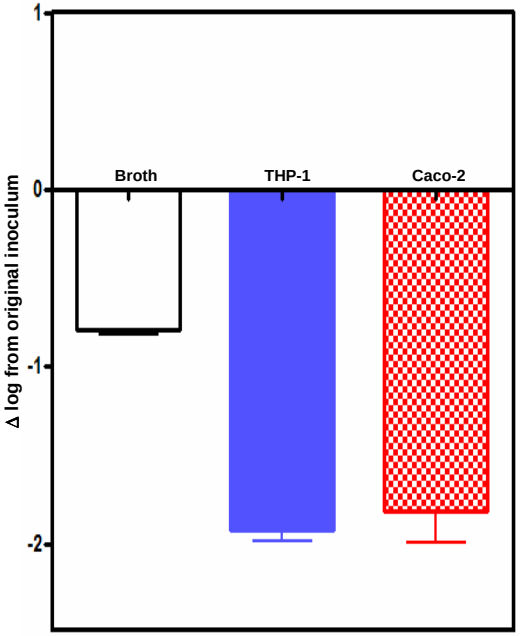
We have recently observed that after 24h, ampicillin shows a bactericidal activity against intracellular forms of *L. monocytogenes* in THP-1 macrophages while it is only static against the extracellular forms (studies in broth) (1). This surprising observation may denote a cooperation between the antibiotic and the cell defense mechanisms.

AIM OF THE STUDY

- To evaluate whether the bactericidal intracellular activity of ampicillin is due to a cooperation with cell defense mechanism by
  - Comparing the intracellular activity of ampicillin in phagocytic (THP-1) and non phagocytic (Caco-2) cells with the extracellular activity.
  - Repressing the immune response of THP-1 macrophages using a series of specific inhibitors.

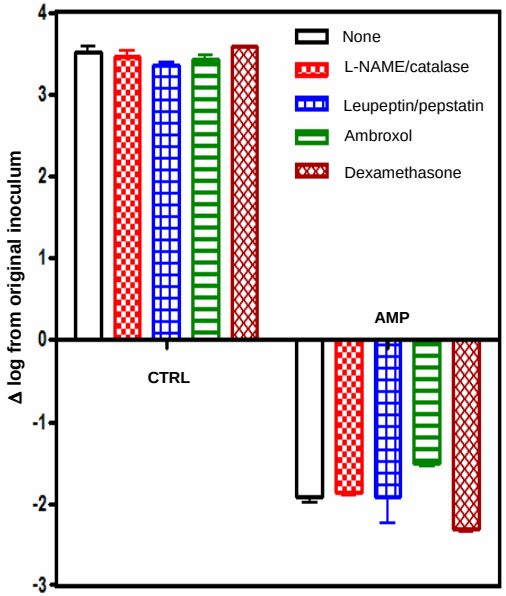
RESULTS

1. Comparison between activity of ampicillin against extracellular and intracellular *Listeria monocytogenes*



1. Change in the number of viable *L. monocytogenes* in broth (TSB) or in infected cells (THP-1 and Caco-2) after 24h incubation in the presence of ampicillin (AMP). The drug is added at an extracellular concentration corresponding to the Cmax measured in the serum of patients (50 mg/L). For experiments with cells, the culture medium was added by 10 % decomplemented fetal calf serum. Data are the mean ± SEM of 3 independent experiments.

2. Effect of inhibitors in THP-1 infected cells



2. Change in the number of viable *L. monocytogenes* in infected cells after 24h incubation in the absence of antibiotic (CTRL) or with ampicillin (AMP). Inhibitors are added 24h before the infection and maintain all the time of infection. The ampicillin is added at an extracellular concentration corresponding to the Cmax measured in the serum of patients (50 mg/L). The culture medium was added by 10 % decomplemented fœtal calf serum.

Based on literature, the agents used would act on the following mechanisms:
- L-NAME/Catalase : inhibition of the oxidative burst (4)
- Leupeptin/pepstatin : lysosomal proteinase inhibitor (5)
- Ambroxol : inhibition of the production of superoxide, hydrogen peroxide, and nitric oxide and of the release of acid phosphatase and lysozyme (6)
- Dexamethasone : inhibition of the antimicrobial activity of mononuclear phagocytes(7)

METHODS

- Extracellular activity was assayed by CFUs counting after 24h exposition to the antibiotic in TSB (1,2).
- Intracellular activity was measured after 24h of incubation of THP-1 human macrophages and of Caco-2 cells infected with an initial inoculum of 5 bacteria/cell. The number of CFUs in cell lysates was determined and the results were expressed by reference to the sample protein content (1,2,3).
- To test the different inhibitors, cells were incubated with 400µM of L-NAME and 1500U/mL of catalase, 100µM of leupeptine and pepstatine, 100µM of ambroxol or 2.5 10^{-7}M of dexamethasone during 24h prior the infection and during the 24h of incubation after infection.

CONCLUSIONS

- In broth, ampicillin is essentially bacteriostatic (0,5 log decrease from the initial inoculum).
- In infected cells, ampicillin is bactericidal (2 log decrease from the initial inoculum), with no noticeable difference between the phagocytic and non phagocytic cell line.
- None of the agents used to reduce the immune response of THP-1 cells is able to modify the intracellular bactericidal activity of ampicillin.

➢ The increased activity of ampicillin against intracellular versus extracellular Listeria monocytogenes is probably not due to a cooperation with cell defense mechanisms, but may be related to an increased susceptibility of the bacteria in the intracellular environment.

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