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

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**ABSTRACT**

**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*

2. Effect of inhibitors in THP-1 infected cells

**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 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.

**REFERENCES**


**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.