

# PMA activation increases accumulation and activity against intracellular *Listeria monocytogenes* in THP-1 cells of ciprofloxacin and moxifloxacin but not of levofloxacin and garenoxacin

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## ABSTRACT

**Objectives :** Quinolones are known to accumulate in eucaryotic cells and to have activity against intracellular *Listeria monocytogenes*. PMA (activator of PKC) differentiates THP-1 monocytes into macrophages but does not induce any listericidal activity in these cells (AAC 40:1225-30).

**Methods :** We measured the cellular accumulation (CC/CE) and the activity against intracellular *L. monocytogenes* in THP-1 human cells (AAC 43:1242-51) activated or not by PMA of 3 fluoroquinolones (ciprofloxacin, moxifloxacin, levofloxacin), and 1 desfluoroquinolone (garenoxacin) at extracellular concentrations equivalent to the C<sub>max</sub> achievable in human serum after conventional treatment.

**Results:** The table shows the CC/CE and the intracellular activity [IA, Δlog (CFU/ mg of cell protein)(5h-0h)] of ciprofloxacin, moxifloxacin, levofloxacin, and garenoxacin.

	Cc/Ce		IA	
	CONTROL	PMA	CONTROL	PMA
ciprofloxacin	4.2 ± 0.1	7.2 ± 0.8 *	-0.2 ± 0.1	-0.8 ± 0.2 *
moxifloxacin	9.3 ± 0.4	13 ± 0.6 *	-1.3 ± 0.1	-2.1 ± 0.3 *
levofloxacin	7.2 ± 0.9	7.1 ± 0.9	-0.9 ± 0.1	-1.1 ± 0.3
garenoxacin	5.0 ± 0.6	4.8 ± 0.6	-1.1 ± 0.1	-1.0 ± 0.1

All values are mean ± SEM (n=3 independent experiments). \* P < 0.05 (Student t test)

PMA activation increases the cellular accumulation of ciprofloxacin and moxifloxacin (1.7 and 1.4x, respectively) and the intracellular activity of ciprofloxacin and moxifloxacin, but does not modify the properties of levofloxacin and garenoxacin.

**Conclusions:** Increases in intracellular activity of CIP and MXF are correlated with an increase in accumulation, which is consistent with their concentration-dependency, but, while properties of LVX and GAR are not modified. Pharmacodynamic properties can be differentially influenced by the state of activation of the macrophages even in the same class of antibiotic.

## INTRODUCTION

*Listeria monocytogenes* is the causative agent of listeriosis. Its penetration, growth in cells and further cell-to-cell spread are the key points of the infection. In spite of the currently available treatments (ampicillin + aminoglycoside), mortality is still high (30%). More active alternative treatments will therefore be welcome.

In our laboratory, we have already shown that quinolones have a bactericidal activity against intracellular *Listeria monocytogenes*. This activity seems to be in correlation with the fact that quinolones are able to accumulate in eukaryotic cells (1, 2, 3).

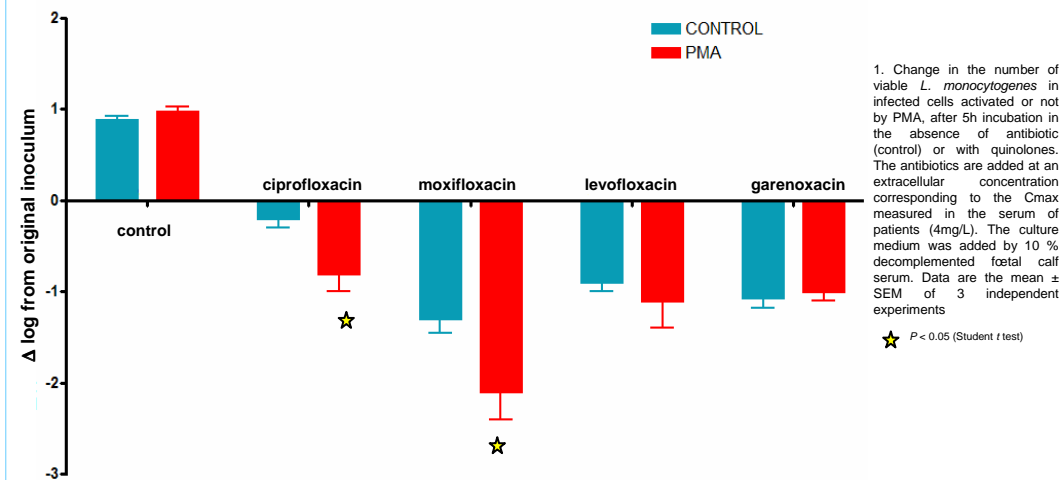
The cell status may also influence the ability of the bacteria to multiply intracellularly and/or the activity of antibiotics. It is for example possible to differentiate THP-1 monocytes in macrophages by incubating them in the presence of PMA (Phorbol 12-Myristate 13-Acetate), an activator of PKC (4).

## AIM OF THE STUDY

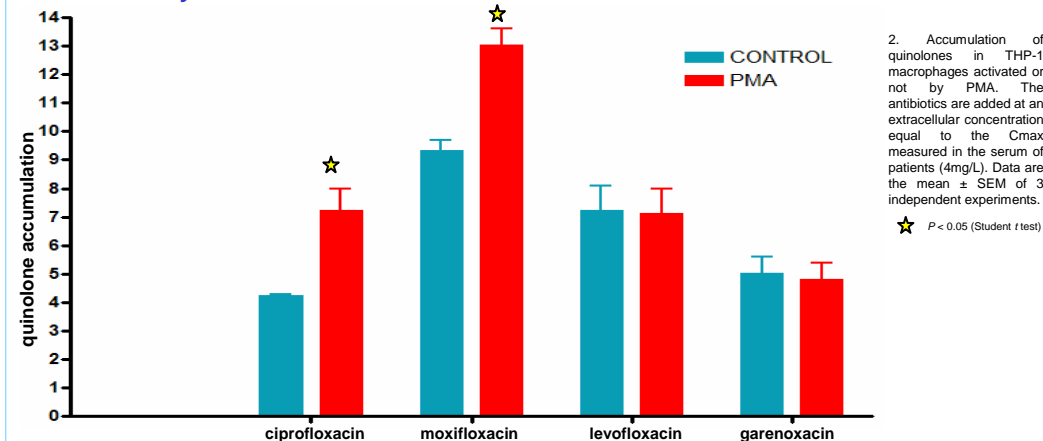
- To determine the intracellular activity of quinolones against *Listeria monocytogenes* in THP-1 cells unactivated or activated by PMA
- To compare the accumulation of quinolones in THP-1 cells with or without activation by PMA

## RESULTS

### 1. Intracellular activity of quinolones in THP-1 macrophages activated or not by PMA



### 2. Comparison between accumulation of quinolones in THP-1 cells unactivated or activated by PMA



## METHODS

- Activation of THP-1 was obtained by the addition of 0,16 μM of PMA during 24h and is followed by the infection (1).
- Intracellular activity was measured after 5 h of incubation of THP-1 human macrophages 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 (3).
- Quinolones were assayed by fluorimetric assay for ciprofloxacin, moxifloxacin and levofloxacin and by radiometric assay for garenoxacin after 2h of incubation. The accumulation is the ratio between the cellular concentration and the extracellular concentration (2).

## CONCLUSIONS

- Quinolones are able to control (ciprofloxacin) or to reduce (levofloxacin < garenoxacin < moxifloxacin) the intracellular inoculum of *Listeria monocytogenes*.
- The activity of ciprofloxacin and moxifloxacin is increased in cells activated by PMA ; enhanced activity is thus related to an increase in their accumulation.
- Neither the activity nor the accumulation of levofloxacin and garenoxacin are affected by PMA.

➤ The data confirm the concentration-dependent character of the intracellular activity of quinolones against *Listeria monocytogenes*. Further studies are needed to elucidate the mechanism by which PMA increases ciprofloxacin and moxifloxacin accumulation.

## REFERENCES

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