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Influence of Oritavancin (ORI) on Phagocytosis, Generation of Reactive Oxygen Species (ROS) and Killing of Intracellular *S. aureus* in Murine and Human Macrophages (MP). Comparison with Azithromycin (AZM) and Vancomycin (VAN)

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Abstract

Objectives: The lipoglycopeptide ORI accumulates in MP (AAC 2004; 48:2853-60) and is highly active against phagocytized *S. aureus* (AAC 2006; 50:841-51). The influence of this accumulation on MP functions has not yet been examined. We have studied the effect of ORI on phagogytosis and production of ROS in parallel to its intracellular activity using VAN (low accumulation) and AZM (high accumulation) as comparators.

Methods: Murine J774 and human PMA-activated THP-1 MP were incubated for 3 h with ORI (0-50 µg/mL; human free C_{max}: 25 mg/L for 1200 mg single dose), VAN or AZM (0-100 mg/L) and used to measure phagocytosis of serum-opsonized *P. aeruginosa* PA01 (CFU counting [ORI MIC > 32 µg/mL]) or fluorescent latex beads (LB), and ROS production (cell-permeant dye CM-H_2DCFDA). Intracellular activity was measured against *S. aureus* ATCC 25923 (MIC ORI 0.06 µg/mL).

Results: ORI did not affect PA01 phagocytosis at any tested concentration. ROS production by J774 MP increased at 50 µg/mL ORI. ORI at 25 and 50 µg/mL reduced LB phagocytosis, mainly in J774 MP. Intracellular killing of *S. aureus* was concentration-dependent, reaching bactericidal effect (3 $\log_{10}$ reduction) at 25 µg/mL ORI.

VAN and AZM did not affect phagocytosis or ROS production (within 10 % of control) and were static against intracellular *S. aureus*.

Parameters studied	Cell type	ORI extracell. conc. (µg/mL)									
		0		1		10		25		50	
PA01 Phagocytosis[a]	J774	100	0.1	99.9	0.1	99.9	0.1	99.9	0.1	99.9	0.1
	THP-1	100.0	2.5	nd		115.9	10.5	99.2	1.6	95.0	2.0
LB Phagocytosis[b]	J774	100.0	7.6	125.3	8.4	106.5	7.1	40.6	17.9	45.3	19.4
	THP-1	100.0	5.5	96.4	10.7	nd		76.1	12.8	73.5	2.9
ROS production[c]	J774	100.0	4.9	97.8	4.1	107.7	6.7	104.7	3.9	189.7	19.4
	THP-1	100.0	5.6	103.5	1.2	104.4	1.1	112.5	5.7	113.7	1.5
intracellular *S. aureus killing* [d,e]	J774	3.0	0.2	2.6	0.1	-0.7	0.2	-3.0	0.2	-4.3	0.1

[a] % of control (10^6 CFU/mg cell. prot)
[b] % of control (10^6 beads/mg of cell. prot)
[c] % of control (25 min)
[d] Δ log cfu at 24 h from time 0
[e] value for THP-1 macrophages at 50 µg/mL: ~ 3 $\log_{10}$ cfu decrease
nd, not determined

Conclusions: At extracellular concentrations up to 25 µg/mL, oritavancin did not affect MP capacity to internalize microbes or the MP oxidative burst. Phagocytosis of inert LB was modestly impacted by ORI in human MP. The bactericidal activity of ORI against intracellular *S. aureus* was confirmed in both MP cell types.

Background and aim

The lipoglycopeptide ORI, which accumulates to high levels in the lysosomes of macrophages[1], shows bactericidal activity towards phagocytised *S. aureus* (including Methicillin- and Vancomycin-Resistant isolates)[2-3] while vancomycin is only poorly effective.

However, in vitro, the high cellular accumulation of oritavancin causes a mixed-lipid storage in lysosomes and related vacuoles[4], which could impair host defenses mechanisms.

The aim of this study was to investigate whether ORI may affect the capacity of phagocytosis and the oxidative burst (production of Reactive Oxygen Species [ROS]) in murine and human macrophages exposed to clinically relevant concentrations of ORI. Intracellular activity of ORI was studied in parallel.

Azithromycin (AZM, high cellular accumulation) and vancomycin (VAN, low cellular accumulation) were used as comparators.

Methods

Cell lines. Murine J774 and PMA-activated human THP-1 cells were incubated for 3h in medium containing increasing concentrations of antibiotic (ORI, 0-50 µg/mL [insolubility of oritavancin in the growth medium prevented testing concentrations above 50 µg/mL]; VAN and AZM, 0-100 µg/mL). For ORI experiments, the culture medium was supplemented with 0.002 % Tween-80 (to avoid absorption of the drug on plastic surfaces)[5].

Phagocytosis of *P. aeruginosa* PA01. Phagocytosis of *P. aeruginosa* (oritavancin MIC > 32 µg/mL) was determined in cells washed free from antibiotic using phosphate buffered saline (PBS) and incubated for 1 h in the presence of the serum-opsonized bacteria (using a bacteria-per-macrophage ratio of 50) to allow phagocytosis. Elimination of adherent but non-phagocytized bacteria was achieved by incubation with 50 µg/mL gentamicin for 45 min. Gentamicin was then eliminated by 3 successive washings with PBS. Cells were finally lysed, harvested by scraping, and enumeration of viable bacteria was performed after appropriate dilution by spreading on TSA plates and overnight incubation at 37°C.

Uptake of red fluorescent latex beads. Uptake of carboxylate-modified red fluorescent latex beads (2 µm in diameter; 270 particles/mL) was assessed by fluorimetry as described previously[6].

Production of intracellular Reactive Oxygen species (ROS). ROS were detected using 5 µM 5-(and-6)-chloromethyl-2'-7'-dichloro-hydrofluorescein diacetate acetyl ester (CM-H2DCFDA; Invitrogen). Briefly, cells were washed free from antibiotic, loaded with the fluorescent probe (5 µM, 37°C, 5 % CO_2) for 30 min, and then reincubated for 30 min in medium (to allow desterification of the probe). Intracellular ROS production was induced by addition of 0.5 % H_2O_2 and fluorescence intensities were measured using emission and extinction wavelength set at 485 and 530 nm, respectively (Packard fluorocount).

ORI intracellular activity towards *S. aureus* ATCC 25923. Cell infection and determination of antibiotic activity was performed as described earlier[7].

Results-1

- Concentration – Effects for Oritavancin

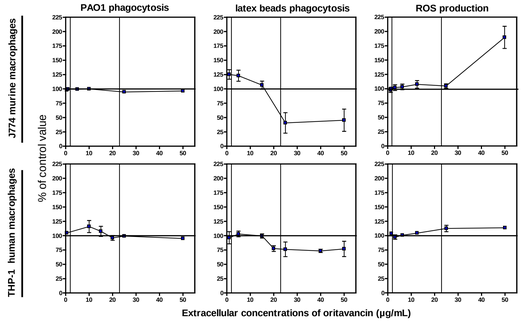

Figure 1. Influence of ORI concentration on phagocytosis of *P. aeruginosa* PA01 [left panel] and of inert Latex beads [middle panel], and on production of ROS [right-panel] in murine (top) and human (bottom) macrophages (3 h exposure to the antibiotic). Data are means SD of three independent determinations. The zones highlighted in blue correspond to the range of free serum concentrations reached in humans (based on PK data for an IV administration of 1200 mg[8].

Exposure of J774 and THP-1 macrophages to increasing concentrations of oritavancin
- did not affect phagocytosis of *P. aeruginosa* PAO1 in both cell types,
- decreased latex beads phagocytosis and increased ROS production, but only at concentrations of the same order of magnitude (for latex beads) or still higher (for ROS production) than the free human Cmax. Yet, these effects were less pronounced in human cells.

- Comparison of Antibiotics at Fixed Concentration

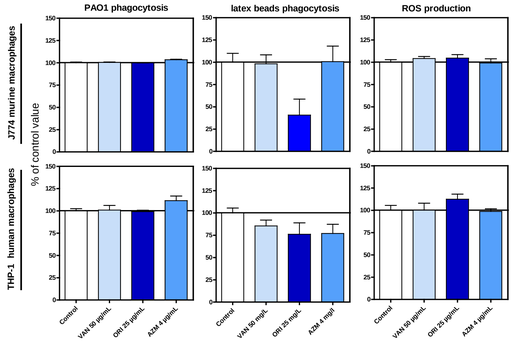

Figure 2. Influence of antibiotics on phagocytosis of *P. aeruginosa* PA01 [left panel] and of inert latex beads [middle panel], and on production of ROS [right panel] in murine (top) and human (bottom) macrophages (3 h exposure to the antibiotic at a fixed, clinically-relevant concentration (human Cmax; 50 mg/L [VAN], 25 mg/L [ORI, free Cmax for an IV administration of 1200 mg] and 4 mg/L [AZM]). Data are means SD of three independent determinations.

Exposure of J774 and THP-1 macrophages to clinically meaningful concentrations of oritavancin, vancomycin, or azithromycin had no effect on the capacity of macrophages to internalize PAO1 or to generate ROS.
Oritavancin markedly decreased the uptake of latex beads in murine macrophages only.

Results-2

- Intracellular activity of antibiotics against *S. aureus*

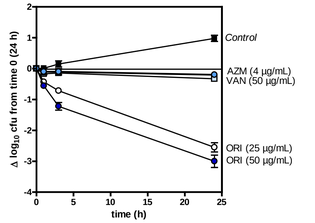

Figure 3. Time-kill curves of AZM (4 µg/mL), VAN (50 µg/mL) and ORI (25 and 50 µg/mL) towards phagocytized *S. aureus* strain ATCC 25923 (murine J774 macrophages). Results are mean SD of three independent determinations.

MICs : AZM, 0.5 µg/mL ; VAN, 1 µg/mL; ORI, 0.06 µg/mL.

In contrast to azithromycin and vancomycin that are only bacteriostatic, oritavancin was bactericidal towards intraphagocytic *S. aureus*

Conclusions

- At clinically meaningful concentrations (25 µg/mL), oritavancin does not affect the capacity of macrophages to internalize microbes (as demonstrated using *P. aeruginosa* PA01) or to generate ROS.
- Oritavancin reduces the phagocytosis of inert latex beads mainly in mouse macrophages and only at high concentration. This occurs however without impairing their accessibility to lysosomes[9]. The clinical significance of this finding, if any, is unclear, since the incidence and severity of adverse events possibly related to immunosuppression in Phase 3 studies were not different between vancomycin and oritavancin. Differences in the effects observed on latex beads and PAO1 phagocytosis may be related to bacterial opsonization (receptor-mediated phagocytosis).
- Oritavancin is bactericidal against intraphagocytic forms of *S. aureus*, in contrast to vancomycin and azithromycin.

References

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