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Cellular pharmacokinetics (accumulation, subcellular distribution, and efflux) of the FabI inhibitor Debio 1452 in J774 mouse macrophages

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Background & Aim

- Debio 1452 (AFN 1252), a first-in-class inhibitor of bacterial fatty acid synthesis, shows selective antibacterial activity against *Staphylococci* [1]. Its prodrug Debio 1450 (afabicin) has completed Phase II in acute bacterial skin and skin structure infections and is presently investigated for the treatment of bone and joint infections [2].
- Intracellular reservoirs of *S. aureus* play a critical role in the persistence of many of these infections [3]. We previously showed that Debio 1452 is active against *S. aureus* infecting phagocytic cells (ECCMID 2016; P1334).
- Our aim was to study the uptake and subcellular disposition of Debio 1452 in macrophages.

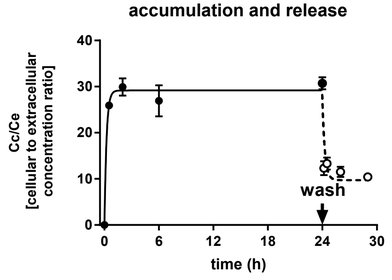
Materials & Methods

- **Kinetics**: J774 mouse macrophages were incubated with 1.4 mg/L ^{14}C-Debio1452 for up to 24h (accumulation), washed and returned to drug-free medium for up to 6h (efflux). Drug concentration was measured by scintillation counting and normalized based on cell protein content.
- **Subcellular localization**: cells were incubated for 90 min with 1 mg/L ^{14}C-Debio 1452 at 37^{O}C and collected after washing at 4^{O}C. They were homogenized, and a cytoplasmic extract (E; free of nuclei and unbroken cells) subjected to high speed isopycnic centrifugation through a sucrose gradient for separation of the main subcellular organelles. Radioactivity, proteins and marker enzymes (lactate dehydrogenase [LDH; cytosol]; N-acetyl-β-hexosaminidase [NAB; lysosomes], and cytochrome *c*-oxidase [CytOx; mitochondria]) were assayed in the collected fractions.

Details on all these procedures have been previously described and are available from [4] and in the references cited therein.
See the Results for a pictorial view of the cell fractionation methods.

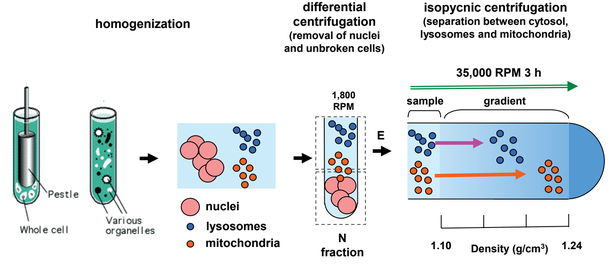
Results

Kinetics of accumulation and efflux



Debio 1452 accumulates quickly in cells (equilibrium reached within 2h) and is also released quickly (at 37°C but in an incomplete fashion (stable binding to intracellular constituents ?).

Intracellular disposition: 1. methods



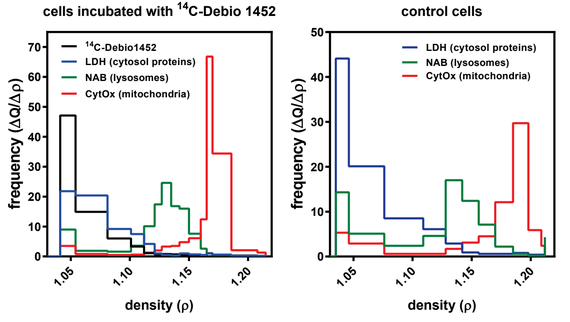
- Separation of nuclei and unbroken cells is monitored by phase contrast microscopy.
- Cytosol and organelles are detected by marker enzymes:
  - cytosol: lactate dehydrogenase (LDH)
  - lysosomes: N-acetyl-β-hexosaminidase (NAB)
  - mitochondria: cytochrome *c*-oxidase (CytOx)

Intracellular disposition: 2. E – N distribution

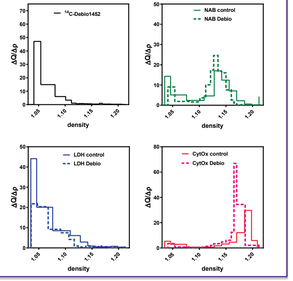
constituent	%	
	E	N
[^{14}C]-Debio 1452	69.4	30.6
LDH (cytosol)	68.9	31.1
NAB (lysosomes)	66.0	34.0
CytOx (mitochondria)	70.5	29.3

Debio 1452 is collected in the N fraction to the same percentage as the marker enzymes of the cytosol and cytoplasmic organelles, ruling out its specific association with nuclei.

Intracellular disposition: 3. cytosol *vs.* organelles



Upper graphs:
Debio 1452 distributes essentially like LDH (cytosol) with no stable association with lysosomes or mitochondria.



Graphs to the right:
Debio 1452 did not affect the distribution of LDH and only minimally altered those of NAB (lysosomes) or of CytOx (mitochondria) suggesting no major change in composition and sucrose permeability of the latter two organelles

Main points and Discussion

- Debio 1452 accumulates in J774 cells to a larger extent than most antibiotic classes (β-lactams, fluoroquinolones, aminoglycosides, vancomycin, or oxazolidinones), except macrolides or lipoglycopeptides [5,6].
- Debio 1452 was not found associated with subcellular organelles. This suggests that it can diffuse and/or redistribute throughout the cell, as previously observed for fluoroquinolones [7], oxazolidinones [8], or gepotidacin [4].
- These findings may explain the activity of Debio 1452 against intracellular *S. aureus* thriving into phagolysosomes.

References

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Acknowledgments and Transparency Declaration

This work was supported by Debiopharm. A.V.W. was a student; F.P. and G.D. are employees of the *Université catholique de Louvain* and of Debiopharm, respectively; FVB is *Directeur de Recherches* of the *Fonds de la Recherche Scientifique* (F.R.S.-FNRS); PMT is emeritus and was unpaid.

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