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P1523 Cellular pharmacokinetics (accumulation, subcellular distribution, efflux) of the FabI inhibitor Debio 1452 in J774 macrophages

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Background: Debio 1452, a first-in-class inhibitor of bacterial fatty acid synthesis, shows selective antibacterial activity against *Staphylococci.* 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. Intracellular reservoirs of *S. aureus* play a critical role in the persistence of such infections. We previously showed that Debio 1452 is active against *S. aureus* infecting phagocytic cells (ECCMID 2016; P1334). The present work aims at studying its cellular disposition in macrophages.

Materials/methods: Kinetics: J774 mouse macrophages were incubated with 1.4mg/L ^{14}C-Debio 1452 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 incubated 90min with 1 mg/L ^{14}C-Debio 1452 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 [cytosol]; N-acetyl-β-hexosaminidase [lysosomes], and cytochrome-*c*-oxidase [mitochondria]) were assayed in the collected fractions.

Results: Debio 1452 rapidly accumulated in cells to reach a plateau at an apparent cellular-to-extracellular concentration ratio of 29 in approx. 1 h (k_{in} [one phase exponential association]: 4.4 h^{-1}). Efflux proceeded also at a fast rate (k_{out} [one phase exponential decay]: 3.6 h^{-1}) but was only partial after 6 h (residual cellular-to-extracellular concentration ratio: 10). Cell-associated Debio 1452 co-distributed with the cytosolic enzyme lactate dehydrogenase, with no evidence of stable association with lysosomal or mitochondrial enzymes.

Conclusions: The apparent cellular accumulation of Debio 1452 is higher than that of most antibiotic classes (β-lactams, fluoroquinolones, aminoglycosides, vancomycin, or oxazolidinones) in the same model. The fact that Debio 1452 was not found associated with subcellular organelles after cell fractionation suggests an ability to diffuse and/or redistribute throughout the cell, as previously observed for fluoroquinolones (JAC 1990;26 Suppl B:27) or oxazolidinones (AAC 2015;59:178-185), which may explain its activity on intracellular *S. aureus* thriving into phagolysosomes.