Accumulation, Release and Subcellular Distribution of the Peptide Deformylase Inhibitor GSK1322322 in Murine Macrophages (J774) and Human Monocytes (THP-1)

F. Peyrusson1, G. Huys1, F. Van Bambeke1, D. Butler2, P.M. Tulkens1

1 Université catholique de Louvain, Bruxelles, Belgium, 2 GSK, Collegeville, PA, United States

Results

Background

Staphylococcus aureus remains a major cause of community and nosocomial infections, due in part to the ability of this organism to acquire resistance mechanisms to most recommended antibiotics (1) as well as to take refuge intracellularly (2). In this context, it is therefore essential (i) to foster the discovery and development of novel antibiotics with mode(s) of action distinct from those in current use, and (ii) to assess the activity of these molecules against intracellular S. aureus.

We presented at the 24th European Congress of Clinical Microbiology and Infectious Diseases (3) data showing that the novel inhibitor of peptide deformylase GSK1322322 (4) (see structure in Figure 1) is active against intracellular S. aureus including strains resistant to other antibiotics.

In the present study, we have examined the cellular accumulation and efflux of GSK1322322 using cultured murine J774 (5) and human (THP-1) macrophages. We also examined its subcellular localization including in infected cells (THP-1 cells only).

Methods

GSK1322322 accumulated rapidly at 37°C (stable cellular to extracellular concentration ratio of 4 to 5 within 5 min), but not at 4°C in or out cells pre-treated at 4°C for 15 min, efflux was complete within 30 min at 37°C (out 1.4, in 0.4). For efflux experiments, cells were resuspended (4°C J774 cells, resuspended or grown-up cells and M200 media for THP-1) in 100 µM GSK1322322 (1-100 µM) in serum-free media (37°C) followed by cell washing and collection and measurement of cell-associated NADH (Subcellular fractionation THP-1: GSK1322322 1 mg/L, 30 min incub. 37°C). Differential pelleting of homogenized cells to collect (i) large granules and nuclei, (ii) large granules (lysosomes, lysosyme), (iii) small granules (endoplasmatic reticulum, Golgi and plasma membrane vesicles), and (iv) final supernatant (lysozome followed by measurement of NADH) in comparison with control enzymes and proteins.

Results

Figure 4: Kinetics of accumulation and efflux of GSK1322322 in macrophages

**Figure 5: Subcellular localization of GSK1322322 in THP-1 macrophages (different confocal microscopy; confocal microscopy)**

**Figure 6: Subcellular localization of GSK1322322 in J774 macrophages (different confocal microscopy)**

**Discussion and Conclusions**

The cell fractionation studies suggest that GSK1322322 accumulated by macrophages (uninfected or infected) is essentially found in the cystos (no evidence of stable association with subcellular organelles or to bacteria). Similar observations were made previously for fluoroquinolones (6) and the oxazolidinone tedizolid (7).

- Although we cannot exclude a post-homogenization degradation of the drug from an intracellular reservoir or from bacteria, the data may actually be consistent with the ability for GSK1322322 to diffuse throughout the cell.
- This may indicate a large intracellular bioavailability of GSK1322322, in line with its demonstrated activity against intraphagocytic S. aureus.

**Background (original)**

GSK1322322 (peptide deformylase inhibitor currently developed for skin and soft tissue infections) is active against the intracellular forms of S. aureus independent of its resistance phenotype to approved antibacterial drugs (1, 2). The drug is a competitive, reversible inhibitor of Peptidylglycine α-Del Peptide deformylase, a cytosolic enzyme that removes the N-terminal glycine from nascent peptides, and a pharmacological and subcellular distribution of GSK1322322 in pharmacologic methods.

**Methods**

Drug (2-17GSK1322322 label in the 2-fluoropyrimidinyl moiety; 99.5% purity); Cells: J774 (adherent; expressing Pgp and MRP) and THP-1 (unadherent; expressing receptor). The drug was incubated with cells for 30 min at 37°C followed by washing of cells with 1-100 µM GSK1322322, followed by measurement of cell-associated NADH. Subcellular fractionation THP-1: GSK1322322 1 mg/L, 30 min incub. 37°C. Differential pelleting of homogenized cells to collect (i) large granules and nuclei, (ii) large granules (lysosomes, lysosyme), (iii) small granules (endoplasmatic reticulum, Golgi and plasma membrane vesicles), and (iv) final supernatant (lysozome followed by measurement of NADH) in comparison with control enzymes and proteins.

**Results**

Figure 4: Kinetics of accumulation and efflux of GSK1322322 in macrophages

**Figure 5: Subcellular localization of GSK1322322 in THP-1 macrophages (different confocal microscopy)**

**Figure 6: Subcellular localization of GSK1322322 in J774 macrophages (different confocal microscopy)**

**Discussion and Conclusions**

The cell fractionation studies suggest that GSK1322322 accumulated by macrophages (uninfected or infected) is essentially found in the cystos (no evidence of stable association with subcellular organelles or to bacteria). Similar observations were made previously for fluoroquinolones (6) and the oxazolidinone tedizolid (7).

- Although we cannot exclude a post-homogenization degradation of the drug from an intracellular reservoir or from bacteria, the data may actually be consistent with the ability for GSK1322322 to diffuse throughout the cell.
- This may indicate a large intracellular bioavailability of GSK1322322, in line with its demonstrated activity against intraphagocytic S. aureus.

**References**


**Funding**

This work was supported by a grant-in-aid from GSK, Collegeville, USA. F. P. is a post-doctoral researcher (F.R.S., FNRS), F. V. B. is a post-doctoral fellow of the “Fondation pour la Recherche Médicale” and F. M. T. is supported by “Fonds de la Recherche Scientifique” (F.R.S., FNRS).