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

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Abstract (original)

Background

GSK1322322 (a peptide deformylase inhibitor currently developed for skin and soft tissue infections) is active against the intraphagocytic forms of *S. aureus* irrespective of its resistance phenotype to approved antistaphylococcal agents (ECCMID 2014, poster eP-185). Our aim was to examine the cellular pharmacokinetics and subcellular distribution of GSK1322322 in phagocytic cells.

Methods

Drug: [^{14}C]-GSK1322322 (label in the 5-fluoropyrimidinyl moiety; 99.5% purity). Cells: J774 (adherent; expressing Pgp and MDR) and THP-1 (suspension) phagocytes. Uptake, release, and effect of efflux inhibitors: 0 to 60 min incub. with GSK1322322 (1-100 mg/L) followed by cell washing, collection and measurement of cell-associated 14C. Subcellular distribution (THP-1; GSK1322322 1 mg/L; 30 min incub. 37°C): differential pelleting of homogenized cells to collect (i) unbroken cells and nuclei; (ii) large granules [mitochondria, lysosomes]; (iii) small granules [endosplamic reticulum, Golgi and plasma membrane vesicles]; and (iv) final supernatant [cytosol]) followed by measurement of ^{14}C in comparison with marker enzymes and proteins.

Results

In THP-1 cells, GSK1322322 accumulated rapidly at 37°C (stable cellular to extracellular concentration ratio of 4 to 6 within 5 min), but not at 4°C or in cells pre-treated at 56°C for 10 min; efflux was complete within 30 min at 37°C but minimal at 4°C. In J774 cells, verapamil or gemfibrozil (P-gp and MRP-inhibitors) did not affect accumulation levels. In both cell types, accumulation was not saturable over a 1-100 mg/L range of extracell. conc. THP-1-associated GSK1322322 was predominantly recovered in the final supernatant (with the cytosolic enzyme lactate dehydrogenase) and its distribution was dissociated from those of cytochrome c-oxidase (mitochondria) and N-acetyl-β-hexosaminidase (lysosomes).

Conclusions

GSK1322322 accumulates in phagocytes but is not associated with subcellular organelles. This may point to its ability to diffuse throughout the cell, as previously observed with fluoroquinolones (JAC 1990;26 Suppl B:27) and the oxazolidinone tedizolid (ICAAC 2012, A1291), two antibiotics active against intracellular *S. aureus* (AAC 2006;50:841; JAC 2009;64:1035).

References

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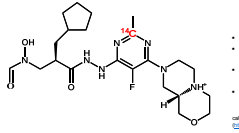
Background and Aims

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) and human (THP-1) macrophages. We also examined its subcellular localization including in infected cells (THP-1 cells only).



- **pKa = 7.10**
- **main species at pH 5 to 7: monocationic**
- **log P = 1.84 (non-ionic species)**
- **log D pH 7.0 = 1.47**
- **logD pH 5.0 = -0.40**

calculated with Reaxys [Reed/Elsevier] (http://www.reaxys.com)

Figure 1: structural formula of [^{14}C]-GSK1322322

Methods

Cell lines: murine J774 macrophages (adherent) and human THP-1 monocytes (suspension) grown in RPMI1640 medium plus 10% bovine fetal serum.

GSK1322322: provided as ^{14}C-labelled (in 5-fluoropyrimidinyl moiety [see Figure 1]; 99.5% purity) by the manufacturer.

Accumulation and efflux studies: cells were incubated up to 60 min with GSK1322322 at concentrations spanning from 1 to 100 mg/L. For efflux experiments, cells where then transferred in drug-free medium for 30 min. Washed cells were collected and lyzed. Cell-associated radioactivity and protein were measured and accumulation expressed as the apparent ratio of the cellular to the extracellular concentrations (taking a cell volume of 3.08 and 5 µL/mg of cell protein for J774 macrophages (5) and THP-1 cells, respectively. Verapamil and gemfibrozil were used as inhibitors of P-glycoprotein (P-gp) and Multi Drug Resistant (MRP) eukaryotic efflux transporters (known to be active in murine J774 macrophages for transport of macrolides (6) and fluoroquinolones (5) respectively.

Cell fractionation studies: cells incubated with [^{14}C]-GSK1322322 were collected, washed in PBS and then in 0.25 M sucrose – 3 mM Na-EDTA – 3mM imidazole pH 7.4, and homogenized in the same medium using a Dounce tissue grinder. Subcellular organelles were separated using differential centrifugation (pelletting at increasing gravity fields) or by centrifugation through sucrose gradients (isopycnic centrifugation), as depicted in Figure 2. Fractions were thereafter assayed for the presence of markers of the organelles of interest and for radioactivity (see [7] for a detailed description) .

Bacterial strain and cell infection: ATCC 25923 (fully susceptible MSSA; GSK1322322 MIC = 1 mg/L) used as depicted in Figure 3 (see [8] for details)

Figure 2: Cell fractionation (4°C)

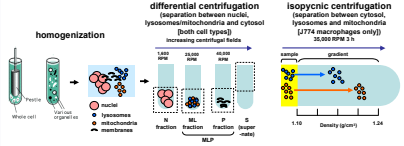


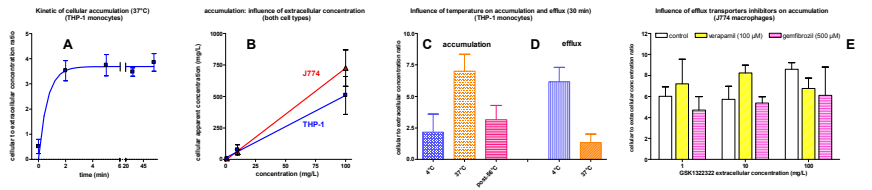
Figure 3: Cell infection

Discussion and Conclusions

- The cell fractionation studies suggest that GSK1322322 accumulated by macrophages (uninfected or infected) is essentially found in the cytosol (no evidence of stable association with subcellular organelles or to bacteria). Similar observations were made previously for fluoroquinolones (9) and the oxazolidinone tedizolid (10).
- Although we cannot exclude a post-homogenization desorption 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* (3).

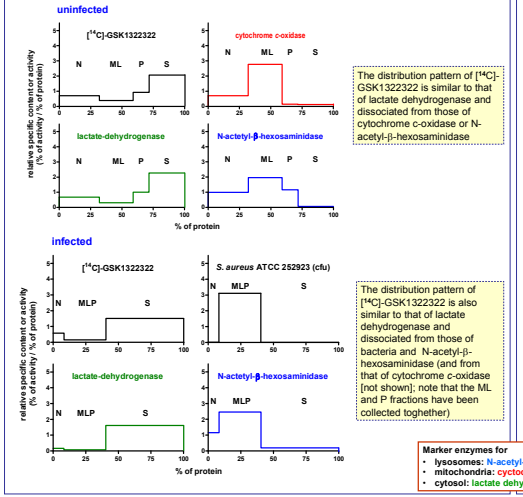
Results

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



Accumulation of GSK1322322 (i) occurred very quickly (equilibrium after 2-5 min [A]), (ii) was non saturable (both cell types) [B]; (iii) impaired at 4°C and in heated (56°C) cells [C]. Efflux was rapid and almost complete at 37°C but not at 4°C [D]. Accumulation was very modestly increased in the presence of verapamil (J774 macrophages) and only at low concentrations [E].

Figure 5: Subcellular localization of GSK1322322 in THP-1 monocytes (differential centrifugation)

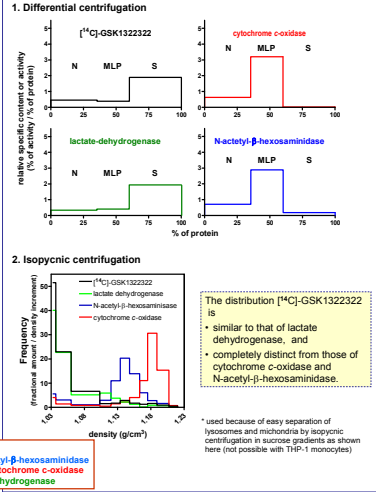


The distribution pattern of [^{14}C]-GSK1322322 is similar to that of lactate dehydrogenase and dissociated from those of cytochrome c-oxidase or N-acetyl-β-hexosaminidase

The distribution pattern of [^{14}C]-GSK1322322 is also similar to that of lactate dehydrogenase and dissociated from those of bacteria and N-acetyl-β-hexosaminidase (and from that of cytochrome c-oxidase [not shown]; note that the ML and P fractions have been collected toghether)

Marker enzymes for
- **lysosomes: N-acetyl-β-hexosaminidase**
- **mitochondria: cytochrome c-oxidase**
- **cytosol: lactate dehydrogenase**

Figure 6: Subcellular localization of GSK1322322 in J774 macrophages *



The distribution [^{14}C]-GSK1322322 is
- similar to that of lactate dehydrogenase, and
- completely distinct from those of cytochrome *c*-oxidase and N-acetyl-β-hexosaminidase.

* used because of easy separation of lysosomes and michondria by isopycnic centrifugation in sucrose gradients as shown here (not possible with THP-1 monocytes)

This poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters