

# Cellular pharmacokinetics of gepotidacin (GSK2140944) in human THP-1 monocytes (THP-1) and murine macrophages (J774)

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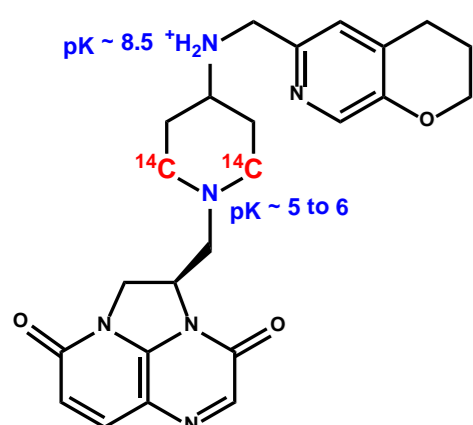
## Background and Aims

*Staphylococcus aureus* remains a major cause of community and nosocomial infections because of acquired resistance mechanisms (1) and ability to take refuge intracellularly (2). Novel antibiotics, therefore, need to be assessed for activity against resistant strains, including after phagocytosis by eukaryotic cells.

Gepotidacin is a novel triazaacenaphthylene antibiotic which inhibits bacterial type IIA topoisomerases (Figure 1). We previously showed that gepotidacin is active against intracellular forms of *S. aureus*, including strains resistant to other antibiotics, in human THP-1 monocytes (3).

The goal of the present study was to characterize the cellular pharmacokinetics of gepotidacin in human monocytes (THP-1; undifferentiated cells growing in suspension) and murine macrophages (J774; differentiated and adherent).

Figure 1: Structure of gepotidacin (GSK2140944)



The figure shows  
(i) the positions of the labelled atoms (<sup>14</sup>C)  
(ii) the calculated pKa's of the two aminofunctions (in blue) assumed to be ionizable in the 5-8 pH range (predominant microspecies: monoionic [68%] at pH 7.0) and dicationic [93%] at pH 5.5).

Sources: structure redrawn from [5]; physicochemical constants: ChemBioDraw (<http://www.cambridgesoft.com/>) and Reaxys® Reed Elsevier (<http://www.reaxys.com>)

## References

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## Methods

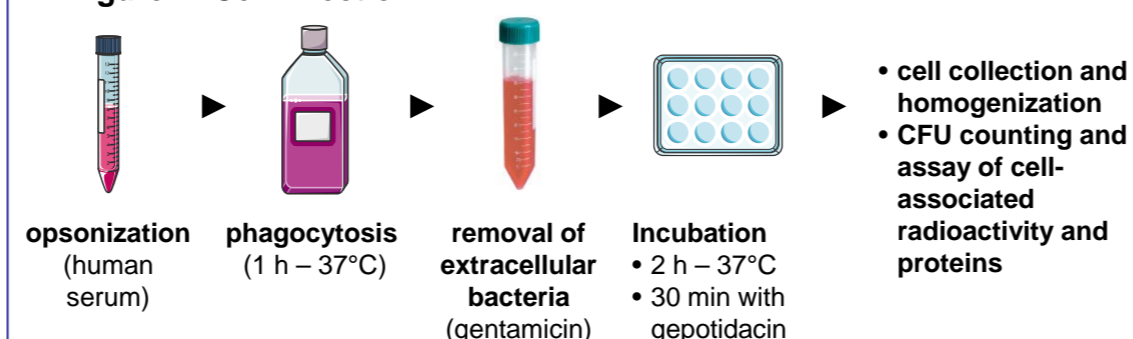
**Cell lines:** murine J774 macrophages (expressing P-gp and MRP efflux transporters) and human THP-1 monocytes

**Gepotidacin:** [<sup>14</sup>C]-gepotidacin and unlabelled compound (99.7% purity).

**Accumulation and efflux studies:** Cells incubated with gepotidacin and collected at the end of the experiment after washing for measurement of (i) cell-associated radioactivity and (ii) total protein content (cell volume: 3.08 and 5 µL/mg of cell protein for J774 macrophages (4) and THP-1 cells, respectively). Verapamil and gemfibrozil were used as inhibitors of P-glycoprotein (P-gp) and Multidrug Resistant Protein (MRP) eukaryotic efflux transporters (4,5).

**Bacterial strain and cell infection:** ATCC 25923 (fully susceptible MSSA; gepotidacin MIC = 0.5 mg/L) used as depicted in Figure 2 (6). Gepotidacin was used at 0.1 mg/L to avoid killing intracellular bacteria.

Figure 2: Cell infection



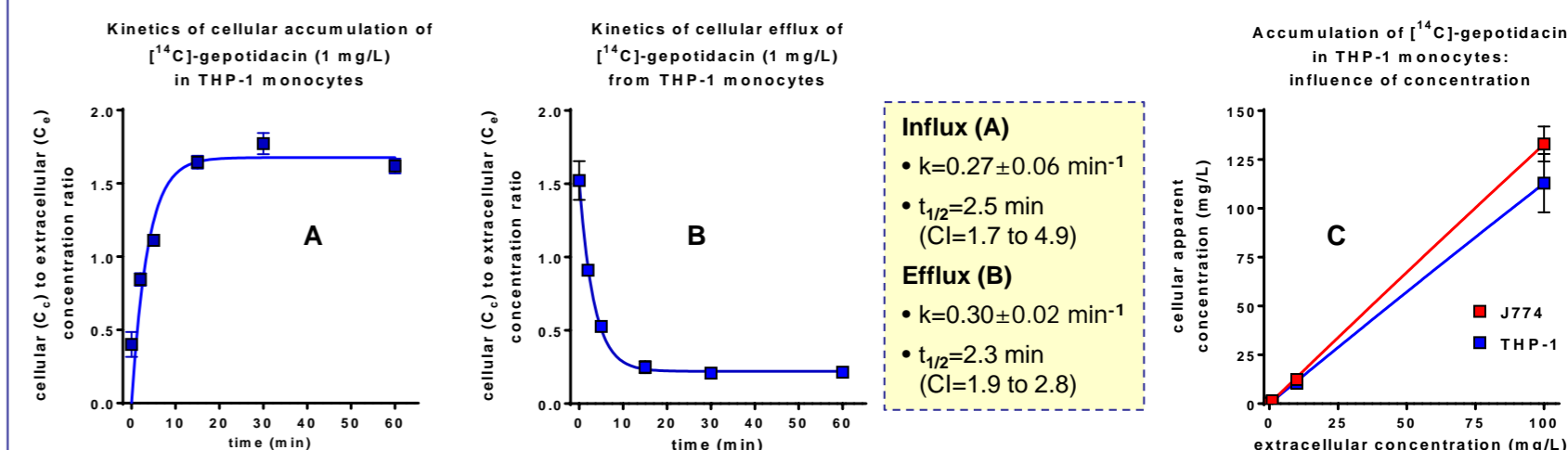
## Discussion and Conclusions

- The kinetic data suggest that gepotidacin enters the cells by passive diffusion (rapid influx and efflux; no saturation), which probably allows it to be bioavailable and to exert its antibacterial activity intracellularly, as demonstrated with *S. aureus* (3).

- Further experiments will need to assess intracellular localization of gepotidacin in both non-infected and infected cells and to measure its ability to reach its bacterial target.

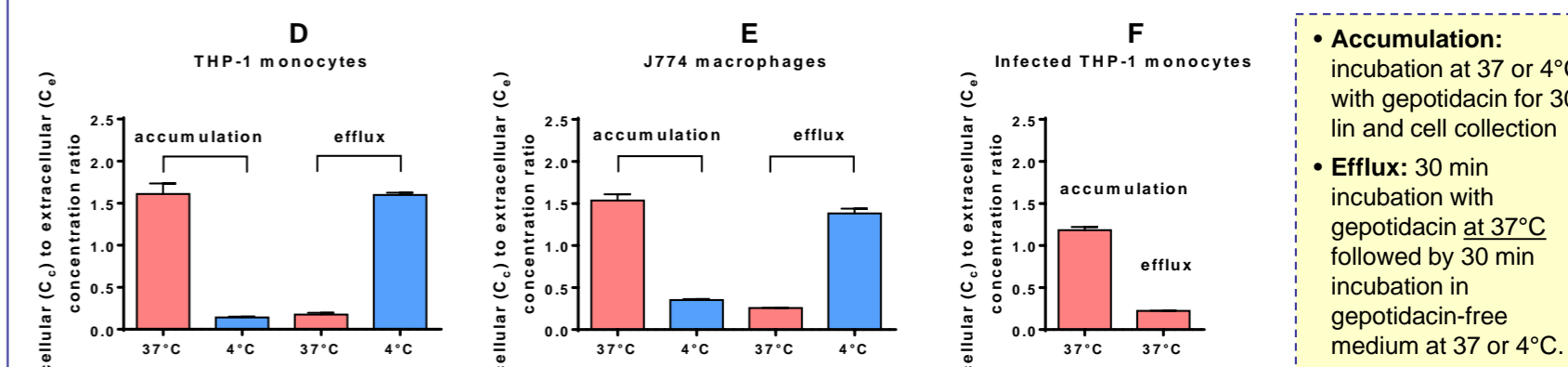
## Results

Figure 3: Kinetics of accumulation and efflux (0-60 min) and effect of concentration



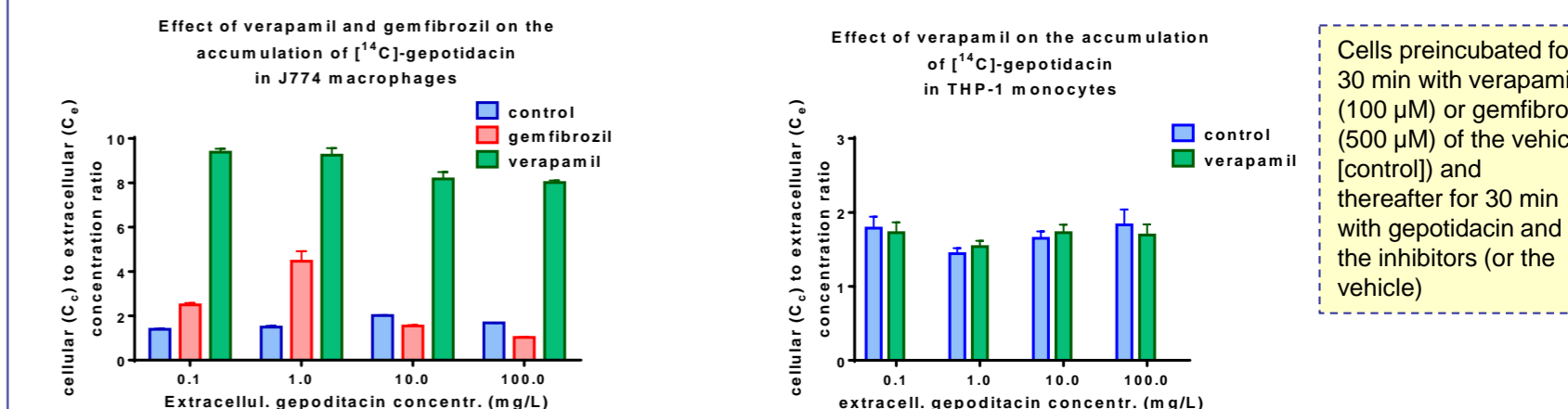
Fast in and out fluxes (equilibrium after 15 min [A] and [B]) and non-saturable accumulation [C]

Figure 4: Influence of temperature and infection on accumulation and efflux



Complete efflux at 37°C -- No accumulation nor efflux at 4°C (D,E) -- Minor effect of infection (F)

Figure 5: Influence of eukaryotic efflux inhibitors on accumulation at 37°C



- J774 macrophages: marked increase in accumulation by verapamil (P-gp-inhibitor) but modest effect of gemfibrozil (MRP-inhibitor) at concentrations  $\leq 1 \text{ mg/L}$ .
- THP1 monocytes: no significant effect of verapamil