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114

Subcellular Distribution and Cell Safety of the Novel Oxazolidinone Tedizolid in Murine J774 and Human THP-1 Macrophages

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Background and Aims

There is a continuous need of discovering and developing new antibiotics to meet the resistance challenge. Tedizolid (TR-700), a novel 5-hydroxy-methyl oxazolidinone [1], shows higher intrinsic activities against Gram-positive pathogens (including MRSA) than linezolid (the first commercialized oxazolidinone) and maintains activity against *cfr*+ linezolid-resistant strains [2-4].

Tedizolid accumulates in approx. 10-fold higher concentrations than linezolid in human THP-1 macrophages *in vitro* [2] as well as in alveolar human macrophages *in vivo* [5]. While this may confer an advantage in terms of activity against intracellular forms of target bacteria [2], it also raises concerns about potential toxicity whether related to physical accumulation of the drug or its mode of action.

Oxazolidinones inhibit bacterial protein synthesis by binding the 50S subunit of ribosomes [6]. Because of structural similarities between mitochondrial and prokaryotic ribosomes [7], linezolid also impairs mitochondrial DNA-dependent protein synthesis in eukaryotic cells [8,9], which represents a limiting factor in its clinical use.

Therefore, we used cell fractionation methods to examine whether the higher accumulation of tedizolid was potentially due to an association with mitochondria. We also examined whether exposure of cells to tedizolid would cause necrosis, as this impacts the interpretation of the cell fractionation studies.

Materials and Methods

Cell fractionation studies

Murine J774 macrophages (*in vitro* model used to study the subcellular localization of antibiotics [10,11]) were incubated with tedizolid (2h; 20 mg/L), collected and homogenized in 0.25 M sucrose using a Dounce tissue grinder to release their subcellular organelles. The latter were separated by differential centrifugation (based on their size) or by isopycnic centrifugation (based on their buoyant density). Fractions were thereafter assayed for:

- markers of the cytosol (lactate dehydrogenase [LDHase]), mitochondria (cytochrome *c*-oxidase [cyt-oxidase]), and lysosomes (N-acetyl-β-glucosaminidase [NAβgase]) as previously described [10,11];
- tedizolid, using LC-MS-MS (Orbitrap™) with extraction in chloroform:methanol (8:4), separation (reverse phase) by isocratic elution (methanol:0.5% acetic acid [1:1 and then 99.5:0.5]; typical tedizolid elution time: 7 min) and selective ion monitoring at m/z 370-372 with linezolid as internal standard.

Cytotoxicity studies

Human THP-1 macrophages (used to study the intracellular activity of tedizolid [2]) were exposed to tedizolid at concentrations up to 200 mg/L (= 800 x its MIC against MRSA [2,12]) for 24h and monitored for the release of LDHase into the medium.

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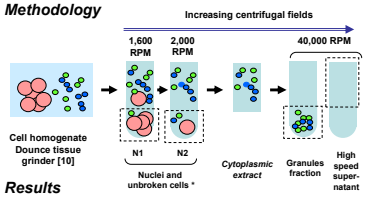
Results

1. Cell fractionation studies

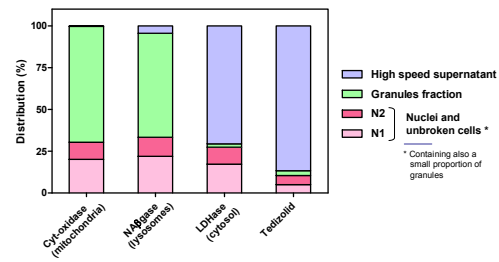
1.1. Differential centrifugation

Methodology



Organelles are separated according to their size leaving only soluble proteins in the final supernatant

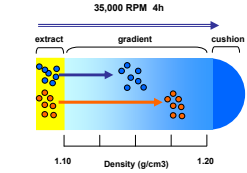
Results



The bulk of tedizolid was recovered from the high speed supernatant with minimal amounts in those fractions that contained the bulk of cytochrome *c*-oxidase (marker for mitochondria).

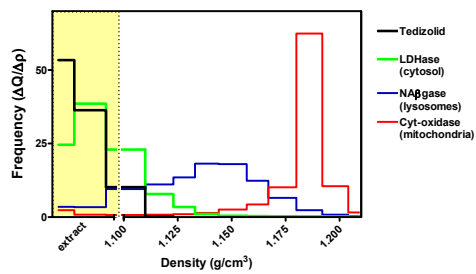
1.2. Isopycnic centrifugation

Methodology



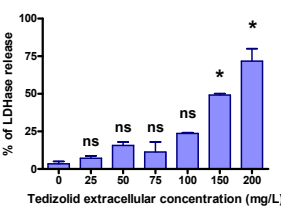
A cytoplasmic extract is deposited on top of a sucrose gradient placed in a swing-out rotor. During centrifugation, organelles migrate and equilibrate at their buoyant density while soluble proteins and low molecular weight molecules remain on top.

Results



The bulk of tedizolid was recovered from the top of the gradient (highlighted area in yellow), indicating no association with mitochondria, which have moved far into the gradient. The modest move of lactate dehydrogenase (LDHase) into the gradient was due its large molecular weight (144 kDa).

2. Cytotoxicity studies



Methodology

THP-1 macrophages were exposed for 24h to tedizolid at increasing concentrations (up to very large multiples of the MIC against the main target organism (MRSA; see [2,12]).

LDH release indicates a major permeabilization of the cell membrane (100 % = exposure to the detergent Triton-X100, causing death of all cells)

Statistical analysis (one-way Analysis of Variance [ANOVA] with Tukey-Kramer Multiple Comparisons Test *vs.* control values (0 mg/L): ns = non significant, * = $p < 0.05$

Tedizolid did not cause significant LDHase release up to a concentration of 100mg/L (250 x the MIC against MRSA), indicating that its "soluble" distribution observed in cell fractionation studies (performed at a concentration of 20 mg/L) was not due to cell damage during the incubation.

Conclusions

Although tedizolid accumulates to a greater extent in macrophages than linezolid [2], it did not show preferential association with mitochondria (a site of linezolid toxicity). We could exclude a cytotoxic effect during incubation to explain this finding.

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