Tedizolid Inhibits Mitochondrial Metabolism and Reversibly Impairs Cytochrome *c*-oxidase Expression in Cultured Human Cells: Impact of a Discontinuous Exposure

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ABSTRACT

Background: Oxazolidinones inhibit mitochondrial DNA-encoded proteins (MEP) synthesis, causing mitochondrial dysfunction (MD) thought to be responsible for myelosuppression, lactic acidosis, and neuropathies. Our aim was to assess the potential of tedizolid (TZD), a more potent oxazolidinone than linezolid (LZD) for MD and decreased expression of MEP in cultured.

Methods: Human THP-1 monocytes and HL-60 promyelocytes were tested for (i) lactate release: glucose consumption molar ratio (Lr:Gc-MR; enzymatic analysis on CMA600), ATP production-linked mitochondrial oxygen consumption rate (OCR) and extracellular acidification rates (ECAR) (Seahorse XF96 bioanalyzer); (ii) comparative expression of cytochrome *c* oxidase subunit I (CYTOX I) and nuclear DNA-encoded succinate dehydrogenase (Western-blot; normalization to Tom20); and (iii) autophagy (increase in level of lipidated microtubule-associated protein LC3 (Western blot; normalization with actin). Treatments: (i) continuous exposure to 3 or 0.5 mg/L (corresponding to human C_{max} and C_{min}); (ii) discontinuous exposure: 12h exposure to C_{max} followed by 12h re-incubation in drug-free medium for up to 4 days.

Results: MD (continuous exposure): (i) HL-60 cells, Lr:Gc-MR: increase from 1.76±0.09 (control) to 2.20±0.03 (C_{min}) and 2.79±0.04 (C_{max}) at 48h; (ii) THP-1 monocytes, OC: decrease from 0.69±0.04 fmol x min^{-1}/cell to 0.45±0.08 (C_{min}) and 0.21±0.04 (C_{max}); ECAR: increase from 0.14±0.04 mpH x min^{-1}/1,000 cells to 0.20±0.02 (C_{min}) and 0.25±0.04 at 72h (all differences: p<0.01 [ANOVA]). CYTOX I expression: (i) 39%, 14% and <1% of controls after 12, 24 and 48 h at C_{max}, with full recovery 48h after transfer to TZD-free medium; (ii) discontinuous exposure: each 12h re-incubation in TDZ-free medium allowed for partial recovery, with mean inhibition over 4 days limited to 52.4±14.3%. TZD did not impair succinate dehydrogenase expression and did not stimulate autophagy.

Conclusions: TZD causes MD probably by impairing MEP expression. As drug wash-out allows for recovery, impairment of MEP by TZD when administered once daily may never progress to levels causing sustained MD. These observations support and rationalize animal data documenting the long-term safety of TZD (Antimicrob Agents Chemother 2015; 59:178-85).

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INTRODUCTION & AIM

- Oxazolidinones bind to ribosomes of both bacteria [1] and mitochondria due to a high degree of homology [2].
- In patients, linezolid (LZD; the first approved oxazolidinone) causes myelosuppression [3], lactic acidosis [4] and neuropathies [5], all of which have been suggested to result from impairment of mitochondrial protein synthesis and ensuing mitochondrial dysfunction [2].
- Tedizolid (TZD) shows lower MICs than LZD due to increased binding to bacterial ribosomes [6] and is also a more potent inhibitor of mitochondrial protein synthesis than LZD [7]. However, TZD is approved at a dose of 200 mg once-daily [8] whereas LZD is commonly administered at 600 mg twice daily [9], which results in a 6-fold difference in total drug daily exposure.
- The aim of this study was therefore to assess the potential of TZD to inhibit the expression of a protein encoded by the mitochondrial genome and to examine its impact on mitochondrial metabolism in cultured human cells while mimicking its conditions of administration to humans.

METHODS

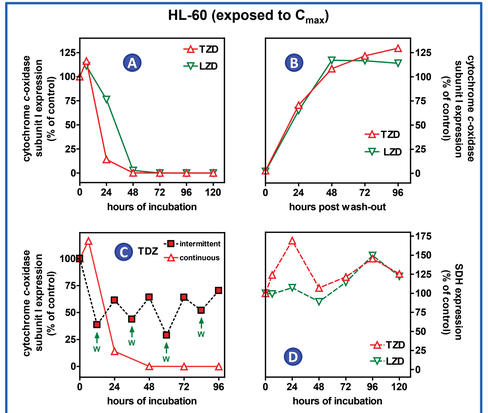
Cells (human):
- HL-60 promyelocytes (progenitors of granulocytes and monocytes)
- THP-1 monocytes (unactivated) with phagocytic capabilities.

Investigations:
- ***Mitochondrial protein expression:*** western blot of cytochrome *c* oxidase subunit I (CYTOX I) and succinate dehydrogenase (SDH), encoded by the mitochondrial and the nuclear genomes, respectively, with normalization using Tom20 (outer membrane protein).
- ***Autophagy:*** increase in level of lipidated protein LC3-II (Western blot; normalization to actin) with or without leupeptin (cathepsin B inhibitor).
- ***Lactate release/glucose consumption molar ratio:*** enzymatic assay using CMA600.
- ***Mitochondrial oxygen consumption rate*** (OCR) and ***extracellular acidification rate*** (ECAR): Seahorse XF96 bioanalyzer (see details at http://www.seahorsebio.com)

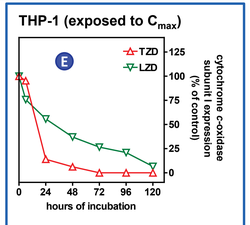
Treatments:
- ***Continuous exposure:*** incubation with 15 or 2.5 mg/L (total concentrations) for LZD and 3 or 0.5 mg/L for TZD, mimicking their corresponding human C_{max} and C_{min} (total concentr).
- ***Discontinuous exposure (TZD only):*** 12h exposure to its C_{max} followed by 12h re-incubation in drug-free medium in order to mimic tedizolid serum half-life (12h) for up to 4 days

RESULTS

Expression of CYTOX I (encoded by mitochondrial genome) and SDH (encoded by nuclear genome)



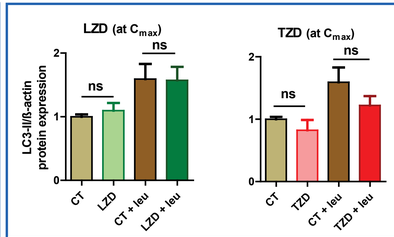
- **A** Continuous exposure causes a complete impairment of CYTOX I expression.
- **B** Transferring cells to fresh medium allows for complete recovery within 48h.
- **C** With intermittent exposure (W=wash-out), impairment by TZD is only partial.
- **D** Expression of SDH (nucleus encoded) is unimpaired by either TZD or LZD.



- **E** THP-1 cells are more susceptible to TZD than to LZD

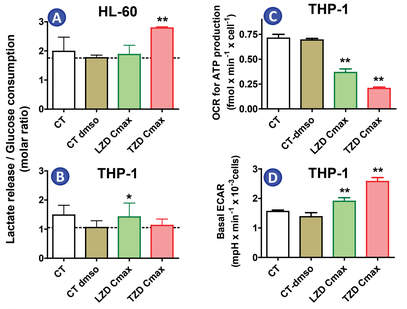
- TZD is more inhibitory of the expression of CYTOX I than LZD at C_{max} [total concentration]. At C_{min} [total concentration], effects were milder but with the same ranking (not shown).
- Recovery of CYTOX I expression is rapid upon wash-out. This explains why intermittent exposure with tedizolid only causes partial impairment of CYTOX I expression.

Expression of LC3II (marker of autophagy) in HL-60 cells after 48h at C_{max}



- No increase in LC3-II over leupeptin control (CT + leu) was observed after 48 h of incubation with either TZD or LZD at C_{max} (total concentration).
- This excludes potential effects of these antibiotics on the degradation of intracellular organelles (autophagy).

Mitochondrial metabolism, respiration and extracellular acidification after 48 to 72h at C_{max}



- Both TZD and LZD impair the mitochondrial metabolism when cells are exposed continuously to the antibiotics at their C_{max} (total concentration), but to different extents.
- Minimal (most non-statistically significant) effects were detected after exposure to C_{min}; not shown).
- TZD effects are overall more marked than those of LZD (except for lactate release/glucose consumption in THP-1 monocytes).

- **A** TZD (48h exposure) significantly increases (p<0.01) lactate / glucose molar ratio in HL-60 cells.
- **B** Only LZD (48h exposure) has a modest but significant increase in lactate / glucose molar ratio in THP-1 cells.
- **C** Both drugs (72h exposure) significantly decrease (p<0.01) the OCR for ATP production in THP-1 cells.
- **D** Both drugs (72h exposure) cause an acidification of the culture medium of THP-1 cells, but more markedly for TZD.

DISCUSSION AND CONCLUSION

In these *in vitro* models, tedizolid causes obvious mitochondrial metabolic dysfunctions, probably related to the impairment of the expression of proteins encoded by the mitochondrial genome. The clinical impact may, however, be mitigated by the fast recovery upon drug wash-out, especially if tedizolid is used for short periods and with a once-daily schedule as recommended in the Prescribing Information.

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