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Mitochondrial alterations induced by oxazolidinone antibiotics in human cultured cells

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Tamara Milosevic,[1] Valéry L. Payen,[2] Pierre Sonveaux,[2] Giulio G. Muccioli,[1] Paul M. Tulkens,[1] Françoise Van Bambeke.[1]

[1] *Louvain Drug Research Institute,* [2] *Institute of Experimental and Clinical Research, Université catholique de Louvain, Brussels, Belgium*

Contact info:
Françoise Van Bambeke
Av. Mounier 73 B1.73.05
1200 Brussels- Belgium
francoise.vanbambeke@uclouvain.be

Introduction

- Oxazolidinone antibiotics (linezolid [LZD], tedizolid [TZD]) inhibit bacterial protein synthesis but also mitochondrial DNA-encoded protein synthesis (MEPS) in eukaryotic cells [1], which has been associated with the development of severe adverse effects (myelosuppression [2], lactic acidosis [3] and neuropathies [4]) upon prolonged treatments.
- TZD shows lowers minimal inhibitory concentrations (MICs) towards susceptible bacteria than LZD due to increasing binding to bacterial ribosomes [5] and is also a more potent inhibitor of MEPS [6].

Aim of the study

- Our aim was to use LZD and TZD to document whether MEPS leads to mitochondrial metabolism impairment and ultrastructural alterations by studying two human cell lines exposed to therapeutic concentrations of these drugs.

Methods

- ***Cells*:** Human promyelocytes (HL-60) and monocytes (THP-1)
- ***Mitochondrial protein expression*:** western blot of cytochrome *c*-oxidase subunit I (CYTOX I) and succinate dehydrogenase (SDH), encoded by the mitochondrial and nuclear genome, respectively, with normalization using Tom 20 (outer membrane protein).
- ***Basal mitochondrial oxygen consumption rate (OCR) and reverse capacity*:** Seahorse XF96 bioanalyzer.
- ***Cytochrome c-oxidase activity*:** measure of the rate of oxidation of reduced cytochrome c (decrease of OD_{550}).
- ***Electron microscopy:*** cells fixed in 2% glutaraldehyde in 0.1M sodium cacodylate, post-fixed in 1% osmium tetroxide, stained *en bloc* with 0.5 % uranyl acetate, and ultrathin sections stained with lead citrate and uranyl acetate and observed at 80 kV
- ***Oxazolidinone cellular concentrations:*** sonicated cell lysates extracted with acetonitrile:methanol (21:4), dried, solubilized in methanol, and subjected to LC-MS analysis (LTQ-Orbitrap mass spectrometer) with [^{2}H]-LZD and -TZD as internal standards
  → Results not shown but demonstrating a ~ 5-fold larger accumulation of TZD over LZD.

Treatments:

- Incubation with 15 mg/L (45 µM) for LZD and 3 mg/L (8 µM) for TZD (total concentration, corresponding to C_{max} in patients receiving approved daily dosages (LZD, 600 mg BID; TZD, 200 mg qD)

References

(1) Nagiec *et al*, Antimicrob Agents Chemother 49, 3896-902 (2005) – PMID 16127068
(2) Hachem *et al*, Clin Infect Dis 37, e8-11 (2003) – PMID 12830431
(3) Apodaca *et al*, N Engl J Med 348, 86-87 (2003) – PMID 12510056
(4) Bressler *et al*, Lancet Infect Dis 4, 528-531 (2004) – PMID 15288827
(5) Im *et al,* Eur J Med Chem 46, 1027-1039 (2011) – PMID 21292356
(6) Flanagan *et al,* Antimicrob Agents Chemother 59, 178-185 (2015) – PMID 25331703

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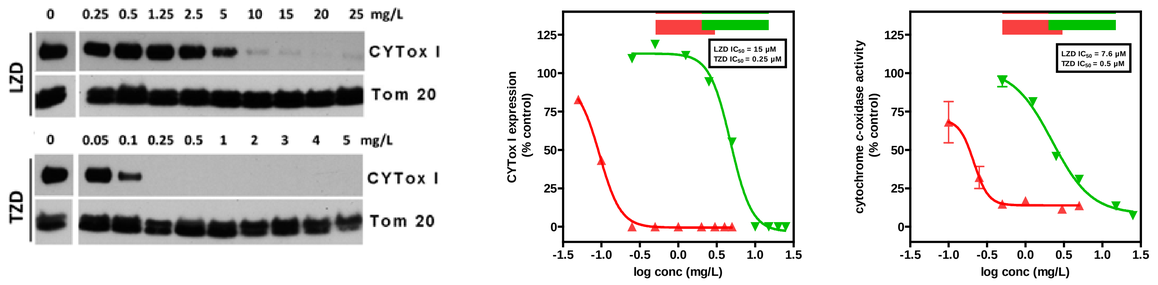
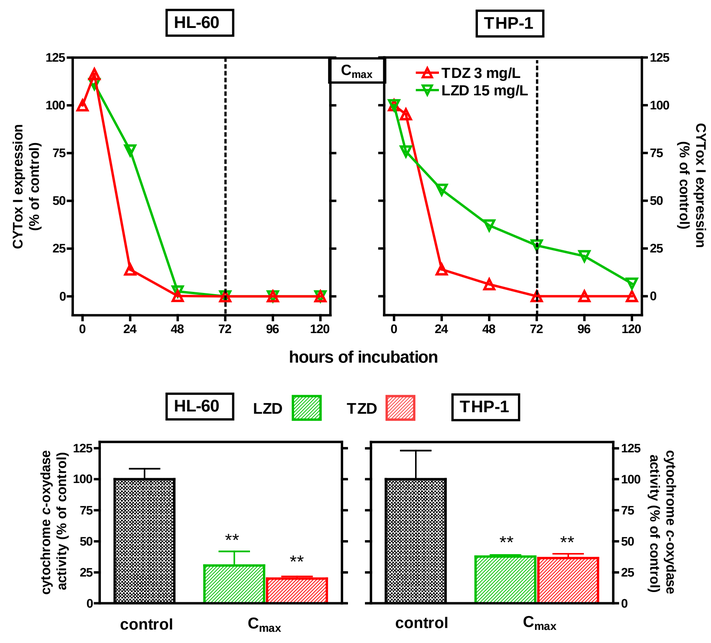
Results

1. CYTox I expression and cytochrome *c*-oxidase activity: effect of concentration (HL-60)



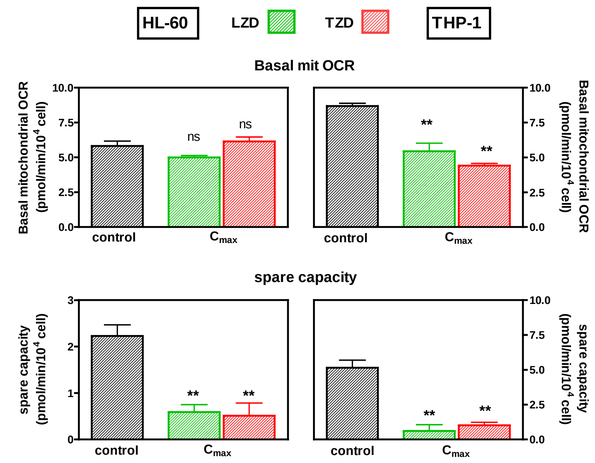
- Both LZD and TZD inhibit the expression of a protein encoded by the mitochondrial genome and the activity of a key enzyme of complex IV in mitochondria. TZD is a more potent inhibitor than LZD for CYTox I expression. The difference between two drugs is less pronounced for cytochrome *c*-oxidase activity inhibition.

2. CYTox I expression and cytochrome *c*-oxidase activity at C_{max}: LZD 15 mg/L (45 µM) TZD 3 mg/L (8 µM)



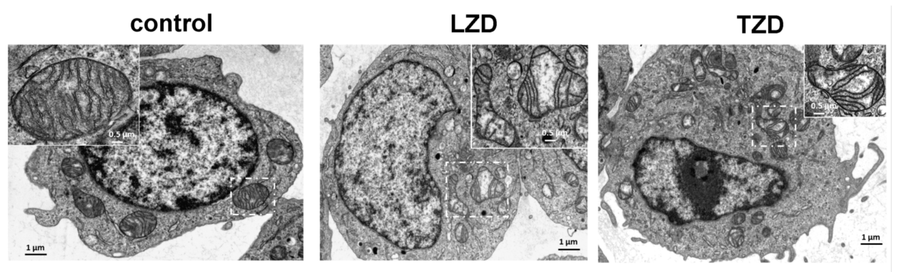
- A fast inhibition of CYTox I expression is observed at C_{max}. THP-1 are less susceptible than HL-60. SDH expression is not impaired (data not shown).
- Cytochrome *c*- oxidase enzyme activity (at 72h) is significantly decreased in both cell lines.

3. Mitochondrial respiration after 72h at C_{max} : LZD C_{max}= 45 µM, TZD C_{max} = 8 µM



- Basal mitochondrial OCR is not affected in HL-60 but slightly decreased in THP-1 cells. Spare capacity is almost completely blocked in both cell lines in the presence of either drugs.

4. Ultrastructural studies (HL-60 cells)



- Both LZD and TZD induce mitochondrial morphological alterations (decrease of inner membrane cristae and swelling of a matrix) consistent with an impairment of inner membrane proteins encoded by the mitochondrial genome.

5. Recovery upon drug withdrawal (not illustrated)

- CYTox I expression, cytochrome c-oxidase activity and spare capacity (for both LZD and TZD) returned to normal values within 72 h upon drug removal.

Summary and Perspectives

- Oxazolidinones exert biochemical, metabolic and ultrastructural toxicities to mitochondria consistent with the preferential inhibition of the synthesis of proteins encoded by the mitochondrial genome.
- Future work should examine the link between mitochondrial dysfunction and the development of the known drug-related toxicities such as myelosuppression and neuropathies.

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