Mitochondrial metabolism impairment induced by oxazolidinones at clinically-relevant concentrations: studies with two human megakaryocytic cell lines (UT7-WT and UT7-MPL)

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Background and Aim

Oxazolidinone antibiotics inhibit protein synthesis in bacteria as well as in mitochondria [1-3]. The latter inhibition has been associated with severe side effects like myelosuppression [4] especially thrombocytopenia.

Our aim was to compare linezolid (LZD) and tedizolid (TZD) for their inhibitory effect on the expression and activity of cytochrome *c*-oxidase and on mitochondrial respiration in two different megakaryocytic cell lines.

Materials & Methods

Cells: **UT7-WT** (wild type; human acute megakaryoblastic leukemia cells [5]) and **UT7-MPL** (transfected by the pMex-ires-GFP HA huMPL vector and expressing a thrombopoietin receptor [TpoR or c-MPL] [6]).

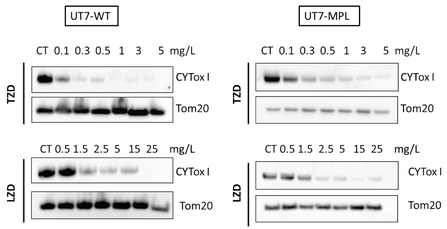
Treatments: incubation with LZD or TZD at increasing total concentrations or at their total C_{max} (as observed in patients treated as per the corresponding EU-approved label (see [3]).

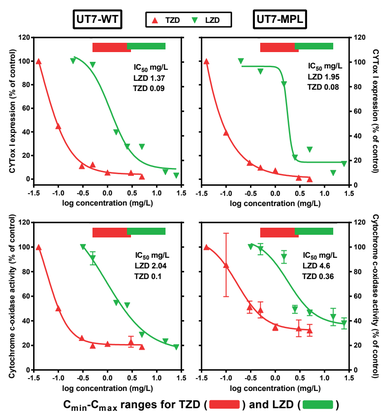
Assays (see [3] for details):

- ***CYTox I*** (subunit of cytochrome c-oxidase; encoded by the mitochondrial genome)*:* Western blot analysis with Tom 20 (encoded by the nuclear genome) as loading control;
- ***Cytochrome c-oxidase activity:*** measure of the rate of oxidation of reduced cytochrome *c* (decrease of OD_{550});
- ***Basal mitochondrial oxygen consumption rate (OCR) and Spare capacity:*** Seahorse XF96 bioanalyzer.

Results

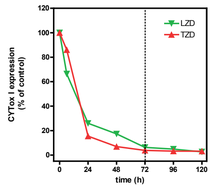
1. CYTox I expression and cytochrome *c*-oxidase activity: concentration-effect





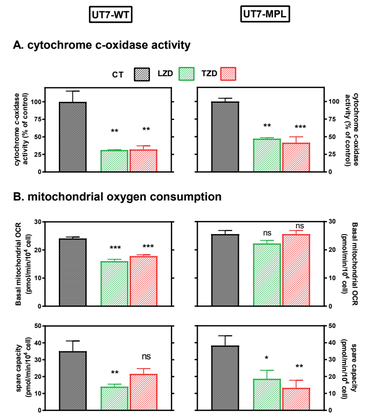
- Both LZD and TZD inhibit the expression of a protein encoded by the mitochondrial genome (CYTox I) and the activity of a key enzyme of complex IV in mitochondria (cytochrome c-oxidase) at therapeutically pertinent concentrations.
- TZD is a more potent inhibitor than LZD for both criteria.

2. CYTox I expression: time-effect at C_{max} (UT7-WT cells)



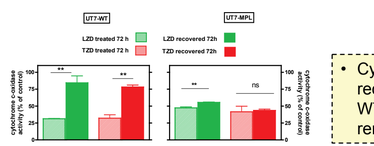
- A fast inhibition of CYTox I expression is observed at C_{max} with the maximal effect between 48 and 72 hours.

3. Mitochondrial metabolic activity after 72h at C_{max}



- Cytochrome *c*- oxidase activity (at 72h) is significantly decreased in both cell lines.
- Basal mitochondrial OCR is reduced in UT7-WT but not in UT7-MPL.
- Spare capacity (denoting the ability of cells to meet an increased energy demand [3]) is reduced in both cell lines.

4. Recovery (cytochrome *c*-oxidase) upon drug withdrawal



- Cytochrome *c*-oxidase activity recovers to about 80% in UT7-WT within 72 h upon drug removal but not in UT7-MPL.

Main points and Discussion

- ✓ Both LZD and TZD cause, at clinically-relevant concentrations, an impairment of the expression of CYTox I, an inhibition of the activity of cytochrome *c*-oxidase, and a decrease of the spare capacity of the mitochondrial respiration in human megakaryocytic cell lines, with less differentiated cells being only slightly more susceptible.
- ✓ TZD is, globally, a more potent inhibitor than LZD in cells continuously exposed to either drug.
- ✓ The lack of recovery upon drug withdrawal in UT7-MPL but not in UTP-WT or in other cell lines not part of the megakaryocyte lineage (see [3]) might explain the preferential and therapeutically limiting effect of oxazolidinones on platelets levels in patients [4,7].

References

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