Optimizing a Dual-Platform Workflow for Predicting Drug-Induced Liver Injury Using Mito Tox Index and Cell Index

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Introduction

Label-free measurement of cellular impedance in Cell Index using Agilent xCELLigence RTCA provides insight into the cytotoxic effects of drugs or compounds via real-time monitoring of cell status, including cell number, viability, and morphology.

The Mito Tox Index (MTI) measured by Agilent Seahorse XF Pro analyzer quantifies the extent to which substances disrupt mitochondrial function by distinguishing between ETC inhibition and uncoupling.

This study aims to optimize and validate a drug safety testing workflow that integrates mitochondrial toxicity assessment using MTI with cell viability assessment via the Cell Index. Fourteen drugs, known to cause varying degrees of hepatotoxic adverse effects, with or without impacting mitochondrial function, were tested.

The cytotoxicity of the drugs was quantitatively evaluated using the Cell Index, while mitochondrial toxicity was assessed using an MTI threshold of 0.3, both in the HepG2 cell line. Drugs were then categorized based on the type of toxicity determined by these two sets of data.

Assay Design

Real-time monitoring in the presence of drug

xCELLigence RTCA MP

Mito Tox assay with an acute drug pretreatment

Seahorse XF Pro

Cytotoxicity evaluation by **Cell Index (CI)** changes

- Real-time monitoring of Cell Index for up to 72 hour at fixed dose (10 µM and 100 µM)
- Classify as toxic if ΔCell Index > 20% using AUC normalized Cell Index for 24- or 72-hour duration
- Identify IC_{50} by dose response analysis for selected drugs

Mitochondrial toxicity evaluation by **Mito Tox Index (MTI)**

- MTI evaluation after acute treatment (1 hour) at fixed dose (10 µM and 100 µM)
- Classify as toxic if $MTI_{inhibition}$ < -0.3 or $MTI_{uncoupler}$ > 0.3
- Identify IC_{50} and EC_{50} by dose response analysis for selected drugs

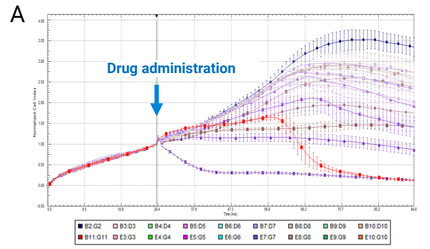
Cytotoxicity vs. Mitochondrial toxicity comparison

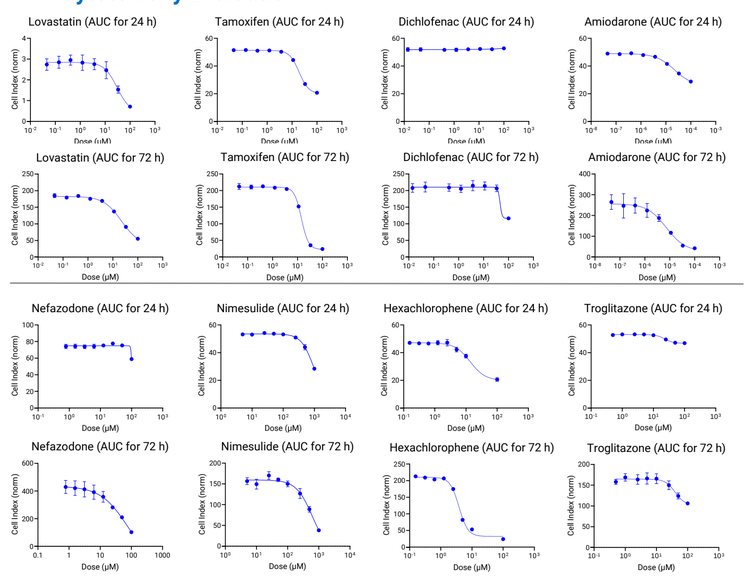
Compare the toxicity in different concentration ranges (e.g. 10x C_{max} vs. 30x C_{max}) and drug exposure durations (e.g. 24 h vs. 72 h)

Figure 1. Toxicity evaluation workflow using dual cell analysis platforms, Agilent xCELLigence RTCA MP and Seahorse XF Pro

Results and Discussion

Cytotoxicity Evaluation





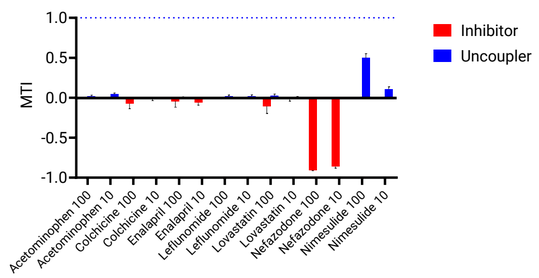
C

Drug Selected	IC_{50} (µM) by AUC for 24 h	IC_{50} (µM) by AUC for 72 h
Colchicine	NA	0.00015
Tamoxifen	19.3	14.4
Diclofenac	NA	45.6
Nefazodone	98.6	76.5
Nimesulide	927	626
Hexachlorophene	13.6	3.84
Amiodarone	22.8	7.31
Lovastatin	30.2	22.1
Troglitazone	23.1	39.6

Figure 2. Cytotoxicity evalution by Cell Index comparison
A. A representative changes in Cell Index of HepG2 cells measured for up to 72 hours in the presence of drugs at 10 or 100 µM.
B. Dose-response of normalized Cell Index in AUC for the indicated time to drugs selected
C. IC_{50} summary of the drugs selected.

Mitochondrial Toxicity Evaluation

A



B

Drugs	10 µM		100 µM	
	$MTI_{Inhibition}$	$MTI_{Uncoupling}$	$MTI_{Inhibition}$	$MTI_{Uncoupling}$
Acetaminophen	0.00	0.04	0.00	0.02
Erythromycin	-0.01	0.01	0.00	0.01
Enalapril	-0.06	0.00	-0.04	0.01
Leflunomide	0.00	0.02	-0.01	0.02
Lovastatin	-0.02	0.01	-0.11	0.02
Colchicine	-0.01	0.00	-0.07	0.00
Tamoxifen	-0.02	0.05	**-1.02**	0.00
Diclofenac	-0.08	0.00	-0.17	0.03
Methotrexate	-0.06	0.00	-0.01	0.00
Amiodarone	-0.03	0.01	**-0.49**	0.04
Nefazodone	**-0.86**	0.00	**-0.91**	0.00
Nimesulide	0.00	0.11	0.00	**0.50**
Hexachlorophene	**-0.91**	0.01	**-1.03**	0.00
Troglitazone	**-1.03**	0.03	**-0.80**	0.02

C

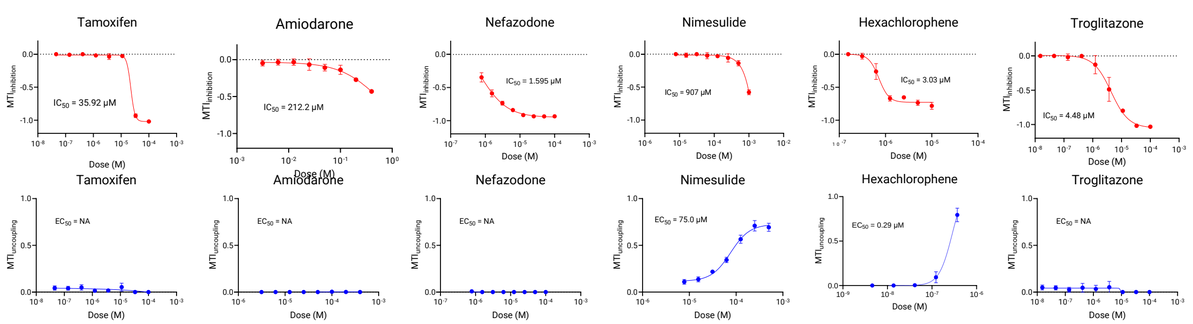


Figure 3. Mitochondrial toxicity evalution by MTI comparison
A. A representative MTI data from HepG2 cells acutely treated for 1 hour with drugs at 10 or 100 µM.
B. Summary of $MTI_{Inhibitor}$ and $MTI_{Uncoupling}$ for drugs tested.
C. Dose-response of MTI to drugs show |0.3| or higher at 100 µM.

Summary

Toxicity Evaluation Results

Drugs	Toxicity at 30x C_{max} MitoTox[1]	CytoTox[2]		Toxicity at 10x C_{max} MitoTox[1]	CytoTox[2]	
Name	|MTI| >0.3	ΔAUC_{24h} > 20%	ΔAUC_{72h} > 20%	|MTI| > 0.3	ΔAUC_{24h} > 20%	ΔAUC_{72h} > 20%
Acetaminophen	NA	NA	NA	Acetaminophen	No	No	No
Erythromycin	NA	NA	NA	Erythromycin	No	No	No
Enalapril	No	No	No	Enalapril	No	No	No
Leflunomide	No	No	No	Leflunomide	No	No	No
Lovastatin	No	No	Yes	Lovastatin	No	No	No
Colchicine	No	No	Yes	Colchicine	No	No	No
Tamoxifen	No	No	Yes	Tamoxifen	No	No	No
Diclofenac	No	No	Yes	Diclofenac	No	No	Yes
Methotrexate	No	No	Yes	Methotrexate	No	No	Yes
Amiodarone	Yes	Yes	Yes	Amiodarone	No	Yes	Yes
Nefazodone	Yes	Yes	Yes	Nefazodone	Yes	No	Yes
Nimesulide[3]	Yes	Yes	Yes	Nimesulide[3]	Yes	No	Yes
Hexachlorophene[3]	Yes	Yes	Yes	Hexachlorophene[3]	Yes	Yes	Yes
Troglitazone	Yes	Yes	Yes	Troglitazone	Yes	Yes	Yes

1) Classified as toxic if ΔCell Index > 20% using AUC normalized Cell Index for 24 or 72-hour duration
2) Classified as toxic if $MTI_{inhibition}$ < -0.3 or $MTI_{uncoupler}$ > 0.3
3) Uncoupler

Nontoxic
No evidence of mitochondrial toxicity
Attention required for mitochondrial toxicity
Toxic

Impact of mitochondrial toxicity

Drug Selected	Cytotoxicity		Mitochondrial Toxicity	
	IC_{50} (µM, 24 h)	IC_{50} (µM,72 h)	$MTI\ IC_{50}$ (µM)	$MTI\ EC_{50}$ (µM)
Tamoxifen	19.3	14.4	39.9	NA
Amiodarone	22.8	7.31	212	NA
Nefazodone	98.6	76.5	**1.60**	NA
Nimesulide	927	626	907	**75.0**
Hexachlorophene	13.6	3.84	**3.03**	**0.29**
Troglitazone	23.1	39.6	**4.48**	NA

Conclusion

By collecting data from both Agilent xCelligence and Seahorse XF technologies, a rich dataset is generated that goes beyond simple cytotoxicity measures.

Cellular impedance measurements provide insights into cell viability, proliferation, and morphological changes in real-time. The ability to track these changes over time results in sensitive and comprehensive data sets.

Mitochondrial toxicity assessment offers quantitative mechanistic information, which may be informative for decisions and predictions on whether drug candidates should or shouldn't be removed from a drug pipeline.

This study highlights the complexity of drug-induced cytotoxicity, particularly in identifying those associated with mitochondrial dysfunction, and the importance of considering both dose and time-dependent effects.

In vitro models using cell lines like HepG2 have limitations in accurately predicting liver toxicity and are often insufficient to be physiologically relevant. The enriched data from dual platforms may offer more comprehensive insights into this complexity. Further research with optimized workflows and improved data integration methods is anticipated to enhance these predictions.

References

1. Huang *at al.* (2021) Assessing Water Cytotoxicity with an Impedance-Based, Real-Time, and Label-Free Cellular Assay. Agilent Technologies, application note, publication number 5994-3712EN
2. Kam *et al.* (2022) A Customized XF Workflow for Detection and Characterization of Mitochondrial Toxicity. Agilent Technologies, application note, publication number 5994-4778EN
3. Schulz, *et al.* (2012) Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Critical Care 16:R136

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