Profiling Toxic Chemicals with a New Liver Cell-based Assay

By Lawrence A. Wiater, Shawn Noble and Barry R. Bochner

Biolog, Inc. Hayward, CA, USA

bbochner@biolog.com or www.biolog.com

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Introduction

We have developed a new and simple cell based assay that can detect mitochondrial toxicity as well as cytotoxic effects and evaluated the assay using the ToxCast set of 320 chemicals. The assay employs a novel liver cell line (LW5-3 cells) and measures IC_{50} values with cells that are provided 8 different carbon/energy substrates. This approach provides more sensitive detection of mitochondrial toxicity and also enables more precise grouping of chemicals based on their mechanism of inhibition of the 8 diverse substrate metabolism pathways.

Abstract

Background: A new liver cell line and simple colorimetric assay was developed for assessing hepatotoxicity of chemicals. The assay measures inhibition of tetrazolium reduction and determines IC_{50} values while cells incubate in eight different media, each containing unique carbon/energy sources. An array of $\log(IC_{50})$ difference (LICD) values was calculated to characterize each chemical independent of its potency. Clustering experiments determined if arrays of LICD values contain enough information to assign chemicals into groups whose members produce similar cellular effects.

Method: LW5-3 liver cells, a HepG2 derivative, in RPMI1640 (without glucose) were dispensed into Biolog PM-M TOX1 MicroPlate wells whose rows contain different carbon/energy sources: glucose, inosine, xylitol, galactose, glucose-1-phosphate, α-ketoglutarate, β-hydroxybutyrate, and pyruvate. After a one day incubation, 2-fold serial dilutions of chemical were added to cultures. After two more days of incubation, Biolog Redox Dye Mix MB containing glucose was added and the rate of the tetrazolium reduction was measured with a Biolog OmniLog®, an automated incubator/reader. LICD array values were determined by assessing IC_{50} values, taking the log of the IC_{50} values, calculating an average $\log(IC_{50})$ value and subtracting it from all eight $\log(IC_{50})$ values. LICD arrays were clustered using Euclidean distance with Ward linkage.

Results: The carbon/energy sources for the cells changed the IC_{50} value for some but not all chemicals. Upon clustering chemical LICD arrays, unique groups were formed whose members had recognizable effects on mitochondrial function. The uncouplers FCCP and CCCP were clustered with fenofibrate, a chemical that induces uncoupler protein 2 in liver cells. Nine of the mitochondrial electron transport inhibitors, exemplified by rotenone, were all placed in the same cluster group. Reproducibility of obtaining LICD arrays was demonstrated using five different chemicals examined in replicate assays that were always placed in their unique cluster group. Based on a similarity cutoff score derived from replicate LICD arrays, eight cluster groups appear to be defined of which four could be annotated as uncouplers, electron transport inhibitors, protein synthesis inhibitors (cycloheximide) and non-specific inhibitors.

Conclusions: Using LICD arrays, structurally diverse chemicals can be grouped by the common mechanism they use to alter liver cell viability and metabolism. Databases built on arrays of LICD values appear to distinguish groups of chemicals whose inhibition of tetrazolium reduction is dependent on the carbon/energy source used to culture cells.

Materials

Biolog PM-M TOX1 Plates
- Versatile 96-well plate that forms eight different media upon cell addition.
- Wells in each row contains one of the following eight metabolites:
- Glucose, Inosine, Galactose, Glucose 1-phosphate, Xylitol, α-Ketoglutarate, β-Hydroxybutyrate and Pyruvate

Cells
- LW5-3 - A proprietary derivative of HepG2 liver cells

Chemicals
- Mitochondrial toxicants including uncouplers and inhibitors
- Non-specific inhibitors
- Protein synthesis inhibitors
- ToxCast 320 chemicals

OmniLog®
- Incubator/Reader
- Holds up to 50 microplates
- User selected temperature
- Reads wells every 15 min
- Newer software being developed for reading every 5 min
- Ideal to monitor tetrazolium assays
- Software available to determine rate of tetrazolium reduction.

Biolog OmniLog®: An automated incubator/reader

PM-M TOX1 Assay

Preparation of Cells and PM-M TOX1 Plate — Prep Time

Harvest cells with trypsin
Suspend liver cells at 187,500/mL in IF-M1 + 2 mM Gln + PS
Dispense into PM-M TOX1 plates at 7,500 cells/well in 40 uL in triplicate
Incubate overnight at 37°C under 5% CO_2-95% air — 2 hr

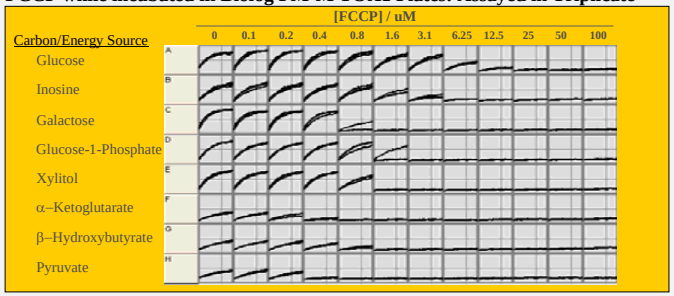
Chemical Addition

Make dilution series in IF-M1 + 2 mM Gln + PS + 5% DMSO
Add 10 uL per well
Incubate for 2 days at 37°C under 5% CO_2-95% air — 3 hr

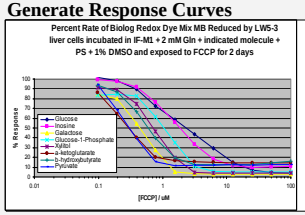
Cell Enumeration

Add 10 uL Biolog Redox Dye MB + 30 mM Glucose
Seal plate with clear film
Measure dye reduction with OmniLog at 37°C over 18 hr — 20 min

Reduction of Biolog Redox Dye MB by LW5-3 Cells after 2 day exposure to FCCP while incubated in Biolog PM-M TOX1 Plates: Assayed in Triplicate



Generate Response Curves



Extrapolate IC_{50} Values and Derive LICD

	Chemical A			Chemical B		
Media	Gluc	Xylitol	Pyr	Gluc	Xylitol	Pyr
IC_{50}	1	10	100	10	100	1000
$\log(IC_{50})$	0	1	2	1	2	3
$\log(IC_{50})$-Ave[$\log(IC_{50})$]	-1	0	1	-1	0	1

- Different potency by IC_{50} values
- But similar differences in $\log(IC_{50})$ values
- $\log(IC_{50})$-Ave[$\log(IC_{50})$] generates LICD values

Conclusions

Biolog's PM-M TOX1 MicroPlate provides a simple and sensitive assay to detect and categorize chemicals that are cytotoxic, or more specifically mitochondrial toxicants. The assay technology introduces several innovations: (1) Use of a novel liver cell line with greater metabolic versatility than HepG2 cells, (2) Use of a novel redox dye chemistry that gives a simple colorimetric readout, (3) Assay of cells with 8 diverse carbon/energy substrates, and (4) Accurate quantitation of metabolic rates using the OmniLog® instrument with capabilities to incubate and read kinetically 50 MicroPlates at a time.

From the set of ToxCast plus other chemicals, 4 cluster groups were discerned based on LICD values:

1. Non-Specific Inhibitors
2. Cycloheximide (Protein Synthesis Inhibitors)
3. Uncouplers
4. Respiratory Inhibitors

Clustering of LICD values from chemicals showed that:

1. Seven of eight chemicals examined in replicate were placed together in the same cluster group
2. Mitochondrial uncoupler protein (UCP) inducer fenofibrate clustered with uncouplers
3. Mitochondrial toxicants that reduce ATP production clustered with uncouplers

Clustering Chemicals By LICD