ALLOWS HEPATOTOXICITY STUDIES

INTRODUCTION

Hepatotoxicity testing in animals predicts only 50% of liver damage observed in clinical context, in part, because of difference in interspecies hepatic metabolism (1). Therefore, a relevant in vitro model mimicking the microenvironment of the human liver for maintaining and promoting hepatocyte functions, is needed for a better hepatotoxicity prediction which could greatly improve the efficiency of drug development.

(1) First dose of potential new medicines to Humans: how animals help, Greaves P. et al. Nature Reviews Drug Discovery. 3: 226-236, 2004

Materials required

- BIOMIMESYS®*Liver*
- HepG2, from ATCC
- Chlorpromazine (TCI Europe)
- Amiodarone (TCI Europe)
- Acetaminophen (TCI Europe)
- Cell Proliferation Reagent WST-1 (Sigma Aldrich)

Matrix properties

Translucent and porous

Method

- Seeding in 2D with 10,000 cells and in 3D with 50,000 cells
- For acute toxicity, cells were exposed to a range of 5 drug concentrations (Table 1) on day 7 for 24h (one dose)
- For chronic toxicity, cells were also exposed to chlorpromazine on days 5, 6 and 7 (3 repeated doses)

Chlorpromazine[C]	0.1 µM	1 µM	10 µM	50 µM	100 µM
Acetaminophen[C]	0.01 mM	0.1 mM	1 mM	10 mM	50 mM

Table 1: Drug concentrations used on HepG2

RESULTS

1. Acute Toxicity

– Cryopreserved human hepatocytes: cHH

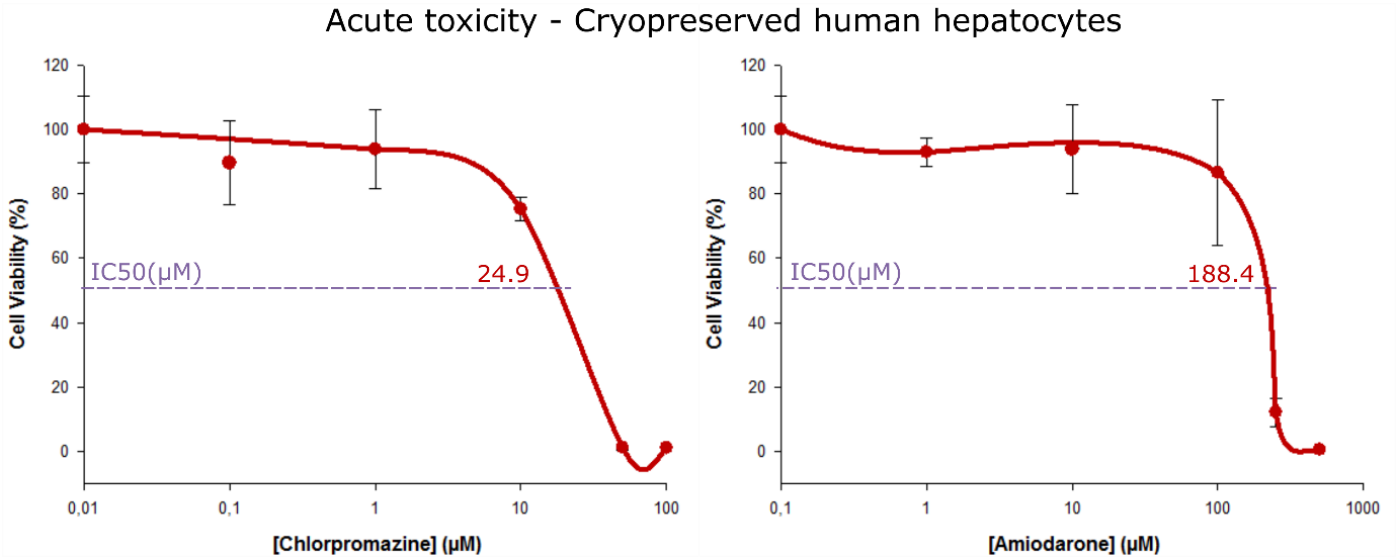

Figure 1: Drug-induced cell death in 3D grown cryopreserved human hepatocytes (2 sets of independent experiments)

Cryopreserved human hepatocytes grown in BIOMIMESYS®*Liver* represent a model for routine drug testing in 96-well format.

– Liver hepatocellular carcinoma : HepG2

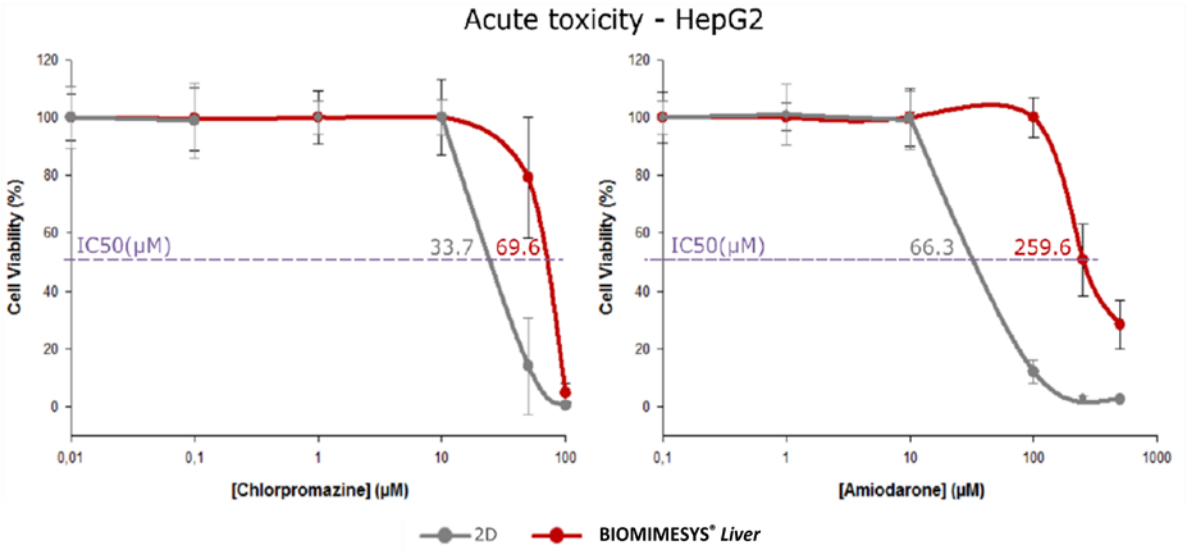

Figure 2: Drug-induced cell death in 2D and 3D grown HepG (2 sets of independent experiments)

HepG2 grown in BIOMIMESYS®*Liver* have higher IC50 compared to 2D conditions.

2. HepG2 – Chronic hepatotoxicity studies

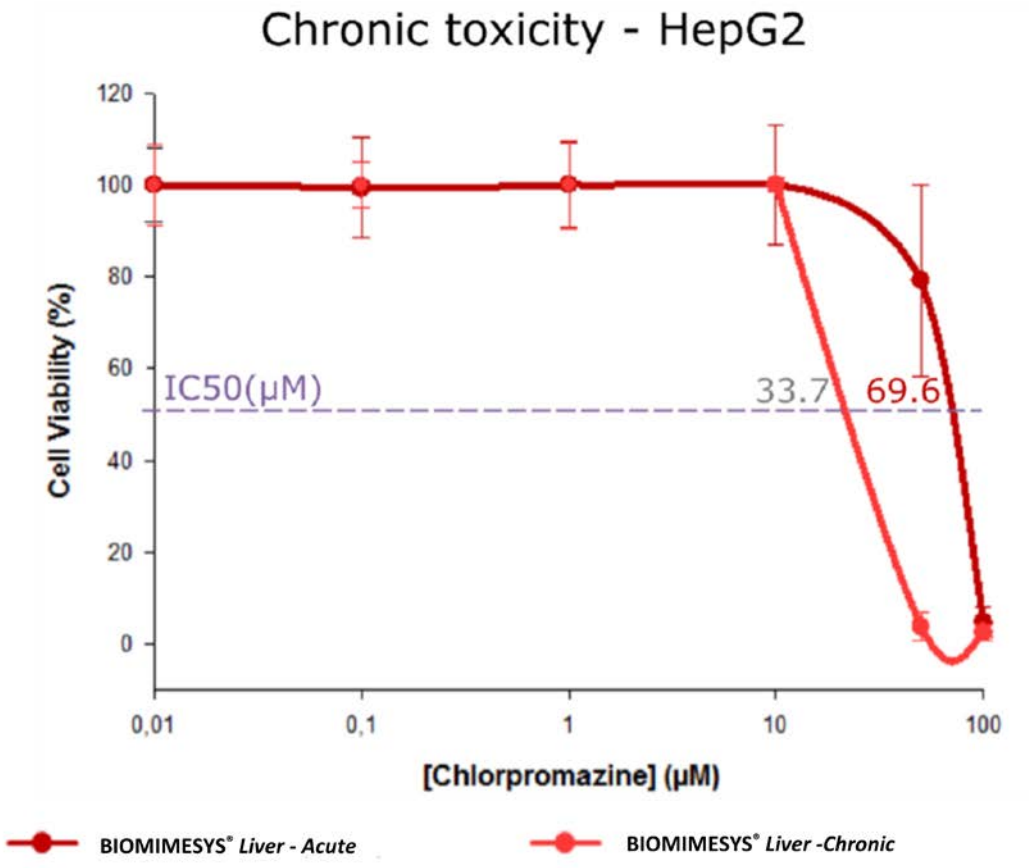

Figure 3: Repeated doses (3) of chlorpromazine induce cell death (2 sets of independent experiments)

Repeated doses of chlorpromazine decrease its IC50 value in acute treatment by a factor of 2.

CONCLUSION

- HepG2 grown in BIOMIMESYS®*Liver* display higher IC50 for chlorpromazine and amiodarone compared to 2D.
- Chronic treatment decreases the chlorpromazine IC50 of HepG2, compared to a single dose treatment.
- IC50 values of HepG2 grown in BIOMIMESYS®*Liver* are close to other 3D models using this cell line (2, 3), confirming the relevance of our scaffold to assess drug-induced hepatotoxicity.

REFERENCE

(2) 3D organotypic HepaRG cultures as in vitro model for acute and repeated dose toxicity studies, Mueller D. Toxicology in vitro. 28: 104-112, 2014

(3) Determination of drug toxicity using 3D spheroids constructed from an immortal human hepatocyte cell line, Fey S.J. and Wrzesinski K. Toxicological Sciences. 127: 403-411, 2012

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