Nov 30, 2017, JSSX Young Investigator's Award

Evaluation of drug-induced liver injury in sandwich cultured hepatocytes

薬物動態を基軸とした薬物誘発性肝毒性評価に関する研究

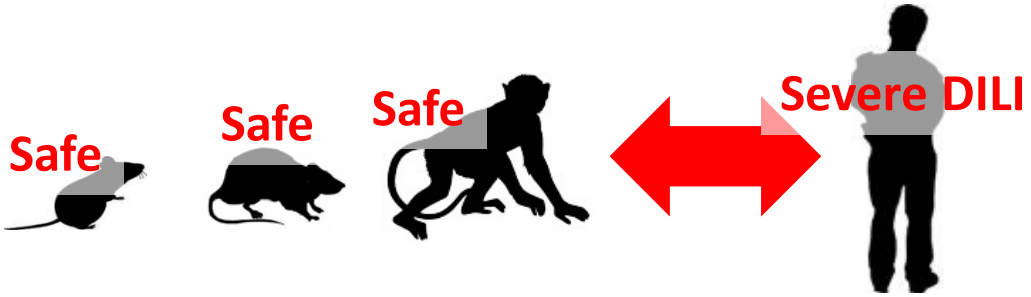
Shuichi Sekine, Ph.D., D.J.S.O.T

Department of Biopharmaceutics

Graduate school of Pharmaceutical Sciences,

CHIBA UNIVERSITY

Prediction of human DILI in preclinical study



Cultured human hepatocytes

= Golden Standard!!

Problems in prediction using "Animal study"

- ☑ High cost and Low throughput
- ☑ Differences in PKs and TDs
- ☑ Poor extrapolation from animal study to DILI risk in human

Problems in prediction using "human hepatocytes"

- ☑ Low reproducibility
- ☑ Loss function after isolation
- ☑ Culture conditions are not always appropriate

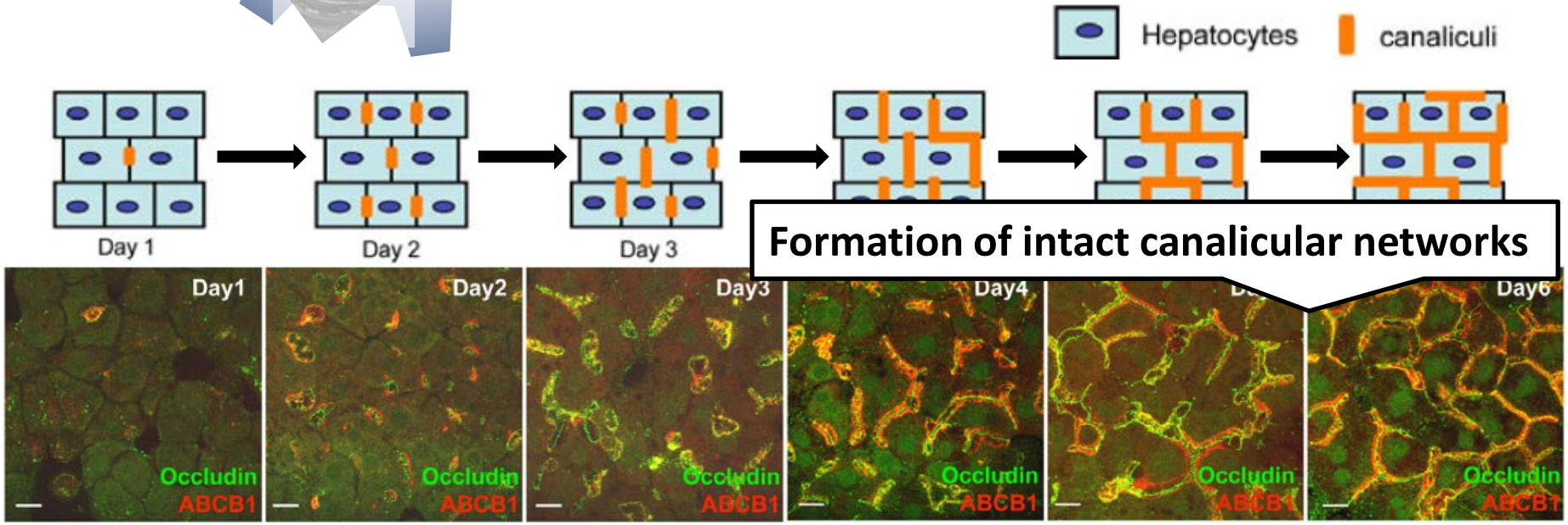
Highly functionalized cell-based assays are needed!!

Sandwich-cultured hepatocytes are a powerful in vitro tool

"Highly functionalized cells"

Sandwich cultured hepatocytes

- ☑ **Biliary efflux function**
- ☑ **Maintaining metabolic capability**
- ☑ **Easy to use and good reproducibility**



Prediction of human DILI in preclinical study

"Highly functionalized cells"

Sandwich cultured hepatocytes

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"Optimization for prediction of DILI"

- ☑ **Onset mechanisms of DILI are multifactorial events!!**
- ☑ **Understanding of DILI onset mechanism is indispensable!**

Inhibition of liver-specific functions associate with severe liver injury!!!

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

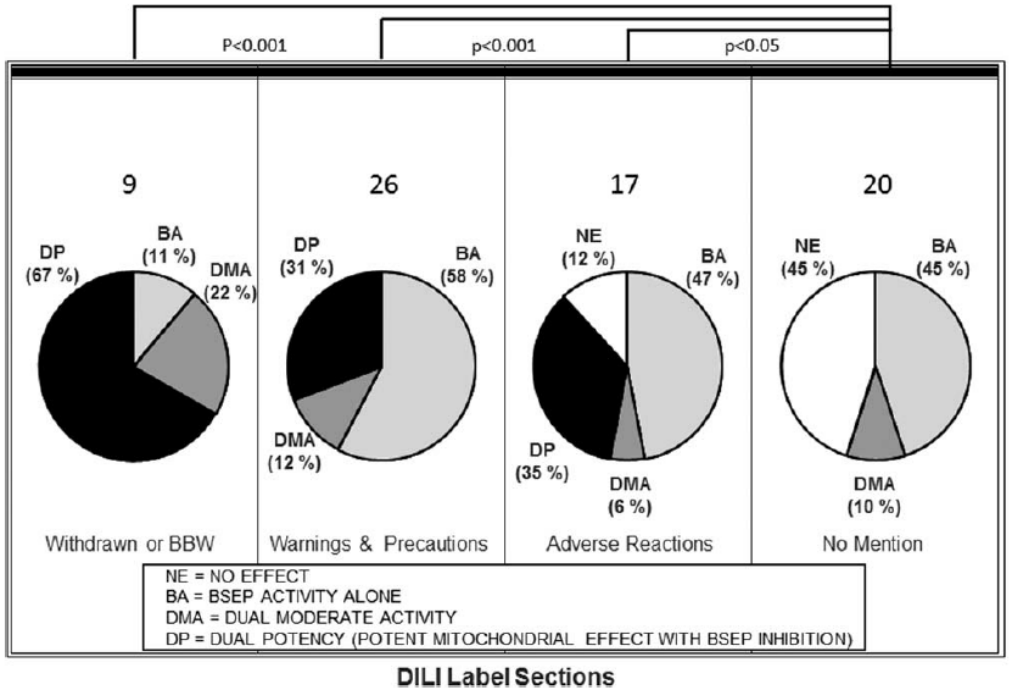
… which lead to loss of energy production

Human Drug-Induced Liver Injury Severity Is Highly Associated With Dual Inhibition of Liver Mitochondrial Function and Bile Salt Export Pump

… which leads to accumulation of toxic bile acids

Michael D. Aleo,[1] Yi Luo,[3] Rachel Swiss,[3] Paul D. Bonin,[2] David M. Potter,[4] and Yvonne Will[3]

Drug-induced liver injury (DILI) accounts for
hepatic failure and is a common reason for withdr
tinuation of a potentially new drug from clinical
individual risk factors contribute to the susceptibi
that are either compound- and/or patient-specific.
nisms linked to DILI include: cytotoxicity, reactive
bile salt export pump (BSEP), and mitochondrial d
dependent protein responsible for the efflux of
hypothesized that humans exposed to drugs that i
and BSEP functional activity are more sensitive to
than drugs that only have a single liability factor
National Center for Toxicological Research Liver
LTKB), the inhibitory properties of 24 Most-DIL
concern drugs were investigated. Drug potency fo
activity was generally correlated across human DIL
with dual potency as mitochondrial and BSEP in
more severe human DILI, more restrictive product
and appear more sensitive to the drug exposure (C
occurs. *Conclusion*: These data affirm that severe
multifactorial, highly associated with combinations
to known mechanisms of DILI (like mitochondria
with patient-specific factors, lead to differences in
associated with clinical DILI. (HEPATOLOGY 2014;60:



Prediction of human DILI in preclinical study

"Highly functionalized cells"

Sandwich cultured hepatocytes

- ☑ **Biliary efflux function**
- ☑ **Maintaining metabolic capability**
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"Optimization for prediction of DILI"

- ☑ **Cholestatic liver injury**
  - ➡ **Bile acids**
- ☑ **Mitochondrial toxicity**
  - ➡ **Oxygen supply**

Evaluation of cholestatic liver injury in SCHs

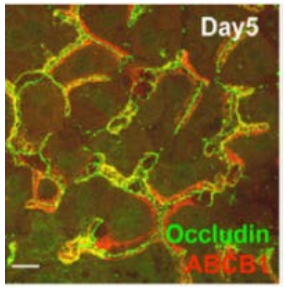
Even if, cholestatic drugs are exposed to sandwich-cultured hepatocytes, it is still difficult to detect cholestatic type liver injury ...

Bile acid	1× standard concentration (μM)
CA	0.30
CDCA	0.50
GCDCA	2.60
DCA	1.10
LCA	0.045
UDCA	0.17
GCA	0.62
GDCA	0.57
TCA	0.070
TCDCA	0.32
TLCA	0.13
TUDCA	0.43

±Bile acids mixture

Cholestatic drugs (50 μM)

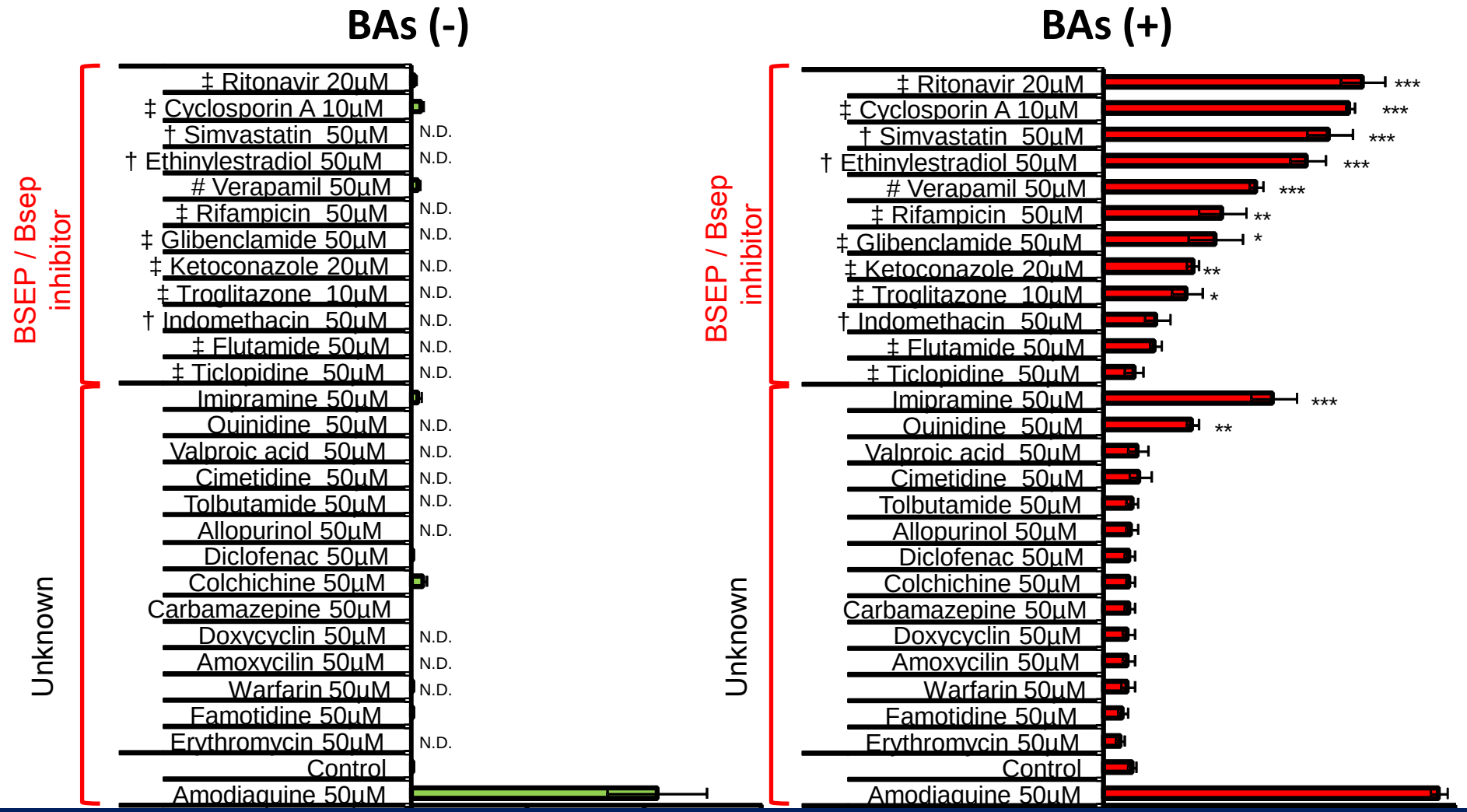
Rat SCH (Day 5)
Human SCH (Day 5)



Cell toxicity (LDH leakage)
24 hr exposure

When cholestatic drugs are exposed with bile acids, toxic bile acids accumulate in the cells and are expected to show toxicity.

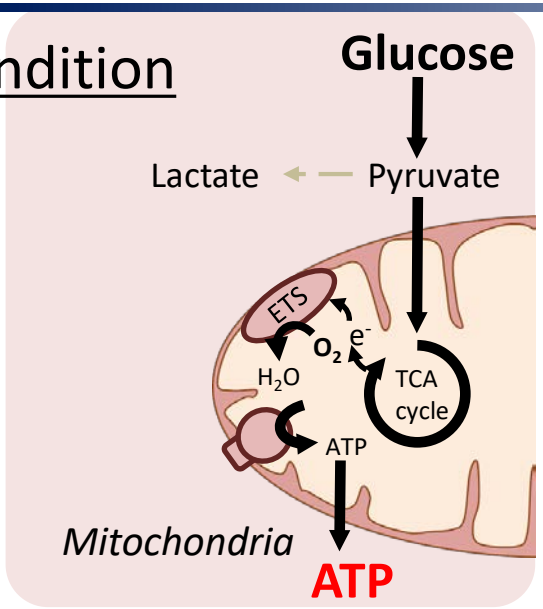
Evaluation of cholestatic liver injury in SCHs



It is possible to evaluate BAs-dependent drug-induced hepatocellular toxicity by cholestatic drugs

Problems in mitochondrial toxicity evaluation in vitro

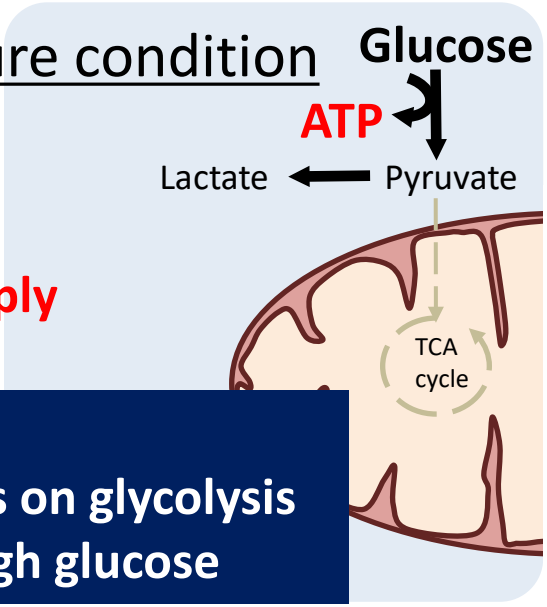
In physiological condition
(In vivo)

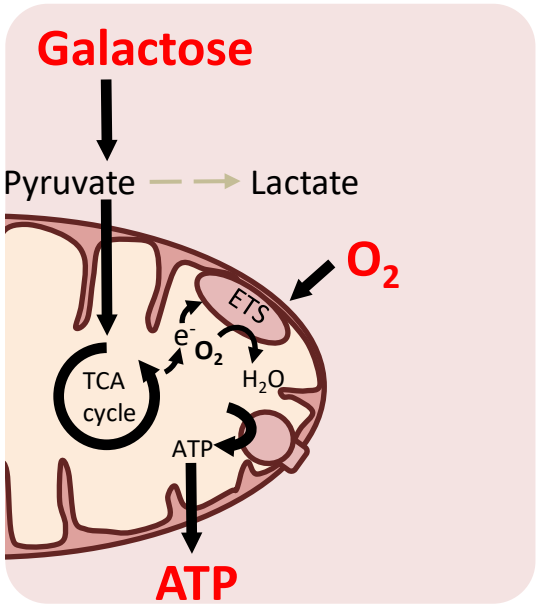


ATP production relies on oxidative phosphorylation in mitochondria

Conventional culture condition
(In vitro)

High Glucose
Limited Oxygen supply

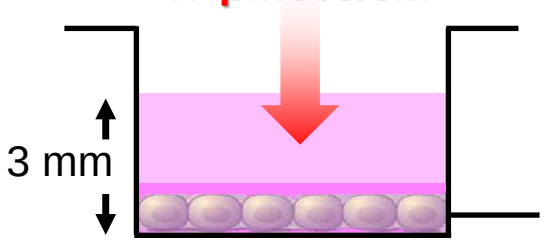




Crabtree effect:
ATP production relies on glycolysis in the presence of high glucose

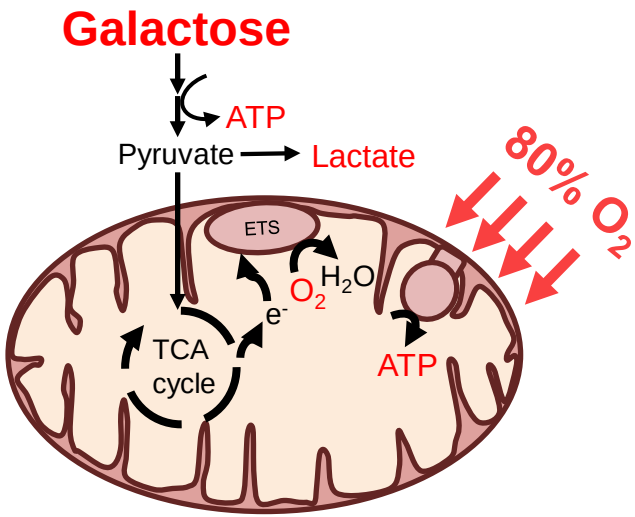
Appropriate oxygen supplement suppresses glycolysis

O_2 supplement through liquid-vapor interface
17 pmol/s/cm²

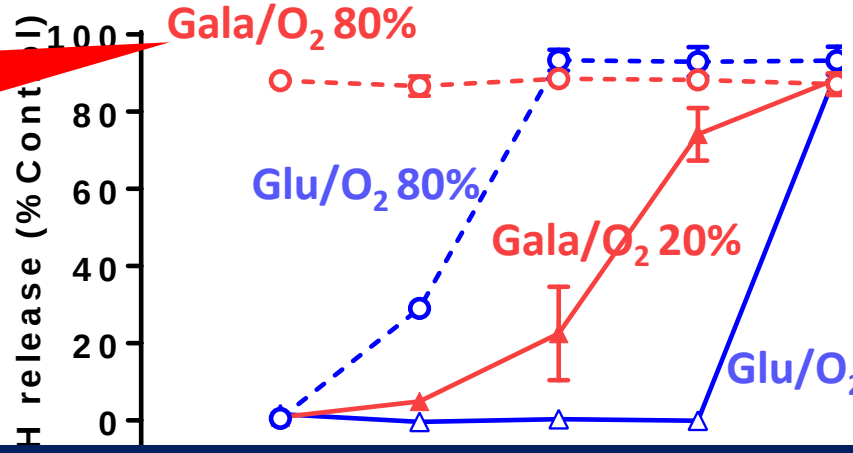


O_2 consumption in physiological condition
40-90 pmol/s/cm²

(*Sakai et al., Liver Stem Cells, 2011*)



Physiologically relevant culture condition



Conventional culture condition

Culture with Galactose medium and appropriate Oxygen supply enhances sensitivity to mitochondrial toxicant in SCHs

Mitochondrial toxicity screening under optimum conditions using rat SCHs

Conventional culture condition

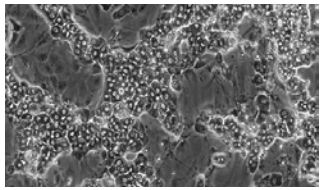
Physiologically relevant culture condition

Drugs	Mechanism of mitochondrial dysfunction	$TC_{50,Glu}$ (μM)	$TC_{50,Gala}$ (μM)	$TC_{50,Glu}$/ $TC_{50,Gala}$
Amiodarone		>500	121	>4.13
Rotenone		1.51	<0.50	>3.02
Phenformin	Lactic acidosis ➡ Withdrawn	267	<150	>1.78
Flutamide	OXPHOS inhibitor	85.3	52.2	1.63
Oligomycin		0.39	0.27	1.42
Antimycin		2.98	2.14	1.39
Ketoconazole		54.3	45.3	1.20
Metformin		>500	>500	1.00
Amitriptyline	Oxidative stress inducer	176	96.5	1.82
Imipramine		215	129	1.67
Nortriptyline		69.6	68.4	1.02
Troglitazone		64.1	63.0	1.02
Benzbromarone	MPT inducer	10.2	10.3	0.99

The distinct toxicities of phenformin and metformin were appropriately evaluated and reflected the rank order of risk in human

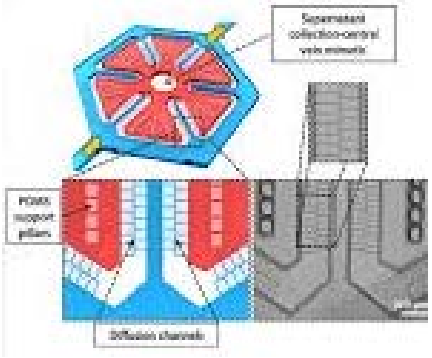
Perspectives

"Highly functional hepatocytes"



☑ **eg. iPS cells, PXB cells, HepaRG cells etc.**

"Highly functional liver"



☑ **New microphysiological system (MPS)**

What can they do??

Understanding their functions and features
(eg. Biliary efflux?, Mitochondrial function?)

How to use them??

Optimization for better prediction of DILI
(eg. Supplement missing items (Bile acids, Oxygen))

Acknowlegments

Prof. Toshiharu Horie
（Teikyo Heisei University）

Prof. Kousei Ito
（Chiba University）

Chiba University

Collaboration and Support

Toru Horie (D3 Institute)
Oshimura Mitsuo (Tottori University)
Kazuki Yasuhiro (Tottori University)
Satoh Daisuke (Tottori University)
Ohe Tomoyuki (Keio University)
KAC Co., Ltd.
Biopredic International
LSI Medience Corporation
Sekisui medical co., ltd