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In Vitro Cholestatic DILI & Mitochondrial Toxicity Studies to Assess Hepatotoxicity

Presented by

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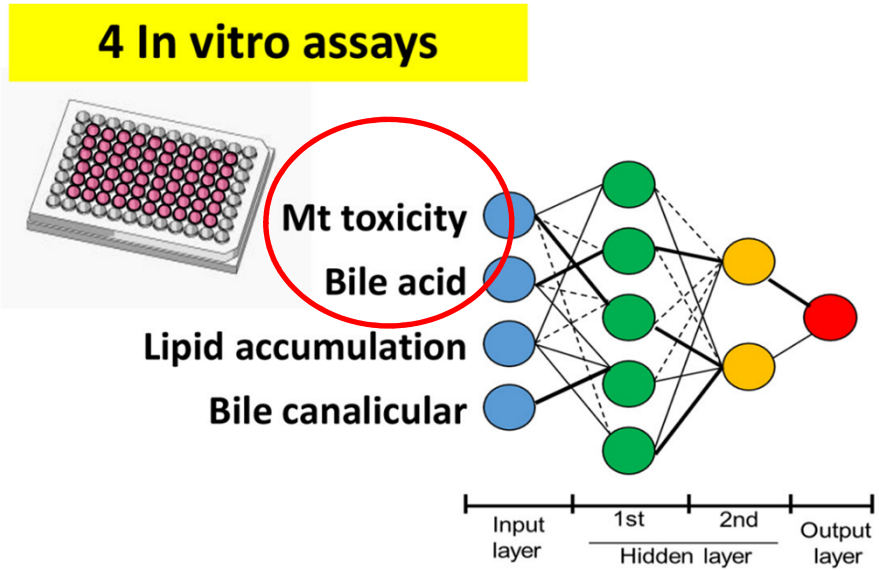
SEKISUI CHEMICAL GROUP

Background

Drug-induced liver injury (DILI) is caused by various developmental mechanisms and it is difficult to accurately predict it in one type of assay. Therefore conducting various experiments and making a comprehensive judgment leads to accurate risk evaluation.

Figure 1. Overview of assays and their interrelationship.

Chemical Research in Toxicology
Volume 25, Issue 8, 20 August 2012

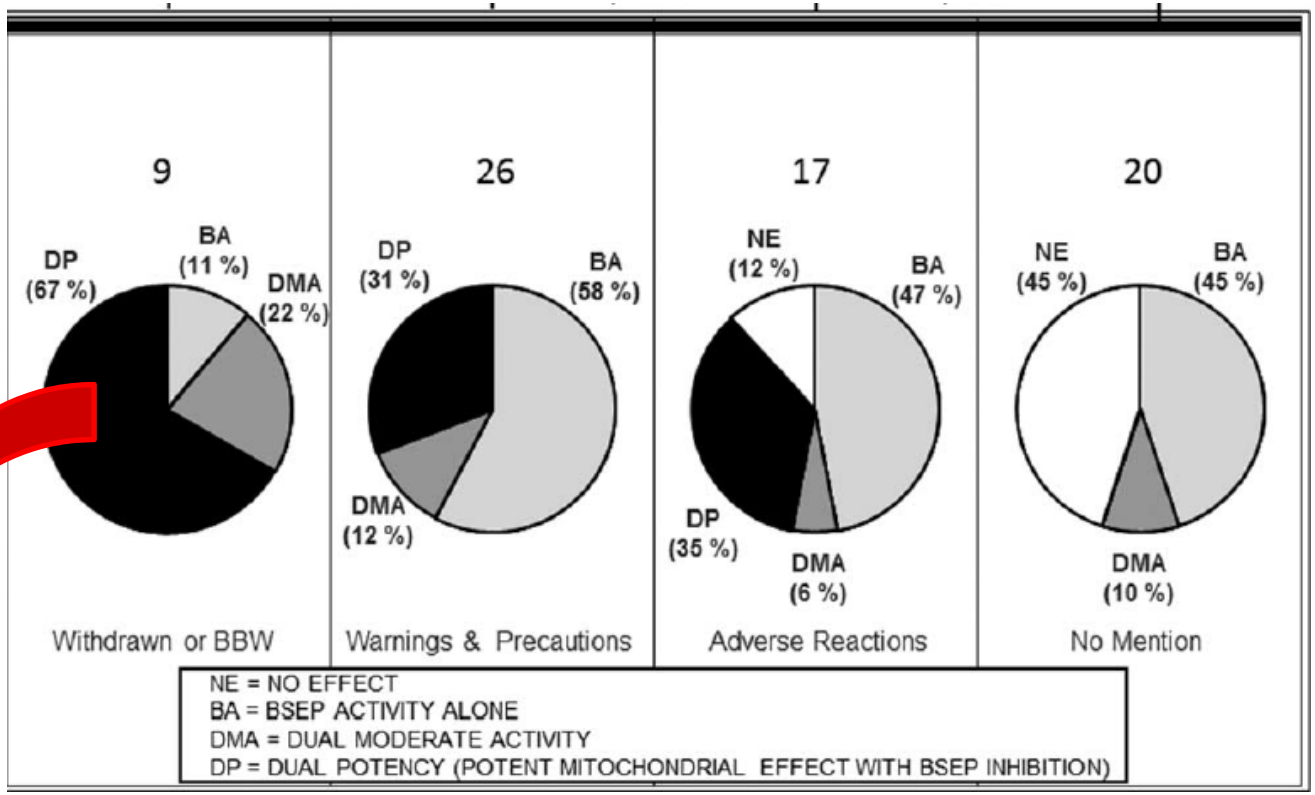


Toxicology and Applied Pharmacology 394 (2020) 114958

A survey of papers conducting comprehensive evaluations reveals that many studies focus on cholestasis and mitochondrial toxicity.

Background

From the point of view of clinical safety information ...



HEPATOLOGY, Vol. 60, No. 3, 2014 (modified)

In many cases, mitochondrial toxicity and bile acid transporter inhibition are observed in drugs that are withdrawn or classified for BBW.

In Vitro Hepatotoxicity Studies

- Cholestatic Drug-Induced Liver Injury
  - ROC Analysis: Predicting Clinical Hepatotoxicity / Cholestasis
  - Functional Assay: Bile Acid-Dependent Hepatotoxicity
- Mitochondrial Toxicity

In Vitro Hepatotoxicity Studies

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- Mitochondrial Toxicity

Cholestatic DILI Assessment

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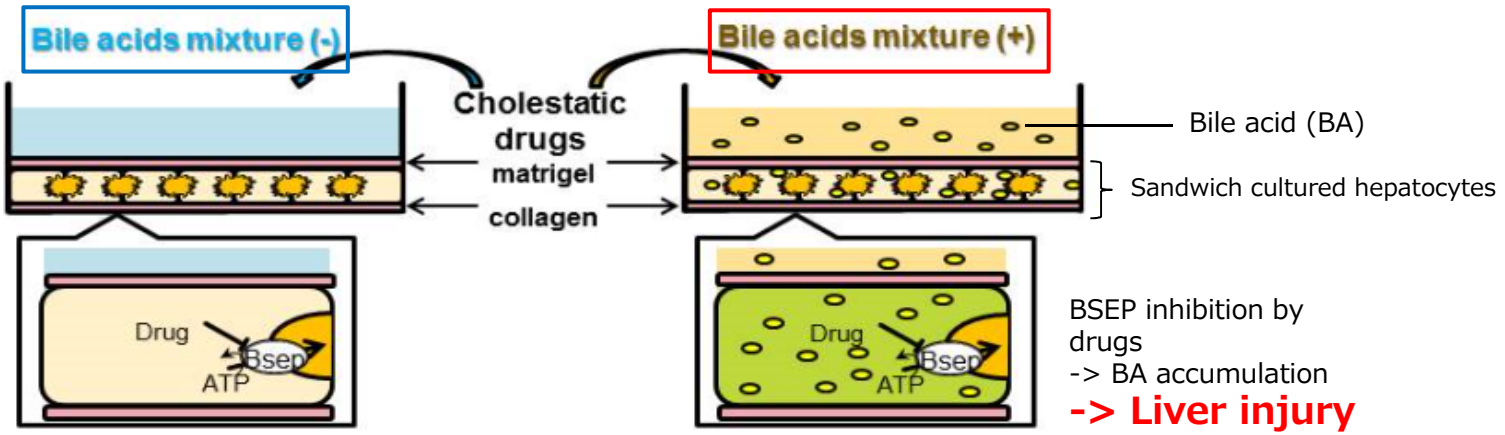
➢BSEP is one of transporters that relates bile acid (BA) excretion
➢BA accumulation in liver occurred when BSEP is inhibited by drugs

= Drug-induced cholestatic liver injury

Ref) Susukida et al., Drug Metab Disp, 2015

SMD has established an in vitro test system that assess the drug-induced cholestatic livery injury by patent* licensing from Chiba University

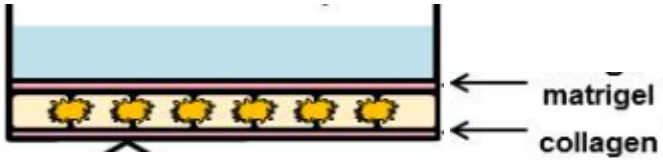
*patent application 2011-152087



Cholestatic DILI Assessment

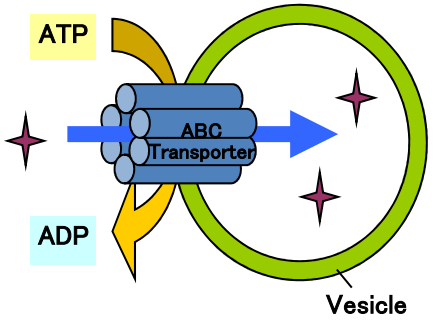
<Features of the sandwich-cultured hepatocytes>

Sandwich-cultured hepatocytes



・Has metabolic capacity
・Has a function to regulate the expression level of transporters and metabolic enzymes

Transporter expression vesicle

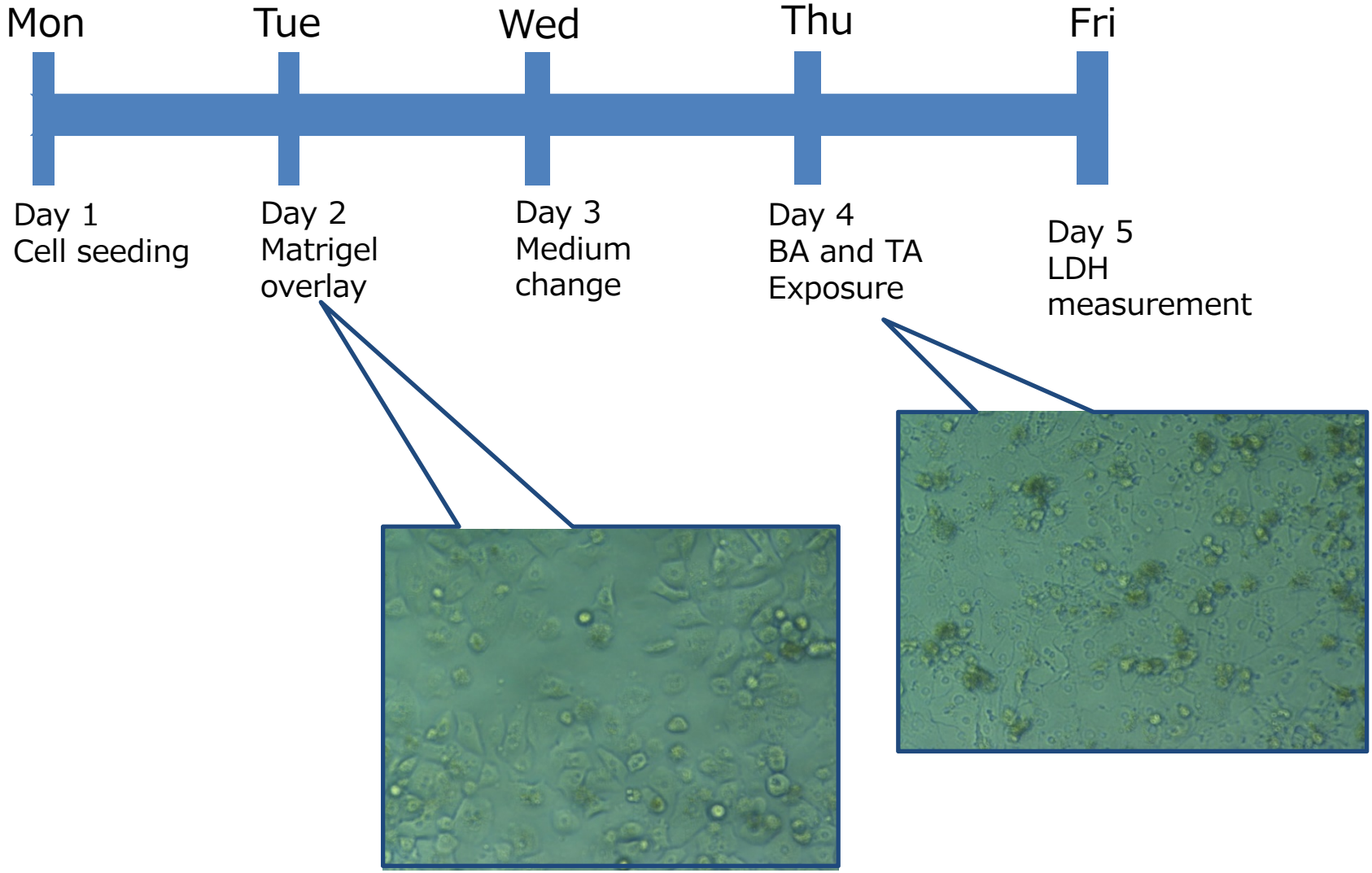


・No metabolic capacity
・No function to regulate the expression level of transporters and metabolic enzymes

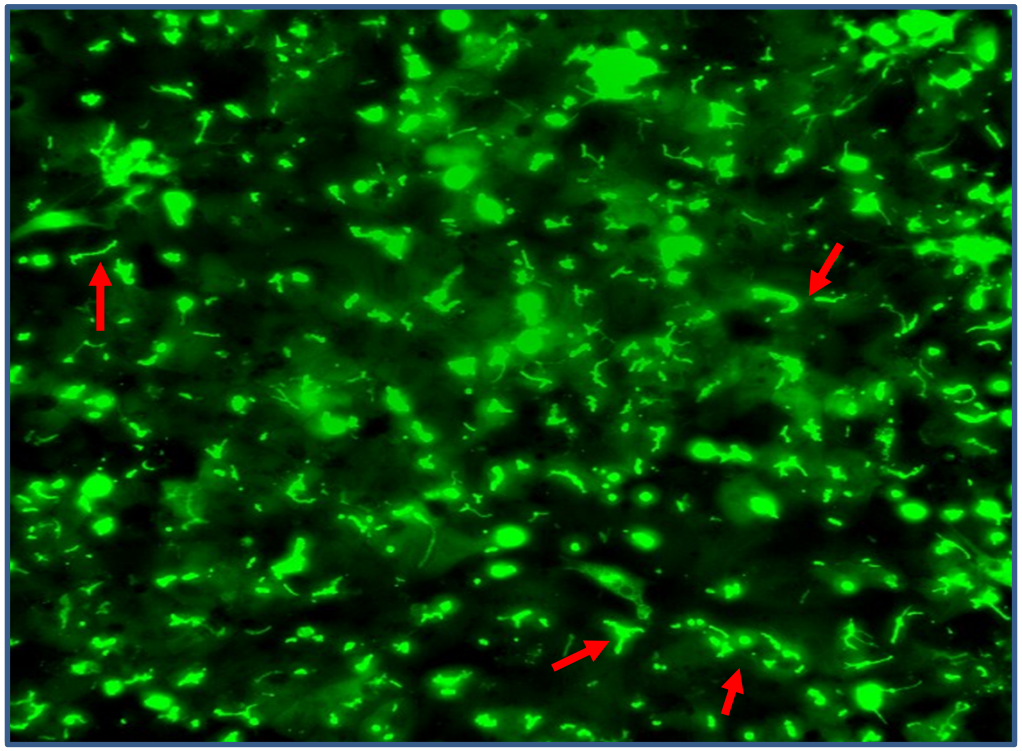
Frozen hepatocyte sandwich culture is thought to be able to evaluate phenomena involving biological mechanisms such as metabolism.

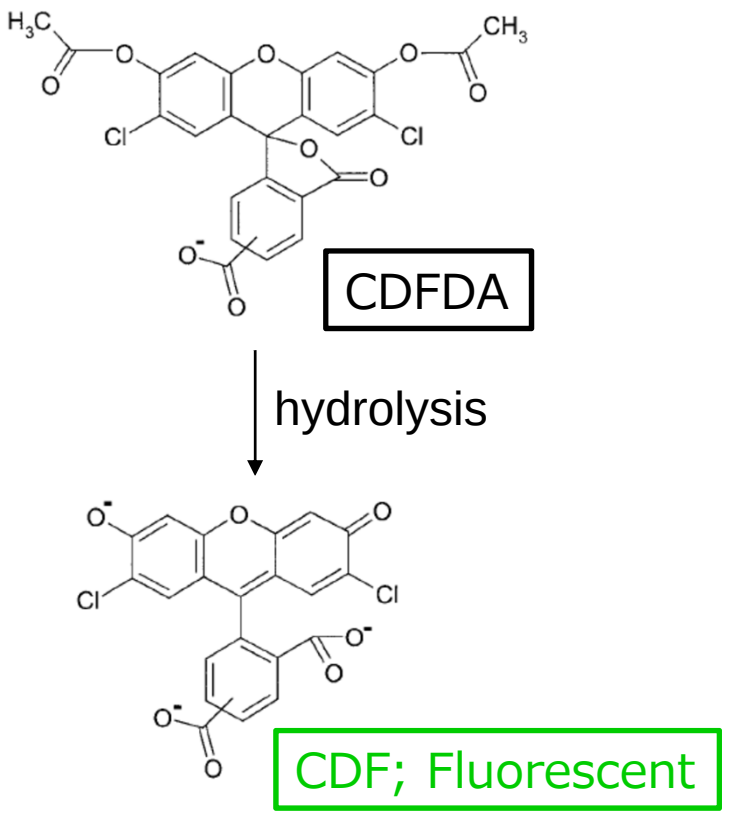
Cholestatic DILI Assessment

<Methodology>



Cholestatic DILI Assessment

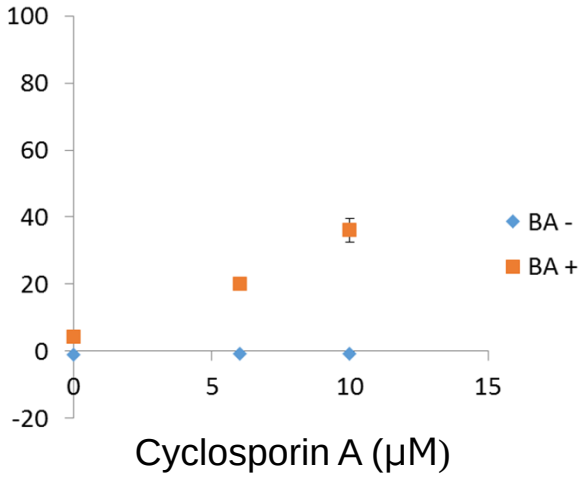




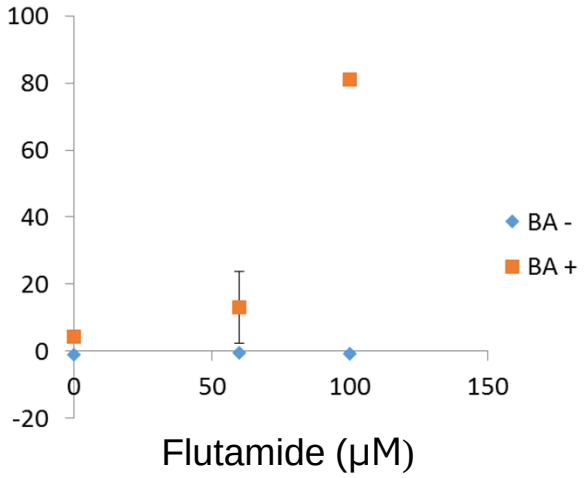
- Day 5 of sandwich cultured hepatocyte
- Bile canaliculi-like structure is visualized by CDFDA (5 (and 6)-carboxy-2,7-dichlorofluorescein diacetate) exposure and obtain photomicrograph by confocal microscope (ImageXpress, Molecular Devices)

Cholestatic DILI Assessment

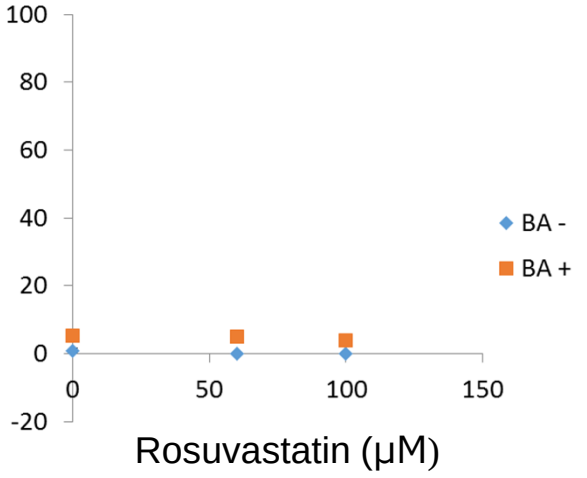
< Validation (positive, negative control evaluation) >



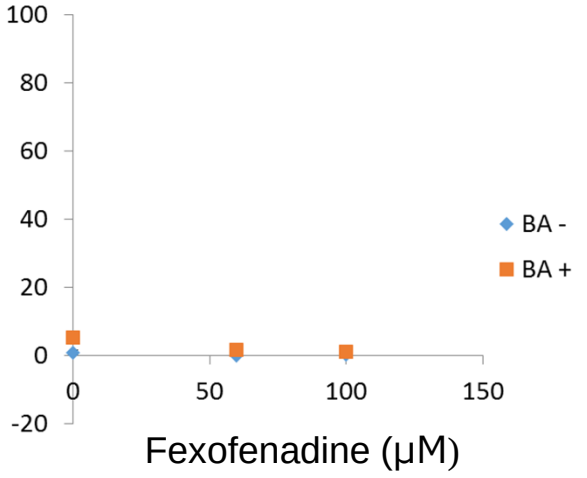
Cyclosporin A (μM)



Flutamide (μM)



Rosuvastatin (μM)



Fexofenadine (μM)

Cholestatic DILI Assessment

<Sample Protocol>

1. ROC (Receiver Operating Characteristics) analysis

- Test substance 1 concentration (100 μM)
- Bile acid presence
- 24 compounds for ROC analysis

(1) Set the cutoff value with the compound for ROC analysis
(2) Positive hepatotoxicity if the LDH value of the test substance exceeds the cutoff value

2. Functional Assay (Bile Acid-Dependent Hepatotoxicity)

- 7 concentrations of test substance
- Bile acid with / without
- Positive control 1 concentration 1 type (Cyclosporin A)
- Negative control 1 concentration 1 type (Fexofenadine)

Procedure
1 Calculate EC_{50} with and without bile acid
2 Evaluate whether there is a difference in EC_{50} with or without bile acid
⇒ If there is a difference, positive for cholestasis

Cholestatic DILI Assessment

ROC (Receiver Operating Characteristics) analysis

Relation between *in vitro* tox test (LDH release) and clinical biomaker (ALP)

Ref) Susukida et al., Drug Metab Disp, 2015

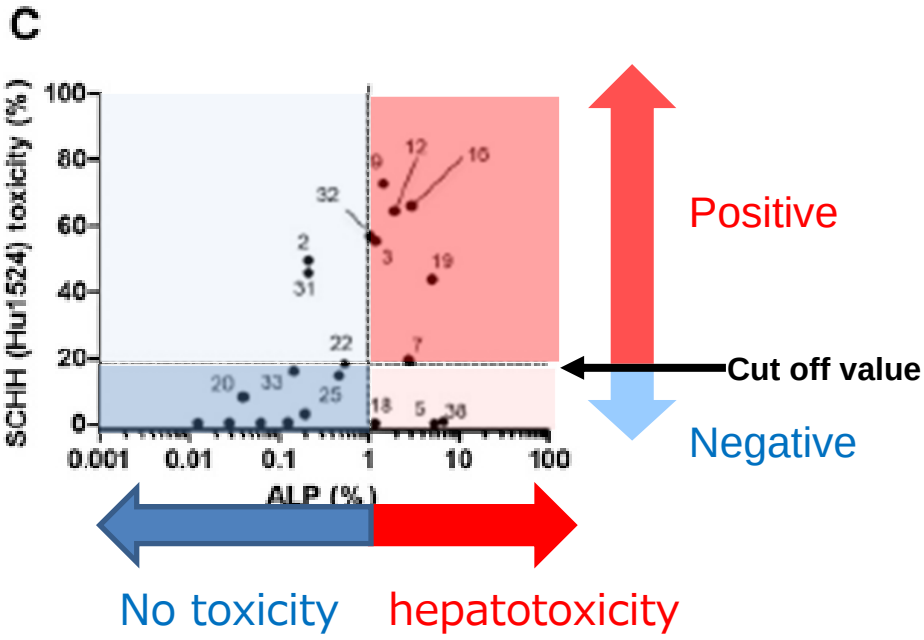
Clinical toxicity information

X

No.	Drug	ALP (%)
1	Acarbose	0.24 (11/4543)
2	Amiodarone	0.22 (3/1352)
3	Atorvastatin	1.26 (72/5702)
4	Bosentan	7.5 (3/40)
5	Carbamazepine	5.54 (18/325)
6	Clarithromycin	0.011 (3/26,923)
7	Clopidogrel	2.87 (65/2268)
8	Clozapine	14.3 (11/77)
9	Cyclosporine A	1.53 (112/7300)
10	Duloxetine	2.90 (36/1242)
11	Ethinyl estradiol	5.28 (36/682)
12	Everolimus	2.00 (25/1247)
13	Famotidine	0.065 (13/20,137)
14	Fexofenadine	0.013 (1/7838)
15	Fluoxetine	0.73 (4/550)
16	Flutamide	3.14 (201/6393)
17	Fluvoxamine	0.73 (7/965)
18	Lamivudine	1.23 (40/3253)
19	Leflunomide	5.21 (19/365)
20	Levofloxacin	0.041 (13/31,810)
21	Losartan	0.029 (11/36,288)
22	Methotrexate	0.55 (22/4038)
23	Mitiglinide	0.84 (13/1555)
24	Naftopidil	0.027 (6/22,013)
25	Pioglitazone	0.48 (23/4776)
26	Pranlukast	0.032 (3/9240)
27	Pravastatin	0.13 (15/11,137)
28	Ranitidine	0.063 (10/15,761)
29	Rosuvastatin	0.20 (18/8997)
30	Sertraline	0.71 (9/1263)
31	Simvastatin	0.22 (23/10,420)
32	Tacrolimus	1.07 (107/10,038)
33	Ticlopidine	0.15 (12/7933)
34	Tranilast	0.13 (32/24,788)
35	Ursodeoxycholic acid	0.12 (12/9880)
36	Valproate	0.35 (19/5366)
37	Valsartan	0.24 (19/7814)
38	Voriconazole	7.00 (7/100)

ALP; >1 % is considered as hepatotoxicity

ROC analysis

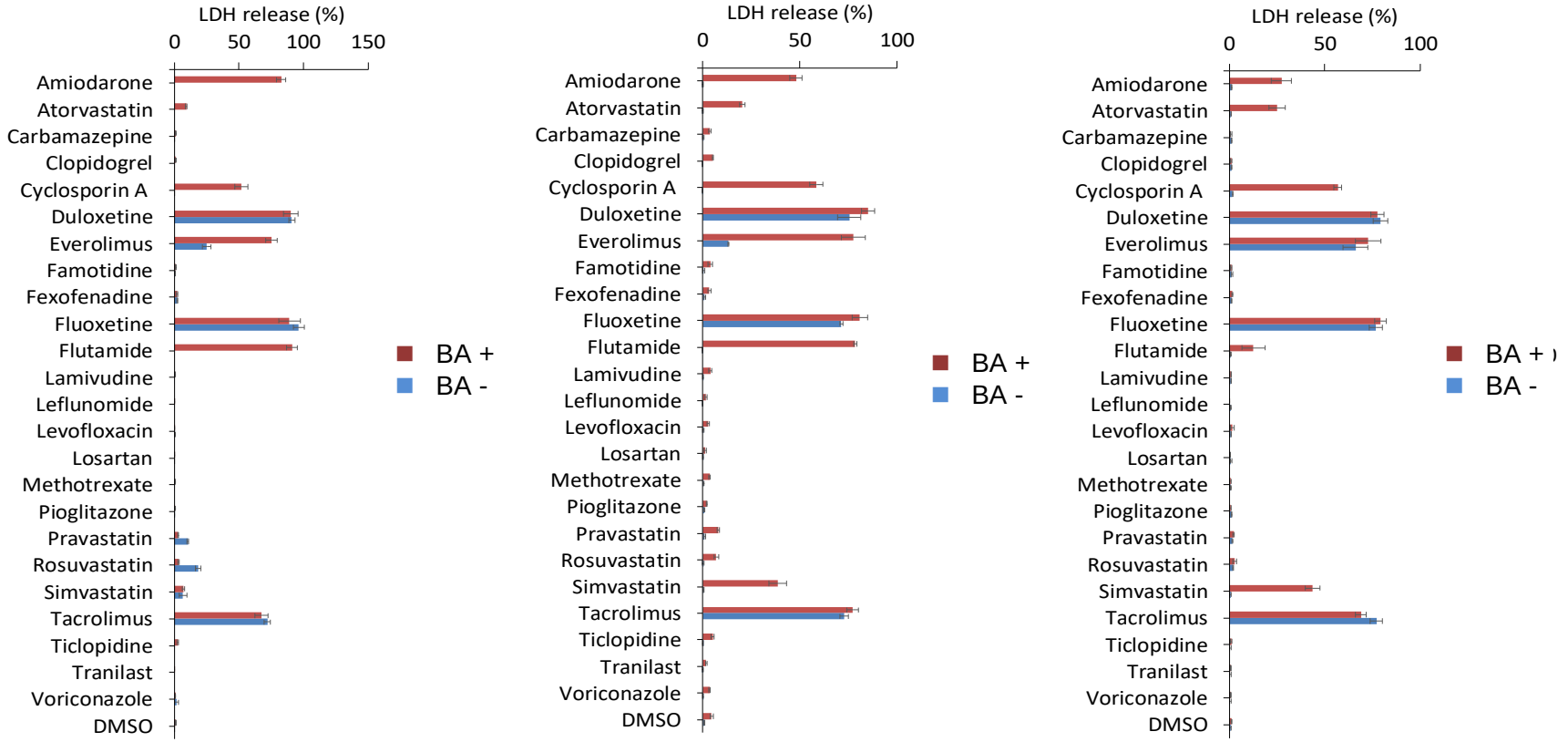


In vitro toxicity cutoff (%)	18.5
Sensitivity (%)	70.0
Specificity (%)	83.3
Area under the ROC curve	0.742
P value	0.0560

Cholestatic DILI Assessment

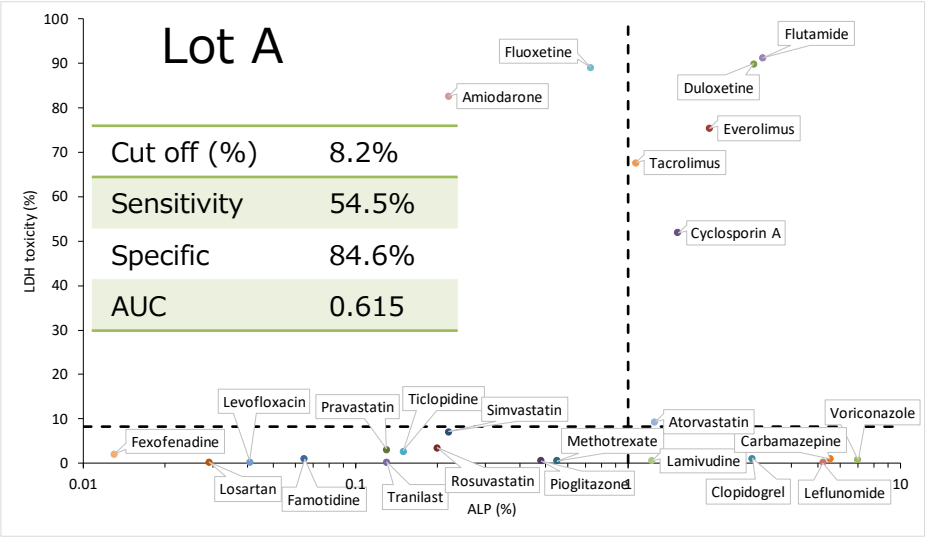
<Toxicity test for 24 chemicals>

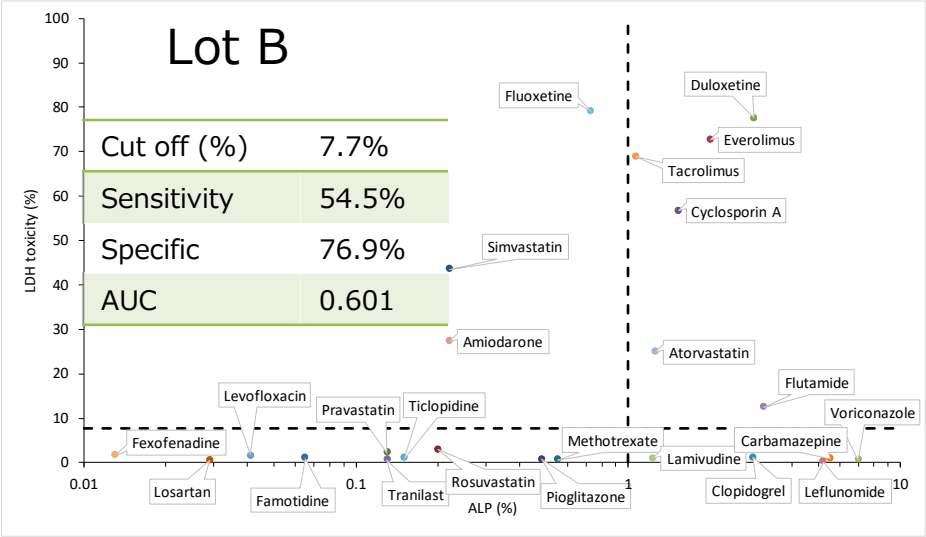
LDH measurement was performed on 3 Lots of hepatocytes after exposure to the drug/bile acid for 24 hours.

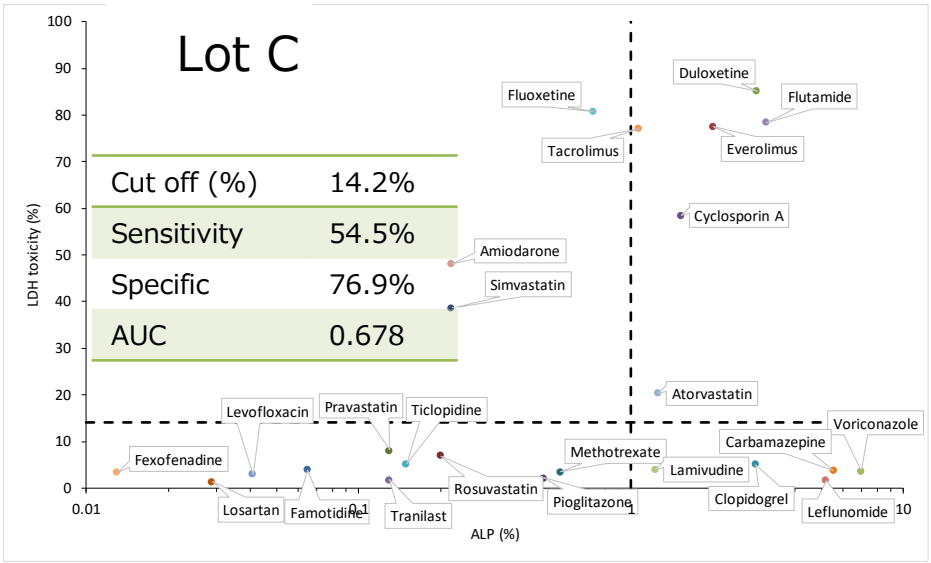


- By adding BA, more toxicity can be detected.
- Similar tendency was seen between lots

Cholestatic DILI Assessment







High reproducibility was observed

- We totally have 5 lot data
- Day-to-day reproducibility data was also obtained (data not shown)

Cholestatic DILI Assessment

Functional Assay (Bile Acid-Dependent Hepatotoxicity)

Normal Study Design

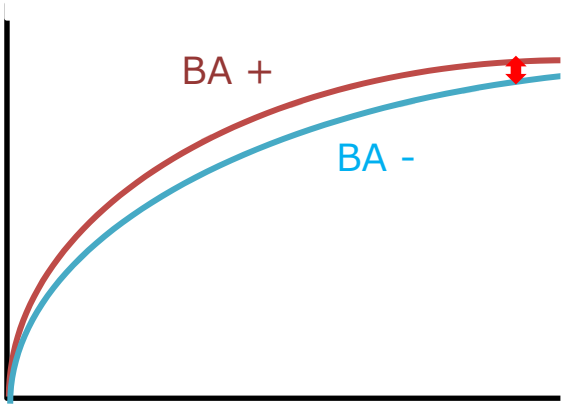
- 7 concentrations of test substance
- Bile acid with / without
- Positive control 1 concentration 1 type (Cyclosporin A)
- Negative control 1 concentration 1 type (Fexofenadine)

Procedure

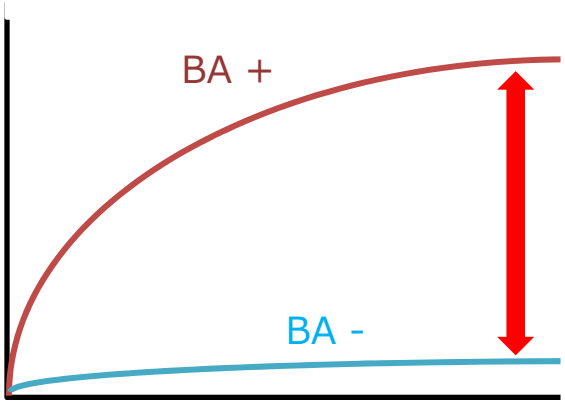
1 Calculate EC_{50} with and without bile acid

2 Evaluate whether there is a difference in EC_{50} with or without bile acid

⇒ If there is a difference, positive for cholestasis



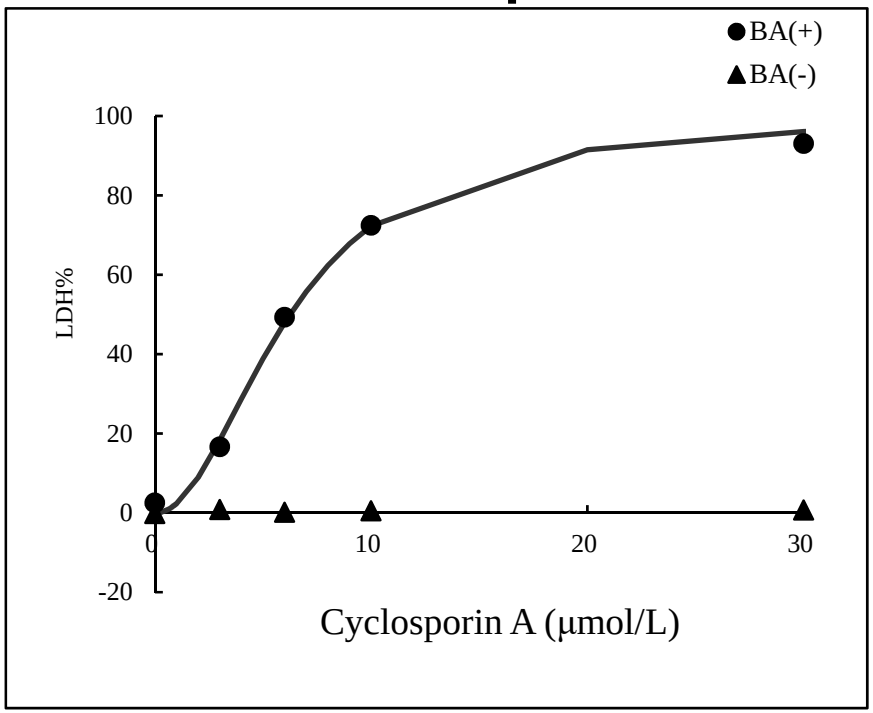
No difference: Negative for cholestasis

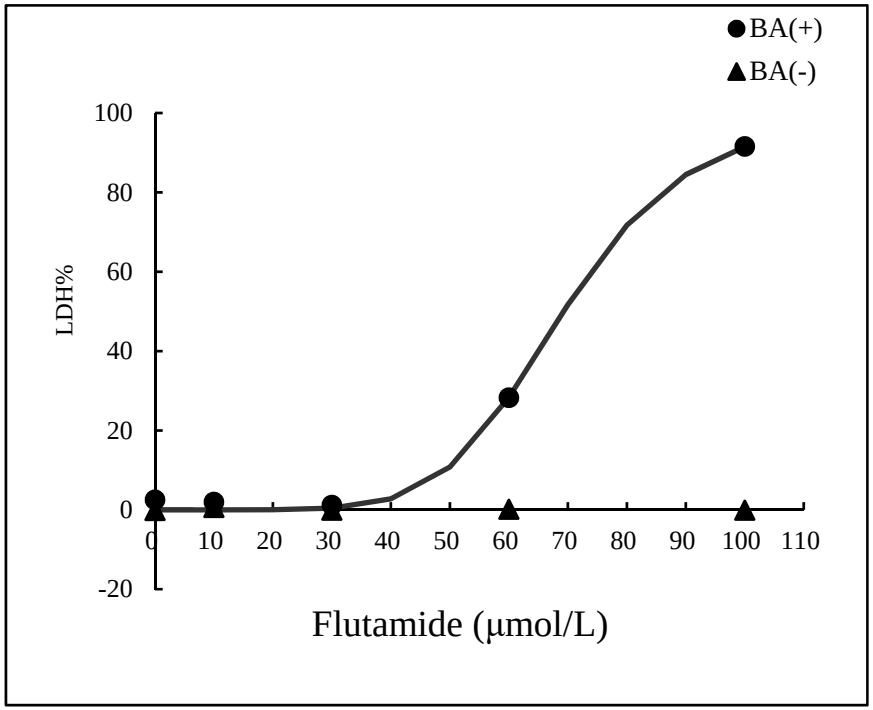


Big difference: Positive for cholestasis

Cholestatic DILI Assessment

<Prediction of the presence or absence of cholestasis>





Differences in toxicity between the presence of bile acids (BA +) and the absence of bile acids (BA-)
⇒ Suspected cholestatic-type hepatotoxicity

Cholestatic DILI Assessment

This study could be helpful for…

- Someone who observed hepatotoxicity in in vivo animal test
  -> To get the data regarding human liver

- Someone who observed strong BSEP inhibition in vesicle study
  -> This study shows an “actual hepatotoxicity”. The data obtained in this study may save NCEs that have BSEP inhibition potency.

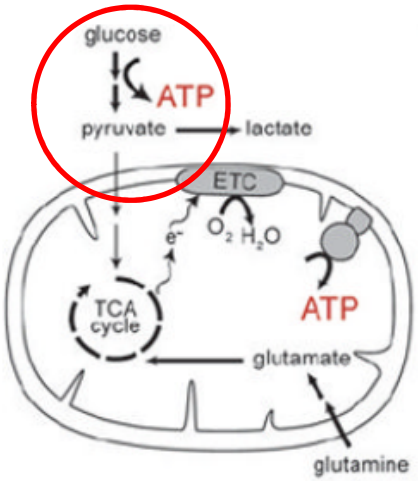
In Vitro Hepatotoxicity Studies

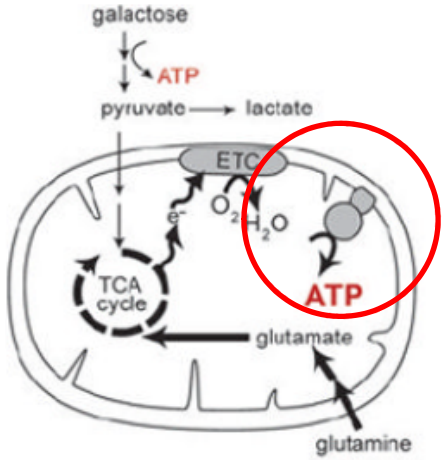
- Cholestatic Drug-Induced Liver Injury
  - ROC Analysis: Predicting Clinical Hepatotoxicity / Cholestasis
  - Functional Assay: Bile Acid-Dependent Hepatotoxicity
- Mitochondrial Toxicity

Mitochondrial Toxicity Assessment SEKISUI

<Overview>

Crabtree effect: In vivo hepatocytes produce ATP by aerobic respiration, but when cultured in a glucose-rich medium (general medium), aerobic respiration is suppressed and ATP is produced mainly in glycolysis. It is possible to shift to a state in which aerobic respiration is dominant by culturing in a galactose medium.





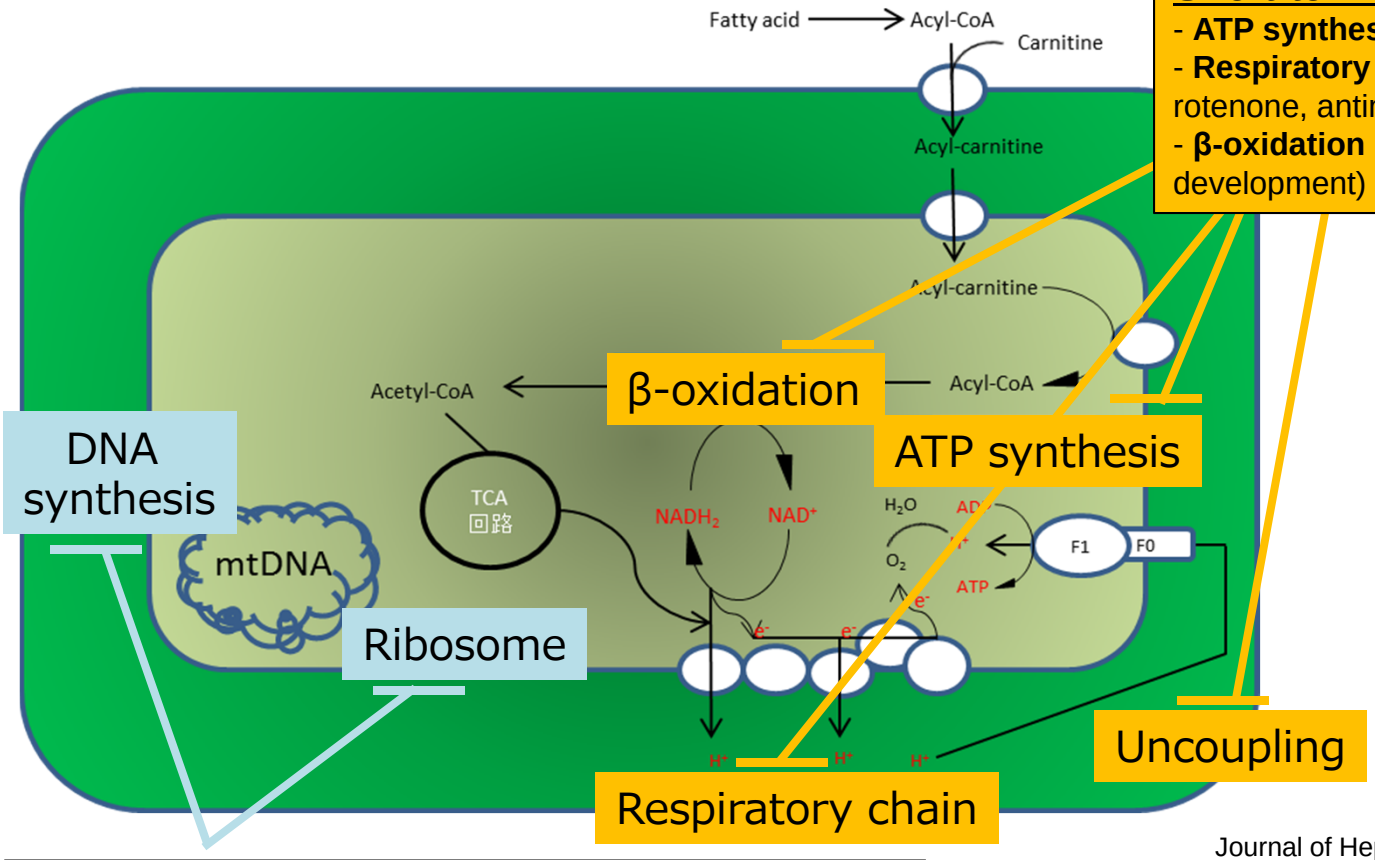
Nat Biotechnol. 2010 March ; 28(3): 249–255

Glucose culture
ATP is mainly produced by glycolysis.
ATP reduction is unlikely to occur even if mitochondrial toxicity occurs

Galactose culture
ATP is produced mainly in mitochondria.
When mitochondrial toxicity occurs, ATP production decreases directly

Mitochondrial Toxicity Assessment

◆ Mechanism of mitochondrial toxicity



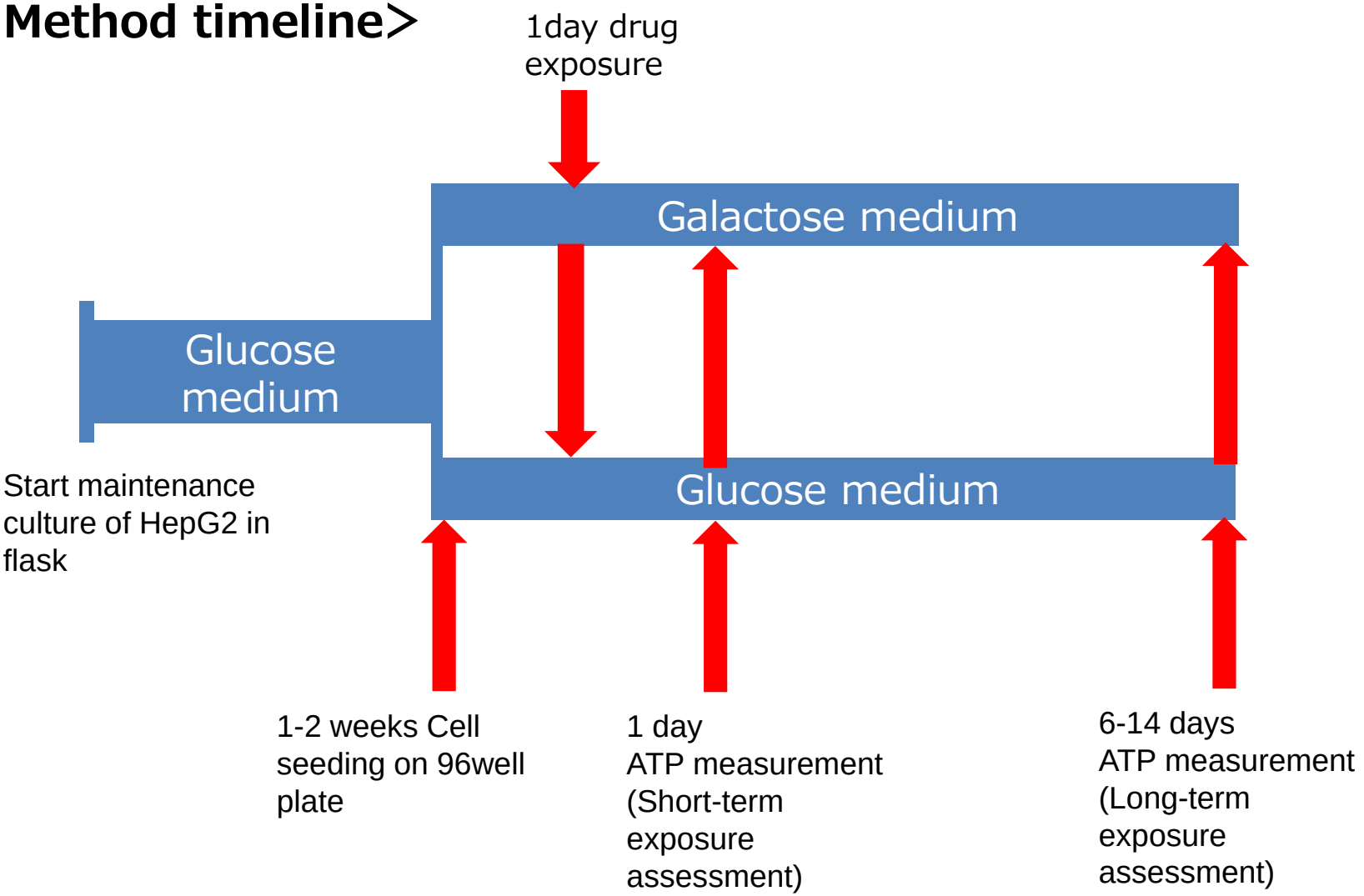
Short-term exposure (1day)
- **ATP synthesis inhibitor**: oligomycin
- **Respiratory chain inhibition, uncoupling**: rotenone, antimycin, FCCP
- **β-oxidation inhibition**: (under development)

Journal of Hepatology 2011 vol. 54 j 773–794

Long-term exposure assessment (6-14 days)
- **Ribosome inhibition**: chloramphenicol, linezolid
- **Inhibition of mtDNA synthesis**: (under development)

Mitochondrial Toxicity Assessment

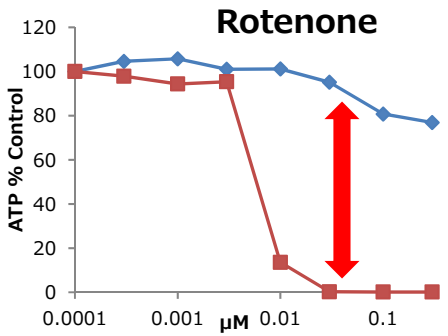
<Method timeline>

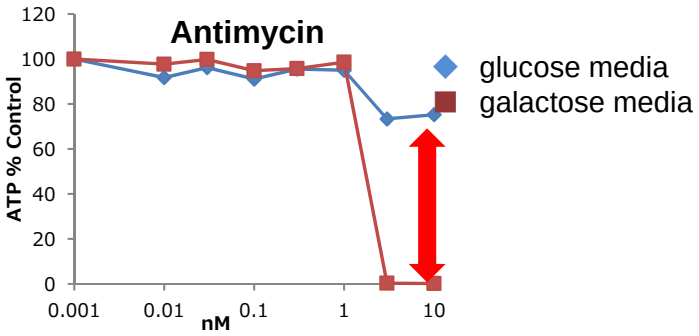


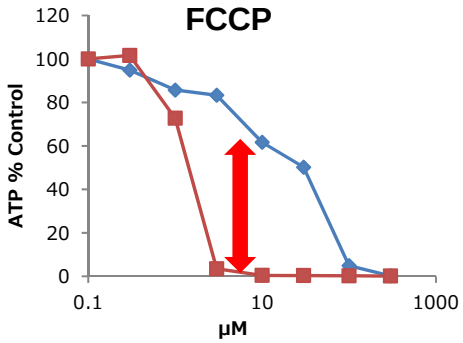
Mitochondrial Toxicity Assessment

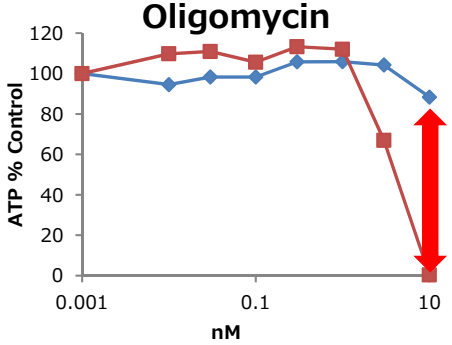
<Short-term exposure assessment>

◆ Respiratory chain inhibition, uncoupling, ATP synthesis inhibition

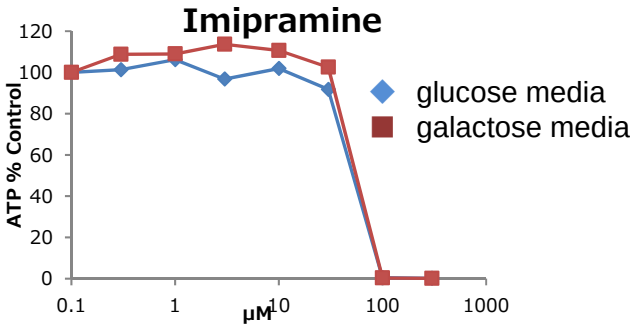


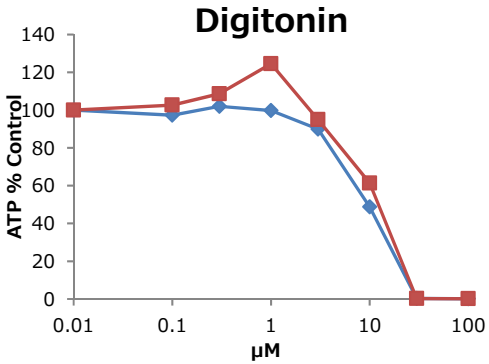






◆ Other cytotoxicity (negative control)





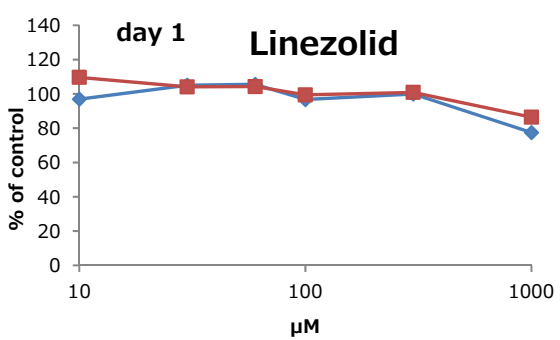
・Differences in toxicity expression in glucose / galactose cultures are observed for mitochondrial toxicity-positive compounds
・If the main toxicity mechanism is different than mitochondrial toxicity, no difference can be seen.

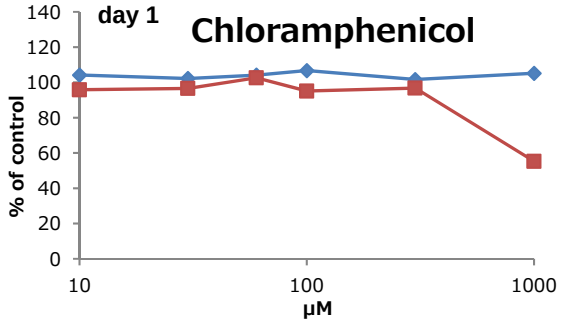
Mitochondrial Toxicity Assessment

<Long-term exposure assessment>

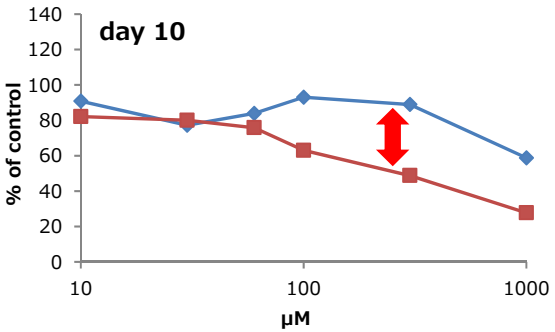
◆ Ribosome synthesis inhibition (long period exposure)

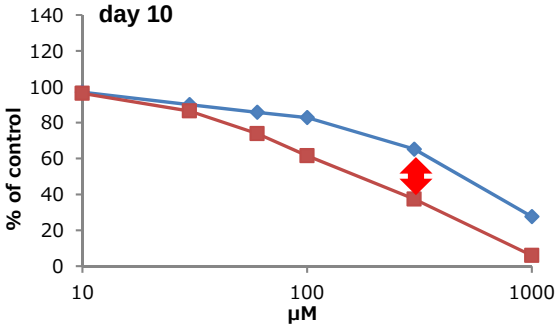
Toxicity is hard to see with short-term exposure





Detection of mitochondrial toxicity by long-term exposure





Toxicity that can only be detected by long-term exposure

Conclusion

- DILI is caused by many and complex mechanism

- Drug-induced liver cholestasis and Mitochondrial toxicity are important for DILI prediction

The Drug Development Solutions Center offers contract services of these two in vitro studies

Thank you for your attention

For questions regarding the presentation, study details, or how to get a quote for contracted Hepatotoxicity Studies please use the **Contact Us** form and a service specialist will get in touch.