Comparison of two cell model systems, primary human hepatocytes and hiPSC-derived hepatocytes to determine the hepatoxicity of three candidate drugs developed for Rheumatoid Arthritis.

[1]Sei Kameoka, [2]Vanessa Ott, [3]Tanja Zabka, [4]Thomas Weiser, [4]Thomas Singer, [1]Kyle Kolaja, and [1]Eric Chiao.

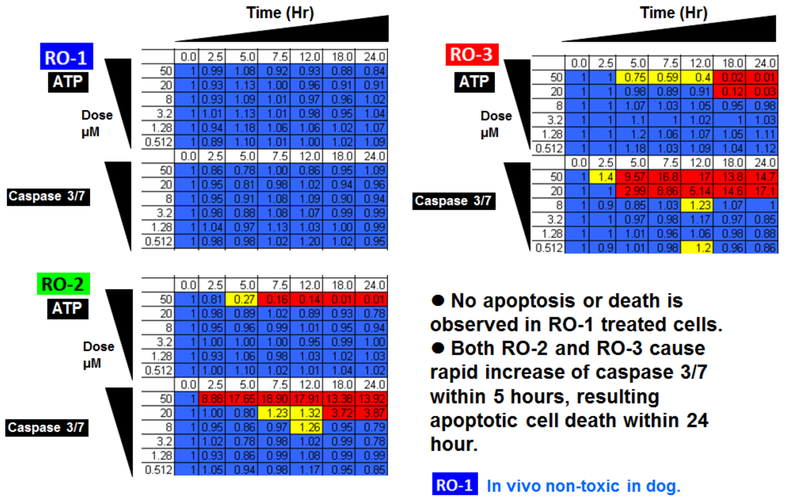
[1]Laboratory of Applied Safety Science, Nonclinical Safety, Hoffmann-La Roche, Nutley, NJ, [2]Cellular Dynamics International, Madison, WI, [3]Genentech, San Francisco, CA, [4]Nonclinical Safety, Hoffmann-La Roche, Basel, Switzerland.

ABSTRACT

In vitro hepatocyte culture serves as a very useful model system to detect potential hepatoxicity without sacrificing animals. However, current in vitro model systems have limitations. Primary human hepatocytes (PHH) are known to exhibit donor-specific phenotypic variability and availability is too limited for high throughput assays. Immortalized hepatoma cell lines, such as HepG2 and HepaRG, offer more reproducible and homogenous culture systems, but interpreting the toxicant sensitivity in cells of tumor origin poses a considerable problem. Using human induced pluripotent stem cell (hiPSC)-derived hepatocytes is an important new tool which offers unlimited supply of euploid cells from single donors. Here, we tested the hepatoxicity of three internal candidate drugs developed for the treatment of rheumatoid arthritis. In vivo, two of these compounds induced canine liver toxicity, while one compound showed no toxicity. This toxicological profile of three compounds is recapitulated in vitro, both in cultures of PHH and the hiPSC-derived hepatocytes. IC50 values of the three compounds were determined by 24 hour ATP assay (>50, 20, 14μM by PHH; >50, 17, 13μM by HLCs) to be nearly identical.

This pilot study shows that despite some difference in metabolism (cytochrome P450 and Phase II enzyme levels) between PHH and hiPSC-derived hepatocytes, stem cell-derived hepatocytes may provide a valuable model system for preclinical drug safety study and disease modeling.

Time course cytotoxicity profile of three RO compounds in primary dog hepatocytes.



RO-1 ATP	0.0	2.5	5.0	7.5	12.0	18.0	24.0
50	1	0.99	1.08	0.92	0.93	0.88	0.84
20	1	0.93	1.13	1.00	0.96	0.91	0.91
8	1	0.93	1.09	1.01	0.97	0.96	1.02
3.2	1	1.01	1.13	1.01	0.98	0.95	1.04
1.28	1	0.94	1.18	1.06	1.06	1.02	1.07
0.512	1	0.89	1.10	1.01	1.00	1.02	1.09

RO-1 Caspase 3/7	0.0	2.5	5.0	7.5	12.0	18.0	24.0
50	1	0.86	0.78	1.00	0.86	0.95	1.05
20	1	0.95	0.81	0.98	1.02	0.94	0.96
8	1	0.95	0.91	1.06	1.09	0.90	0.94
3.2	1	0.98	0.88	1.08	1.07	0.99	0.99
1.28	1	1.04	0.97	1.13	1.03	1.00	0.99
0.512	1	0.98	0.98	1.02	1.20	1.02	0.95

RO-2 ATP	0.0	2.5	5.0	7.5	12.0	18.0	24.0
50	1	0.81	0.27	0.16	0.14	0.01	0.01
20	1	0.98	0.89	1.02	0.89	0.93	0.78
8	1	0.95	0.96	0.99	1.01	0.95	0.94
3.2	1	1.00	1.00	1.00	0.95	0.99	1.00
1.28	1	0.93	1.06	0.98	1.03	1.02	1.03
0.512	1	1.00	1.10	1.02	1.01	1.04	1.02

RO-2 Caspase 3/7	0.0	2.5	5.0	7.5	12.0	18.0	24.0
50	1	8.88	17.65	18.90	17.91	13.38	13.92
20	1	1.00	0.80	1.23	1.32	3.72	3.87
8	1	0.95	0.86	0.97	1.26	0.95	0.79
3.2	1	1.02	0.78	0.98	1.02	0.99	0.76
1.28	1	0.93	0.86	0.99	1.08	0.99	0.99
0.512	1	1.05	0.94	0.98	1.17	0.95	0.85

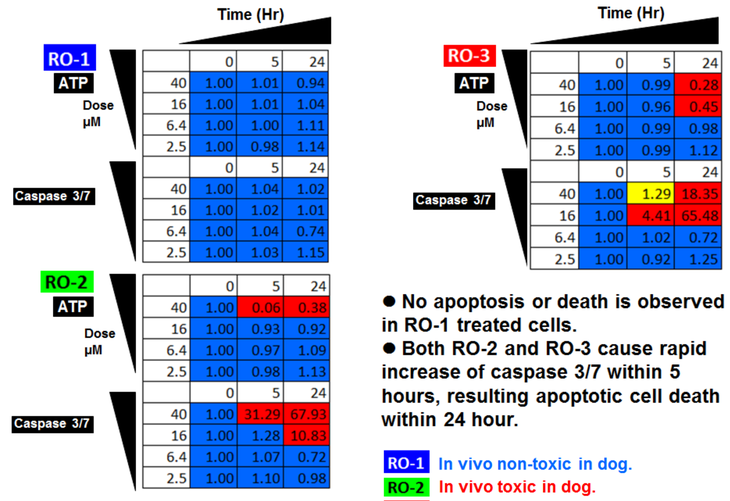
RO-3 ATP	0.0	2.5	5.0	7.5	12.0	18.0	24.0
50	1	1	0.75	0.59	0.4	0.02	0.01
20	1	1	0.98	0.89	0.91	0.12	0.03
8	1	1	1.07	1.03	1.05	0.95	0.98
3.2	1	1	1.1	1	1.02	1	1.03
1.28	1	1	1.2	1.06	1.07	1.05	1.11
0.512	1	1	1.18	1.03	1.09	1.04	1.12

RO-3 Caspase 3/7	0.0	2.5	5.0	7.5	12.0	18.0	24.0
50	1	1.4	9.57	16.8	17	13.8	14.7
20	1	1	2.99	8.86	5.14	14.6	17.1
8	1	0.9	0.85	1.03	1.23	1.07	1
3.2	1	1	0.97	0.98	1.17	0.97	0.85
1.28	1	1	1.01	0.96	1.06	0.98	0.88
0.512	1	0.9	1.01	0.98	1.2	0.96	0.86

- **No apoptosis or death is observed in RO-1 treated cells.**
- **Both RO-2 and RO-3 cause rapid increase of caspase 3/7 within 5 hours, resulting apoptotic cell death within 24 hour.**

RO-1 In vivo non-toxic in dog.
RO-2 In vivo toxic in dog.
RO-3 In vivo toxic in dog.

Time course cytotoxicity profile of three RO compounds in iCell Hepatocytes.



RO-1 ATP	0	5	24
40	1.00	1.01	0.94
16	1.00	1.01	1.04
6.4	1.00	1.00	1.11
2.5	1.00	0.98	1.14

RO-1 Caspase 3/7	0	5	24
40	1.00	1.04	1.02
16	1.00	1.02	1.01
6.4	1.00	1.04	0.74
2.5	1.00	1.03	1.15

RO-2 ATP	0	5	24
40	1.00	0.06	0.38
16	1.00	0.93	0.92
6.4	1.00	0.97	1.09
2.5	1.00	0.98	1.13

RO-2 Caspase 3/7	0	5	24
40	1.00	31.29	67.93
16	1.00	1.28	10.83
6.4	1.00	1.07	0.72
2.5	1.00	1.10	0.98

RO-3 ATP	0	5	24
40	1.00	0.99	0.28
16	1.00	0.96	0.45
6.4	1.00	0.99	0.98
2.5	1.00	0.99	1.12

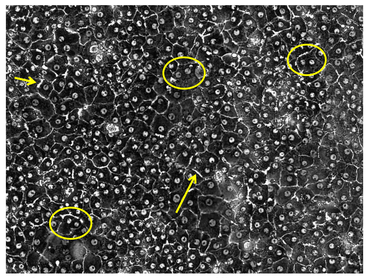
RO-3 Caspase 3/7	0	5	24
40	1.00	1.29	18.35
16	1.00	4.41	65.48
6.4	1.00	1.02	0.72
2.5	1.00	0.92	1.25

- **No apoptosis or death is observed in RO-1 treated cells.**
- **Both RO-2 and RO-3 cause rapid increase of caspase 3/7 within 5 hours, resulting apoptotic cell death within 24 hour.**

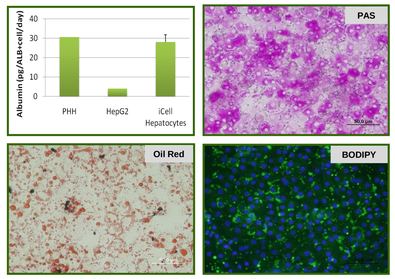
RO-1 In vivo non-toxic in dog.
RO-2 In vivo toxic in dog.
RO-3 In vivo toxic in dog.

Properties of hiPSC-derived hepatocytes

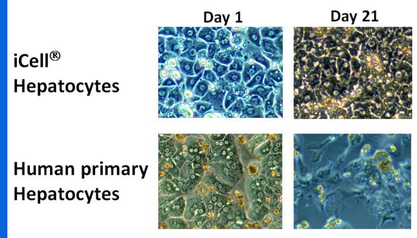
(iCell® Hepatocytes)



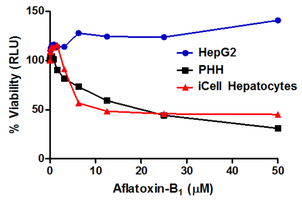
- Adherent monolayer
- Round nucleus
- Distinct nucleoli
- High cyto/nuclear ratio
- Bi-nucleation (circles)
- Bile canaliculi (arrows)



- Albumin secretion similar to primary human hepatocytes
- Store glycogen (Periodic acid-Schiff (PAS) staining)
- Produce Lipid (Oil Red and BODIPY staining)

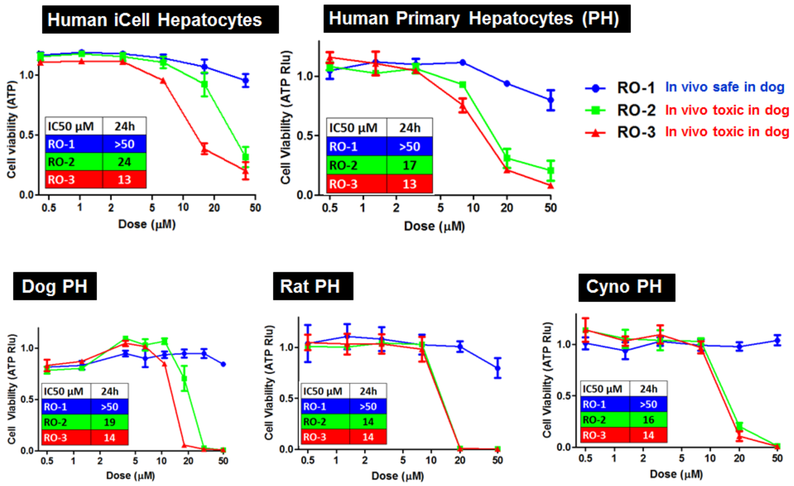


Viable over extended culture periods.



Aflatoxin B_1 metabolism/activation requires metabolically competent cells (Data courtesy of Promega)

Comparison of IC50 of three RO compounds in iCell Hepatocytes and primary hepatocytes of four species.



Conclusion:

Albumin secretion, morphology and sensitivity to Aflatoxin B_1 of iCell Hepatocytes are similar to primary hepatocytes.

But drug toxicity is a manifestation of dynamic interactions between many cellular functions and drugs. To determine if iCell Hepatocytes is a suitable model for toxicity testing, we tested if iCell Hepatocytes responded to known hepatoxic compounds in a similar way to primary hepatocytes. iCell Hepatocytes recapitulated the toxic profile observed in both primary hepatocytes and animal studies, suggesting they may serve as a good in vitro model system.

	RO-1	RO-2	RO-3
In Vivo (dog)	Safe	Toxic	Toxic
iCell Hepatocytes	Safe	Toxic	Toxic
Primary Hepatocytes	Safe	Toxic	Toxic