EVALUATION OF THE EFFECTS OF INFLAMMATORY CYTOKINES ON DRUG-INDUCED LIVER INJURY (DILI) IN HUMAN HEPATOCYTES

In Vitro ADMET Laboratories, LLC

For more information, contact:
Albert P. Li, Ph. D.
E-mail: lialbert@invitroadmet.com

Albert P. Li, Doshi Utkarsh and Qian Yang
APSciences Inc. and In Vitro ADMET Laboratories LLC, 9221 Rumsey Road Suite 8, Columbia, MD 21045

Introduction:

Drug-induced liver injury (DILI) is a major challenge in pharmaceutical development. It is a key reason for clinical trial failure and has led to withdrawal of marketed drugs.

Inflammation is believed to be a key risk factor for DILI. Animal studies as demonstrated enhanced drug-induced liver toxicity by proinflammatory agents.

We report here our investigation on the effects of 10 proinflammatory cytokines on the in vitro cytotoxcity of a known hepatotoxic drug, troglitazone (TRO), in primary human hepatocytes.

Materials and Methods

Human hepatocytes: Plateable cryopreserved human hepatocytes were used in this study

Cytokines: Ten cytokines: IL1b, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, TNFβ, INFα 2b, INFγ were evaluated. They were obtained from R&D Systems, Biovision, PBL InterferonSource, and Antigenix America.

Cytotoxicity Evaluation: The hepatocytes were plated in collagen-coated 384-well plates for the evaluation of troglitazone cytotoxicity in the presence and absence of each of the cytokines. The hepatocytes were thawed and plated for 4 hours at a cell density of 5000 cells/well in 10 μL followed by the addition of an equal volume of treatment medium containing 2X of the desired final concentrations of troglitazone and cytokines. After an overnight incubation, cellular ATP contents were determined using a commercially available ATP kit consisting of lysis buffer and ATP detection reagent (PerkinElmer, Boston). Cytotoxicity is indicated by a decrease in cellular ATP content.

Cytotoxicity was expressed as % viability calculated using the following equation:

% viability = ATP (treatment)/ATP (negative control) x 100%

Conclusions

Troglitazone demonstrated the expected, dose-dependent cytotoxicity in cultured human hepatocytes, thereby suggesting that its known hepatotoxicity could be evaluated in vitro.

Co-treatment of human hepatocytes with troglitazone with each of the 10 proinflammatory cytokines did not lead to higher cytotoxicity as expected.

The results suggest that cytokines per se do not potentiate the hepatotoxicity of troglitazone. Inflammation may still be a risk factor for troglitazone induced liver failure, but other in vivo factors (e.g. cytotoxic antibodies) may be involved.

An interesting finding is that IFNα apparently has protective effects. Further investigation is needed to understand this apparent protective effect. A working hypothesis is the alteration of drug metabolizing capacity of the hepatocytes by IFNα.

Experiment 1: Evaluation of relative noncytotoxic TRO concentrations to investigate if cytokines could potentiate cytotoxicity

Finding: None of the cytokines demonstrate significant potentiation of TRO cytotoxicity

μM TRO	No Cytokines	IL1b		IL6		TNFα		IL4		IL8		IL10		IL2		TNFβ		IFNγ		IFNα	
		0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml	0.1 ng/ml	5 ng/ml
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3.125	100.72	117.00	107.54	104.00	96.72	95.67	94.72	109.25	106.13	102.55	107.95	94.14	89.85	126.09	122.75	100.44	99.83	114.24	119.91	108.58	111.25
6.25	106.94	117.08	105.70	97.99	93.29	97.90	79.34	119.47	85.84	92.15	100.79	104.14	84.33	123.77	92.41	93.37	84.01	110.22	101.64	107.19	103.99
12.5	76.89	117.29	94.25	97.51	83.60	92.99	84.00	113.18	88.79	92.25	107.06	85.04	89.56	122.20	117.14	73.49	90.15	108.67	108.74	99.84	106.84
25	42.55	85.99	68.96	61.33	56.27	66.63	65.89	84.48	79.32	62.15	69.60	53.31	51.10	74.77	46.37	60.53	60.90	71.01	74.23	71.41	82.09
50	2.88	6.10	2.10	3.39	3.45	4.18	6.89	4.97	3.91	3.31	4.02	4.68	3.44	4.44	4.11	3.47	3.15	4.44	3.84	3.16	5.17

Experiment 2: Evaluation of cytotoxic TRO concentrations to further investigate the protective effects at 5 ng/mL of cytokines

Finding: That cytokines do not potentiate TRO cytotoxicity is confirmed

	RELATIVE VIABILITY (%)																							
μM TRO	No Cytokines		IL-1b		IL-6		TNFα		IL-4		IL-8		IL-10		IL-2		TNFβ		IFNγ		IFNα	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
0	100.00	17.20	100.00	19.15	100.00	10.85	100.00	31.71	100.00	17.86	100.00	15.49	100.00	11.70	100.00	8.58	100.00	9.50	100.00	5.95	100.00	20.24
2.5	104.50	21.23	101.18	11.34	99.92	10.41	119.05	11.53	111.61	9.15	113.80	20.52	124.39	8.79	99.78	11.20	123.36	22.01	122.64	12.12	103.06	18.83
5	124.61	9.10	115.58	6.25	98.13	7.17	123.07	3.84	124.97	10.82	103.38	6.04	127.81	18.63	127.80	19.83	115.64	11.32	116.71	4.34	114.25	17.57
10	113.63	9.40	97.01	18.04	87.66	11.75	110.26	7.63	126.23	16.56	88.40	12.63	113.11	20.88	104.73	4.34	120.94	16.17	87.22	35.94	88.39	12.04
20	16.11	7.75	18.00	7.54	13.23	5.28	19.10	4.54	22.43	7.34	15.01	4.23	21.42	10.60	10.42	4.41	11.03	4.48	11.60	4.15	12.69	3.26
30	5.70	0.75	6.20	0.89	5.18	0.45	6.70	1.00	10.11	1.00	6.75	0.90	7.49	0.68	5.54	1.02	6.53	0.62	6.91	0.24	8.55	1.13
40	4.21	0.28	4.31	0.44	3.68	0.15	4.76	0.43	7.44	0.77	4.47	0.19	5.00	0.61	3.64	0.29	5.00	0.24	4.98	0.36	5.88	0.67

Experiment 3 Further evaluation of the protective effects of the cytokines using 20 μM TRO and varying concentrations of cytokines

Findings: Again, no sensitization but apparent protective effects observed for interferon alpha (INFα)

	RELATIVE VIABILITY (%)																					
Concentration *(ng/mL)*		IL1b		IL6		TNFα		IL4		IL8		IL10		IL2		TNFβ		IFNγ		IFNα	
IFNα	*Cytokines*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*
0	0	4.28	0.32	3.31	0.21	2.96	0.13	3.09	0.30	3.63	0.27	3.70	0.35	2.92	0.14	2.30	0.13	2.79	0.48	1.94	0.11
0.5	3.125	4.06	0.66	3.47	0.84	2.98	0.15	3.19	0.90	4.34	0.61	4.06	0.73	3.75	0.39	3.20	0.43	2.76	0.21	2.61	0.09
1.0	6.25	3.30	0.22	3.87	0.29	3.24	0.21	2.90	0.45	4.01	0.04	4.02	0.31	4.00	0.69	3.11	0.26	3.17	0.25	2.63	0.47
2.1	12.5	3.61	0.62	3.89	0.73	2.99	0.67	3.46	0.83	3.81	0.22	4.09	0.55	5.22	1.86	2.77	0.22	3.80	0.59	3.54	1.34
4.2	25	3.05	0.29	3.69	0.57	2.97	0.24	2.67	0.23	3.62	0.18	2.95	0.22	3.69	0.66	2.61	0.18	3.07	0.43	4.29	1.46
8.3	50	2.59	0.20	3.72	0.80	3.05	0.31	2.79	0.45	3.77	0.15	2.79	0.13	4.00	1.58	2.70	0.43	3.04	0.13	3.38	1.31
16.7	100	2.39	0.32	5.10	1.73	3.20	0.26	2.65	0.67	3.60	0.63	2.53	0.17	3.53	1.59	2.63	0.23	3.08	1.97	1.89	0.62

Results normalized to viability at 20 μM troglitazone in the absence of cytokines

Concentration *(ng/mL)*		IL1b		IL6		TNFα		IL4		IL8		IL10		IL2		TNFβ		IFNγ		IFNα	
IFNα	*Cytokines*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*	*mean*	*sd*
0	0	100.00	7.42	100.00	6.30	100.00	4.40	100.00	9.67	100.00	7.40	100.00	9.38	100.00	4.91	100.00	5.59	100.00	17.09	100.00	5.79
0.5	3.125	94.87	15.50	104.76	25.44	100.77	4.91	103.30	28.95	119.59	16.80	109.64	19.75	128.30	13.21	139.20	18.84	99.09	7.68	134.58	4.69
1.0	6.25	77.16	5.05	116.95	8.77	109.39	7.05	93.66	14.59	110.38	1.05	108.68	8.32	136.72	23.65	135.08	11.13	113.58	8.97	135.67	24.01
2.1	12.5	84.38	14.60	117.33	21.97	100.91	22.62	111.88	26.72	105.04	6.12	110.56	14.77	178.47	63.58	120.31	9.48	136.31	21.13	182.87	69.37
4.2	25	71.27	6.84	111.35	17.26	100.15	8.03	86.27	7.46	99.87	5.03	79.78	5.91	126.28	22.57	113.40	7.76	110.32	15.27	221.36	75.24
8.3	50	60.59	4.62	112.19	24.13	102.85	10.41	90.27	14.67	103.76	4.09	75.43	3.54	136.77	54.05	117.61	18.49	108.91	4.76	174.40	67.63
16.7	100	55.74	7.56	154.09	52.14	108.00	8.77	85.72	21.69	99.31	17.39	68.22	4.61	120.75	54.46	114.47	10.10	110.42	70.66	97.47	31.85