Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

A liver-immune coculture array for predicting systemic drug-induced skin sensitization

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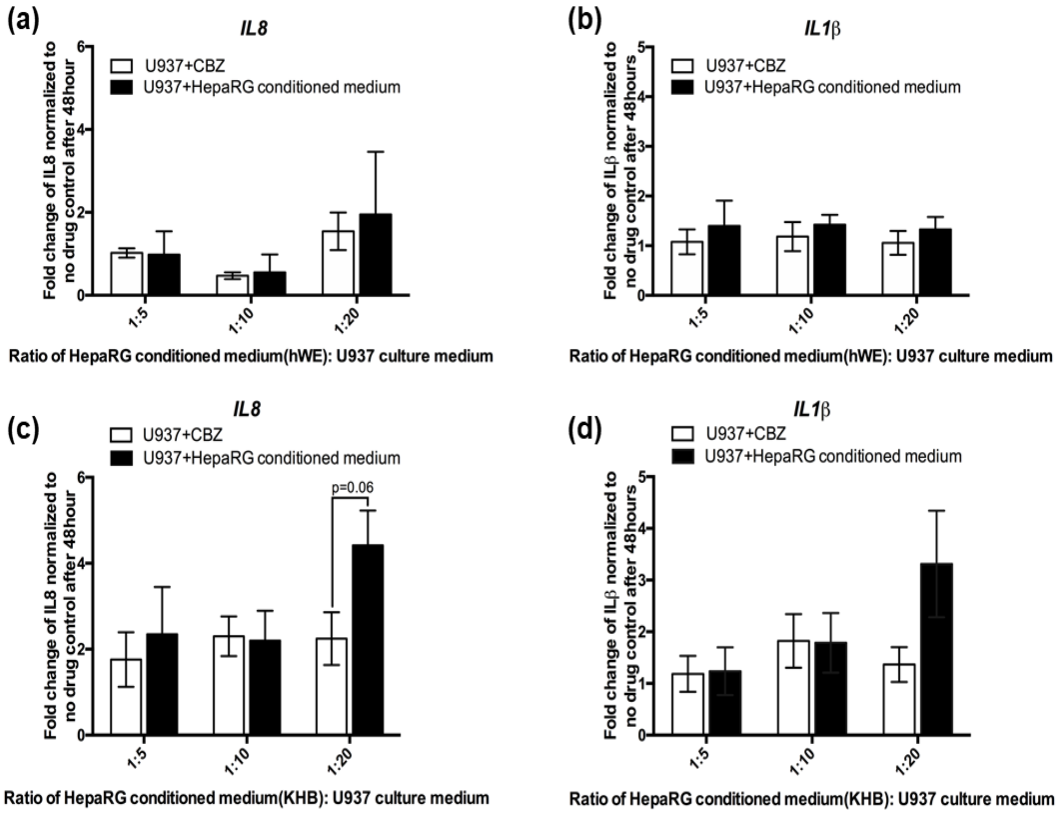
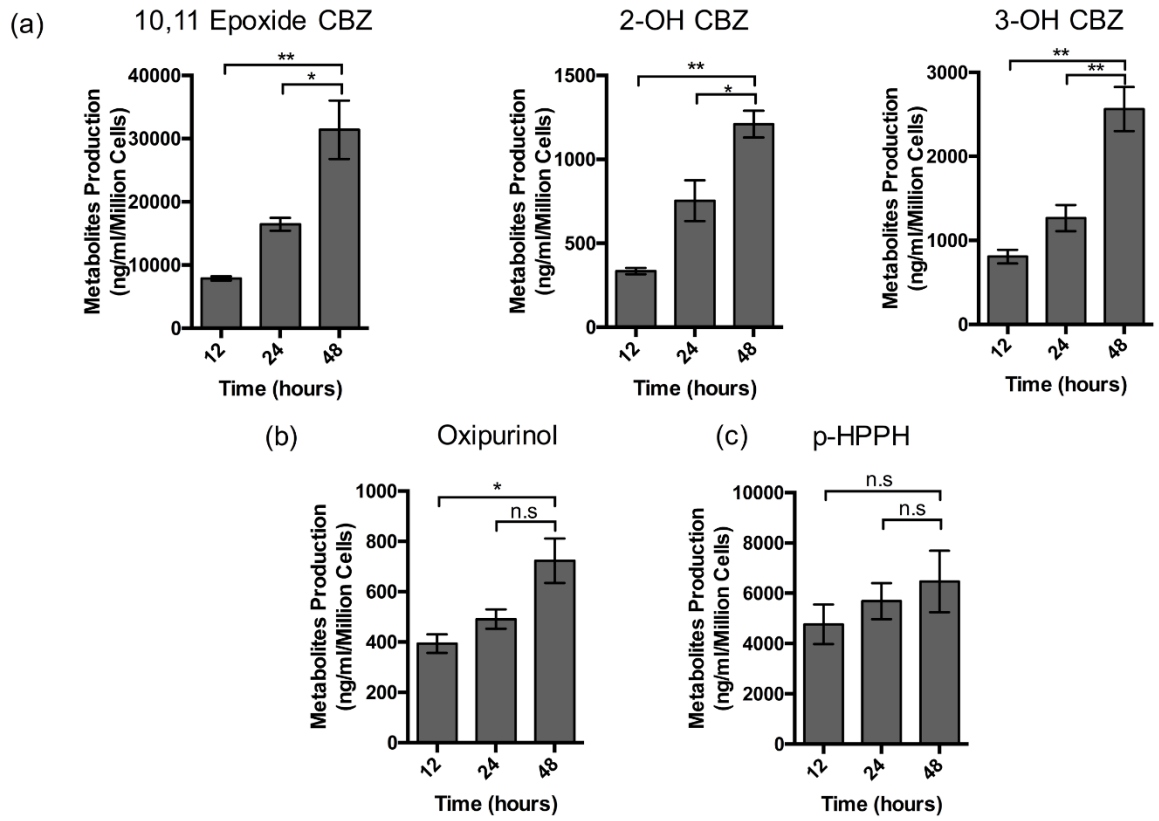


SI Fig. 1. Hepatocyte conditioned medium attenuates U937 activation response. Gene expression changes in U937 cells after 48 hours of incubation with either carbamezapine (CBZ) or HepaRG-hepatocyte conditioned medium containing CBZ reactive metabolites. Conditioned medium was collected from HepaRG-derived hepatocyte cultures that were incubated with 100 μM CBZ for 6 hours in human William E (a, b) and Krebs-Henseleit Buffer (KHB) (c, d) to generate the reactive drug metabolites. The HepaRG conditioned medium was supplemented to U937 cultures at different ratios of HepaRG conditioned medium: U937 culture medium (1:5, 1:10 and 1:20 as shown in the black bars). The white bars denote the monoculture of U937 with the parent drug (CBZ) in a corresponding ratio of human William E (a, b) or KHB (c, d) to U937 culture medium to ensure a fair comparison. Data are average ± SEM of 2 independent experiments for human William E and 3 independent experiments for KHB. Asterisks denote the statistical significant differences (Student T test, *$p<0.05$).



SI Fig. 2. Generation of drug metabolites by HepaRG-derived hepatcocytes monolayer. (a) carbamazepine(CBZ)'s reactive metabolites: 10,11-epoxide carbamazepine (CBZ-E), 2 hydroxy carbamazepine (2-OH CBZ), 3 hydroxy carbamazepine (3-OH CBZ) (b) phenytoin's reactive metabolites, 5-(4'-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) (c) allopurinol's metabolites, oxipurinol. Data are average ± SEM of 3 independent experiments. Asterisks denote statistical significant differences (Student T test, *$p<0.05$; **$p<0.01$).

SI Table 1. List of primers that were used in this study

Table 1a Human Hepatocytes Primer

Primer	Forward	Reverse
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG
CYP1A2	GGGCCGGCCTGACCTCTACA	CAGGGGGTTCCCGGAGGAGG
CYP3A4	AAGTCGCCTCGAAGATACACA	AAGGAGAGAACACTGCTCGTG
CYP2B6	AGACGCCTTCAATCCTGACC	CCTTCACCAAGACAAATCCG
HNF4A	AGAGCAGGAATGGGAAGGAT	GCAGTGGCTTCAACATGAGA
PXR	CCAGGACATACACCCCTTTG	CTACCTGTGATGCCGAACAA
Albumin	ACACAAGCCCAAGGCAACAA	TATCGTCAGCCTTGCAGCAC

Table 1b Human U937 (APC Activation) Primer

Primer	Forward	Reverse
IL8	ATGACTTCCAAGCTGGCCGTGGCT	TCTCAGCCCTCTTCAAAAACTTCTC
IL1B	ACAGATGAAGTGCTCCTTCCA	GTCGGAGATTCGTAGCTGGAT
CD86	GTATTTTGGCAGGACCAGGA	GCCGCTTCTTCTTCTTCCAT

SI Table 2. The primary and secondary antibody that used in this study

Cell Types	Markers	Primary Antibody	Secondary Antibody
HHS	CYP3A4	rabbit anti-CYP3A4(1:100) (Abcam, Cambridge, UK)	Alexa Fluor® 555 donkey anti-rabbit IgG (1:1000) (Life Technologies)
	CK19	mouse anti-CK19(1:100) (Abcam, Cambridge, UK)	Alexa Fluor® 647 donkey anti-mouse IgG(1:1000) (Life Technologies)
	Albumin	Anti-Human Serum Albumin antibody (FITC) (Abcam, Cambridge, UK)	
U937	CD86+	Rabbit anti-CD86 antibody (Abcam, Cambridge, UK)(1:100)	Goat anti rabbit IgG (Alexa Fluor® 488) (1:1000) (Life Technologies)
	DAPI	Life Technologies (1:1000)	

SI Table 3. The molecular weight of fluorescein sodium salt, parent drugs and metabolites that used in this study

Items	**Molecular Weight**
Fluorescein sodium salt	376.27
Carbamazepine	236.269
Carbamazepine Metabolites (2OH CBZ, 3OH CBZ, 10,11 Epoxide CBZ)	252.273
Phenytoin	252.268
Phenytoin Metabolites 5-(4'-hydroxyphenyl)-5-phenylhydantoin	268.27
Allopurinol	136.11
Oxipurinol	152.11