ASSESSING THE *IN VITRO IN VIVO* CORRELATION OF SMALL MOLECULE METABOLISM IN THREE LONG-TERM PRIMARY HUMAN HEPATOCYTE CULTURE MODELS

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ABSTRACT

Isolated human hepatocytes in suspension (SHH) are routinely used as an *in vitro* tool to investigate hepatic metabolism. However, the use of SHH is limited by a relatively short incubation time (≤ 6 h), which can be inadequate to form metabolites for slowly metabolized compounds. Long-term primary human hepatocyte culture models such as HepatoPac, spheroids, and most recently the Tri-Culture system (TCS), have been developed to address this challenge. **The objective of this study is to compare the performance of these three models and SHH as *in vitro* tools to generate Phase I and II metabolites observed *in vivo*.** BI 425809, BI-A, and TAK-041 were investigated due to their absent or low metabolism in SHH and their diverse circulating metabolites in humans. When BI 425809 was incubated with HepatoPac, spheroids, or TCS, 8 out of 11 circulating metabolites were identified. One unique *in vivo* pathway was amide hydrolysis of M526. Both M232 and M312, formed via hydrolysis of the amide bond in M526, were detected in spheroids and TCS, but not in SHH or HepatoPac. In human plasma, 2 oxidative and 1 oxidative-glucuronide metabolites of BI-A were identified. Interestingly, the two-step oxidative-glucuronide metabolite, which was not observed in SHH, was detected in incubations with all three long-term culture models. TAK-041 undergoes multistep and sequential downstream metabolism in humans, but there was no metabolite formed in SHH[1]. Notably, all three long-term culture models can produce sequential *in vivo* metabolites such as cysteine *S*-conjugate and glutathione adduct-derived thiol metabolites. Overall, the three long-term culture models investigated generated clinically relevant metabolites at measurable levels. All three systems generated metabolites more extensively than SHH, which is relevant for slowly metabolized compounds, and thus are suitable tools to predict *in vivo* metabolism.

METHODS

	SHH	Human HepatoPac	Hepatocyte spheroids	TCS
Incubation time	6 h	0, 24, 48, 96, 168 h	0, 48, 96, 168 h	0, 96, 168 h
Serum	5% HSA	0-5% FBS	0	< 1% FBS
Donors	Multi	Multi	Single	Single
Hepatocytes/ mL	1 million	87,500	221,200	450,000
Hepatocytes/ well	250,000	35,000	55,300	225,000

CONCLUSIONS

- Three long-term primary human hepatocyte culture models:
  - Generally, form more metabolites than incubations with SHH.
  - Can predict *in vivo* metabolism of slowly and extensively metabolized compounds.
  - May be suitable to biosynthesize *in vivo* metabolites.
- Single donor hepatocyte systems (spheroids and TCS) can generate relevant *in vivo* metabolites as in the multi donor system (HepatoPac).
- Ease of use, potential limitations, and cost effectiveness need to be carefully considered (Table 4).
- For certain studies, SHH may be adequate, while a long-term hepatocyte culture models may be more suitable for other types of studies.

RESULTS

Figure 1: Proposed metabolic pathways of BI 425809.

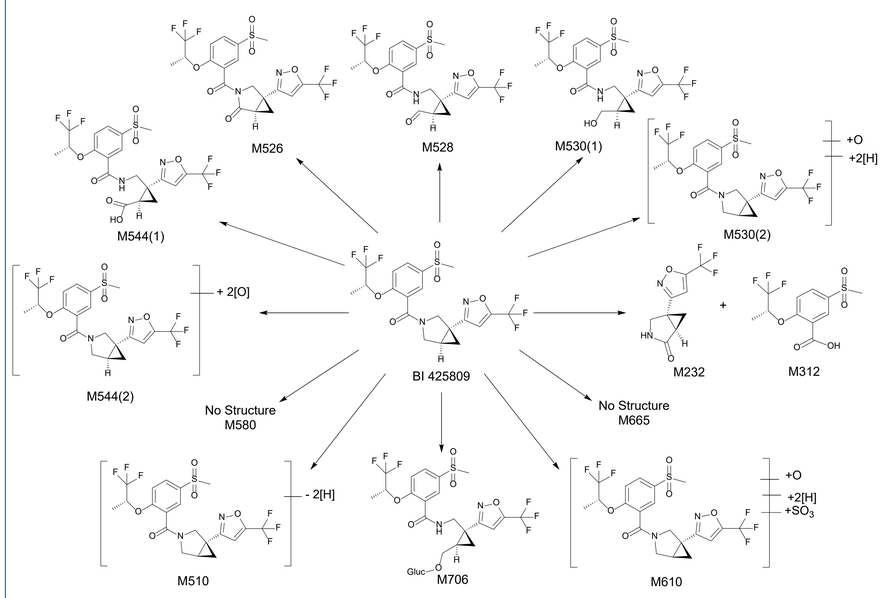


Figure 2: Proposed metabolic pathways of BI-A.

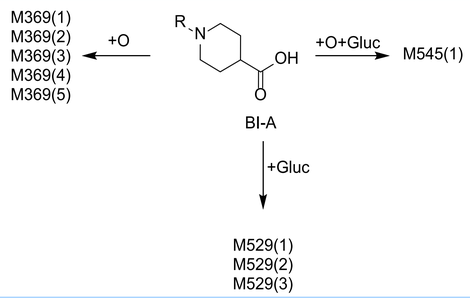
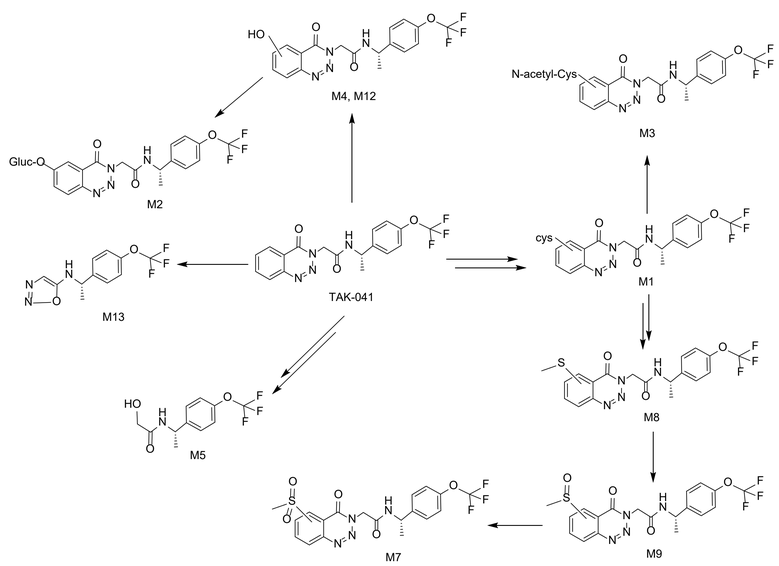


Figure 3: Proposed metabolic pathways of TAK-041[1].



[1]Kamel A. et al. DMD (2021) 49:121-132.

Figure 4: Percent of parent depletion in HepatoPac, spheroids and TCS after 7 days of incubation.

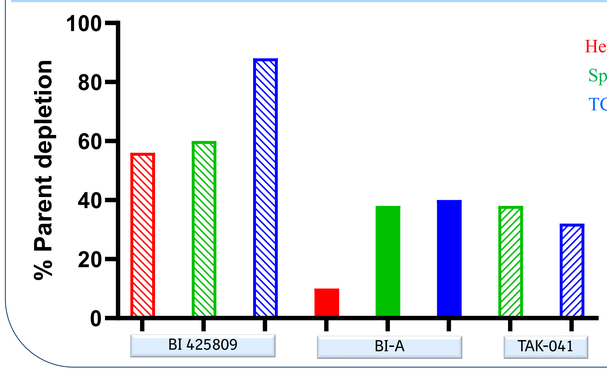


Table 1: BI 425809 and its metabolites identified in human plasma and hepatocytes.

Analyte	Biotransformation	SHH, 6 h (% TDRM MS)	HepatoPac, 168 h (% TDRM MS)	Spheroids, 168 h (% TDRM MS)	TCS, 168 h (% TDRM MS)	hADME plasma (% total ^{14}C)
BI 425809	--	99	35	54.0	30.2	41
M232	Amide hydrolysis of M526 (amine)	--	--	2.8	9.3	12
M312	Amide hydrolysis of M526 (acid)	--	--	2.8	9.3	2.6
M510	-2[H]	--	--	3.4	6.2	0.4
M528	+[O]	--	--	4.0	1.1	0.8
M526	+[O]-2[H]	--	3.5	0.4	4.2	6.5
M530(1)	+[O]+2[H]	<1	24	12	6.8	34
M544(1)	+2[O]	--	19	18	22	0.8
M544(2)	+2[O]	--	--	--	--	0.6
M580	+$C_3H_7ON_2$	--	--	--	--	0.7
M665	+$C_6H_7ON_3$	--	--	--	--	0.4
M706	+[Gluc], +[O]+2[H]	--	16	1.8	6.0	1.5
M610	+[SO_4]+2[H]	--	2.6	--	--	--
M530(2)	+[O]+2[H]	<1	--	1.3	5.0	--

-- = not detected; Gluc=glucuronic acid; TDRM= total drug related material; MS= mass spec.

Table 2: BI-A and its metabolites identified in human plasma and hepatocytes.

Analyte	Biotransformation	SHH, 6 h	HepatoPac, 168 h (% TDRM MS)	Spheroids, 168 h (% TDRM MS)	TCS, 168 h (% TDRM MS)	Human plasma (% TDRM MS)
BI-A	--	Observed	99	97	80	96
M369(1)	+[O]	Observed	--	--	--	--
M369(2)	+[O]	Observed	0.06	0.22	0.97	Trace level
M369(3)	+[O]	Observed	0.40	2.3	7.1	0.81
M369(4)	+[O]	Observed	0.14	0.17	0.84	--
M369(5)	+[O]	Observed	0.013	0.05	0.03	--
M529(1)	+[Gluc]	--	0.042	0.10	0.51	--
M529(2)	+[Gluc]	Observed	0.049	0.14	0.71	--
M545(1)	+[Gluc], +[O]	--	0.22	0.52	10	3.6

Table 3: TAK-041 and its metabolites identified in human plasma and hepatocytes.

Analyte	Biotransformation	SHH, 2 h[1]	HepatoPac, 14 day[1]	Spheroids, 168 h (% TDRM MS)	TCS, 168 h (% TDRM MS)	Human plasma[1]
TAK-041	--	Observed	Observed	75	69	Observed
M1	+[Cys]	--	Observed	13	17	Observed
M2	+[Gluc], +[O]	--	Observed	11	12	Observed
M3	+[*N*-Acetyl-Cys]	--	Observed	--	--	Observed
M4/M12	+[O]	--	Observed	0.8	1.0	Observed
M5	-[$C_7H_3N_3$]	--	Observed	Trace level	1.6	--
M7	Methylsulfone conjugation	--	Observed	Trace level	0.3	Trace level
M8	Methylsulfide conjugation	--	Observed	--	--	Trace level
M9	Methylsulfoxide conjugation	--	Observed	Trace level	Trace level	Trace level
M13	Ring closure and hydrolysis	--	Trace level	Trace level	Trace level	--

-- = not detected; Gluc=glucuronic acid; TDRM= total drug related material; MS= mass spec.; trace level < 0.3 % TDRM

Table 4: Comparison of *in vitro* models investigated regarding familiarity, cost, ease of use, and caveats.

In vitro model	Familiarity/ Historical Data	Cost per study	Ease of use	Known caveats
Suspension Hepatocytes (SHH)				• Short incubation time (≤ 6h). • Internalization of efflux transporters.
Micropatterned Hepatocytes (HepatoPac)				• Reduced aldehyde oxidase activity. • Background mouse fibroblasts. • Low hepatocyte count.
Spheroids				• Substantial drop in CYP activities after seeding. • Limited knowledge regarding non-CYP450 activities.
Tri-Culture system (TCS)				• Limited knowledge regarding non-CYP450 activities. • Unknown cell types and media composition.