Development of a Convenient *In Vitro* Method for Predicting Metabolism and Disposition of Acrylamide Covalent Drugs in Humans

Ruixing Li[1], Rong Yang[1], Hong Zhang[1], Weiqun Cao[1], Mingshe Zhu[1,2], Guoxiang Huang[1], Yi Tao[1], Liang Shen[1]

1 DMPK Service Department, WuXi AppTec, Shanghai, China , 200131

2 MassDefect Technologies, Princeton, NJ 08540, USA

药明康德 WuXi AppTec

Poster#: P109

Abstract

Targeted covalent inhibitors are a new type of drug modality that covalently bind to an active site of the targeted enzyme or receptor, usually undergoing oxidative metabolism, GSH conjugation and the formation of protein adducts in humans.

Currently, there is a lack of systematic and efficient *in vitro* method to comprehensively investigate and predict both metabolism and disposition of such drugs in humans.

Two acrylamide covalent drugs (Abivertinib and Osimertinib) were used as model compounds. An *in vitro* methodology was designed and utilized for investigating oxidative metabolism, protein binding and GSH conjugations of two acrylamide covalent drugs. This approach integrated metabolite profiling in human liver microsomes (HLM) with and without GSH, along with the exploration of potential covalent binding in human serum albumin (HSA) with and without GSH.

A convenient *in vitro* method has been successfully developed for predicting the metabolism and disposition of acrylamide covalent drugs in humans.

Objectives

The major objective of this study was to develop an efficient and comprehensive *in vitro* approach for predicting *in vivo* metabolism and disposition pathways of acrylamide covalent drugs.

Methods

Incubation systems:

- In liver microsomes: Two acrylamide drugs (abivertinib and osimertinib) were incubated at 10 μM in human liver microsomes with and without GSH for 0.5 h.
- In serum albumin: Two covalent drugs were incubated at 1 μM in human serum albumin with and without GSH for 0, 2 and 6 h.
- For buffer control, the matrix was replaced by phosphate buffer (PB).

Sample and data analysis:

The sample analysis was performed by Thermo Q-Exactive™ Plus with Waters® ACQUITY UPLC® System.

Results

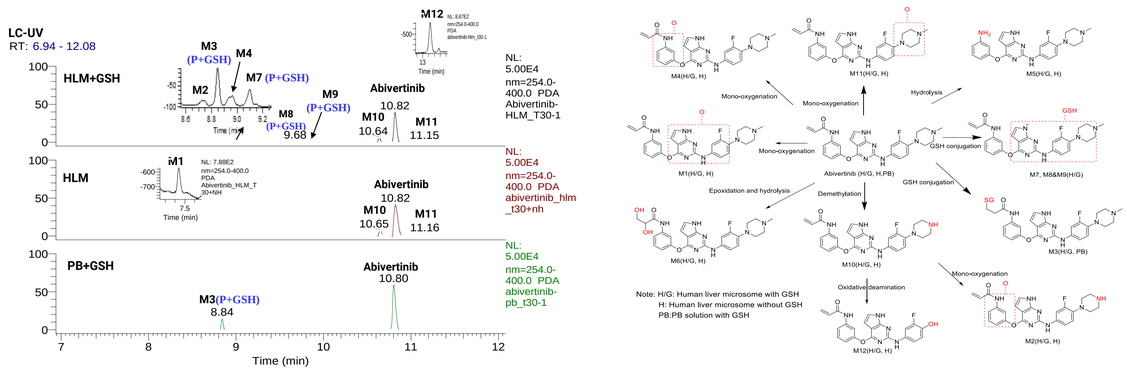

Figure 1. LC-UV Chromatograms (left) of abivertinib/its metabolites and proposed metabolic pathways (right) of abivertinib in human liver microsomes

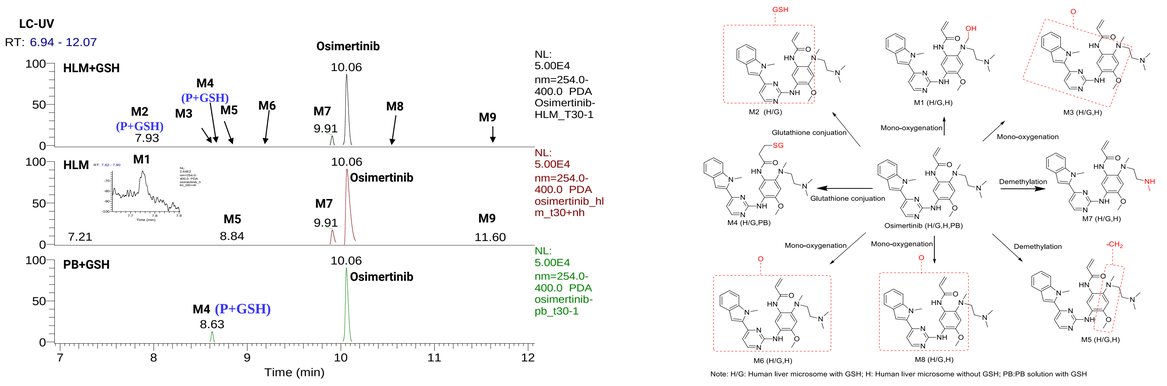

Figure 2. LC-UV Chromatograms (left) of osimertinib and proposed metabolic pathways (right) of osimertinib in human liver microsomes with and without GSH

Table 1. Comparison of human metabolism pathway between *in vitro* and *in vivo* of Abivertinib and osimertinib

#Code	Metabolic Pathways	*In vivo*	*In vitro*
Abivertinib	NA	+++	+++
M2	N-oxidation	+	+
M4	Amide hydrolysis	+	+
M5	Oxygenation	+	+
M7	&Reduction	+	ND
MII-1*	Acetylcysteine conjugation	++	++
MII-2*	Cysteine conjugation	+	
MII-6	Acetylcysteine conjugation of M4	+	ND

#Code	Metabolic Pathways	*In vivo*	*In vitro*
Osimertinib	NA	+++	+++
M1	Oxygenation	+	+
M2	Dealkylation	+	+
AZ7550	Demethylation	++	++
AZ5104	Demethylation	++	+
M17	Oxygenation	+	+
M20	Oxygenation	+	+
M23	Oxygenation of M2	+	+
M8*	Glycine-cysteine conjugation	+	++
M21*	Cysteine conjugation	+	
M18	Glucuronidation of M17	+	ND

Note: #: the code from the reported human *in vivo* data *: Further hydrolyzed metabolites of GSH adducts; &: Gut microbiota mediated metabolite; +++: Relative abundance of over 50%; ++: Relative abundance of 10%~50%; +: Relative abundance of less than 10%.

Table 2. Remaining% of free drugs and GSH adduct formation of abivertinib and osimertinib in human serum albumin with and without GSH

Remaining% of Abiverinib and Osimertinib (n=2)						
Incubation time (h)	Abiverinib			Osimertinib		
	HSA	HSA+GSH	PB+GSH	HSA	HSA+GSH	PB+GSH
0	100.00	100.00	100.00	100.00	100.00	100.00
2	99.53	89.33	50.28	44.97	40.71	36.33
6	89.33	70.15	8.12	8.19	7.74	2.58
GSH adduct formation of Abiverinib and Osimertinib (n=2)						
Incubation time (h)	Abiverinib			Osimertinib		
	HSA	HSA+GSH	PB+GSH	HSA	HSA+GSH	PB+GSH
0	ND	0.00	1.53	ND	0.00	0.00
2	ND	8.78	72.61	ND	19.92	88.73
6	ND	16.07	95.98	ND	67.97	90.51

The major metabolic pathways of abivertinib and osimertinib were oxygenation and dealkylation in liver microsomes with and without GSH after incubated 0.5 h. Meanwhile, several GSH adducts were found in liver microsomes and PB with GSH (Figure1 and Figure 2). And their metabolic pathways (oxidation, GSH conjugation) between *in vitro* and *in vivo* human are comparable (Table 1).

As the result showed in Table 2, the remaining of abiverinib and osimertinib were decreased and their GSH adducts were increased in human serum albumin with GSH after incubated 2 to 6 h. Compared with abiverinib, osimertinib was more readily binding to HSA in HSA samples with and without GSH. In the PB buffer, both drugs were mainly conjugated with GSH and the free drugs were less than 10%.

The covalent binding of abiverinib and osimertinib to albumin can be estimated indirectly by comparing the remaining % and GSH adducts in HSA, HSA with GSH and PB with GSH. Meanwhile, the binding trend of the two drugs in *in vitro* is consistent with *in vivo* results reported by Yiqian Liu. et al. [1] and Paul A. Dickinson et al.[2]

Conclusion

- Similar to the metabolism and disposition observed in many conventional covalent drugs, the two acrylamide covalent drugs underwent oxidative metabolism, formed GSH conjugates and protein adducts *in vitro*.
- The metabolic pathways and protein binding for abiverinib and osimertinib, as determined through the newly established *in vitro* approach, were consistent with the those observed in humans. Furthermore, same conclusion was obtained from the other three acrylamide covalent drugs evaluated in our lab.
- The established *in vitro* approach is useful for predicting metabolic pathways and protein binding of acrylamide covalent drugs in humans.

References

[1] Yiqian Liu, Lianke Liu, Lingxiang Liu, et al. A phase I study investigation of metabolism, and disposition of [14C]-anlotinib after an oral administration in patients with advanced refractory solid tumors. Cancer Chemotherapy and Pharmacology.2020, 85, 907–915.

[2] Paul A. Dickinson, Mireille V Cantarini, Jo Collier, et al. Metabolic Disposition of Osimertinib in Rats, Dogs, and Humans: Insights into a Drug Designed to Bind Covalently to a Cysteine Residue of Epidermal Growth Factor Receptor. Drug Metab Dispos. 2016,44,1201-1212.

Contact