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In vitro **ADME & PK**

Carboxylesterase (CE) Reaction Phenotyping

Background Information

'Of the enzymes involved in phase I reactions, cytochrome P450 enzymes play a pivotal role in drug metabolism (i.e. approximately 75% of clinically used drugs), followed by esterases, which contribute to the metabolism of 10% of the clinical, therapeutic drugs that contain ester, amide and thioester bonds.'

[1]Fukami T. and Yokoi T. (2012), *Drug Metab Pharmacokinet* **27(5)**; 466-477

- Human carboxylesterases (CE) are Phase I drug-metabolising enzymes of the serine hydrolase superfamily. They hydrolyse a variety of ester containing drugs and prodrugs.
- hCE1 and hCE2 are the most extensively studied of the human CEs. They differ in their substrate specificity and tissue distribution. hCE1 is expressed in many organs especially in the liver, with low expression in the gastrointestinal tract. hCE2 protein is also expressed in many extrahepatic tissues, especially in the gastrointestinal tract and at lower levels in the liver.
- To improve oral bioavailability, ester prodrugs are often designed so they can be hydrolysed by carboxylesterases either in the intestine or the liver with the intention of liberating the active drug.
- Cyprotex's carboxylesterase reaction phenotyping assay identifies if your compound is a substrate for carboxylesterase enzymes.

Protocol

Test Systems*
hCE1-b, hCE1-c, hCE2 expressed enzymes, or Human liver/intestinal microsomes incubated with and without CE inhibitor

Test Article Concentration
1 µM (different concentrations available)

Time Points
0, 5, 15, 30, 45 min

Positive Control Substrates
Trandolapril (hCE1 substrate)
Irinotecan (hCE2 substrate, monitoring for formation of 7-ethyl-10-hydroxycamptothecin)

Test Article Requirements
100 µL of a 10 mM DMSO solution (or equivalent amount in solid)

Analysis Method
LC-MS/MS

Data Delivery
Parent compound remaining at each time point for each isoform
Half life
Standard error of half life

* Alternative species or enzyme sources (i.e. plasma, blood) are available on request. We also have experience in evaluating other esterase mediated metabolism such as paraoxonase and butyrylcholinesterase metabolism.

'...successful design of ester-containing drugs will be greatly improved by further detailed analysis of the mechanism of action, substrate recognition and expression of human CES isozymes. Furthermore, development of an *in vitro* evaluating system for absorption and metabolism of prodrugs will assist with discovery of a suitable prodrug.'[2]

Figure 1

Assessment of CE probe substrates trandolapril (hCE1 substrate), oseltamivir (hCE1 substrate) and procaine (hCE2 substrate) in different enzyme sources.

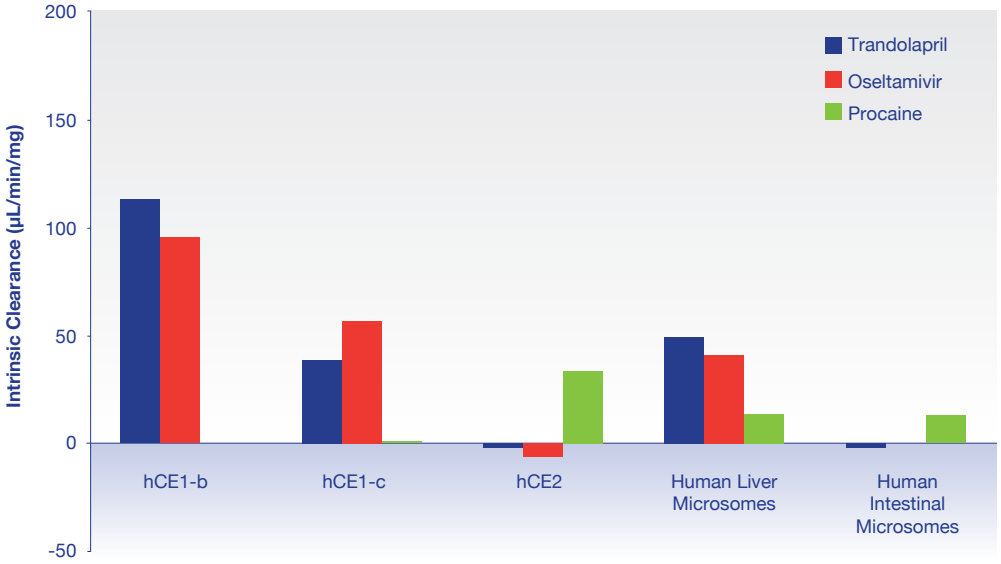
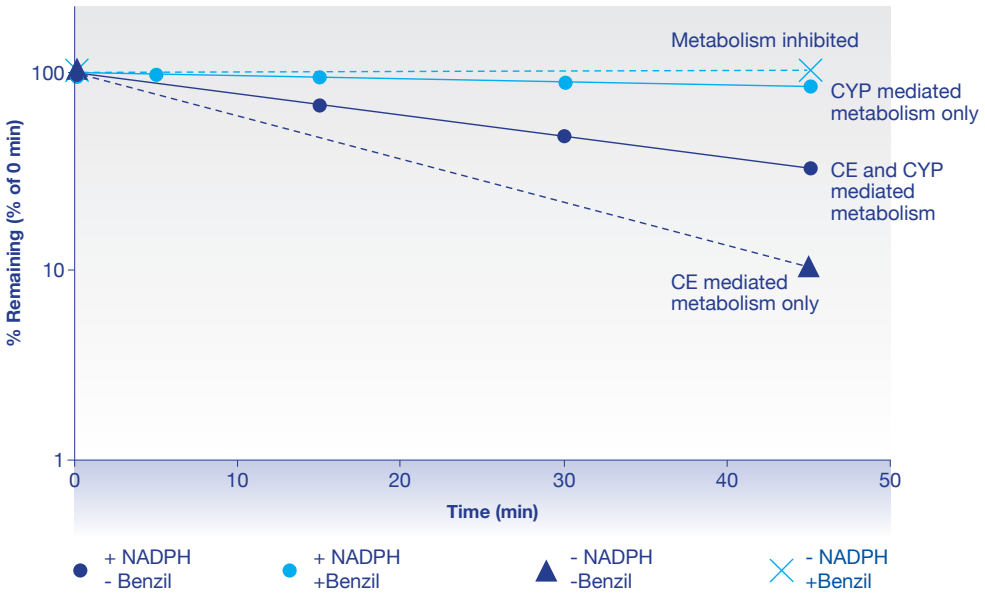


Figure 2

Human liver microsomal intrinsic clearance of the hCE1 substrate, trandolapril, in the presence of NADPH over a 45 min time course and in the absence of NADPH at 45 min, incubated with and without benzil (non-specific CE inhibitor).



By evaluating the metabolism of trandolapril in the presence and absence of the cofactor NADPH and in the presence and absence of benzil, it is possible to identify the relevance of CYP and non-CYP (i.e., carboxylesterase) mediated metabolism. In the absence of CYP activity, trandolapril is more rapidly cleared via carboxylesterase metabolism.

References

[1] Fukami T and Yokoi T. (2012) The emerging roles of human esterases. *Drug Metab Pharmacokinet* **27(5)**;466-477

[1] Imai T. (2006) Human carboxylesterase isozymes: catalytic properties and rational drug design. *Drug Metab Pharmacokinet* **21(3)**;173-185