active drug by functionally prominent and nonspecific hydrolases such as peptidases, phosphatases, and esterases including acetylcholinesterase, paraoxonase, butyrylcholinesterase, and in particular, carboxylesterase. Carboxylesterases (CESs) are predominantly involved in hydrolase activity in the liver and the small intestine of human and various preclinical species. CESs efficiently catalyzes the hydrolysis of a variety of esters and amides such as cocaine, heroin, irinotecan, and temocapril. CESs are ubiquitously distributed and their potential to become saturated or for their substrates to become involved in drug–drug interactions is considered to be negligible, although not impossible. Therefore, CESs are attractive targets for various ester and amide prodrugs of hydroxyl, phenolic, carboxyl and amino group containing compounds. Several prodrugs also rely on cytochrome P450 (CYP) enzymes in their bioactivation process. The rest of the text in this chapter focuses on hydrolases, particularly, carboxylesterase, and to lesser extent on CYPs.

The majority of CESs fall within two isozyme families, CES1 and CES2 which are characterized by their substrate specificities, tissue distribution, and gene regulation. In humans, CES1 is highly expressed in the liver, and distributed throughout many tissues with the notable exception of the intestine. Human CES2, in contrast, is highly expressed in the small intestine, the kidney, and the colon, but at much lower levels than CES1 in the liver. Although there is 40%–50% sequence homology between CES1 and CES2, differences in hydrolysis of their substrates have been observed. CES1 prefers compounds with large acyl and small alcohol groups, whereas CES2 prefers hydrolyzing compounds with large alcohol and small acyl groups. For example, oseltamivir is predominantly hydrolyzed by CES1, while irinotecan, with its bulky alcohol group, is hydrolyzed almost exclusively by CES2 (Figure 10.18).

In addition to substrate specificities, significant differences in CES expression between mammalian species should be considered when selecting animal models for preclinical studies of prodrugs and translating preclinical data in humans. Similarly, with dog and monkey, human plasma does not contain CES which is highly expressed in rabbit, mouse, and rat plasma. The esterase expression and hydrolyzing pattern of dog plasma has been found to be closest to that of human plasma which are both ubiquitously expressing other hydrolyzing enzymes paraoxonase and butyrylcholinesterase. Hydrolase activity in the small intestine of humans and rats is similar with exclusive expression of CES2 and similar expression along the whole length of the intestine. Interestingly, no hydrolase activity has been found in dog small intestine. The human liver contains both CES isozymes like various preclinical species with similar or less hydrolase activity depending on species compared. Using human recombinant enzymes or human tissue extracts can reduce the interspecies variability and identify tissue-specific hydrolysis.

While ubiquitous distribution of hydrolase enzymes can be utilized in effective bioconversion of ester prodrugs, premature hydrolysis during absorption process of hydrolytically susceptible prodrugs can significantly diminish their bioavailability. Reduced absorption has been observed with several ester prodrugs of penicillins, cephalosporins, and angiotensin-converting enzyme inhibitors. In general, these ester prodrugs have bioavailabilities of around 40%–60% which can be

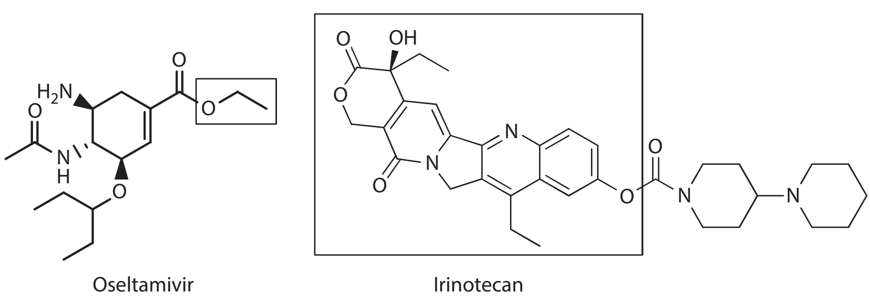

FIGURE 10.18 Human CES1 mainly hydrolyses a substrate with a small alcohol group (oseltamivir) while human CES2 mainly hydrolyses a substrate with a large alcohol group (irinotecan).