Title page

Conclusive Identification of the Oxybutynin-hydrolyzing Enzyme in Human Liver

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Running title page

Running title: CES1 as the principal enzyme for oxybutynin hydrolysis

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Non-standard abbreviations used in this paper:
BNPP, bis-(p-nitrophenyl) phosphate; CES, carboxylesterase; CPGA, 2-cyclohexyl-2-phenylglycolic acid; HLC, human liver cytosol; HLM, human liver microsomes; NDGA, nordihydroguaiaretic acid.
Abstract

The aim of this study was to conclusively determine the enzyme responsible for the hydrolysis of oxybutynin in human liver. Hydrolysis in human liver microsomes (HLMs) and cytosol (HLC) followed Michaelis-Menten kinetics with similar $K_m$ values. In recombinant human carboxylesterase (CES)-expressing microsomes, CES1 was much more efficient than CES2 and yielded a $K_m$ value more comparable to that found in HLMs or HLC than did CES2. A correlation analysis using a set of individual HLMs in which both CESs acted independently showed that the hydrolysis rate of oxybutynin correlated significantly with a CES1 marker reaction, clopidogrel hydrolysis, but not with a CES2 marker reaction, CPT-11 hydrolysis. Chemical inhibition studies using bis-(p-nitrophenyl) phosphate, clopidogrel, nordihydroguaiaretic acid, procainamide, physostigmine, and loperamide revealed that the effects of these compounds in HLMs, HLC, and recombinant CES1-expressing microsomes were similar, while those in CES2-expressing microsomes were clearly different. These results strongly suggest that CES1, rather than CES2, is the principal enzyme responsible for the hydrolysis of oxybutynin in human liver.
Introduction

Oxybutynin hydrochloride is an antimuscarinic agent administered for overactive bladder (Appell et al., 2003; Guay, 2003), and is primarily metabolized in humans via cytochrome P450 3A4, which yields a dealkylated, pharmacologically active form, N-desethyloxybutynin (Mizushima et al., 2007). It is also hydrolyzed to a pharmacologically inactive metabolite, 2-cyclohexyl-2-phenylglycolic acid (CPGA) (Abramov and Sand, 2004) (Fig. 1).

Carboxylesterases (CESs, EC 3.1.1.1) belong to the esterase superfamily involved in the hydrolysis of ester-bearing molecules like oxybutynin (Hosokawa et al., 1995; Hosokawa et al., 2007; Hosokawa, 2008). Two CES isozymes, CES1 and CES2, are expressed in both the microsomal and cytosolic fractions of human liver (Imai, 2006; Ross and Crow, 2007), and each has been implicated as the oxybutynin-hydrolyzing enzyme in separate studies. Takai et al. investigated the hydrolytic activity of purified CES proteins on various drugs (Takai et al., 1997) and demonstrated that oxybutynin was hydrolyzed by CES2 (pI 4.5) with $K_m$ and $V_{max}$ values of 1.1 mM and 0.36 $\mu$mol/min/mg, respectively. Meanwhile, hydrolysis by CES1 (pI 5.3) was below the detection limit (1.0 nmol/min/mg). In contrast, Takahashi et al. investigated the hydrolysis kinetics of oxybutynin in human liver microsomes (HLMs) and cytosol (HLC), and reported $K_m$ values between 75 and 120 $\mu$M (Takahashi et al., 2008). They performed a correlation analysis using imidapril as a marker substrate for CES1 activity, and results showed a significant correlation between the formation of imidaprilat and CPGA, indicating that CES1 likely hydrolyzes oxybutynin as well as imidapril. They also reported preliminary data suggesting that the formation of CPGA was inhibited by bis-(p-nitrophenyl) phosphate.
(BNPP), a well-known CES inhibitor (Heymann and Krisch, 1967; Eng et al., 2010), but not by loperamide, a CES2 inhibitor (Rivory et al., 1996; Quinney et al., 2005), although no concrete data regarding the potency of inhibition was given.

Although the hydrolytic potential of CES proteins can be examined using purified protein assays, $K_m$ values should be compared with those in HLMs and HLC to assess involvement of CESs in human liver tissue fractions. Additionally, when using a correlation analysis, one cannot define the contribution of each isozyme unless it can be shown that their activities are independent from each other. To resolve this controversy, and identify the oxybutynin-hydrolyzing enzyme in human liver, we performed systematic in vitro experiments. First, we investigated kinetics for oxybutynin hydrolysis in HLMs, HLC, and recombinant human CES-expressing microsomes. Then, adopting clopidogrel and irinotecan (CPT-11) as marker substrates for CES1 and CES2 respectively, we conducted a correlation analysis using a set of 16 individual HLMs. Finally, chemical inhibition studies in human liver tissue fractions and recombinant CESs were conducted using BNPP and several other potential CES inhibitors (clopidogrel, nordihydroguaiaretic acid (NDGA), procainamide, physostigmine (eserine) and loperamide) (Schegg and Welch, 1984; Rivory et al., 1996; Quinney et al., 2005; Shi et al., 2006; Takahashi et al., 2009).
Materials and Methods

Chemicals and Reagents

Oxybutynin hydrochloride, clopidogrel carboxylic acid, clopidogrel-d4 carboxylic acid (internal standard for clopidogrel carboxylic acid quantitation), CPT-11 hydrochloride trihydrate, and SN-38 were purchased from Toronto Research Chemicals (Toronto, ON, Canada). CPGA was obtained from Wako Pure Chemicals (Osaka, Japan). Benzilic acid (internal standard for CPGA quantitation), camptothecin (internal standard for SN-38 quantitation), NDGA, eserine, procainamide, and loperamide were purchased from Sigma (St. Louis, MO, USA). Clopidogrel monosulfate was obtained from LKT Laboratories, Inc. (St. Paul, MN, USA). BNPP was obtained from Nacalai Tesque, Inc. (Osaka, Japan). Pooled and individual HLMs (Reaction Phenotyping Kit® ver. 7) and pooled HLC were purchased from XenoTech LLC (Kansas City, KS, USA). Recombinant human CES1 (CES1-b/CES1A1) - and CES2-expressing microsomes (prepared from baculovirus-infected High Five® insect cells) were obtained from BD Gentest (Woburn, MA, USA) (Wang et al., 2011). All other chemicals and reagents used were commercially available, and guaranteed of purity.

Hydrolysis Assays

The assays were performed according to Tang et al. (Tang et al., 2006). Briefly, hydrolysis of oxybutynin was carried out at 37 °C in 100 μL of 0.05 mol/L Tris-HCl buffer (pH 7.6). After pre-incubation at 37 °C for 5 min, the reaction was initiated by adding an oxybutynin solution prepared using the same buffer. In chemical inhibition studies, the inhibitor solution prepared using the same buffer was added just prior to the pre-incubation step. Some inhibitor solutions were prepared by dissolving with
DMSO before diluting with the buffer solution. In these cases, the final DMSO concentration in the reaction mixture was 0.2%, which was confirmed to have no effect on the hydrolysis of oxybutynin. The reaction was terminated by adding 150 μL acetonitrile (ACN) with 1% (v/v) formic acid containing internal standard (IS) for subsequent HPLC analysis. After centrifugation at 1870 × g for 10 min at 4 °C, the supernatant was mixed with the HPLC mobile phase. All assays were performed in duplicate.

Bioanalysis

The formation of hydrolysates, CPGA, clopidogrel carboxylate and SN-38 was determined using HPLC tandem mass spectrometry. The system comprised a Prominence HPLC system (Shimadzu, Kyoto, Japan) with a Synergi Fusion-RP 100A 50 × 2.00 mm, 2.5-micron, column (Phenomenex, Torrance, CA, USA) and a QTRAP® 5500 (Applied Biosystems/MDS Sciex, Foster City, CA, USA). The HPLC mobile phase was a combination of 0.1% formic acid (A) and ACN (B). Samples were injected onto the column at a flow rate of 0.4 mL/min. The gradient program was 30%-80% B in 3 min, 80%-30% B in 3.1 min and 30% B in 6 min for CPGA, and 15%-60% B in 3 min, 60%-15% B in 3.1 min, and 15% B in 6 min for clopidogrel carboxylate and SN-38. The sample rack and column temperatures were maintained at 10 and 45 °C, respectively. Quantitation of CPGA was performed in negative ion multiple reaction monitoring (MRM) mode by applying the following precursor to product transitions: CPGA m/z 233→189, and benzilic acid (IS) m/z 227→183. Quantitation of clopidogrel carboxylate and SN-38 was performed in positive ion MRM mode by applying the following precursors to the product transitions:
clopidogrel carboxylate $m/z$ 308→198, d4-clopidogrel carboxylate (IS) $m/z$ 312→202, SN-38 $m/z$ 393→349, and camptothecin (IS) $m/z$ 349→305. The data were processed using Analyst 1.5.1 software (Applied Biosystems/MDS Sciex).

**Kinetics Studies**

Hydrolysis kinetics studies were conducted in pooled HLMs, HLC and recombinant CES-expressing microsomes with oxybutynin concentrations of 2.5 to 250 μM. Investigation of higher concentrations was not feasible due to limitations of solubility. The final protein concentrations for HLMs, HLC, CES1 and CES2 were 0.05, 0.1, 0.05, and 0.1 mg/mL, respectively. The reaction time was 15 min. CPGA concentration was measured as noted above. Hydrolysis rate versus substrate concentration data were fitted to a single component Michaelis-Menten equation using GraphPad Prism software ver. 5.03 (GraphPad Software, San Diego, CA, USA) to estimate $K_m$ and $V_{max}$. The intrinsic clearance (CL$_{int}$) was calculated by dividing $V_{max}$ by $K_m$.

**Correlation Analysis**

The hydrolysis rates of clopidogrel (5 μM), CPT-11 (1 and 100 μM), and oxybutynin (10 μM) were investigated in 16 individual HLMs. Their final microsomal protein concentrations were 0.02, 0.25, and 0.05 mg/mL, and their reaction times were 20, 30, and 15 min, respectively. The independence of CES1 and CES2 activity was investigated via linear regression analysis of the hydrolysis rates of clopidogrel and CPT-11. Linear regression analysis of hydrolysis rates of oxybutynin and these CES marker substrates (clopidogrel and CPT-11 [1 μM]) was also performed. GraphPad Prism software ver. 5.03 was used for linear regression analysis and to calculate the
coefficients of determination ($r^2$) and p values. A p value < 0.05 was considered significant.

**Chemical Inhibition**

Formation of CPGA was investigated in pooled HLMs, HLC, and recombinant CES-expressing microsomes in the presence of BNPP (10 μM), clopidogrel (5 and 50 μM), NDGA (10 and 100 μM), procainamide (30 and 300 μM), eserine (2 and 20 μM), and loperamide (5 and 50 μM). The substrate concentration was 10 μM. The final protein concentrations for HLMs, HLC, CES1 and CES2 were 0.05, 0.2, 0.05, and 0.1 mg/mL, respectively. The reaction time was 30 min. The relative hydrolytic activity was calculated by normalizing with respect to the amount of CPGA formed in the inhibitor-free sample.
Results

Kinetics Studies

The formation of CPGA in HLMs and HLC showed single component Michaelis-Menten kinetics as indicated by the Eadie-Hofstee plots (Fig. 2, A and B). Kinetics parameters are summarized in Table 1. In HLMs, $K_m$, $V_{max}$, and $CL_{int}$ were 22 $\mu$M, 130 pmol/min/mg protein, and 5.9 $\mu$L/min/mg protein, respectively, and in HLC, values were 13 $\mu$M, 110 pmol/min/mg protein, and 8.2 $\mu$L/min/mg protein, respectively. The formation of CPGA in recombinant CES1-expressing microsomes also followed Michaelis-Menten kinetics (Fig. 2C) with $K_m$, $V_{max}$, and $CL_{int}$ values of 17 $\mu$M, 310 pmol/min/mg protein, and 18 $\mu$L/min/mg protein, respectively. Hydrolysis by recombinant CES2 was extremely low, and formation of CPGA at an oxybutynin concentration of 2.5 $\mu$M was below the detection limit (1 ng/mL). Thus, fitting was conducted using data from 5 to 250 $\mu$M (6 points) (Fig. 2D), and yielded $K_m$, $V_{max}$ and $CL_{int}$ values of 62 $\mu$M, 32 pmol/min/mg protein and 0.51 $\mu$L/min/mg protein, respectively.

Correlation Analysis

While clopidogrel is a CES1-specific substrate (Tang et al., 2006), CPT-11 is a dual CES substrate whose hydrolysis is catalyzed by both CES1 and CES2 at high substrate concentrations but is hydrolyzed predominantly by CES2 at low concentrations (Slatter et al., 1997). Therefore, hydrolysis rates of CPT-11 at different concentrations were measured to ensure that we could assess CES1 and CES2 activity independently. The hydrolysis rates of clopidogrel (5 $\mu$M) in 16 individual HLMs varied 18-fold (0.50 to 9.2 nmol/min/mg protein). The hydrolysis rates of CPT-11 at 1 and 100 $\mu$M in the
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same set of HLMs varied 7- and 6-fold (0.041 to 0.28 pmol/min/mg protein and 0.48 to 2.9 pmol/min/mg protein), respectively. Linear regression analysis revealed that at a high concentration of CPT-11 (100 μM), hydrolysis rates of clopidogrel and CPT-11 were significantly correlated ($r^2 = 0.4044; p = 0.0081$; Fig. 3B), but at a low level of CPT-11 (1 μM), this correlation disappeared ($r^2 < 0.0001; p = 0.9494$; Fig. 3A). This indicates that at 1 μM, CPT-11 can be treated as CES2-specific. Oxybutynin (10 μM) hydrolysis levels in the same set of HLMs varied 12-fold (24 to 290 pmol/min/mg protein). As shown in Fig. 4A, an excellent correlation was observed between the hydrolysis rates of oxybutynin and clopidogrel ($r^2 = 0.8396; p < 0.0001$), but not between oxybutynin and CPT-11 (1 μM) ($r^2 = 0.01589; p = 0.6418$; Fig. 4B).

Chemical Inhibition

Results are illustrated in Fig. 5. The hydrolysis of oxybutynin (10 μM) was inhibited by more than 96% in the presence of BNPP (10 μM) in all cases. At 5 μM, clopidogrel inhibited hydrolysis by less than 11% in all cases. At 50 μM, hydrolytic activity decreased by more than 84% in HLMs, HLC, and CES1-expressing microsomes, but only decreased by 53% in CES2-expressing microsomes. In the presence of NDGA (10 μM), activities in HLMs, HLC, and CES1-expressing microsomes decreased by 77% to 87%, while that in CES2-expressing microsomes was reduced only by 29%. With an elevated concentrations of NDGA (100 μM), hydrolysis in HLMs, HLC, and CES1-expressing microsomes dropped by more than 97%, while that in CES2-expressing microsomes fell to 24%. Procainamide, at both 30 and 300 μM, inhibited hydrolysis less than 14% in all cases. Eserine at both 2 and 20 μM blocked less than 17% of activity in HLMs, HLC, and CES1-expressing microsomes but more.
than 74% in CES2-expressing microsomes. In the presence of loperamide (5 μM), hydrolysis in HLMs, HLC, and CES1-expressing microsomes was inhibited less than 12%, while inhibition in CES2-expressing microsomes was higher, at 58%. At elevated concentrations of loperamide (50 μM), inhibition in HLMs, HLC, and CES1-expressing microsomes increased to between 36% and 47%, and that in CES2-expressing microsomes increased to 80%.
Discussion

Two previous studies involving the CES isozyme responsible for the hydrolysis of oxybutynin in human liver have yielded contradictory results, with one demonstrating that CES1 was responsible while the other cited CES2. However, results in the present study conclusively demonstrated through systematic in vitro examinations that the isozyme in question was CES1.

As a first step, we investigated the hydrolysis kinetics in pooled HLMs and HLC along with kinetics in recombinant human CES-expressing microsomes. Hydrolysis of oxybutynin in HLMs and HLC followed single component Michaelis-Menten kinetics, with lower $K_m$ values (22 and 13 μM, respectively) compared with reported values (75 to 120 μM) (Takahashi et al., 2008). Although the precise reason for this discrepancy remains unknown, it might be due to differences in reaction conditions, as the reaction mixture prepared by Takahashi et al. used 100 mM potassium phosphate buffer (pH 7.4). Despite these conflicting findings, clinically relevant concentrations of oxybutynin are indeed likely to be much lower than any $K_m$ values, based on its maximum plasma concentration in clinical doses (less than 151 ng/mL [0.4 μM]) and plasma protein binding rate (>99%) (Guay, 2003). The $V_{max}$ and $CL_{int}$ values in the present study were similar in HLMs and HLC, which might be surprising given that expression of CESSs in HLMs has been reported to be higher than in HLC (Ross and Crow, 2007). However, values in the present study might be possible because $V_{max}$ values were within ranges reported previously (Takahashi et al., 2008).

Between the recombinant CESs, the $K_m$ value of CES1 (17 μM) was more comparable to those found in HLMs and HLC than that for CES2 (62 μM). Further,
assuming that expression levels of recombinant CES1 and CES2 are similar, $V_{\text{max}}$ and $CL_{\text{int}}$ values indicate that CES1 is more potent than CES2 in hydrolyzing oxybutynin. In HLMs, protein expression of CES1 has been reported to be markedly higher than of CES2 (1070 and 23.0 pmol/ing microsomal protein, respectively) (Godin et al., 2007; Ross and Crow, 2007). Taken together, these results suggest the contribution of CES1 to the hydrolysis of oxybutynin in HLMs to be much higher than that of CES2.

To test this hypothesis, we performed a correlation analysis using a set of 16 individual HLMs. In some previous studies, statistically significant correlations were observed between the CES marker activity and the activity of the test substance (Yamaori et al., 2006; Takahashi et al., 2008; Hagihara et al., 2009). However, to conclude whether CES1 or CES2 is involved in HLMs, the independence of the different CES isozyme activities must be established in advance. Therefore, we investigated the independence of CES1 and CES2 activities using clopidogrel and CPT-11 as respective marker substrates. Clopidogrel is exclusively hydrolyzed to its carboxylate by CES1 (Hagihara et al., 2009; Farid et al., 2010), with a $K_m$ of 58 μM in HLMs (Tang et al., 2006). CPT-11 is hydrolyzed to SN-38 (Satoh et al., 1994; Haaz et al., 1997), and catalyzed predominantly by CES2 at low concentrations (under 5 μM) in HLMs (Slatter et al., 1997; Xu et al., 2002; Takahashi et al., 2009).

In the present study, regression analysis showed an insignificant correlation between the hydrolysis rates of clopidogrel at 5 μM and CPT-11 at 1 μM (Fig. 3A), indicating that use of a correlation analysis to differentiate between CES1 and CES2 activity is feasible. Further, the significant correlation observed between the hydrolysis rates of clopidogrel and CPT-11 at 100 μM indicates not only the superior contribution of CES1 to the hydrolysis of CPT-11 at 100 μM, but also the importance of the
CPT-11 concentration when used as a CES2 marker substrate (Fig. 3B). In the same individual HLMs, the hydrolysis of oxybutynin correlated well with the hydrolysis rate of clopidogrel but poorly with that of CPT-11 at 1 μM (Fig. 4), clearly suggesting that the major isozyme responsible for oxybutynin hydrolysis in HLMs is CES1, not CES2.

Finally, chemical inhibition studies were conducted in HLMs, HLC, and recombinant CESs using 6 compounds (BNPP, clopidogrel, NDGA, procainamide, eserine and loperamide) (Fig. 5). BNPP is a well-known irreversible, non-selective CES inhibitor (Heymann and Krisch, 1967; Eng et al., 2010). The hydrolysis of oxybutynin by recombinant CESs was completely inhibited in the presence of BNPP (10 μM). At the same concentration, hydrolysis in HLMs and HLC was almost entirely inhibited, suggesting a predominant contribution of CESs to the hydrolysis of oxybutynin in human liver tissue fractions. Clopidogrel has been reported to be a potential CES1 inhibitor. The hydrolysis of oseltamivir (50 μM), another CES1-specific substrate with a $K_m$ value of 180 μM, is greatly inhibited in the presence of clopidogrel (50 μM) by as much as 90% in CES1-transfected 293T cells (Shi et al., 2006). NDGA and procainamide have been reported to be reversible CES1 inhibitors with $K_i$ values ranging from 2.9 to 13 μM and 29 to 35 μM, respectively (Takahashi et al., 2009). In the present study, the hydrolysis of oxybutynin in both human liver tissue fractions and recombinant CES1 was strongly inhibited by clopidogrel (50 μM) and NDGA (10 and 100 μM). In contrast, these compounds could not inhibit CES2-mediated hydrolysis to the same degree. No obvious inhibition by procainamide (30 and 300 μM) was observed in any fractions. We also investigated the effects of procainamide on the hydrolysis of clopidogrel in HLMs and recombinant CES1,
finding less than 3% inhibition (data not shown). Thus, whether or not procainamide is a useful CES1 inhibitor remains unknown.

Eserine and loperamide are known to be potent reversible CES2 inhibitors with $K_i$ values ranging from 0.20 to 1.6 $\mu$M (Takahashi et al., 2009) and 1.5 $\mu$M (Quinney et al., 2005), respectively. Although both eserine and loperamide also inhibit CES1, the effects of these inhibitors on CES2 are more potent (Quinney et al., 2005; Takahashi et al., 2009). In the present study, the hydrolysis of oxybutynin by CES2 was strongly inhibited by eserine (2 and 20 $\mu$M) and loperamide (5 and 50 $\mu$M), but inhibition was modest in the other fractions. Taken together, results from our present studies show that the effects of these chemicals on oxybutynin hydrolysis in HLMs, HLC, and recombinant CES1 were comparable but differed from findings in CES2-expressing microsomes.

Here, we examined the enzymes potentially responsible for the hydrolysis of oxybutynin in human liver. Kinetic studies showed comparable $K_m$ values between human liver tissue fractions and recombinant CES1. The hydrolysis rates of oxybutynin in HLMs correlated well with a CES1-marker activity but poorly with that of CES2. Chemical inhibition studies showed similar effects on oxybutynin hydrolysis in human liver tissue fractions and recombinant CES1, but effects in CES2-expressing microsomes differed substantially. In conclusion, these results conclusively demonstrate that CES1 is the principal oxybutynin hydrolyzing-enzyme in human liver.
Authorship contributions

Participated in research design: Sato and Miyashita

Conducted experiments: Sato

Contributed new reagents or analytic tools: Sato

Performed data analysis: Sato

Wrote or contributed to the writing of the manuscript: Sato, Miyashita, Iwatsubo, and Usui
References


Footnotes

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Figure Legends

**Fig. 1.** Chemical structures of oxybutynin and 2-cyclohexyl-2-phenylglycolic acid (CPGA).

**Fig. 2.** Hydrolysis kinetics of oxybutynin in HLMs (A), HLC (B), recombinant CES1 (C) and CES2 (D) microsomes. The Eadie-Hofstee plots are presented in the inset.

**Fig. 3.** Correlation between the hydrolysis rates of clopidogrel and CPT-11 in HLMs. Linear regression analysis of the hydrolysis rates of clopidogrel (5 μM) vs. CPT-11 (1 μM) (A) and CPT-11 (100 μM) (B).

**Fig. 4.** Correlation between the hydrolysis rates of oxybutynin and CES marker substrates in HLMs. Linear regression analysis of the hydrolysis rates of oxybutynin (10 μM) vs. clopidogrel (5 μM) (A) and CPT-11 (1 μM) (B).

**Fig. 5.** Inhibitory effects of BNPP (10 μM), clopidogrel (5 and 50 μM), NDGA (10 and 100 μM), procainamide (30 and 300 μM), eserine (2 and 20 μM), and loperamide (5 and 50 μM) on the hydrolysis of oxybutynin (10 μM) in human liver tissue fractions and recombinant CESs.
### TABLE 1. Kinetics parameters of oxybutynin hydrolysis in HLMs, HLC, and recombinant human CESs

<table>
<thead>
<tr>
<th></th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (pmol/min/mg protein)</th>
<th>$CL_{int}$ (μL/min/mg protein)</th>
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<tbody>
<tr>
<td>HLM</td>
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<tr>
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<td>32</td>
<td>0.51</td>
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Fig. 2

A

Hydrolysis rate (pmol/min/mg protein)

Oxybutynin (μM)

B

Hydrolysis rate (pmol/min/mg protein)

Oxybutynin (μM)

C

Hydrolysis rate (pmol/min/mg protein)

Oxybutynin (μM)

D

Hydrolysis rate (pmol/min/mg protein)

Oxybutynin (μM)
Fig. 3

A

Hydrolysis rate of CPT-11 (1 µM) (pmol/min/mg protein)

Hydrolysis rate of clopidogrel (nmol/min/mg protein)

$\displaystyle r^2 < 0.0001, p = 0.9494$

B

Hydrolysis rate of CPT-11 (100 µM) (pmol/min/mg protein)

Hydrolysis rate of clopidogrel (nmol/min/mg protein)

$\displaystyle r^2 = 0.4044, p = 0.0081$
Fig. 4

A

Hydrolysis rate of clopidogrel (nmol/min/mg protein)

Hydrolysis rate of oxybutynin (pmol/min/mg protein)

$r^2 = 0.8396, p < 0.0001$

B

Hydrolysis rate of CPT-11 (1 µM)

Hydrolysis rate of oxybutynin (pmol/min/mg protein)

$r^2 = 0.01589, p = 0.6418$
Fig. 5

![Graph showing the percentage of control for various compounds at different concentrations.](image-url)

- BNPP 10 µM
- Clopidogrel 5 µM
- Clopidogrel 50 µM
- NDGA 10 µM
- NDGA 100 µM
- Procainamide 30 µM
- Procainamide 300 µM
- Eserine 2 µM
- Eserine 20 µM
- Loperamide 5 µM
- Loperamide 50 µM

Legend:
- HLMs
- HLC
- CES1
- CES2

% of control