Contributions of Arylacetamide Deacetylase and Carboxylesterase 2 to Flutamide Hydrolysis in Human Liver

Received January 4, 2012; accepted March 23, 2012

Introduction

Flutamide, an antiandrogen drug, is widely used for the treatment of prostate cancer. The major metabolic pathways of flutamide are hydroxylation and hydrolysis. The hydrolyzed metabolite, 5-amino-2-nitrobenzotri fluoride (FLU-1), is further metabolized to N-hydroxy FLU-1, an assumed hepatotoxicant. Our previous study demonstrated that arylacetamide deacetylase (AADAC), one of the major serine esterases expressed in the human liver and gastrointestinal tract, catalyzes the flutamide hydrolysis. However, the enzyme kinetics in human tissue microsomes were not consistent with the kinetics with recombinant human AADAC. Thus, it seemed that AADAC is not the sole enzyme responsible for flutamide hydrolysis in human. In the present study, we found that recombinant carboxylesterase (CES) 2 could hydrolyze flutamide at low concentrations of flutamide. In the inhibition assay, the flutamide hydrolysis activities at a flutamide concentration of 5 μM in human liver and jejunum microsomes were strongly inhibited by a selective CES2 inhibitor, 10 μM loperamide, with the residual activities of 22.9 ± 3.5 and 18.6 ± 0.7%, respectively. These results suggest that CES2 is also involved in the flutamide hydrolysis in human tissues. Using six individual human livers, the contributions of AADAC and CES2 to flutamide hydrolysis were estimated by using the relative activity factor. The relative contribution of CES2 was approximately 75 to 99% at the concentration of 5 μM flutamide. In contrast, the relative contribution of AADAC increased in parallel with the concentration of flutamide. Thus, CES2, rather than AADAC, largely contributed to the flutamide hydrolysis in clinical therapeutics.

Materials and Methods

Chemicals and Reagents. Flutamide, FLU-1, and rifampicin were purchased from Wako Pure Chemical Industries (Osaka, Japan). 25-Desacetyl rifampicin (25-deacetylrifampicin), irinotecan hydrochloride (CPT-11), and 7-ethyl-10-hydroxy camptothecin (SN-38) were purchased from Toronto Research Chemicals (Toronto, Canada). Ethopropazine was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used in this study were of analytical or the highest quality commercially available.

Human Tissues. HLM (pooled, n = 50), human liver cytosol (HLC) (pooled, n = 22), and human jejunum microsomes (HJM) (pooled, n = 10) were purchased from BD Gentest (Woburn, MA) and Tissue Transformation Technologies (Edison, NJ), respectively. Six individual human liver samples were obtained from individuals undergoing liver transplantation. The study was approved by the Institutional Review Board of the National Institute of Biomedical Innovation (NIBIO). The experimental design and sample collection were in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice in Japan. All donors provided written informed consent.

This study was supported by the Japan Society for the Promotion of Science [Grant-in-Aid for Young Scientists (B) 21790418]. Article, publication date, and citation information can be found at http://dmd.aspetjournals.org. http://dx.doi.org/10.1124/dmd.112.044537.

Supplemental material to this article can be found at: http://dmd.aspetjournals.org/content/suppl/2012/03/23/dmd.112.044537.DC1

ABBREVIATIONS: FLU-1, 5-amino-2-nitrobenzotri fluoride; AADAC, arylacetamide deacetylase; HLM, human liver microsomes; CES, carboxylesterase; CPT-11, irinotecan hydrochloride; HLC, human liver cytosol; HJM, human jejunum microsomes; HLH, human liver homogenates; RAF, relative activity factor; \(I_{\text{inlet}}, u_{\text{max}}\), the maximum value of the unbound concentration at the inlet to the liver.

The online version of this article (available at http://dmd.aspetjournals.org) contains supplemental material.
were supplied by the National Disease Research Interchange (Philadelphia, PA) through the Human and Animal Bridging Research Organization (Chiba, Japan). The use of the human livers was approved by the Ethics Committee of Kanazawa University (Kanazawa, Japan). The human liver homogenates (HLH) were prepared according to our previous report (Iwamura et al., 2012).

**Flutamide, Rifampicin, and CPT-11 Hydrolyase Activities.** The flutamide hydrolyase activity was determined according to Watanabe et al. (2009). The concentrations of enzyme sources were as follows: HLM, HLC, HJM, and SF21 cell homogenates expressing human AADAC, CES1, and CES2 (Fukami et al., 2010; Watanabe et al., 2010), 0.4 mg/ml; and HLC, 1.0 mg/ml. An inhibitory analysis of flutamide hydrolysis was performed using loperamide, a potent CES2 inhibitor (Quinney et al., 2005). The concentrations of loperamide and flutamide were 10 and 5 μM, respectively. For kinetic analyses of the flutamide hydrolyase activity, the parameters were estimated from the fitted curves using a computer program (KaleidaGraph; Synergy Software, Reading, PA) designed for nonlinear regression analysis.

The rifampicin hydrolyase activity, which is a marker of AADAC enzyme activity, was determined according to our previous study (Nakajima et al., 2011). The concentration of enzyme sources (HLH and SF21 cell homogenates expressing human AADAC) was 0.5 mg/ml. The substrate concentration of rifampicin was 50 μM.

The CPT-11 hydrolyase activity, which was used as a marker of CES2 enzyme activity, was determined according to Takahashi et al. (2009) except for the mobile phase. The concentration of enzyme sources (HLH and SF21 cell homogenates expressing human CES2) was 0.2 mg/ml. The mobile phase was 25% acetonitrile and 75% of 10 mM KH2PO4 containing 3 mM 1-heptanesulfonic acid sodium salt.

**Contributions of AADAC and CES2 to Flutamide Hydrolyase Activity in HLH.** AADAC is expressed in liver microsomes, whereas CES2 is expressed in liver microsomes and cytosol (Xu et al., 2002). Therefore, their contributions to flutamide hydrolysis in human liver were analyzed using individual HLH samples. The percentage contributions of AADAC and CES2 to the flutamide hydrolyase activity in individual HLH were estimated by applying the relative activity factor (RAF) as the ratio of activity values, as described previously (Watanabe et al., 2010). In this study, the RAF values for AADAC (RAF\(_{\text{AADAC}}\)) were determined as the ratios of the rifampicin hydrolysis activities in HLH to the value by recombinant AADAC. The RAF values for CES2 (RAF\(_{\text{CES2}}\)) were determined as the ratios of the CPT-11 hydrolysis activities in HLH to the value by recombinant CES2. Using the RAF, the predicted enzyme activities by AADAC (V\(_{\text{AADAC}}\)) and CES2 (V\(_{\text{CES2}}\)) in HLH were expressed as follows: 

\[
V_{\text{AADAC}} = V_{\text{AADAC}}^{\text{VHLH}} \times \text{RAF}_{\text{AADAC}}
\]

\[
V_{\text{CES2}} = V_{\text{CES2}}^{\text{VHLH}} \times \text{RAF}_{\text{CES2}}
\]

where \(V_{\text{AADAC}}^{\text{VHLH}}\) and \(V_{\text{CES2}}^{\text{VHLH}}\) are the flutamide hydrolyase activities by recombinant AADAC and CES2, respectively.

The contributions of AADAC and CES2 to the flutamide hydrolyase activities in HLH were calculated using the following equations: Contribution of AADAC (%) = \(\frac{V_{\text{AADAC}}}{V_{\text{AADAC}}^{\text{VHLH}}} \times 100\) and Contribution of CES2 (%) = \(\frac{V_{\text{CES2}}}{V_{\text{CES2}}^{\text{VHLH}}} \times 100\), where the \(V_{\text{AADAC}}\) or \(V_{\text{CES2}}\) are the flutamide hydrolyase activities in HLH.

**Statistical Analysis.** Statistical analyses between multiple groups were performed using one-way analysis of variance, followed by Tukey’s post hoc test. \(P < 0.05\) was considered statistically significant.

**Results and Discussion**

The Eadie-Hofstee plot for the flutamide hydrolyase activity in one individual HLM sample was biphasic (Supplemental Fig. 1), whereas the activity by recombinant AADAC was fitted to the Michaelis-Menten equation (Kobayashi et al., 2012). The CL\(_{\text{int}}\), in vivo values were calculated from wet weight for jejunum (411 g) and jejunum mucosal mass (150.8 ± 17.8 μM, V\(_{\text{max}}\) = 114.56 ± 36.4 nmol·min\(^{-1}·mg\)\(^{-1}\); CES2, K\(_{m}\) = 123.7 ± 13.3 μM, V\(_{\text{max}}\) = 518.1 ± 14.0 nmol·min\(^{-1}·mg\)\(^{-1}\) (data not shown)). The activity at 20 μM in HLM was not inhibited by 10 μM ethopropazine, an inhibitor of butyrylcholinesterase (Yamaori et al., 2006), and was not activated by 1 mM CaCl\(_2\), which is required for exerting paraxonase activity (Kuo and La Du, 1998) (data not shown). Thus, we first found the involvement of human CES2 in the flutamide hydrolysis. Considering the chemical structure of flutamide, this result was consistent with the notion that the preferential substrates for CES2 are compounds bearing a small acyl moiety and a bulky alcohol or amino groups (Imai et al., 2006).

Data for the flutamide hydrolyase activity by recombinant human CES2 were fitted to the substrate inhibition equation with the K\(_{i}\), V\(_{\text{max}}\), and K\(_{m}\). The CL\(_{\text{int}}\) value calculated from V\(_{\text{max}}\)/K\(_{m}\) was 4.2 ± 0.2 μl·min\(^{-1}·mg\) protein\(^{-1}\). It is not surprising that data for flutamide hydrolyase activity by recombinant human CES2 were fitted to the substrate inhibition equation. The prasugrel, a thienopyridine prodrug, hydrolyase activity by human CES2 was also inhibited at high concentrations, although data for its hydrolysis were not fitted to a single substrate inhibition equation (Williams et al., 2008). In our previous studies (Watanabe et al., 2009; Kobayashi et al., 2012), the flutamide hydrolyase activity in HLM was fitted to the Michaelis-Menten equation, and the Eadie-Hofstee plot was not obviously biphasic. Therefore, we mistakenly believed that AADAC was the major enzyme responsible for flutamide hydrolysis. However, when using other HLM samples (from a single donor), the Eadie-Hofstee plot was clearly biphasic (Supplemental Fig. 1). The expression ratio of CES2 and AADAC might cause a different Eadie-Hofstee plot.

To confirm the involvement of CES2 in flutamide hydrolysis, the inhibitory effects of loperamide, a potent CES2 inhibitor (Quinney et al., 2005), on the flutamide hydrolyase activities by recombinant CES2, AADAC, HLM, HLC, and HJM were analyzed (Fig. 1C). The activity by recombinant CES2 was potently inhibited by 10 μM loperamide (percentage of control: 7.7 ± 1.5%), whereas that by recombinant AADAC was weakly inhibited (percentage of control: 74.7 ± 3.2%). In HLM, HLC, and HJM, the activities at the flutamide concentration of 5 μM were decreased to 22.9 ± 3.5, 15.7 ± 4.0, and 18.6 ± 0.7% of the controls by 10 μM loperamide, respectively. These results suggest that CES2 was largely involved in the flutamide hydrolysis at 5 μM in HLM, HLC, and HJM. In HLM, CES1 and CES2, but not AADAC, are expressed. From the finding that the activity in HLC was strongly inhibited by loperamide, soluble CES2 in cytosol would catalyze the flutamide hydrolysis similarly to microsomal membrane-bound CES2. In addition, this result also supports no involvement of CES1 in flutamide hydrolysis, although CES1 is highly expressed in liver (Watanabe et al., 2009).

The in vivo intrinsic clearance (CL\(_{\text{int}}\), in vivo) values of flutamide hydrolysis in HLM and HJM were estimated using the following equation (Soars et al., 2002): 

\[
\text{CL}_{\text{int}} \text{ in vivo} = [\text{CL}_{\text{int}}] \times \frac{[\text{microsomal protein/tissue (mg/g)]}}{[\text{tissue/body weight (g/kg)]}}
\]

The CL\(_{\text{int}}\) values of flutamide hydrolysis in HLM and HJM were approximately 1.8 and 12.9 μl·min\(^{-1}·mg\) protein\(^{-1}\), respectively (Kobayashi et al., 2012). The CL\(_{\text{int}}\) in vivo values were calculated using microsomal protein/liver of 40 mg/g and the liver weight of 1500 g (Hakooz et al., 2006), and microsomal protein/jejunum mucosa of 20.5 mg/g and the jejunum mucosa weight of 65.8 g [calculated from wet weight for jejunum (411 g) and jejunum mucosal mass (16% of total wet weight)] (Paine et al., 1997; Cabitt et al., 2009). According to these values, the CL\(_{\text{int}}\), in vivo values of HLM and HJM...
respectively), the RAFAADAC and RAFCES2 values in individual HLH (Table 1). The total contributions of Hydrolase Activity in HLH
Contributions of AADAC and CES2 to Flutamide
AADAC and CES2 to flutamide hydrolysis were 79.6 to 170.4% in
by AADAC (Nakajima et al., 2011). Data for the activity were fitted
recent study found that rifampicin hydrolysis is specifically catalyzed
RAF values for AADAC and CES2 were calculated. The results of our
would be low in the body.
be denied. The contributions of other tissues to flutamide hydrolysis
thermore, the involvements of the representative esterases in plasma,
compared with those in human liver and jejunum microsomes. Fur-
study (Kobayashi et al., 2012) found that flutamide hydrolase activ-
yond the liver and intestine. However, the results of our previous
higher than that of jejunum.
were calculated to be approximately 108.0 and 17.4 ml/min, respecti-
In addition, enzymes involved in drug hydrolysis are present be-
ond the liver and intestine. However, the results of our previous
study (Kobayashi et al., 2012) found that flutamide hydrolase activ-
ities in human kidney and pulmonary microsomes were extremely low
compared with those in human liver and jejunum microsomes. Fur-
thermore, the involvements of the representative esterases in plasma,
butyrylcholinesterase and paraoxonase, in flutamide hydrolysis could
be denied. The contributions of other tissues to flutamide hydrolysis
would be low in the body.
To predict contributions of AADAC and CES2 quantitatively, the
RAF values for AADAC and CES2 were calculated. The results of our
recent study found that rifampicin hydrolysis is specifically catalyzed
by AADAC (Nakajima et al., 2011). Data for the activity were fitted
the substrate inhibition equation with the maximum activity at 200
μM (Nakajima et al., 2011). Thus, this study used 50 μM rifampicin
as the marker substrate for AADAC. As a marker of CES2 enzyme
activity, the CPT-11 hydrolyase activity at 2 μM was measured in a
similar way to that in our previous study (Fukami et al., 2010).
Using the rifampicin and CPT-11 hydrolyase activities by recombi-
nant AADAC and CES2 (41.77 and 2.99 pmol·min⁻¹·mg protein⁻¹,
respectively), the RAF_{AADAC} and RAF_{CES2} values in individual HLH
were calculated as shown in Table 1. By applying these RAF values,
the contributions of AADAC and CES2 at three flutamide concentra-
tions (5, 50, and 200 μM) in HLH were estimated according to the
equations under Contributions of AADAC and CES2 to Flutamide
Hydrolase Activity in HLH (Table 1). The total contributions of
AADAC and CES2 to flutamide hydrolysis were 79.6 to 170.4% in
individual HLH. A lot of data for total contributions exceed 100%.
The possible causes were as follows: 1) although CPT-11 hydrolysis
is considered to be mostly catalyzed by CES2 at a low concentration
of CPT-11, CES1 might also participate with the reaction with lower
catalytic efficiency compared with CES2, resulting in the overestima-
tion of RAF_{CES2} values. However, in our previous study (Watanabe et
al., 2010), CPT-11 hydrolase activity by CES1 at a concentration of 2
μM was undetectable. Furthermore, above our report (Watanabe et
al., 2010) also showed that the CPT-11 hydrolyase activity at 2 μM in
HLM was potently inhibited by loperamide with IC50 value of 0.46
μM. The possible causes were as follows: 1) although CPT-11 hydrolase
activity in liver homogenates might be decreased, resulting in the overestima-
tion of total contributions, although the relative contributions of AADAC and CES2 were not significantly affected.

![Image](1082.png)

**Fig. 1.** A, flutamide hydrolase activities by recombinant human CES1 and CES2. The SF21 cells expressing these enzymes were incubated with 20 μM flutamide. B, kinetic analysis of flutamide hydrolysis activity by recombinant human CES2 expressed in SF21 cells. Total cell homogenates of SF21 cells were incubated with 0.002 to 0.75 mM flutamide. Each data point represents the mean ± S.D. of triplicate determinations. C, inhibitory effect of loperamide on flutamide hydrolase activity. Flutamide hydro-
lase activities by recombinant human CES2, AADAC, HLM, HLC, and HJM were deter-
mined at the substrate concentration of 5 μM. The concentration of loperamide was 10 μM.
The control activities by CES2, AADAC, HLM, HLC, and HJM were 17.2 ± 2.3 pmol·
min⁻¹·mg protein⁻¹, 9.1 ± 0.2 pmol·min⁻¹·mg protein⁻¹, 8.6 ± 0.3 pmol·min⁻¹·mg
protein⁻¹, 2.1 ± 0.0 pmol·min⁻¹·mg protein⁻¹, and 64.4 ± 1.5 pmol·min⁻¹·mg
protein⁻¹, respectively. Each column represents the mean ± S.D. of triplicate determinations. *,
P < 0.05, **, P < 0.01, and †††, P < 0.001 compared with recombinant CES2. †††, P < 0.001 compared with recombinant AADAC.

- **Table 1.** Contributions of AADAC and CES2 to Flutamide Hydrolysis Activity in HLH

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>AADAC</th>
<th>CES2</th>
<th>RAFAADAC</th>
<th>RAFCES2</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>28.9</td>
<td>2.6</td>
<td>41.77</td>
<td>2.99</td>
</tr>
<tr>
<td>50</td>
<td>41.2</td>
<td>2.3</td>
<td>62.7</td>
<td>3.02</td>
</tr>
<tr>
<td>200</td>
<td>51.3</td>
<td>1.8</td>
<td>89.2</td>
<td>3.70</td>
</tr>
</tbody>
</table>

The mean absolute value of the mean relative contributions was approximately 108.0 and 17.4 ml/min, respectively. Thus, the contribution of liver to flutamide hydrolysis would be higher than that of jejunum.
HLH at 5, 50, and 200 μM flutamide were 11.7/88.3, 53.0/47.0, and 89.4/10.6%, respectively. Sample 1 showed the flutamide hydrolase activity at 5 μM with a similar value to those of the other samples, but it showed lower activity than the others at higher concentrations of flutamide (Fig. 4A). The results of a later study found that this sample has a polymorphic allele of AADAC gene, which causes the decreased enzyme activity (Shimizu et al., 2012).

The maximum value of the unbound concentration at the inlet to the liver ($C_{inlet,u,max}$) (Fukami et al., 2010) was estimated using the following equation: $\text{inlet,u,max} = f_a \times |C_{max}| + (\text{Dose} \times k_a \times$).

<table>
<thead>
<tr>
<th>Hydrolase Activity</th>
<th>RAF</th>
<th>Flutamide Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>CPT-11</td>
<td>AADAC</td>
</tr>
<tr>
<td>Concentration</td>
<td>pmol/min/mg protein</td>
<td>μM</td>
</tr>
<tr>
<td>5</td>
<td>3.1</td>
<td>4.5</td>
</tr>
<tr>
<td>50</td>
<td>87.6</td>
<td>10.6</td>
</tr>
<tr>
<td>200</td>
<td>389.0</td>
<td>200</td>
</tr>
</tbody>
</table>

### Liver homogenate

<table>
<thead>
<tr>
<th>pmol/min/mg protein</th>
<th>μM</th>
<th>pmol/min/mg protein</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>1.23</td>
<td>0.024</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>1.23</td>
<td>0.024</td>
</tr>
<tr>
<td>50</td>
<td>10.6</td>
<td>19.7</td>
<td>150.7</td>
</tr>
<tr>
<td>200</td>
<td>10.5</td>
<td>89.0</td>
<td>53.1</td>
</tr>
</tbody>
</table>

### FIG. 2

A, flutamide hydrolase activities in individual HLH. HLH (0.4 mg/ml) were incubated with 5, 50, and 200 μM flutamide. Gray and black columns represent the activities by AADAC and CES2, respectively, based on the data of percentage contributions. The extrapolation of percentage contributions of AADAC and CES2 were determined by the RAF using HLH and recombinant human AADAC and CES2 from the homogenates of Sf21 cells. To determine the RAF values, 50 μM rifampicin and 2 μM CPT-11 were used as the marker substrates of AADAC and CES2, respectively.

B, relative contributions of AADAC (gray) and CES2 (black) to flutamide hydrolysis in individual HLH. Based on the contributions shown in Table 1, total contributions were normalized to 100%. Each column represents the mean of duplicate determinations.
$F_a/Q_u$, where $F_a$ is the unbound fraction in the plasma, $C_{max}$ is the maximum concentration in the blood, $K_i$ is the first-order rate constant for gastrointestinal absorption, $F_a$ is the fraction absorbed from the gastrointestinal tract into the portal vein, and $Q_h$ is the hepatic blood flow rate (1450 ml/min). Because there are no $F_a$ data for flutamide, a value of 1.0 was used for $F_a$ to avoid underestimating the $Q_u$, max of flutamide. The $Q_u$, max value of flutamide was estimated to be 56 nM after oral dosing at 125 mg, the value of which is much lower than the concentration of flutamide used in this study (5–200 μM). Therefore, CES2 is considered a major enzyme in flutamide hydrolysis in liver in clinical therapeutics, although AADAC also shows flutamide hydrolyase activity. The activity by recombinant CES2 was fitted to the substrate inhibition equation with the highest activity at 20 μM and almost no activity at 750 μM. However, because the $Q_u$, max of flutamide was estimated to be 56 nM after oral dosing at 125 mg, this substrate inhibition pattern of CES2 would not have an effect on the hydrolysis potency of flutamide in vivo.

The small intestine is also involved in the first-pass metabolism after oral dosing. Although the contribution of the liver to flutamide hydrolysis was estimated to be higher than that of the intestine, it is conceivable that the small intestine is also important tissue for its hydrolysis. Data of the flutamide hydrolyase activity in HLM seemed to be fitted to the Michaelis-Menten equation, whereas those in H9262 were clearly fitted to the combined Michaelis-Menten equation/substrate inhibition equation (Kobayashi et al., 2012). Thus, CES2 would contribute to flutamide hydrolysis in human jejunum at a higher rate than in human liver.

Previous reports showed a 15- and 3-fold variation in the CES2 expression in the cytosolic and microsomal fractions of 13 liver samples (Xu et al., 2002). Zhu et al. (2000) reported that dexamethasone and rifampicin could induce human CES2 mRNA expression, probably by the activation of pregnane X receptor. In addition, several functionally deficient CES2 alleles were found in the Japanese population (Kubo et al., 2005). Thus, there may be inter- or intraindividual variations in CES2 expression and function that affect the flutamide pharmacokinetics. The flutamide-hydrolyzed metabolite, FLU-1, is further metabolized to N-hydroxy FLU-1 by human CYP3A4, and N-hydroxy FLU-1 is thought to be associated with hepatotoxicity (Ohbuchi et al., 2009). Further study is needed to clarify the association of CES2 and AADAC with flutamide-induced hepatotoxicity.

In conclusion, we found that human CES2, as well as AADAC, is involved in the flutamide hydrolysis, and CES2 is the principal enzyme in flutamide hydrolysis at a therapeutic dose. Because flutamide hydrolysis is associated with induced hepatotoxicity, this study provides useful information about the safe use of flutamide in a clinical setting.

Acknowledgments

We acknowledge Brent Bell for reviewing the manuscript.

Authorship Contributions

**Participated in research design:** Kobayashi, Fukami, Nakajima, and Yokoi.

**Conducted experiments:** Kobayashi and Shimizu.

**Contributed new reagents or analytic tools:** Kobayashi.

**Performed data analysis:** Kobayashi and Fukami.

**Wrote or contributed to the writing of the manuscript:** Kobayashi, Fukami, and Yokoi.

**Drug Metabolism and Toxicology,**

Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Japan

**Yuki Kobayashi**

Tatsuki Fukami

Mai Shimizu

Miki Nakajima

Tsuyoshi Yokoi

**References**


Kuo CL and La Du BN (1998) Calcium binding by human and rabbit serum paraoxonases. *Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakuma-machi, Kanazawa, Japan* Yuki Kobayashi

Yatsuyoshi Yokoi

Address correspondence to: Dr. Tsuyoshi Yokoi, Drug Metabolism and Toxicology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kakumamachi, Kanazawa 920-1192, Japan. E-mail: tyokoi@kenrou.kanazawa-u.ac.jp

Downloaded from dmd.aspetjournals.org at ASPET Journals on June 27, 2017

1084 KOBAYASHI ET AL.