Isolation and characterization of arylacetamide deacetylase in cynomolgus macaques

Yasuhiro UNO1)*, Masakiyo HOSOKAWA2) and Teruko IMAI3)

1) Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., Wakayama 642–0017, Japan
2) Laboratory of Drug Metabolism and Biopharmaceutics, Faculty of Pharmaceutical Sciences, Chiba Institute of Science, Choshi, Chiba 288–0025, Japan
3) Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862–0973, Japan

(Received 25 September 2014/Accepted 24 January 2015/Published online in J-STAGE 7 February 2015)

NOTE Pharmacology

ABSTRACT. Arylacetamide deacetylase (AADAC), a microsomal serine esterase, hydrolyzes drugs, such as flutamide, phenacetin and rifampicin. Because AADAC has not been fully investigated at molecular levels in cynomolgus macaques, the non-human primate species widely used in drug metabolism studies, cynomolgus AADAC cDNA was isolated and characterized. The deduced amino acid sequence, highly homologous (92%) to human AADAC, was more closely clustered with human AADAC than the dog, rat or mouse ortholog in a phylogenetic tree. AADAC was flanked by AADACL2 and SUCNR1 in the cynomolgus and human genomes. Moreover, relatively abundant expression of AADAC mRNA was found in liver and jejunum, the drug-metabolizing organs, in cynomolgus macaques, similar to humans. The results suggest molecular similarities of AADAC between cynomolgus macaques and humans.

KEY WORDS: arylacetamide deacetylase, cynomolgus monkey, drug metabolism, genome, tissue expression

doi: 10.1292/jvms.14-0496; J. Vet. Med. Sci. 77(6): 721–724, 2015

Arylacetamide deacetylase (AADAC) comprises the serine esterase superfamily, along with carboxylesterase (CES) [4], and is responsible for the hydrolysis of various xenobiotics, including clinically important drugs, such as flutamide, phenacetin and rifampicin, in humans [2]. AADAC is involved in the occurrence of renal failure and hematotoxicity in some patients who take flutamide or phenacetin and thus is an important drug-metabolizing enzyme [2]. AADAC partly shares the substrate selectivity with CES enzymes probably due to their structural and functional similarities; for example, flutamide is hydrolyzed by AADAC and CES2 in human liver [6]. Human AADAC, rat Aadac and mouse Aadac mRNAs are expressed in liver and small intestine, but human AADAC mRNA is not expressed in kidney whereas rat and mouse Aadac mRNAs are [5, 8, 11]. Moreover, human AADAC hydrolyzes flutamide, phenacetin and rifampicin, whereas rat and mouse Aadac enzymes hydrolyze flutamide and phenacetin, but not rifampicin [2]. Therefore, species differences are evident for AADAC-dependent drug metabolism between rodents and humans.

Cynomolgus macaques are frequently used in drug metabolism and toxicity studies due to their evolutionary closeness to humans. Our and other groups have identified and characterized a number of drug-metabolizing enzymes, including cytochromes P450, flavin-containing monoxygenases and glutathione S-transferases, and found generally similar molecular characteristics of these enzymes between cynomolgus macaques and humans [14, 16, 17, 20]. Despite the importance of cynomolgus macaques in drug metabolism studies, AADAC has not been fully investigated partly due to the lack of molecular information on the cynomolgus AADAC. In this study, therefore, cynomolgus AADAC cDNA was characterized by analysis of genomic organization, gene structure, the primary sequence structure, phylogeny and tissue expression pattern.

Cynomolgus AADAC cDNA was originally isolated as an expressed sequence tag, which was generated from cynomolgus liver [21]. Cynomolgus AADAC cDNA sequence identified in this study was deposited to GenBank under accession number KJ922601. Sequence analyses were carried out using DNASIS Pro (Hitachi Software, Tokyo, Japan) and Gene tyx system (Software Development, Tokyo, Japan). Multiple alignment of amino acid sequences was carried out using the ClustalW program, and a phylogenetic tree was created by the neighbor-joining method. A homology search was performed using BLAST (National Center for Biotechnology Information, Bethesda, MD, U.S.A.). The human and cynomolgus macaque genome data were analyzed using BLAT (UCSC Genome Bioinformatics) and Sequence Viewer (National Center for Biotechnology Information), respectively. Amino acid sequences used were from GenBank, including human AADAC (NP_001077), human CES1c (NP_001257), dog Aadac (XP_534309), rat Aadac (NP_065413) and mouse Aadac (NP_075872). Human AADAC cDNA sequence (NM_001086) used was also from GenBank. In addition, cynomolgus AADAC cDNA sequence from this study and the deduced amino acid sequence were also used for the analyses.

Sequence analysis revealed that cynomolgus AADAC had 399 amino acid residues containing the primary sequence structures characteristic of AADAC, such as the His-Gly-Gly box and the active site motif (Gly-X-Ser-X-Gly)
similar to human AADAC, rat Aadac and mouse Aadac (Fig. 1). Together with 343Asp and 373His, Ser189 of the active site motif forms the catalytic triad essential for the catalytic process, and these three residues are also conserved in cynomolgus AADAC, suggesting that cynomolgus AADAC is most likely functional. Cynomolgus AADAC had high sequence identities of amino acid (92%) and cDNA (96%) to human AADAC, substantially higher than dog AADAC, rat Aadac or mouse Aadac (Table 1). A phylogenetic tree of AADAC amino acid sequences showed that cynomolgus AADAC was most closely clustered with human AADAC as compared with dog AADAC, rat Aadac or mouse Aadac (Fig. 2). These results suggest the evolutionary closeness of AADAC between cynomolgus macaques and humans.

The analysis of the genome data found a single AADAC gene, flanked by AADACL2 and SUCNR1, in the cynomolgus and human genomes (data not shown). Therefore, cynomolgus AADAC was located in the genomic regions corresponding to the human ortholog. Cynomolgus AADAC (~16.2 kb) contained 5 coding exons, and the size of each of these AADAC exons was the same between cynomolgus macaques and humans; 138, 223, 70, 172 and 597 bp for exons 1–5, respectively (Fig. 3). All the cynomolgus and human AADAC introns begin with the dinucleotide GU and end with AG, consistent with the consensus sequences for splice junctions in eukaryotic genes. These results indicated similar genome and gene structures of AADAC in cynomolgus macaques and humans.

Single AADAC gene in the cynomolgus and human genomes is in contrast to the multiple CES1 and CES2 genes which have been identified in the genomes of various species [3]. The cytochrome P450 family of drug-metabolizing enzymes has evolved by gene duplication and gene loss during evolution [10]. Similarly, the multiple CES1 and CES2 genes might have evolved by gene duplication to increase their abilities to detoxify diverse xenobiotics taken. In contrast, a single gene of AADAC in various species might reflect the roles of this enzyme to metabolize mainly endogenous substrates.

To determine tissue expression patterns of cynomolgus AADAC mRNA, total RNA was extracted from brain, lung, heart, liver, kidney, adrenal gland, jejunum, testis, ovary and uterus tissues, which were collected from six cynomolgus macaques (three males and three females from Indochina, 4–5 years of age, 3–5 kg) as described previously [13]. Pooled samples of these six animals were used to measure mRNA expression. The study was reviewed and approved by the Institutional Animal Care and Use Committee (Shin Nippon Biomedical Laboratories, Ltd., Tokyo, Japan). Quantitative polymerase chain reaction (qPCR) was performed as described previously [13] with the following modifications. PCR was carried out in a total volume of 25 µl using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, U.S.A.) with the ABI Prism 7500 sequence detection system (Applied Biosystems) according to the manufacturer’s protocol. PCR reactions contained a twenty-fifth volume of each of the reverse transcription product with amplification efficiency of 97% for AADAC mRNA. The primers were used at a final concentration of 600 nM, including mf&hCES5 (5qrt1) 5′-TGCAGGAGGGAATTTAGCTG-3′ and mf&hCES5 (3qrt1) 5′-AGAGGCTGAAGGGCAGGATA-3′. The relative expression level was determined by BLAST.
normalization of the raw data to the 18S ribosomal RNA level based on three independent amplifications, as described previously [13].

The analysis showed that among these tissue types, cynomolgus AADAC mRNA was most abundantly expressed in liver, followed by jejunum (Fig. 4), important organs for drug metabolism, similar to human AADAC mRNA [5]. Similarly, human AADAC protein is also expressed in liver and small intestine [8, 23]. In contrast, rodent Aadac mRNA is expressed in liver, jejunum and kidney, but human AADAC mRNA is not expressed in kidney [5]. The results indicated expression of cynomolgus AADAC mRNAs in liver and jejunum, important organs for drug metabolism, and the tissue expression pattern of cynomolgus AADAC mRNA is more similar to that of human AADAC mRNA compared with that of rodent Aadac mRNA.

AADAC is homologous to CES enzymes to some extent and contained the primary sequence structures important for the enzyme function, including active site triad residues and the His-Gly-Gly-Gly box [11] (Fig. 1). AADAC metabolizes the substrates that contain a large alcohol group and small acyl group, such as flutamide, similar to CES2 [6], indicating that AADAC partly has the function similar to CES enzymes. However, unlike CES enzymes, AADAC is retained on the lumen side of the endoplasmic reticulum, because the signal anchor sequence in the N-terminus is not cleaved [1].

Human AADAC enzyme hydrolyzes flutamide, phenacetin and rifampicin, whereas rat and mouse Aadac enzymes hydrolyze flutamide and phenacetin, but not rifampicin [5]. If substrate specificity of cynomolgus AADAC is similar to that of human AADAC, together with the similar tissue expression pattern of AADAC mRNA as described earlier, cynomolgus macaques might serve as a better animal species in an AADAC-dependent drug metabolism investigation. It is of great importance to investigate hydrolysis properties of cynomolgus AADAC to assess its functional similarity to human AADAC.

In human AADAC, several genetic variants have been identified, including AADAC*3, the enzyme of which shows substantially lower intrinsic clearance of flutamide, phenacetin and rifampicin [9]. Genetic polymorphisms have not been investigated in cynomolgus AADAC. However, cynomolgus macaques possess a heterogeneous genetic background, similar to humans, and thus are expected to have genetic variants. Indeed, numerous genetic variants have been identified in cynomolgus macaques, including the genes encoding drug-metabolizing enzymes, such as cytochromes P450 [12, 15, 18, 19, 22]. The genetic variants would alter enzyme activity of AADAC, possibly resulting in the inter-individual variations of an AADAC-dependent drug metabolism. Metabolic polymorphisms, most likely mediated by AADAC, have been reported for rhesus macaques [7], closely related to cynomolgus macaques. The high- and low-affinity enzymes were purified from extensive metabolizer while only the low-affinity enzyme was from poor metabolizer, and the high-affinity enzyme appeared to account for metabolic polymorphisms. It is of great interest to investigate genetic variants in cynomolgus AADAC.

In conclusion, cynomolgus AADAC, highly homologous to human AADAC, was characterized in this study. Sequence analysis showed the sequence similarity of cynomolgus AADAC to the human ortholog more than that
of the dog, rat or mouse ortholog. Cynomolagus AADAC had similar gene structure and genomic organization to human AADAC. Moreover, cynomolagus AADAC mRNA was relatively abundantly expressed in liver and jejunum, similar to human AADAC mRNA, likely reflecting the roles as drug-metabolizing enzymes. The results suggest overall molecular similarities of AADAC in cynomolgus macaques and humans and the potential roles of cynomolagus AADAC for drug metabolism and toxicity.

ACKNOWLEDGMENTS. We sincerely thank Mr. Masahiro Utoh for his support of this work, Dr. Shotaro Uehara for his technical assistance and Mr. Lance Bell for his advice on English writing.

REFERENCES

1. Frick, C., Atanasov, A. G., Arnold, P., Ozols, J. and Odermatt, A. 2004. Appropriate function of 11β-hydroxysteroid dehydrogenase type 1 in the endoplasmic reticulum lumen is dependent on its N-terminal region sharing similar topological determinants with 50-kDa esterase. *J. Biol. Chem.* 279: 31131–31138. [Medline] [CrossRef]

2. Fukami, T. and Yokoi, T. 2012. The emerging role of human esterases. *Drug Metab. Pharmacokinet.* 27: 466–477. [Medline] [CrossRef]

3. Holmes, R. S., Wright, M. W., Laulederkind, S. J., Cox, L. A., Hosokawa, M., Imai, T., Ishibashi, S., Lehner, R., Miyazaki, M., Perkins, E. J., Potter, P. M., Redinbo, M. R., Robert, J., Satoh, T., Yamashita, T., Yan, B., Yokoi, T., Zechner, R. and Maltais, L. J. 2010. Recommended nomenclature for five mammalian carboxylesterase genes: human, mouse, and rat genes and proteins. *Mamm. Genome* 21: 427–441. [Medline] [CrossRef]

4. Hosokawa, M. 2008. Structure and catalytic properties of carboxylesterase isozymes involved in metabolic activation of prodrugs. *Molecules* 13: 412–431. [Medline] [CrossRef]

5. Kobayashi, Y., Fukami, T., Nakajima, A., Watanabe, A., Nakajima, M. and Yokoi, T. 2012. Species differences in tissue distribution and enzyme activities of arylacetamide deacetylase in human, rat, and mouse. *Drug Metab. Dispos.* 40: 671–679. [Medline] [CrossRef]

6. Kobayashi, Y., Fukami, T., Shimizu, M., Nakajima, M. and Yokoi, T. 2012. Contributions of arylacetamide deacetylase and carboxylesterase 2 to flutamide hydrolysis in human liver. *Drug Metab. Dispos.* 40: 1080–1084. [Medline] [CrossRef]

7. Kusano, K., Seko, T., Tanaka, S., Shikata, Y., Ando, T., Ida, S., Hosokawa, M., Satoh, T., Yuzuruha, T. and Horie, T. 1996. Purification and characterization of rheus monkey liver amido hydrolases and their roles in the metabolic polymorphism for E6123, a platelet-activating factor receptor antagonist. *Drug Metab. Dispos.* 24: 1186–1191. [Medline]

8. Probst, M. R., Beer, M., Beer, D., Jenö, P., Meyer, U. A. and Gasser, R. 1994. Human liver arylacetamide deacetylase. Molecular cloning of a novel esterase involved in the metabolic activation of arylamine carcinogens with high sequence similarity to hormone-sensitive lipase. *J. Biol. Chem.* 269: 21650–21656. [Medline]

9. Shimizu, M., Fukami, T., Kobayashi, Y., Takamiya, M., Aoki, Y., Nakajima, M. and Yokoi, T. 2012. A novel polymorphic allele of human arylacetamide deacetylase leads to decreased enzyme activity. *Drug Metab. Dispos.* 40: 1183–1190. [Medline] [CrossRef]

10. Thomas, J. H. 2007. Rapid birth-death evolution specific to xenobiotic cytochrome P450 genes in vertebrates. *PLoS Genet.* 3: e67. [Medline] [CrossRef]

11. Trickert, J. L., Patel, D. D., Knight, B. L., Saggerson, E. D., Gibbons, G. F. and Pease, R. J. 2001. Characterization of the rodent genes for arylacetamide deacetylase, a putative microsomal lipase, and evidence for transcriptional regulation. *J. Biol. Chem.* 276: 39522–39532. [Medline] [CrossRef]

12. Uehara, S., Murayama, N., Yamazaki, H. and Uno, Y. 2012. CYP2C76 non-synonymous variants in cynomolagus and rhesus macaques. *Drug Metab. Pharmacokinet.* 27: 344–348. [Medline]

13. Uno, Y., Fujiwara, H., Kito, G., Kamataki, T. and Nagata, R. 2006. CYP2C76, a novel cytochrome P450 in cynomolagus monkey, is a major CYP2C in liver, metabolizing tolbutamide and testosterenone. *Mol. Pharmacol.* 70: 477–486. [Medline] [CrossRef]

14. Uno, Y., Iwasaki, K., Yamazaki, H. and Nelson, D. R. 2011. Macaque cytochrome P450: nomenclature, transcript, gene, genomic structure, and function. *Drug Metab. Rev.* 43: 346–361. [Medline] [CrossRef]

15. Uno, Y., Murayama, N., Kunori, M. and Yamazaki, H. 2013. Characterization of microsomal glutathione S-transferases MGST1, MGST2, and MGST3 in cynomolagus macaque. *Drug Metab. Dispos.* 41: 1621–1625. [Medline] [CrossRef]

16. Uno, Y., Murayama, N., Kunori, M. and Yamazaki, H. 2013. Systematic identification and characterization of glutathione S-transferases in cynomolagus macaque. *Biochem. Pharmacol.* 86: 679–690. [Medline] [CrossRef]

17. Uno, Y. and Osada, N. 2011. CpG site degeneration triggered by the loss of functional constraint created a highly polymorphic macaque drug-metabolizing gene, *CYP1A2*. *BMC Evol. Biol.* 11: 283. [Medline] [CrossRef]

18. Uno, Y., Sakuraba, H., Uehara, S., Kamataki, T. and Nagata, R. 2009. A null allele impairs function of *CYP2C76* gene in cynomolagus monkeys: a possible genetic tool for generation of a better animal model in drug metabolism. *Drug Metab. Dispos.* 37: 14–17. [Medline] [CrossRef]

19. Uno, Y., Shimizu, M. and Yamazaki, H. 2013. Molecular and functional characterization of flavin-containing monooxygenases CYP1A2, CYP1A1, CYP2A, CYP2B, and CYP2C in cynomolgus macaques. *PLoS Genet.* 9: e1003267. [Medline] [CrossRef]

20. Uno, Y., Suzuki, Y., Watanabe, A., Sakamoto, Y., Sano, H., Osaka, N., Hashimoto, K., Sugano, S. and Inoue, I. 2008. Expressed sequence tags from cynomolgus monkey (*Macaca fascicularis*): a systematic identification of drug-metabolizing enzymes. *FEBS Lett.* 582: 351–358. [Medline] [CrossRef]

21. Uno, Y., Uehara, S. and Yamazaki, H. 2011. Discovery of genetic variants in *CYP1D1*: implication for functional integrity of *CYP1D1* in cynomolagus macaques and rhesus macaques. *Drug Metab. Pharmacokinet.* 26: 627–631. [Medline] [CrossRef]

22. Watanabe, A., Fukami, T., Nakajima, M., Takamiya, M., Aoki, Y. and Yokoi, T. 2009. Human arylacetamide deacetylase is a principal enzyme in flutamide hydrolysis. *Drug Metab. Dispos.* 37: 1513–1520. [Medline] [CrossRef]