Characterization of dog CYP2D15 : cDNA cloning, heterologous expression using baculovirus and drug-metabolism studies

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The P4502D (CYP2D) subfamily plays an important role in the metabolism of over 30 clinically used drugs. Since dogs are commonly used as experimental animals in pharmacokinetic and metabolic studies for the development of new drugs, it is important to understand the properties of the CYP2D subfamily in dogs. In this study, to characterize dog CYP2D15, the author carried out the cloning of cDNA belonging to dog CYP2D subfamily, heterologous expressionof CYP2D protein using baculovirus and the study of drug metabolizing enzyme activities of this P450.

A male dog liver cDNA library was acreened with human CYP2D6 cDNA as a probe. The full length clone (CFL-114) contained 1631 nucleotides and an open reading frame encoding 500 amino acid residues. The cloned cDNA was identical to the CYP2D15 that has already been reported. Dog CYP2D15 was moderately similar in deduced amino acid sequence to the bovine, human and monkey CYP2D subfamilies (74.8–76.4%), While showing lower similarities to the rat, mouse and guince pig CYP2D subfamilies (60.6–70.2%).

Dog CYP2D15 was expressed in Sf9 cells with a recombinant baculovirus. Infection of SF9 insect cells with a recombinant dog CYP2D15-virus resulted in the successive expression of a protein that crocc-reacted with a polyclonal antibody against a dog CYP2D15-specific peptide. The difference spectrum of CO-complex of reduced P450 of the infected cell microsomes had a maximal absorbance at 449 nm. The specific content of P450 was calculated to be 0.56 nmol/mg of Sf9 cell microsomal protein. The expressed dog CYP2D15 showed high catalytic activity for the hydroxylations of bunitrolol and imipramine at low substrate concentration(10 μM). Quinine inhibition studies suggest that hydroxylations of bunitrolol and imipramine are mainly catalyzed by CYP2D15 in dog liver microsomes. The expressed CYP2D15 also showed high catalytic activity for imipramine N-demethylation. Since quinine inhibited only moderately the activity of imipramine Ndemethylation by both the expressed CYP2D15 and dog liver microsomes, the relations between C'YP2D15 and imipramine N-demethylation in dog liver microsomes was unclear. Western blot analysis suggested that the dog CYP2D15 contents were less tha 4% of the total liver P450 content, assuming that 100% of expressed CYP2D15 incorporated heme.

The author has studied the regio-and stereoselectivity of ring-hydroxylation and N-desisopropylation of S(−)-and R(+)-propranolol, using dog liver microsomes and the expresssed dog CYP2D15 in insect calls. In dog liver microsomes, 4-hydroxylation was the most preferred pathway in S(−)-propranolol oxidation, While N-desisopropylation was the preferred pathway in R(+)-propranolol oxidation. S(−)-propranolol was the preferred substrate over R(+)-propranolol for 4-and 5-hydroxylations, while R(+)-propranolol was the preferred substrate for N-desisopropylation at the higher substrate concentrations. The expressed CYP2D15 had high catalytic activities toward 4-, 5-

hydroxylation, as well as N-desisopropylation of both enantiomers. At the substrate concentrations used, 4-hdroxylation was the most preferred pathway for the metabolism of both enantiomers, and S(−)-propranolol was the preferred substrate over R(+)-propranolol for all three monooxygenations catalyzed by CYP2D15, Anti-CYP2D15 peptide antibody strongly inhibited 4-and 5-hydroxylation of both enantiomers in dog liver microsomes, While it did not inhibit N-desisopropylation of both enantiomers in dog liver microsomes. These suggest that CYP2D15 is highly responsible for the stereoselective 4-and 5-hydroxylations of propranolol in dog liver microsomes.

Debrisoquine is well known as a substrate for the CYP2D subfamily, and debrisoquine 4-hydroxylase activity is often used as a marker for CYP2D activity in polymorphism studies. The present study has been the first to show that the dog liver microsomes are unablle to catalyze sebrisoquine 4-hydroxylation at the low substrate concentration. The author demonstrated that the lack of debrisoquine 4-hydroxylation is not due to the lack of the CYP2D subfamily in dog liver microsomes; instead, dog CYP2D15 has little catalytic ability for debrisoquine 4-hydroxylation at the low concentration of the substrate.

The author studied testosterone metabolism catalyzed by CYP2D subfamilies. Human CYP2D6 highly catalyzed testosterone 6b-hydroxylation and 17-oxidation. Dog CYP2D15 showed high testosterone 17-oxidation. Rat CYP2D2, which highly catalyzed bunitrolol 4-hydroxylation, did not show testosterone metabolism. In contrast, rat CYP2D1 showed testosterone 7a-hydroxylation, while it did not catalze bunitrolol 4-hydroxylation.

In summary, the author expressed dog CYP2D15 at high levels using a baculovirus expression system, and charcterized dog CYP2D15 in detail. This study indicates that the expressed CYP2D15 had high catalytic activities and a unique substrate specificity from other CYP2D subfamilies. The author suggested that the expressed CYP2D15 was proven to be useful in studies on the role of CYP2D15 in dog liver drug metabolism. The author also demonstrated that there is a various regioselectivity in testosterone metabolism catalyzed by CYP2D subfamilies.

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