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Research report

Presence of splice variant forms of cytochrome P4502D1 in rat brain but not in liver

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Abstract

Cytochromes P450 (P450), a family of heme-containing proteins, is involved in the oxidative metabolism of both foreign and endogenous compounds. Although liver is quantitatively the major organ involved in the metabolism of most xenobiotics, there is increasing evidence that these enzymes are present in extrahepatic tissues, such as lung, kidney, brain, etc and they may contribute to the in situ metabolism of xenobiotics in these organs. The possible relationship between genetic polymorphism seen in P4502D6 and incidence of neurodegenerative diseases, such as Parkinson's disease, has prompted the characterization of P4502D enzymes in rat brain. In the present study, we demonstrate that P4502D1 (the rat homologue of human P4502D6) is constitutively expressed in rat brain and the mRNA and protein are localized predominantly in neuronal cell population in the olfactory bulb, cortex, cerebellum, and hippocampus. An alternate spliced transcript of CYP2D1 having exon 3 deletion was detected in rat brain but not in liver. Deletion of exon 3 causes frame shift and generates a stop codon at 391 bp relative to the start codon ATG leading to premature termination of translation. Thus, Northern blotting and in situ hybridization represent contributions from functional transcripts and alternate spliced variants that do not translate into functional protein. Further, the splice variant having partial inclusion of intron 6 detected in human brain was not detected in rat brain indicating that alternate spliced gene products of P450 enzymes are generated in species-specific and tissue-specific manner.

Theme: Disorders of nervous system
Topic: Neuropsychiatric disorders

Keywords: Brain; Drug metabolism; Cytochrome P450; CYP2D; Monooxygenase

1. Introduction

Cytochrome P450 (E.C. 1.14.14.1; P450) and associated monooxygenases, a family of heme proteins, are the principal class of drug metabolizing enzymes. They are encoded by a supergene family and the member proteins exist in multiple forms having distinct yet overlapping substrate specificities. Multiple forms of P450, which are selectively induced or inhibited by a variety of drugs, are known to exist in liver, the major organ involved in P450-mediated metabolism [8]. In recent years, there is increasing evidence that these enzymes are present in extrahepatic tissues such as lung, kidney, and brain and that they may contribute to the metabolism of drugs and activation of carcinogens and toxins in situ in the target tissue [3,13,23]. P450-mediated metabolism of psychoactive drugs directly in the brain can lead to local pharmacological modulation at the site of action and result in variable drug response. The inter-individual variability in hepatic metabolism of drugs

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caused by genetic polymorphism exhibited by some forms of P450, such as P4502D6, is reflected in the plasma levels of administered drugs. But plasma drug levels often show poor correlation with therapeutic effect [14] suggesting that metabolism within the brain could influence the therapeutic outcome regardless of hepatic clearance and plasma drug levels. A moderate difference in the pharmacokinetics of psychoactive drugs often leads to dramatic pharmacodynamic effects suggesting that metabolism in situ within the brain could play a significant role [5].

Over the past decade, studies from our laboratory and others have demonstrated the presence of a competent microsomal P450 system in the rodent [1,13,34] and human [23,29] brain and its ability to metabolize a variety of xenobiotics. The appearance of multiple forms of P450 in brain and their selective inducibility by a variety of drugs and xenobiotics has also been identified [1,2,33,36]. Significant differences are seen in the regulation and function brain P450 enzymes compared to liver [27,35,37]. For example, drugs, such as alprazolam, are metabolized variably in liver and brain wherein relatively larger amount of the active metabolite is generated in rat brain compared to liver [27]. These observations have indicated the possible existence of unique P450 isoforms in brain that are different from the well-characterized hepatic P450s.

CYP2D is one of the major forms of P450 present in both rat [17] and human brain [6]. In rats, five genes belonging to the CYP2D family (CYP2D1, CYP2D2, CYP2D3, CYP2D4, CYP2D5) have been described [9,15,25], whereas in humans, one gene (CYP2D6) and several pseudogenes (CYP2D7 and CYP2D8) are known [24]. In humans, 5–10% of Caucasians exhibit defects in CYP2D6 alleles with resultant decreased rates of metabolism of CYP2D6 substrates [10,11]. Sprague–Dawley rats have a variant 2D1 allele, 2D1v, whereas Dark Agouti rats have no detectable expression of 2D1 mRNA in the liver [21].

Although CYP2D6 mRNA is mainly expressed in human liver, it has also been detected in human brain [32];

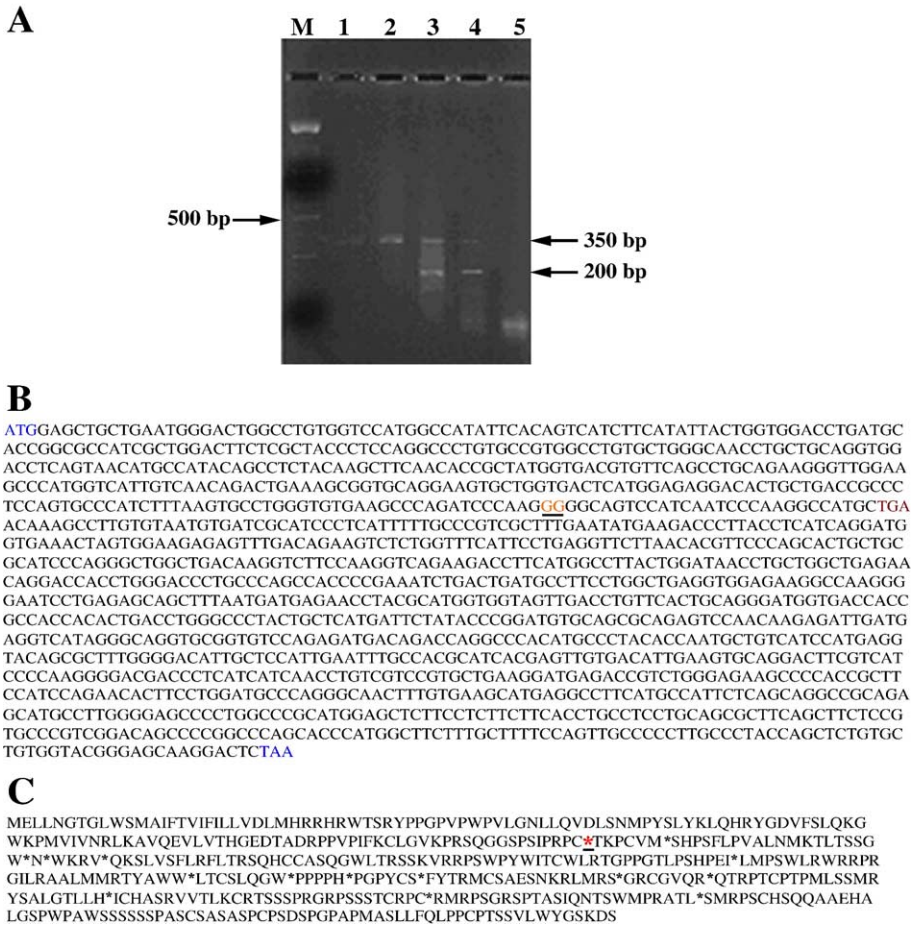

Fig. 1. Analysis of presence of exon 3 deletion in rat brain by RT-PCR. (A) RT-PCR analyses using specific primers for detecting the presence of exon 3 deleted splice variants in rat brain shows the presence of the splice variant form in rat brain. Lanes 1 and 2—RT-PCR product (approximately 350 bp) using rat liver cDNA. Lanes 3 and 4—PCR amplified product obtained using rat whole brain cDNA showing the presence of additional lower band (200 bp). Lane 5—negative control performed without DNA template. 'M' represents 0.5 Kb DNA ladder. (B) The mRNA sequence of CYP2D1 constructed from the sequence data of the 200 bp RT-PCR product as depicted above. (C) The translated protein sequence of the exon 3 deleted form of rat brain showing the premature termination of translation at amino acid 131 (represented by asterisk).