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Cytochrome P450 in Normal Human Brain and Brain Tumours

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The cytochromes P450 are a multi-gene family of constitutive and inducible haem-containing enzymes with a critical role in the oxidative metabolism of a wide range of environmental chemicals, including carcinogens, toxins and therapeutic drugs [1,2]. In addition, P450s may have a role in cell regulation, in view of their involvement in the metabolism of physiological chemicals active in cellular signalling. The major xenobiotic metabolizing families of P450 (CYP1, CYP2 and CYP3) have been well characterised in liver, which is the primary organ involved in the metabolism of xenobiotics. Individual forms of P450 have also been identified in a variety of extrahepatic tissues, including kidney, lung and small intestine [3], and the presence of P450s in particular extrahepatic tissues is important in influencing the tissue-specific toxicity of many xenobiotics.

A range of compounds that are metabolised by P450 are potentially neurotoxic [4]. Furthermore, P450 metabolism of endogenous compounds within brain may contribute to endogenous brain function. Several forms of P450 have already been identified in rodent brain and these studies have indicated that there is differential expression of individual P450s in particular regions of brain. However, there has been very limited study of individual P450s in human brain. In this study, we have investigated the expression of individual P450 mRNAs in normal human brain by reverse transcriptase polymerase chain reaction, and the cellular localisation of P450s have been investigated in normal brain and brain tumours by immunohistochemistry.

RNA was isolated from different regions of normal human brain, cDNA synthesised and the cDNA used in a reverse transcriptase polymerase chain reaction [5]. PCR products were analysed by agarose gel electrophoresis and direct sequencing. Immunohistochemistry with an alkaline phosphatase technique was performed using formalin fixed wax embedded sections of normal human brain and primary brain tumours [6].

CYP1A1 mRNA was detected in cerebellum, mid brain, basal ganglia, frontal cortex and temporal cortex, but was not detected in either the medulla or pons; meanwhile, CYP1A2 mRNA was detected only weakly in the basal ganglia and not in any of the other regions of brain. The presence of CYP1B1 mRNA was identified only

in the medulla. CYP2C8 mRNA was detected in cerebellum, midbrain, basal ganglia, and frontal and temporal cortices, but was not identified in medulla, pons or cerebellum. CYP2D6 was detected in only the mid brain, while CYP2E1 mRNA was present only in the medulla. CYP3A4 mRNA was identified in both basal ganglia and frontal cortex, while CYP3A5 mRNA was identified in mid brain, basal ganglia and - very weakly - frontal cortex.

In normal brain, immunoreactivity was observed for CYP1A, CYP2C9, CYP2E1 and CYP3A. Immunoreactivity was mainly present in neuronal cell bodies. All the tumours studied were astrocytomas and, by immunohistochemistry, CYP1A was present in 96% of tumours, CYP1B1 was present in 87% of tumours. CYP2A6 was found in 20% of tumours, CYP2C was found in 17% of tumours, CYP2E1 was found in 13% of tumours. Immunoreactivity for CYP3A was identified in 43% of tumours. In all cases, positive immunoreactivity for P450 was identified in tumour cells and generally in positively-staining cells the immunoreactivity was strong.

The P450s have a critical role in the activation and deactivation of many toxic foreign chemicals, and the relative expression of specific forms of P450 in a particular tissue is important in determining the toxicity of an individual chemical. It is therefore important to determine the expression of different forms of P450 in specific areas of the brain, to further understand the effect of toxic substances in brain. In rodent brain, several forms of P450 have been found to be constitutively present, with specific expression of different forms of P450 in particular areas of brain [7].

In this study, the presence of individual forms of P450 mRNA in different regions of normal human brain has been investigated by reverse transcriptase polymerase chain reaction. Normal human brain tissue can only be obtained at post-mortem and this has limited the direct study of P450 in human brain. All the cases included in this study were obtained from patients who had no evidence of neurological disease and whose brains appeared normal at post-mortem. Although the samples of brain tissue were obtained at post-mortem, amplification for β-actin indicated that the RNA that had been isolated from all the brain samples had not undergone any significant degradation and was suitable for investigating the presence of individual forms of P450. All the P450 forms in this study were identified in at least one area of brain and each P450 showed a distinct distribution in different regions of brain.

The presence of individual forms of P450 was also identified in brain tumours and the majority of brain tumours showed the presence of individual subfamilies of the CYP1 family. The presence of individual forms of P450 (particularly CYP1A and CYP3A) has previously been identified in several different types of malignant tumour, and the presence of members of the CYP1 family in brain tumours provides further evidence for the

expression of specific forms of P450 in tumours. The presence of P450 in brain tumours could be important therapeutically for the development of anti-cancer drugs, which are activated by those forms of P450.

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