Brain cytochrome P450 as a new target for the treatment of neurological and psychiatric diseases. Studies with human neuronal cells and transgenic animals

The cytochrome P450 monooxygenases (CYP) are recognized as the unique defense system comprising specific enzymes which help us to protect our wellbeing. Studies of the last years indicate that brain cytochrome P450 (CYP) is involved in the synthesis of dopamine and serotonin, neurotransmitters engaged in neurological and psychiatric diseases. *In vitro* investigations using cDNA-expressed CYP2D6 or liver microsomes revealed that CYP2D6 can catalyze formation of dopamine and serotonin *via* tyramine hydroxylation or 5-methoxytryptamine *O*-demethylation, respectively. The *in vitro* comparative studies showed that the efficiency of human CYP2D6 to synthesize dopamine/serotonin is much higher than that of rat CYP2D's, and the occurrence of these reactions in brain microsomes was shown in our laboratory. Later *in vivo* research demonstrated the CYP2D-mediated synthesis of dopamine from endogenous and exogenous tyramine in rat brain. Similarly, serotonin formation from exogenous 5-methoxytryptamine in rat brain was demonstrated *in vivo* using the brain microdialysis model, and the reaction was supported by peripheral administration of melatonin. Moreover, an increased serotonin level in the brain of CYP2D6-transgenic mice was shown, however, the number of human *CYP2D6* genes introduced into the mouse genome was not defined.

The ability of CYP2D to synthesize dopamine and serotonin in the brain has been documented, however, the relative contributions of those alternative pathways to the total neurotransmitter synthesis in the brain *in vivo* require further investigation. In particular, the contribution of CYP2D6 to the synthesis of neurotransmitters in human brain needs special attention, as the enzyme could serve as a new pharmacological target for enhancement of brain levels of serotonin and dopamine. And this would help to attenuate the symptoms of depression or neurodegenerative diseases (Parkinson's disorders). The human gene *CYP2D6* is highly polymorphic thus delivering enzyme of different catalytic activity, which may influence personality traits (anxiety, impulsivity). Our recent studies indicate that the activity of brain CYP2D is changing in rat brain during aging and serotonin deficiency, which may relate to neurodegenerative process and depression. These observations reflect functional role of CYP2D in the brain, which might be used as a therapeutic target.

The aim of the project is to evaluate significance of CYP2D6-mediated synthesis of dopamine and serotonin to the total neurotransmitter synthesis in human neuronal cells with dopaminergic or serotonergic profile *(in vitro)*, and in rat brain with overexpression of human *CYP2D6* (*in vivo*):

Task 1 –The genotyping of human cell lines *via* sequencing. Determination of the level of *CYP2D6* gene expression and protein function (mRNA, enzyme protein and activity) in WT (wild type) cell lines.

Task 2 – Development of human neuronal cell lines with *CYP2D6* gene knockout (*CYP2D6-KO*) and overexpression (*CYP2D6+OX*). Functional studies of the CYP2D6 enzyme activity toward dopamine and serotonin synthesis in *WT*, *CYP2D6-KO*, and *CYP2D6+OX* cell lines will be performed.

Task 3 – Development of *in vivo* rat model with overexpression of human gene *CYP2D6* in the brain. Functional studies of human CYP2D6 enzyme in rat brain will be performed.

Task 4 – A role of brain-derived and liver-derived 5-methoxytryptamine (a CYP2D6 substrate) in the synthesis of serotonin by brain CYP2D6 will be estimated in rats with gene knockout of brain tryptophan hydroxylase (*TPH2*) or peripheral tryptophan hydroxylase (*TPH1*). The studies will be performed in cooperation with Max-Delbrück-Center for Molecular Medicine (Berlin).

This research will contribute to evaluation of the significance of the human enzyme CYP2D6 as a novel target for therapy of neurological and psychiatric disorders. Provide evidence of the cerebral activity of CYP2D6-catalyzed alternative pathways of dopamine and serotonin synthesis in human neuronal cells. In addition, new experimental models for the identification of human cytochrome CYP2D6 activity in neuronal cells and in CYP2D6-humanized living brain will be developed.