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Recombinant expression of cytochrome P450-2D6 and its application in tamoxifen metabolism

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Introduction: Breast cancer is the leading cause of most fatal incidences in women worldwide. It is mostly induced by hormone oestrogen which stimulates the DNA to proliferate cells into cancerous cells. Tamoxifen is administered as a pro-drug for both treatment and prevention of breast cancer in women and men who are oestrogen positive. Tamoxifen metabolites function by binding and competing with oestrogen for binding to oestrogen receptors thus blocking the development of cancerous cells. Cytochrome p450-2D6 (*CYP2D6*) is one of the main enzymes in the tamoxifen metabolism. There is an inter-individual difference in response to tamoxifen treatment due to polymorphism in this enzyme. Therefore there is a need to diagnose whether patients taking tamoxifen are able to metabolize the drug. The currently used assay for patient's tamoxifen metabolism have limitations including non-specific, time consuming and cost effective. This study aimed to develop a *CYP2D6* based electrochemical biosensor which will test the metabolism of tamoxifen in breast cancer patients.

Methods: Physico-chemical parameters of *CYP2D6* were determined to pave way for *CYP2D6* gene amplification, cloning into pTrcHis TOPO vector, over-expression in *E.coli* followed by purification, in order to obtain an active recombinant *CYP2D6*. Tamoxifen metabolism by *CYP2D6* was assayed using UV-Visible and emission spectra and validated by electrochemical techniques using *CYP2D6* based-biosensor.

Results and Discussion: *CYP2D6* is a 50.05397 kDa insoluble trans-membrane protein that is slightly acidic in nature (pI = 6.21). The *CYP2D6* gene was successfully amplified as characterized by a 1.375bp band and subsequently cloned into pTrcHis TOPO vector. The protein was overexpressed in TOP10 *E. coli* cells, extracted and purified under denatured condition since it was expressed in the inclusion bodies. The protein was successfully refolded to its active form as determined by the P450 GLO *CYP2D6* assay. *CYP2D6* is characterized by a Soret band at 215 nm (UV-Vis) and 425 nm (emission spectra). Electrochemical assays indicated that the enzyme is active and has the ability to metabolize tamoxifen into its active forms at a potential of 0.6 V. Therefore, these results are adequate to be applied in the development of *CYP2D6* based sensor for tamoxifen metabolism during breast cancer therapy.

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