ALTERNATIVE TO ANIMAL TESTING FOR NEW DRUGS: STRUCTURAL, FUNCTIONAL AND ELECTROCHEMICAL STUDIES OF MONKEY CYP2C20 AND DOG CYP2D15

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Currently, numerous preclinical studies must be conducted on animals including primates, in order to obtain FDA approval of new drugs. In Europe alone, around 10.7 million animals are used per year, 50% of which are for research and 10% for toxicological experiments. In view of the large numbers of animals involved, the goal of this research is to reduce and ultimately replace animal testing with alternative *in vitro* methods.

In this work two key animal models, monkey and dog are taken as a test study for developing a new electrochemical platform where drug metabolising enzymes of these species, cytochromes P450 2C20 and 2D15, are immobilised. The genes encoding for 2C20 and 2D15 were successfully cloned in pCW vector and expressed in *E.coli* leading to 11 mg of pure protein per litre of culture. Both enzymes were characterised spectrophotometrically and their catalytic activity was assayed. In the case of 2C20, the turnover of paclitaxel (a mitotic inhibitor used in the treatment of ovarian and breast cancers metabolised by human 2C8) led to a Km of 81±28 μM and a Vmax of 73±11 nmol/min/nmol. For the purified 2D15 protein, binding assays with debrisoquine (an anti-hypertensive drug used as a marker substrate for human 2D6) resulted in a Kd value of 30±3 μM. Finally, the two enzymes were immobilised on glassy carbon electrodes with the cationic polymer poly(diallyldimethylammonium chloride) and mid-point potential of -0.310±10 mV and -0.370±15 mV (versus Ag/AgCl) were measured using cyclic voltammetry for 2C20 and 2D15, respectively.

The data from turnover, product characterisation and electrochemistry support the feasibility of the goal of this project, the creation of an alternative method to animal testing for toxicological studies.