UNIVERSITI TEKNOLOGI MARA

CLONING AND EXPRESSION OF CYTOCHROME P450 2C9: AN ENZYME FOR DRUG METABOLISM

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Introduction

CYP2C9 is one of the major enzymes present in the liver. Many of the drugs available in the market such as warfarin, phenytoin and NSAIDS go trough this enzymes for metabolism. Thus there is a need to develop an *in vitro* enzymes system that study substrates or chemical compounds which alter CYP2C9 enzyme activity.

Method

The pCW2C9 and pACYC-OxR was cloned inside competent cells of *Escherichia coli at 25°C, 30°C and 37°C*. The lengths of incubation time were 24 hours, 48 hours and 72 hours. The proteins expressed were confirmed and evaluated through immunoblotting.

Result

The CYP2C9 and OxR protein was successfully expressed by recombinant *E.coli* through biotechnology technique. The immunoblotting showed the correct protein of size were 35 kDa and 80 kDa.

Conclusion

The bacteria system in recombinant technology can be used to further investigate the type of traditional and modern drugs and food that are substrates or inhibitors of CYP2C9 and thus offer drug- herbs and drug- drug interaction study.

CHAPTER 1

INTRODUCTION

CYP2C9 is a family member of cytochrome P450 2C families (Nelson, 1996) and found abundant in liver. In fact, CYP2C9 is one of the most important enzymes in the liver where most of drugs undergo metabolism in liver through this enzyme. The CYP2C9 involves in phase I metabolism where it give one or more oxygen species to a substrate, thus make the substance more polar and easily excreted from the body. In clinical practice, when two or more drugs are administered at the same or overlapping times, there is always a concern for drug-drug interactions. Although interactions can be pharmacokinetic or pharmacodynamic in nature, in many cases, the interactions have a pharmacokinetic basis (Guengerich 1997). There are many underlying mechanisms responsible for pharmacokinetic interactions that can be understood in terms of alterations of CYP-catalyzed reactions.

The major reasons for drug-drug interactions involving CYP enzymes are induction, inhibition, and possibly stimulation, with inhibition appearing to be the most important in terms of known clinical problems (Guengerich 1997). The inhibition of CYP enzymes can result in the undesirable elevation of plasma drug concentrations, leading to toxicity or therapeutic failure. A good understanding of the underlying mechanisms involving in such drug-drug interactions can avoid toxicity or therapeutic failure by a corresponding reduction or increment of the therapeutic doses of a targeted drug, or close monitoring of