UNIVERSITI TEKNOLOGI MARA

NMR-BASED IDENTIFICATION OF CARBAMAZEPINE METABOLISM THROUGH CYP2D6 ENZYME

NORSURYAHANI BINTI RAHMAN

Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

Faculty of Pharmacy

November 2008

ACKNOWLEDGEMENTS

This project cannot be completed without help by many individuals. I am deeply indebted to those individuals who rose the occasion and provided extra time to help in the completion of this project. I am particularly thankful to Dr Syed Adnan Ali Shah for his kindness, supervision, guidance and especially for his comments and suggestions throughout the completion of this project. The efforts given by him in supervising me is much appreciated.

I would also like to express my gratitude to AP Dr Teh Lay Kek, Mr Lee Wee Leng and Mr Tommy Julianto on their helps and guides regarding this project. Their supervisory and editorial task drove me to be more dedicated throughout the completion of this project.

Not forgetting, all the staffs of Pharmacogenomics as well as Institute of Chemistry and Herbal Remedies (iKUS) laboratories that really being helpful and supportive during the experimental session. Without them, it is impossible for me to locate the position of chemicals and the right ways to use equipments.

Special thanks are also given to Khalidah Maruan and Raudhatun Saadia Ithnin that works concomitantly with me in this research. All information, discussions, reminders and others are really appreciated. In their absence, this project may not be completed.

The appreciation is also granted to my beloved family. I would like to thank them for their tolerance of my absences, physically and emotionally. I am blessed and strengthened by their unconditional support and love, and for their sharing of the belief that my efforts are worthwhile and useful.

Last but not least, to all individuals which involved either directly or indirectly in this project, your kindness will always be appreciated. Thank you very much

TABLE OF CONTENTS

ABSTRACT

This project is aimed to identify metabolites of carbamazepine by action of CYP2D6 enzyme. Carbamazepine has numbers of side effects. It is believed that metabolites of carbamazepine are responsible for the occurrence of its side effects although the mechanism underlying the reactions is not known. Thus, this research project will investigate carbamazepine metabolism by using biological catalyst. Different concentrations of carbamazepine were used with different concentrations of CYP2D6 enzyme in two differents pH of media. After incubation, the reaction mixtures were extracted using chloroform. The residues were subjected to analysis using chromatographic and spectroscopic technique. The transformed metabolites of carbamazepine were purified through thin layer chromatography (TLC) and structure metabolites were elucidated through nuclear magnetic resonance (NMR) spectroscopy. As a result of carbamazepine metabolism by CYP2D6 enzyme, the metabolites being produced are 10,11-dihydroxycarbamazepine and 10,11-dihydroxy-10-hydroxycarbamazepine. Even though the compounds are not main metabolites in metabolism of carbamazepine as stated in various literatures, we managed to identify new potential compounds that may have possible contribution in various toxicity problem of carbamazepine.

CHAPTER 1

INTRODUCTION

Carbamazepine (CBZ) (proprietary name Tegretol) (5H-dibenzo [b,f]azepine-5-carboxamide) is an anticonvulsant drug. It is used primarily in the treatment of epilepsy, bipolar disorder, Attention Deficit Hyperactive Disorder (ADHD), schizophrenia and trigeminal neuralgia. Carbamazepine may depress activity in the nucleus ventralis of the thalamus or decrease synaptic transmission or decrease summation of temporal stimulation leading to neural discharge by limiting influx of sodium ions across cell membrane and thus stimulates the release of anti diuretic hormone (ADH) and potentiates its action in promoting reabsorption of water, chemically related to tricyclic anti depressants (TCA).

CYP2D6 is one of the members of cytochrome P450 family where cytochrome P450 enzymes catalyze the oxidative metabolism of xenobiotics as well as endogenous substances. CYP2D6 itself metabolized a large number of drugs. CYP2D6 is being produced more in the presence of carbamazepine. It results in increased metabolism of carbamazepine itself.

Metabolites of carbamazepine by action of CYP2D6, which are chemically reactive, are believed to be responsible for the occurrence of its side effects although the mechanism underlying the reactions is not known. However only few researches were published related to involvement of CYP2D6 in metabolism of carbamazepine.