UNIVERSITI TEKNOLOGI MARA

DEVELOPMENT OF A GENOTYPING METHOD FOR TPMT*2 GENE POLYMORPHISM DETECTION IN RELATED TO AZATHIOPRINE MEDICATION

NUR SYAHIRAH NADHIRAH BINTI MOHD RADZUN

BACHELOR OF PHARMACY (Hons.)

2016

ACKNOWLEDGMENT

Alhamdulillah, all praise to Allah S.W.T for the wisdom he bestowed upon me and without His blessings and grace, this research would not have been possible.

Deepest gratitude and special thanks to my supervisor, Prof. Dr. Hamid Fauzi for his guidance, advices, valuable comments and provisions that benefited much in completing this research.

I also would like to thanks to Ms Miss Serene and Ms Norodiyah Othman and all other post-graduate students in Brain Laboratory at Faculty Pharmacy for their encouragement and willing to spend their time to help me when needed.

I want to express my appreciation to all volunteers who were willingly to give their blood sample for this research. This research would not complete without their help.

I also would like to thank to my family and friends who give endless support through out the research. May Allah bless us all.

Thank you.

TABLE OF CONTENTS

ABSTRACT

Most medications show wide interpatient variability either in efficacy or toxicity. These interindividual variation result in part from polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters and others. For some genetic polymorphisms, such as thiopurine S-methyltransferase (TPMT), has an effect on the pharmacokinetics of medications, such that individuals who inherit or has an enzyme deficiency must be treated with different doses of the Azathioprine. This type of individual can not be treated by using conventional doses as it can develop certain toxicity. The aim of this study is to develop genotyping method of polymerase chain reaction (PCR) in detection of nonsynonymous coding SNP rs11800462 *TPMT* 2* gene. Primer for first and second PCR method were designed for this research. In this study, PCR was optimized by adjustment of PCR parameter which is annealing temperature. However, due to limitation of time, other parameter such as concentration of primer, concentration of $MgCl_2$, and concentration of DNA can not be optimized. Optimization of PCR of *TPMT*2* gene polymorphism is important as it opens way for further studies to look into significant association of *TPMT*2* gene polymorphism in Malaysian population.

CHAPTER 1

INTRODUCTION

1.1 Overview

Azathioprine (AZA) or the commercial name, IMURAN is prescribed drugs for a immunosuppressant, drug that is use to lower down or suppress body's immune system and widely be used over 30 years after its introduction. It is commonly used to treat patients who have inflammatory bowel disease (IBD), severe rheumatoid arthritis, childhood acute lymphoblastic leukemia and used during organ transplant to prevent of acute rejection (M. Chrzanowska *et al.,* 2001).

AZA undergoes two main inactivation pathways which one is thiol methylation, which is catalyzed by the enzyme thiopurine S-methyltransferase (TPMT) and methyl-6-MP (6-MeMP) is an inactive metabolite from this pathway. Another pathway is oxidation, which is catalyzed by xanthine oxidase (XO) and the product is 6-thiouric acid. As stated, TPMT is important in the metabolism of all thiopurines and is one of the major enzymes that inactivates mercaptopurine. AZA is a prodrug, thus to exert its immunosuppressant action, it needs to be metabolised to active form which is in 6-thioguanine nucleotides (6-TGN). However, pharmacogenomic studies discovered that polymorphism in TPMT gene can cause the variable and there are some cases that lead to adverse drug reaction. As a result of catabolism of AZA to the active metabolite 6-TGNs, which is aided by