CHAPTER I

INTRODUCTION

Theophylline is commonly used in the treatment of bronchospasm with both asthma and chronic obstructive lung disease. It is presently used to prevent apnea and bradycardia in infants (Shannon et al., 1975 ; Ellis , Koysooko and Levy , 1976 ; Wyatt , Hendeles and Weinberger , 1978 ; Rowe et al., 1988). Because of its narrow therapeutic range and variation in biological half life. (Weinberger and Bronsky , 1974 ; Jacobs , Senior and Keszler , 1976) , theophylline is then become one of the drug recommended to be monitored in patients to achieve the best therapeutic effect with least adverse reactions (Sadee and Beelem , 1980). Its optimum therapeutic range is reported to be 10-20 mcg /ml (Jenne et al., 1972 ; Piafsky and Ogilvie , 1975 ; Hendels , Weinberger and Johnson , 1978). Serious side effects can be observed when plasma theophylline concentration is greater than 20 mcg /ml ; such as nausea , vomitting , abdominal pain , gastrointestinal bleeding , headache , insomnia , anxiety , vertigo and palpitations. Severe overdosage may also lead to maniacal behaviour , diuresis , dilirium , tachycardia , seizures , brain damage and death . (Shannon et al., 1975 ; McDonald and Landerson , 1978 ; Mathews , Schneiweiss and Cersosimo Zwillich ,

1986 ; Zwillich et al., 1987 ; Reynol , 1989 ; Wilson et al., 1991). In contrary , with plasma concentration less than 10 mcg /ml the bronchospasm condition in patients could not be relieved.

In order to monitor drug dosage in patients , drug plasma concentration related to its therapeutic response have to be determined before calculating the next doses. So the methods of drug analysis have to be convenient , rapid and easily to be conduct. That' s why various immunoassays become demanding. For theophylline , three different immunoassay methods have been reported , they are RIA (radioimmunoassay) , FPIA (fluorescence polarization immunoassay) and EMIT (enzyme-multiplied immunoassay technique) . These immunoassay methods were named according to the hapten - labeled used. Therefore , Tritium labeled -theophylline was used in RIA (Neese and Soyka , 1977). Fluorescence Umbelliferone and enzyme Glucose - 6 - phosphate dehydrogenase (G-6-PDH) labeled - theophylline were used in FPIA (Li , Benovic and Burd , 1981 ; Loomis and Frye , 1983) and EMIT , respectively (Gushow , Chang and Gotcher , 1982 ; Singh et al., 1977).

By comparison , RIA requires special set - up space for

radioactive materials and expensive equipments. The strict regulatory controls on the use of radioactive materials tend to exclude RIA from many small laboratories, hospital or clinical laboratories. FPIA method, known as Tdx have to be used with its specific reagent kits and instruments. The fixed - cost per analysis is rather high. For enzyme - immunoassay as EMIT (Gushow, Chang and Gotcher, 1982 ; Singh et al., 1977) which was commercially available before Tdx , used only simple spectrophotometer. However, reagent kits have to be specific and imported as well as that for Tdx . Therefore, from these point of views , there is a merit of interest to study the possibility of preparing necessary reagents for developing the enzyme - immunoassay method of theophylline in the future.

Actually, various enzymes are available for labelling hapten such as horseradish peroxidase (HRP), glucose oxidase , lysozyme, alkaline phosphatase, malate dehydrogenase, beta-galactosidase etc. In this study, HRP was selected to be the labeled enzyme because of its high reactivity, suitable for linkaging with haptens. Besides, it is not too expensive and is available in a highly purified form (Paul and Stigbrand, 1970; Erlanger, 1973 ; Tijssen, 1985)

Thus, the main objective of this study is to prepare

HRP labeled theophylline and to determine the possibility of using this labeled compound to develop the new enzyme - immunoassay of theophylline in the future.

The significance of the study

1. This study will provide the HRP labeled theophylline which would be available for enzyme - immunoassay of theophylline in the future.

2. The determined method of synthesizing theophylline derivatives for labelling HRP would be the valuable idea for labeling other drugs.

3. This study will also produce specific antibody for theophylline which would possibly be utilized in the other related researchs for theophylline.