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Pharmacokinetics of Propranolol in B-Thalassemia/Hb E

[1]J. Tankanitlert, [2]P. Yamanont, [3]T. Inthong, [3]P. Wilairat, [4]S. Fucharoen and [2]N.P. Morales
[1]Department of Pharmacology, Phramongkutklao College of Medicine, Bangkok, Thailand
[2]Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok, Thailand
[3]National Doping Control Centre, Mahidol University, Rama 6 Road, Bangkok, Thailand
[4]Thalassemia Research Center, Institute of Molecular Bioscience,
Mahidol University, Nakornpathom, Thailand

Abstract: Propranolol is widely used for the treatment of heart diseases in thalassemic patients. Hyperbilirubinemia and pathophysiological changes in the patients may alter pharmacokinetics of the drug. To define the pharmacokinetics of propranolol in thalassemic patients compared to with healthy volunteers, ten thalassemic patients and healthy subjects were enrolled in this study. All subjects received a single oral dose of 40 mg propranolol. Blood samples were collected after dosing. Propranolol and propranolol-lucuronide concentrations in plasma were determined using HPLC method. Pharmacokinetic parameters of the drug were calculated using one-compartment model. Compared with healthy subjects, thalassemic patients showed the statistically significant increase of maximum concentration, area under concentration time curve and longer half-life of propranolol. In addition, the area under concentration time curve of propranolo-glucuronide was significantly lower in the patients compared with those of healthy subjects. Blood pressure as well as heart rate were recorded during the study period. The significant reduction of systolic and diastolic blood pressure were found in the patients more than in healthy subjects. The results suggested that starting dose of propranolol should be low in thalassemic patient.

Key words: Propranolol, thalassemia, pharmacokinetics, volunteers, plasma, Thailand

INTRODUCTION

Beta-thalassemia is an inherited hemolytic anemia resulting from genetic abnormalities within globin genes. Coinheritance of β-thalassemia with Hemoglobin E (HbE) results in a compound heterozygous condition known as β-thalassemia/HbE disease (β-thal/HbE) commonly found in Southeast Asia including Thailand (Rees *et al*., 1998; Fucharoen *et al*., 2000).

Accelerated erythrocyte destruction in thalassemia often leads to hyperbilirubinemia which is associated with UDP-glucuronosyltransferase 1A polymorphisms (Borgna-Pignatti *et al*., 2003; Premawardhena *et al*., 2001). Hyperbilirub inemia may affect pharmacokinetics of drugs used in thalassemic patients. Iron overload caused from increased red cell destruction, gut iron absorption and chronic blood transfusion is also commonly found in thalassemia.

An accumulation of iron occurs in many organs including the liver which is a vital organ for drug metabolism. Other pathological changes in β-thal/Hb E which are include consequences of chronic anemia, requiring increased cardiac output and that eventually may produce tachycardia, hypoxia, hypertension and congestive heart failure which is a major cause of death in thalassemia in the 2nd decade of life (Aessopos *et al*., 2008; Wood, 2009). Propranolol is a non-selective beta-adrenergic receptor blocking drug widely used in the treatment of cardiovascular diseases as well as for a number of other indications such as hyperthyroidism, tremor and migraine. The drug was metabolized by oxidation, N-dealkylation and the glucuronidation pathway. The oxidation product; active metabolite (4-OH propranolol) undergoes subsequent glucuronidation into phenol compound (4-OH propranolol-glucuronide) (Ludden, 1991).

Even though the pharmacokinetics of propranolol has been studied quite extensively, the doses of the drugs given to thalassemic patients are the same as the doses used in non-thalassemic patients. The doses may be either too large or too small leading to ineffectiveness or causing toxic effects to the patients. Since, there are significant alterations of various factors may modify the pharmacokinetics of propranolol, it is imperative to

Corresponding Author: Jeeranut Tankanitlert, Department of Pharmacology, Phramongkutklao College of Medicine, 315 Rajavithi Road, Phrayathai, 10400 Bangkok, Thailand

analyze the disposition of this drug in the patients. The purpose of this study was to elucidate the pharmacokinetic characteristics of propranolol in thalassemic patients in comparison with healthy subjects.

MATERIALS AND METHODS

Reagents and standard: Propranolol (Inderal®, ZENECA Ltd., Macclesfield Cheshirse, UK) and pronetheral reference standards and The *Escherichia coli* beta-glucuronidase type IX-A were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography-grade methanol and acetonitrile were purchased from Labscan Analytical Sciences (Bangkok, Thailand). All other chemicals were commercially purchased and were analytical reagent grade.

Subjects: Thalassemic patients (5 women and 5 men) and ten healthy subjects (5 women and 5 men) were enrolled in this study. All subjects were between 25 and 50 years of age and between 30 and 70 kg of body weight. Exclusion criteria were the use of any prescription or nonprescription medication, except for folic acid a week before and throughout the study and pregnancy. The subjects underwent clinical examination, blood chemistry and pregnancy tests for female subjects prior to the study. The study protocol was approved by the Ethics Committee on Human Experimentation at Ramathibodi Hospital, Mahidol University (Bangkok, Thailand) and all subjects gave their written informed consent before participating.

Pharmacokinetic study: All subjects received a single oral dose of 40 mg propranolol after an overnight fast. A low protein meal was provided 2 h after the drug administration. Through the venous catheter, 3 mL of blood samples were collected before and at 15, 30, 60, 90, 150, 210, 330 and 450 min after dosing. Blood samples were centrifuged at 3000 rpm for 15 min and the separated plasma samples were stored at -20°C until analysis.

Blood sample analysis

Determination of Propranolol (P) and Propranolol-Glucuronide (PG): The frozen plasma samples were thawed at room temperature and centrifuged at 3000 g for 15 min. An aliquot of 500 μL of plasma was extracted by solid extraction cartridges (SepPak®; C18). Extracts were evaporated to dryness under nitrogen at 37°C and the residues were reconstituted in 200 μL of potassium dihydrogen phosphate/methanol at the volume ratio of 90/10. Then 5 μL of 20 ng mL^{-1} pronetherol as internal standard was added into the samples and then analyzed for nonconjugated forms of propranolol using HPLC consisting of a waters 600 pump, fluorescence detector waters 966 and autosampler injector waters 717 and water symmetry C18 column (3.9 nm×150 cm) (Water Corp., Milford, MA., USA). The excitation wavelength was set at 230 nm and the emission wavelength at 340 nm. The mobile phase consisted of 100 mM potassium dihydrogen phosphate, pH 3 and acetonitrile at the volume ratio of 70:30 buffer to acetonitrile. The flow rate was 1.0 mL min^{-1}. Peak areas were integrated with Millenium 3.2 software (Water Corp., Milford, M A., USA).

Determination of total propranolol was done after converting the glucuronide to parent propranolol by *E. coli* beta-glucuronidase type IX-A; 480 μL of plasma was mixed with 20 μL of β-glucuronidase (12,500 U mL^{-1} in 1.0 M HEPES buffer pH 7) and then incubated at 37°C for 16 h. After the reaction, plasma samples were prepared and injected into HPLC as described before.

The precision and accuracy were assessed at five different concentrations of propranolol (5, 20, 40, 80 and 100 ng mL^{-1} for plasma assay). The intraday and interday coefficients of variation of the plasma assays were 0.2-14.2 and 2.6-14.5% , respectively. The accuracy of the plasma determined by recovery analysis ranged from 81.2-98.7%. The lower limits of detection was 5 ng mL^{-1}.

Pharmacokinetic analysis: Pharmacokinetic parameters of the drug were calculated using one-compartment open model for oral administration. Peak plasma concentrations (C^{max}) and the time to reach peak concentrations (T^{max}) of propranolol were determined by observation of individual concentration-time data.

A terminal elimination rate constant (Ke) was calculated by linear least-squares regression of the log-transformed plasma concentration-time data. The terminal elimination half-life (T1/2) was calculated as 0.693/Ke.

The area under the curve for the time at which the final measurable concentration (C_{last}) was obtained ($AUC_{0\text{-}last}$) was calculated by the trapezoidal rule. The AUC from the final time point to time infinity ($AUC_{last\text{-}\infty}$) was calculated as the ratio of the C_{last} to ke. The AUC from time zero to infinity ($AUC_{0\text{-}\infty}$) was calculated by addition of $AUC_{0\text{-}last}$ and $AUC_{last\text{-}\infty}$. The apparent oral Clearance (CL/F) of propranolol was calculated as the ratio of administered dose to $AUC_{0\text{-}\infty}$. The apparent volume of distribution (Vd/F) was calculated by equation: Vd/F = Cl/Ke.

Statistical analysis: All pharmacokinetic and pharmacodynamic data were expressed as the mean value ±SEM. One-way Analysis of Variance (ANOVA) were used as appropriate to analyze the data using SPSS software (Version 17; SPSS Inc., Chicago, IL, USA). $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Ten thalassemic patients were recruited to this study. No significant differences were observed in age and body weight between the studied groups. Blood chemistry such as AST, ALT, total bilirubin and direct bilirubin in thalassemic patients were significantly different from healthy subjects (Table 1). Plasma profiles of propranolol are shown in Fig. 1. The concentration of propranolol reached its peak within 3 h of the ingestion and the time required to reach peak plasma concentration (T^{max}) was not different between groups.

Table 1: Clinical characteristics of thalassemic patients and healthy subjects

Characteristic	Healthy subjects	Thalassemic patients
Subjects (M/F)	5/5	5/5
Age (years)	38.6±10.1	40.6±4.300
Weight (kg)	48.6±12.3	41.6±6.100
Hb (12-18 g dL^{-1})	15.0±0.90	6.3±2.000*
Hct (30-54%)	40.1±2.60	20.2±5.000
ALP (50-136 U L^{-1})	65.6±3.40	115.3±32.60*
AST (15-37 U L^{-1})	15.0±4.50	58.3±23.00*
ALT (30-65 U L^{-1})	58.3±5.60	109.3±23.40*
Albumin (34-50 g L^{-1})	34.5±2.10	31.2±5.000
Serum ferritin (30-300 ng mL^{-1})	98.5±20.6	3517.9±423.9**
TB (0-17.1 µmo L^{-1})	12.5±0.50	64.3±1.200**
DB (0-5 µmol L^{-1})	2.4±0.40	7.6±0.200*
BUN (6-12 mg dL^{-1})	13.3±0.90	12.0±0.800
Serum creatinine (0.5-1.5 mg dL^{-1})	0.56±0.5	0.60±0.08

Data are given as mean±SEM; Hb = Haemoglobin, Hct = Haematocrit, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase; ALT = Alanine aminotransferase, TB = Total Bilirubin, DB = Direct Bilirubin, *p<0.05 versus healthy subjects,**p<0.001 versus healthy subjects

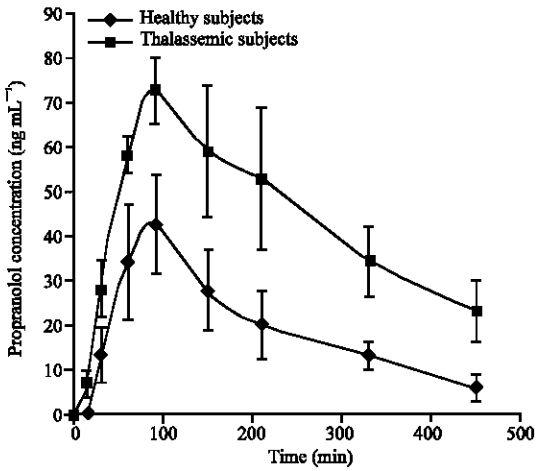

Fig. 1: Time profile of plasma propranolol level after oral 40 mg dose in thalassemic patients and healthy subjects. Each point represents mean±SEM

All pharmacokinetic parameters of propranolol are shown in Table 2. The area under concentration-time curve for the time at which the final measurable concentration ($AUC_{0\text{-last}}$) was >80 % of the AUC from time 0-∞ ($AUC_{0\text{-}\infty}$).

Compared to with healthy subjects, significantly higher C^{max} (41.7±7.9 vs. 76.3±12.1 ng mL^{-1}) and $AUC_{0\text{-}\infty}$ (135.2±15.5 vs. 337.67±46.71 ng/mL/h) was observed in thalassemic subjects. The biphasic decline in the drug concentration was observed.

Lower elimination rate constant of propranolol in thalassemic subjects (0.24±0.03 h^{-1}) was observed versus those of healthy subjects (0.32±0.03 h^{-1}) leading to significantly longer half-life of propranolol (218.0±15.0 min) (Table 2). An apparent volume of distribution in thalassemic patients was lower than in healthy subjects (2.7±0.5 and 6.8±1.6 mL kg^{-1}, respectively). Apparent clearance was not different between groups.

Plasma profiles of propranolol-glucuronide were also biphasic decline in both groups. The maximum plasma concentration of propranolol-glucuronide of each group was observed after the time required to reach peak plasma concentration of propranolol.

Significantly lower AUC of propranolol-glucuronide in thalassemic patients (1250.2±455.6 ng/mL/h) compared with those of healthy subjects (2317.4±348.8 ng/mL/h) was noted (Fig. 2). Blood pressure and heart rate were measured throughout the study period.

In corresponding to the plasma propranolol level, the significant reduction of systolic blood pressure was observed in thalassemic patients and healthy subjects while the significant reduction of diastolic blood pressure was found in the thalassemic patients more than in healthy subjects (Fig. 3).

The fall of heart rate was observed in thalassemic patients less than in healthy subjects (Fig. 4). The significant reduction of pulse pressure was observed

Table 2: Pharmacokinetic parameters of propranolol in thalassemic patients and healthy subjects

Parameters	Healthy subjects	Thalassemic patients
C^{max} (ng mL^{-1})	41.7±7.900	76.30±12.1*
T^{max} (min)	110.0±16.20	128.00±15.0
T1/2 (min)	139.2±28.00	210.30±20.1*
$AUC_{0\text{-}\infty}$ (min ng mL^{-1})	135.2±15.50	337.70±46.7*
Ke (1 h^{-1})	0.32±0.03	0.24±0.03
Vd/F (mL kg^{-1})	6.8±1.600	2.70±0.50*
CL/F (mL min^{-1})	39.4±10.30	40.00±5.70

Data are presented as mean±SEM, *p<0.05

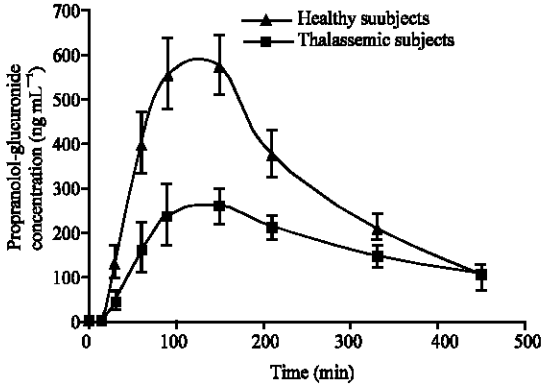

Fig. 2: Time profile of plasma propranolol-glucuronide level after oral 40 mg dose in halassemic patients and healthy subjects. Each point represents mean±SEM

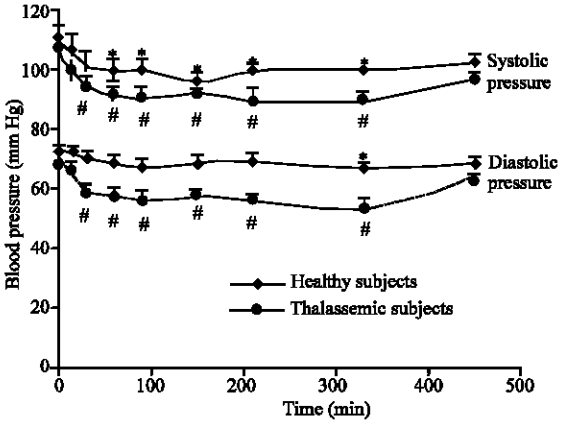

Fig. 3: Time profile of the change of systolic and diastolic blood pressure after oral 40 mg dose in thalassemic patients and healthy subjects. Each point represents mean±SEM

at 15, 60 and 450 min after drug administration in thalassemic subjects and 30, 60 and 150 min after drug administration in thalassemic subjects (Fig. 5).

The results of this study revealed statistically significant changes in many pharmacokinetic parameters of thalassemic patients compared with healthy subjects after single oral dose of propranolol. After reaching the circulation, the drug is removed at a rate which depends upon hepatic blood flow. Chronic anemia in thalassemia usually increases the cardiac output to cope with oxygen demand of vital organs when hemoglobin level is <7 g dL^{-1} (Aessopos *et al*., 2007; Walker, 2002; Lekawanvijit and Chattipakorn, 2009).

All the subjects had hemoglobin levels <7 g dL^{-1}, therefore hyperdynamic state presented in these patients increased tissue perfusion and may have increased the

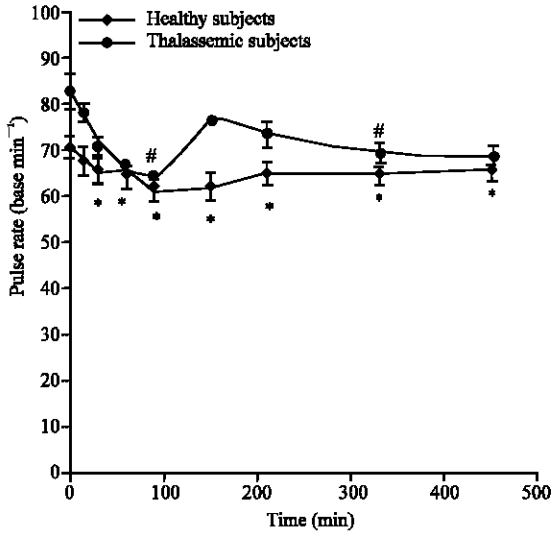

Fig. 4: Time profile of the change of heart rate after oral 40 mg dose in thalassemic patients and healthy subjects. Each point represents mean±SEM. #p<0.05 compared with the baseline values of each group for thalassemic patients. *p<0.05 versus baseline values of each group for normal subjects

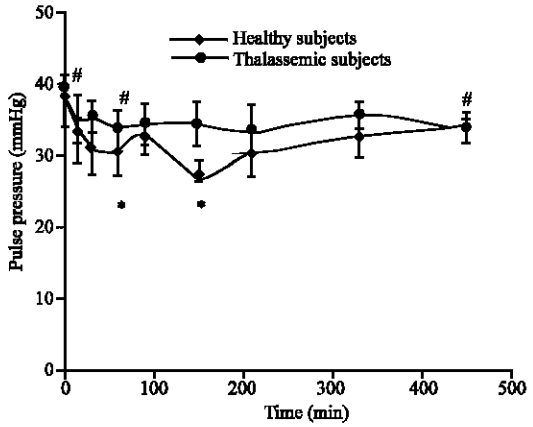

Fig. 5: Time profile of the change of pulse pressure after oral 40 mg dose in thalassemic patients and healthy subjects. Each point represents mean±SEM. #p<0.05 compared with the baseline values of each group for thalassemic patients. *p<0.05 versus baseline values of each group for normal subjects

extraction ratio of the flow-limited drugs like propranolol. Thus, the drug should be more removed. In contrast, the higher C^{max} and AUC of propranolol were observed in the result. This may have resulted from many factors such as hyperbilirubinemia or dysfunction of the liver in the patients. Hyperbilirubinemia was observed in all patients

recruited in this study which can displace the drug protein binding (Weisiger *et al*., 2001). In addition, the UGT1A1 polymorphism associating with hyperbilirubinemia was in strong Linkage Disequilibrium (LD) with UGT1A9 polymorphism (Saito *et al*., 2009). It is well known that propranolol is a substrate for UGT1A9 (Yu *et al*., 2010), so the decrease of propranolol-glucuronide in the study may have resulted from this polymorphisms. Because iron accumulation in the liver can causes fibrosis and cirrhosis in the patients (Jean *et al*., 1984; Galanello and Origa, 2010), liver dysfunction from iron overload could be considered.

An elevation of ALT and AST of the patients recruited in this study reflects damages to the hepatic cells. This is also confirmed by the decrease of propranolol-glucuronide leading to prolong half-life of the drug. So far, the results are in agreement with those of Watson *et al*. (1987) who could show a similar pharmacokinetic characteristics of propranolol in patients with cirrhosis.

Propranolol is a highly lipophilic and wildly distributed throughout the body. The distribution of the drug into body tissues will depend upon the relative amounts of the different types of tissues. A previous study showed that the lipophillic beta blocker diffuses less efficiently into adipose tissue than into lean tissue (Cheymol *et al*., 1997).

In addition, lipid peroxidation in the cell membrane may result in cell membrane dysfunction (Amer *et al*., 2004; Rachmilewitz *et al*., 1976). It is likely that the reduction of lean tissue mass and lipid peroxidation in the cell membrane in thalassemic patients can decrease the volume of distribution of propranolol. The hemodynamic effects of propranolol in the patients were investigated concomitantly with its pharmacokinetics in the present study.

Propranolol is a competitive antagonist at beta-adrenoceptors and its pharmacologic effects are therefore dose-related. In corresponding to the plasma propranolol level, the significant reduction of systolic blood pressure was observed in both groups but significant reduction of diastolic pressure was found in thalassemic subjects more than in healthy subjects.

The data were slightly different from the study of Wojcicki *et al*. (2003) which demonstrated no significant correlation between plasma propranolol levels and its blood pressure lowering effect in healthy volunteers and hyperlipidaemic patients. The decrease of heart rate was less observed in thalassemic subjects than healthy subjects which can be described from reflex after the significant reduction of diastolic blood pressure. In addition, the reduction in mean pulse pressure occurred earlier in tha lassemic subjects. It is thus suggested that there may be an alteration of the controlling of vascular response in the patients.

CONCLUSION

This study demonstrated that pathophysiological changes in thalassemic patients modified pharmacokinetic and pharmacodynamic parameters including higher C^{max}, AUC and proplonged half-life of propranolol and a significant reduction of systolic and diastolic blood pressure. Therefore, the patients should be carefully monitored for a reduction of blood pressure after the drug administration.

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