DISCUSSION

The rising cost of medication and hospitalization in Thailand has led to a wider usage of cheaper generic drug products. However, the quality, safety and efficacy of such products are of great concern for the protection of and to meet the demand of the consumers. Substitution of a generic drug product for an innovator requires that the product must not only be pharmaceutical equivalence, but also bioequivalence. Moreover, the Thai FDA now demands the bioequivalence studies between the generic products and the innovator preparations and the result should be submitted for drug approval and marketing.

The pharmacokinetic parameters which represent the rate and extent of drug absorption are subjected to be determined in order to assess the bioequivalence (1, 2). The basic is that if the two formulations exhibit similar drug concentration-time profiles in the blood, they should exhibit the similar therapeutic effects (1, 2). Bioequivalence studies rely on pharmacokinetic parameters measurement such as T_{max}, C_{max} and AUC. The AUC determines the extent of systemic drug absorption or drug bioavailability, whereas, T_{max} and C_{max} determine the rate of drug absorption (1, 2). The two formulations will be proved bioequivalence if there are no significant differences in these parameters (1, 2). To compare the pharmacokinetic parameters, the data will be analyzed by non compartmental analysis and a 90%CI for the quantity of $\mu_T - \mu_R$ will be constructed (1, 2). The 90% CI for the differences in the mean of the log-transformed data of AUC and C_{maz} will be calculated using ANOVA and the antilogs of the confidence limits obtained constitute the 90% CI for the ratio of the test and reference products (1, 2). To reach a conclusion of bioequivalence, the 90% CI should be contained within the interval of 0.8-1.25 (1, 2).

Donepezil HCl is a highly selective inhibitor of the enzyme acethycholine esterase (AChE) approved for the treatment of Alzheimer's disease (AD) (30, 31). The inhibition activity of donepezil is specific, noncompetitive and reversible for this

enzyme (32). The structure of donepezil consists of N-benzylpiperidine and an indanone moiety, which demonstrate a greater selectivity for AChE in the brain than for butyrylcholinesterase (pseudocholinesterase) an enzyme that is widely distributed in plasma and peripheral tissues (14, 33). Animal studies have shown that donepezil exhibits tissue selectivity; it significantly inhibits AChE in the brain but causes little inhibition of AChE in smooth, striated, or cardiac muscle (32, 34). Clinical studies in human report that donepezil is effective in the treatment of cognitive impairment and memory loss in patients with mild to moderate AD (14, 31, 35). However, the efficacy and safety of donepezil are dose-dependent which relates to its involves the increase in the concentration of Ach through inhibition of its hydrolysis by AChE (14, 15). An increase in Ach concentration may result in improvement of patients' symptoms or may cause cholinomimetic adverse effects. Common adverse effects of donepezil include diarrhea, nausea, vomiting, insomnia, headache, muscle cramp, fatigue and anorexia (10, 12, 14, 15). In order to reduce the risk of adverse effects, the dose of donepezil should be started with a 5-mg daily for at least 6 weeks prior to increasing the dose up to 10 mg (10, 12, 14, 15).

It is well recognized that Alzheimer's disease is now becoming one of a major health problem in Thailand. Since the cost of the innovator donepezil product is rather high, and the patent of the drug is expired, the bioequivalence testing of a generic donepezil manufactured in Thailand is urgently required. This study aimed to investigate the bioequivalence of the generic donepezil HCl and the reference Aricept® and was to determine the pharmacokinetic data of the drug in 20 healthy Thai volunteers. This study was conducted as a single-dose, two-way, crossover design. The drug was administered under fasting and due to a long half-life of donepezil the washout period between each treatment was 3 weeks.

The measurement of plasma donepezil was performed by using HPLC with ultraviolet (UV) detection (36, 37). This method required only a simple apparatus, therefore, a number of studies reported the determination of donepezil in blood sample and tablets by this method (36, 37). The chromatogram is detected using a UV detector set at 315 nm (36, 37). The method of plasma preparation in the literature was a liquid-liquid extraction, whereas in this study, the solid-phase

extraction (SPE) method was developed and found to be more convenient and less time consume.

In this study, the pharmacokinetic parameters of donepezil between the test and the reference preparations were compared. After dose administrations, the plasma concentrations of donepezil from both preparations increased rapidly and attained the peak levels at 2 h post dose (range 1.0-3.0 h). There was no significant difference of the T_{max} between the two preparations and the mean [90%CI] for the T_{max} difference was 0.05 hr [(-0.19)-0.29 hr], within the bioequivalence range of ± 0.41 hr (20% of T_{max} of the reference). The T_{max} from our study was faster than those values of 3-5 h previously reported (13, 16 ,17-20), however, were comparable to the data from Aricept® product monograph (2007) which gave T_{max} values of 2 h (range 1-8 h), in the comparative bioavailability studies of 5-mg Aricept® VS 5 mg Aricept RDT®. Donepezil is well absorbed with a relative oral bioavailability of 100% (10, 15). The mean values (± SD) of the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for the test VS the reference were 20.42 ± 4.5 ng/mL VS 18.93 ± 3.82 ng/mL, 1051.44 ng.h/mL ± 206.87 VS 983.58 ± 199.40 ng.h/mL and 1375.01 ± 369.01 ng.h/mL VS 1277.47 ± 328.51 ng.h/mL, respectively. The values of C_{max} and AUC from our study were two to three times higher than those values reported in the literature [C_{max} 7.7-10.1 ng/mL (13, 18)] and product monograph (C_{max} of 8.58 ± 24.36 ng/ml and $AUC_{0-\infty}$ of 433.7 ± 31.52 ng.h/ml).

In previous clinical trials, significant correlations have been demonstrated between the plasma concentration of donepezil and the percentage of AChE inhibition (37, 38). The 50% inhibition of AChE activity is obtained at a plasma drug concentration of 15.6 ng/mL, and the inhibition plateaus at plasma concentration of higher than 50 ng/mL (37, 38). Therefore, plasma drug concentration could be a useful tool to predict clinical outcome of donepezil in the treatment of AD (37, 38). From our study, a single dose of 5 mg donepezil produce a higher peak and a higher AUC of drug concentration, thus, this dose should be a suitable dose for Thai patients.

The average half-life ($t_{1/2}$) of donepezil between the test and the reference were also similar (91.5 h, range 59.1-164 h VS 90.7 h, range 62.5-148 h), however the $t_{1/2}$ obtained from our study were longer than the values previously reported (50-70 h) (16, 39). Correspondingly, the volume of distribution (Vd/F) and the clearance (CL/F) between the two preparations were not significantly different (7.88 $\pm$ 1.28 L/Kg VS 8.48 $\pm$ 1.41 L/Kg and 1.03 $\pm$ 0.22 ml/min/kg VS 1.12 $\pm$ 0.29 ml/min/kg, respectively).

The large Vd of donepezil (average 7-8 L/Kg or 490-527 L) may be due to its highly lipid-soluble that binds avidly to the tissue. This characteristic limits the use of hemodialysis or hemoperfusion in case of poisoning. The CL/F of donepezil from this study is very slow (1.1 ml/min/kg) compared with the value previously reported (2.2 ml/min/kg) and was about 20 times less than the rate of hepatic blood flow (19-20 ml/min/kg). The large Vd and the slow clearance rate resulted in a characteristic long elimination half-life of the drug.

Donepezil undergoes first pass metabolism and is metabolized in the liver by CYP 450 isoenzymes 2D6 and 3A4 thereafter glucuronidation (10, 19, 20). The rate of metabolism is slow and does not appear to be saturated (19, 20). Study in patient with stable alcoholic cirrhosis, the clearance of a single dose of donepezil was decreased by 20% relative to that in healthy age and sex-matched subjects (29). With the exception of peak plasma concentrations, there were no statistically significant differences in the pharmacokinetics of donepezil between the groups, and dosage modification in patients with impaired hepatic function was not required. Study in patients with moderate to severe renal function impairment also found no clinically significant changes in the clearance and the pharmacokinetics of donepezil and dosing modification in these patients was not required (13).

Elimination pathway of donepezil had been studied following administration of ^{14}C-labeled donepezil (23). The plasma radioactivity expressed as a percent of the administered dose, was present primarily as intact donepezil (53 %), 6-O-desmethyl donepezil (11%), which has been reported to inhibit AChE to the same extent as donepezil *in vitro* and was found in the plasma at concentrations about 20 % of parent

compound (23). Approximately 57% and 15% of the total radioactivity was recovered in urine and feces, respectively, over a period of 10 days, while 28% remained unrecovered and about 17% of the dose recovered in the urine as unchanged drug (23).

The pattern of concentration-time profiles of donepezil is complex. As illustrated by the graph, the decline in drug concentration has two phases. The initial phase last over a period of 24 h, during which the concentration of a drug declines relatively rapidly. This is followed by a more prolonged phase of gradual declined in the drug concentration until 7 days post dose. The initial phase is called α phase of redistribution. The rapid declined in drug concentration in this phase is caused mainly by the prompt redistribution of a drug from the small central compartment of well-perfused tissues (e.g. brain and heart) into a much larger peripheral compartment of more poorly-perfused tissues (e.g.fat, muscle) rather than by actual clearance of the drug from the body. The second phase is called the β phase of elimination, in which the declined in drug concentration is caused predominantly by the body clearance. Therefore, the concentration-time profile of donepezil is best described mathematically by two-compartment model.

The clinical important is that the patients may experience adverse effects such as dizziness, headache and insomnia due to a rapid initial distribution of a drug to the brain. Previous study reported that the plasma level of unchanged donepezil declined rapidly and the brain level of radioactivity declined almost in parallel with the plasma level of unchanged donepezil (23). The ratio of donepezil to total radioactivity in brain was 86.9 to 93.0%. No heterogeneous localization of radioactivity was recognized in the brain and the concentration in each part of the brain was 1.74 to 2.24 times the plasma concentration (23). The steady state volume of distribution of donepezil is 12 L/kg (14, 15). Donepezil is approximately 96 % bound to human plasma proteins (14, 15). The distribution of donepezil in various body tissues has not been definitively studied.

However, in a mass balance study conducted in healthy male volunteers, 240 hours after the administration of a single 5 mg dose of ^{14}C-labeled donepezil

hydrochloride, approximately 28% of the label remained un-recovered (23). This suggests that donepezil and/or its metabolites may persist in the body for more than 10 days (23). Although the $t_{1/2}$ of denepezil is as long as 2-3 days, the drug should be divided and given once-a-day dosing at bed time to lessen the adverse effects (15). If insomnia is a problem, this medication may be taken during the day with or without food (15).

Bioequivalence analysis showed no significant differences in the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ between the two preparations based on the 90% CI for the ratios $\frac{\text{Test}}{\text{Reference}}$ of 0.99-1.17, 1.02-1.13 and 1.02-1.14, respectively. These values were within the bioequivalence range of 0.8-1.25. From the ANOVA, the inter-subject variability in the AUC and C_{max} were observed (p=0.000 and 0.037, respectively). These findings were expected since some volunteers exhibited either extremely high AUC (volunteers No 4 and 19) and C_{max} (volunteers No 1, and 5) whereas some volunteers exhibioted extremely low AUC (volunteers No 4 and 14) and C_{max} (volunteers No 17 and 18). However this study was conducted as a cross-over design and the pharmacokinetic parameters were measured in the same subject, intra-subject variability would be minimized since each subject would serve as his own control. The % CV estimated from S^2 obtained from the ANOVA after logarithmic transformed of the $AUC_{o-t,}$ $AUC_{o-\infty}$ and C_{max} were 9%, 10% and 15%, respectively. According to the nomograms of Diletti, the power of tests for AUC and C_{max} obtained from this study were > 90% and 80%, respectively.