FORMULATION OF ORALLY DISINTEGRATING TABLET OF DONEPEZIL AND ITS COMPARATIVE BIOAVAILABILITY IN HEALTHY VOLUNTEERS

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AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration between all authors. This study was designed and coordinated by author YH as the principle investigator. Authors YH, TS and LYP wrote the protocol and performed the blood sampling. Authors WL and LYY measured the drug plasma concentration. Authors RMM and ESH developed the formulation and performed the in-vitro dissolution. Author BP gathered the initial data and performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

ABSTRACT

Donepezil is clinically used for treatment of the symptoms of Alzheimer’s disease. It has a very bitter taste and taste masking techniques should be used in orally disintegrating tablets to enhance patient compliance. Many techniques are available to mask the bitter taste of drugs. The objective of the study was to formulate donepezil using ion exchange resin in orally disintegrating tablet and to compare its bioavailability with Aricept® Evess ODT as the reference product as well as to evaluate the in vitro release studies. The in vitro dissolution studies were carried out in three different media. Twenty male volunteers were included in this single-dose, open-label, randomized, two-way crossover study with an overnight fasting and three-week wash out period. Blood samples were drawn up to 72 h following drug administration. Plasma concentration of donepezil was determined by liquid chromatography-tandem mass spectrometry method. Pharmacokinetic data was available for 14 volunteers. No significant differences in pharmacokinetic parameters were detected between both formulation (p-value >0.05). The 90% confidence intervals (CI) for AUC0-72h and Cmax were 93.65%-104.16% and 91.39%-107.32%, respectively. The result of this study indicates that the different technique of taste masking used in the test formulation exhibits pharmacokinetic profiles comparable to reference product in healthy volunteers.

Keywords: Bioavailability; donepezil; orally disintegrating tablet; pharmacokinetics; taste masking.

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1. INTRODUCTION

The oral route of administration is considered as the most widely accepted route. But the most evident drawback of the commonly used oral dosage forms like tablets and capsules is difficulty in swallowing, leading to the patient incompliance particularly in case of pediatric and geriatric patients, but it also applies to people who are ill in bed and to those active working patients who are busy or travelling, especially those who have no access to water [1,2].

Over the past three decades, orally disintegrating tablets (ODTs) have gained considerable attention as a preferred alternative to conventional tablets and capsules due to better patient compliance. Orally disintegrating tablets are appreciated by a significant segment of populations particularly who have difficulty in swallowing. It has been reported that dysphagia (difficulty in swallowing) is common among all age groups and more specific pediatric, geriatric population along with institutionalized patients, psychiatric patients and patients with nausea, vomiting, and motion sickness complications. ODTs with good taste and flavor increase the acceptability of bitter drugs by various groups of population. Various techniques available for masking bitter taste of drugs include taste masking with ingredients such as sweeteners and amino acids; and taste masking by polymer coating; ion-exchange resin complexation; spray-drying technique; formation of inclusion complexes with cyclodextrins; microencapsulation technique; making multiple emulsions; and taste masking with gelatin, gelatinized starch, liposomes, lecithins or lecithin-like substances, surfactans, salts or polymeric membranes [3,4].

An orally disintegrating tablet (ODT) formulation of donepezil has recently been developed. Donepezil HCl (also known as E2020 or Aricept® the registered trademark of Eisai Co. Ltd, Tokyo, Japan), a chemically distinct and highly selective inhibitor of the enzyme acetylcholinesterase (AChE) has recently been approved for marketing in the USA, Canada and several European countries, including the UK, for treatment of the symptoms of mild-moderate Alzheimer’s disease. Donepezil is a basic medicine that having unpleasant taste. The structure of donepezil is shown in Fig. 1. Because ODTs dissolve or disintegrate in the patient’s mouth, the drug will be partially dissolved in close proximity to the taste buds. A pleasant taste inside the mouth becomes critical for patient acceptance. Aricept® Evess ODT used a method for reducing bitter taste which comprises the step of adding a gelling agent selected from a κ-carrageenan [5,6]. The present study investigates the use of complex with ion exchange resin for reducing bitter taste in orally disintegrating tablet and evaluates its bioavailability compared to the originator. Resin is being used for taste masking of tramadol, quinine sulfate, diphenhydramine, levamisole, ranitidine and ciprofloxacin [4].

Fig. 1. Chemical structure of donepezil

2. MATERIALS AND METHODS

2.1 Donepezil Formulations

The test formulation of donepezil ODT was manufactured by PT. Novell Pharmaceutical Laboratories and was prepared using polacrillin potassium as ion exchange resin, mannitol as directly compressible diluent and crosspovidone as super disintegrant and suitable flavor and sweetener. The resinate formation was carried out using polacrilin potassium with optimal parameters like drug resin ratio, pH of complexation medium, and time of complexation on drug loading efficiency. The originator product (Aricept® Evess ODT) was used as reference formulation.

2.2 In vitro Drug Release Testing

Dissolution tests were performed using a Hanson type SR8-Plus dissolution tester (Hanson Research Co., USA) and samples were assayed by a HPLC method developed in house. Experiments were performed using USP apparatus 2 (paddle) rotating at 50 rpm in 900 mL dissolution medium of pH 1.2, 4.5, and 6.8. The medium was maintained at 37±0.5°C. In all experiments, 5 mL dissolution samples were withdrawn at 5, 10, 15, 20, 30, and 45 minutes. The cumulative percentages of the drug released were calculated and were analyzed through a model independent approach using a similarity factor (f₂) to compare the dissolution profiles.

2.3 Subjects and Study Design

A single-dose, open-label, randomized, two-way crossover study with an overnight fasting and three-week wash out period was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human subjects and Good Clinical Practice (GCP). The study protocol was reviewed by the Committee of The Medical Research Ethics of the Faculty of Medicine,
University of Indonesia (Jakarta, Indonesia) and was approved by the National Agency of Drug and Food Control (Jakarta, Indonesia). All participants signed a written informed consent after they had been informed of the nature and details of the study.

Twenty volunteers were selected among Indonesia residents and participated in this study. Volunteers were selected after passing a clinical screening procedure which included physical examination, ECG and clinical laboratory tests: hemoglobin, hematocrit, WBC, platelets, WBC differential, blood urea nitrogen, sGPT, sGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, total cholesterol, creatinine, urine analysis, pregnancy test (for female subjects) and negative results of HBsAg, anti HBC and anti HIV. Volunteers were excluded if they had history of hepatic, gastrointestinal or renal disease, an allergy to any medicines containing donepezil and piperidine derivates, asthma or obstructive lung disease, consumed alcohol or other medications for a long period of time, donate or lost >450 mL of blood within 3 months prior to the screening of the study. All volunteers were required not to use any drugs for at least two weeks prior to the study until completion of the study. They also refrained from ingesting alcohol, caffeine, chocolate, tea or coke-containing beverages at least 48 h before each dosing and until last blood sampling.

Volunteers were randomized to one of the two sequences to receive the formulations according to randomization scheme. Volunteers were confined to clinical unit of Clinisindo Laboratories one night before study to assure the fasting condition (10 h before drug administration). On the study day, each volunteers received one tablet of either product on the dose. No food was allowed until 4 h after dose administration. Standard meals were served at 4 h and 11 h, snack was served at 8 h after drug administration.

Volunteers were remained at the clinical unit of Clinisindo Laboratories for 24 h after drug administration and were not allowed to take strenuous exercise during the sampling days. Blood pressure, heart rate, body temperature and adverse events were monitored during blood sampling.

2.4 Bioanalytical Method

Donepezil plasma concentration was determined using LC-MS/MS (API 3200) method with TurboIonSpray mode and loratadine was used as an internal standard (IS).

Briefly, plasma samples (500 µL) were added with an internal standard and 200 µL of sodium hydroxide 1N. After mixing, 3 mL of ethyl acetate was added. The mixture was vortex-mixed for 1 minute and centrifuged at 3000 rpm for 10 minutes. The organic phase was transferred into evaporated tube and evaporated to dryness under vacuum for 20 minutes. The residue was reconstituted with 250 µL of acetonitrile:water (1:1). A 10 µL aliquot was injected into the LC-MS/MS system for analysis.

The analytical separation was performed on a Synergi 4µ POLAR-RP-80A, 50 x 2.0 mm, 4 µm (Phenomenex®, Torrance, CA, USA) preceded by a guard column AQ C18, 4 x 2.0 mm (Phenomenex®, Torrance, CA, USA). Mobile phase was 0.1% formic acid in acetonitrile and 0.1% formic acid in water set as gradient. Flow rate used was 0.6 mL/min. Column temperature was maintained at 40°C. Multiple reaction monitoring (MRM) in positive ion mode was used to monitor transitions at m/z 380.1 → 91.1 and m/z 383.1 → 337.0 for donepezil and the IS.

The assay had been validated in terms of selectivity, sensitivity, linearity, accuracy and precision, recovery, matrix effect and carry-over according to the Guideline on bioanalytical validation, EMA 2011 [7]. This method also has been verified before being used in this study.

The best linear fit and least-squares residual for the calibration curve was achieved with 1/x² weighing factor. The standard calibration curve for donepezil was ranged from 0.1-50 ng/mL. The lower limit of quantification was 0.1 ng/mL and the precision and accuracy obtained at LLOQ were -13.53% and 6.39%.

The mean recoveries of donepezil and IS were 86.03% - 90.93% and 97.23%. The matrix effect was also investigated. The CV of the IS-normalized MF calibration curve was achieved with 1/x² weighing factor. The standard calibration curve for donepezil was ranged from 0.1-50 ng/mL. The lower limit of quantification was 0.1 ng/mL and the precision and accuracy obtained at LLOQ were -13.53% and 6.39%.

The mean recoveries of donepezil and IS were 86.03% - 90.93% and 97.23%. The matrix effect was also investigated. The CV of the IS-normalized MF calculated from the 6 lots of matrix of low and high concentrations were 8.17% and 10.22%.

The stability study showed that donepezil in plasma was stable at room temperature for 6 hours, at -20°C for 61 days and after three freeze-thaw cycles. The stability auto-sampler showed that donepezil was stable after reconstitution for 24 hours.

2.5 Outcomes and Assessments

For the assessment of PK parameters [i.e., maximum concentration (C_max), time to maximum concentration (t_max) and area-under-the plasma concentration curve (AUC_{0-72h})] for donepezil venous blood samples (5 mL) were collected at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 48, and 72 h after drug administration in the Li-heparin tubes.
Safety and tolerability were assessed by adverse events and vital signs. The taste masked evaluation also done by asking the volunteers.

2.6 Statistical Methods

The PK parameters were assessed by non-compartmental analysis. Bioequivalence was investigated by determining the 90% confidence limits for the log-transformed ratio (test product/reference product) for the parameter AUC and \( C_{\text{max}} \). All statistical analyses were performed using EquiTest version 2.0 software (Statistical Solution, Cork, Ireland).

3. RESULTS

3.1 In vitro Drug Release Testing

The release profiles of the two formulations in different medium are shown in Fig. 2. Each data point represents an average of 12 measurements for each formulation. In medium HCl 0.1N over 85% of the drug was dissolved within 15 minutes for both formulations.

3.2 Study Population

A total of twenty male volunteers were enrolled and randomized in the study. Five volunteers withdrew from the treatment due to an adverse event and one subject was excluded as a non-compliance subject. The mean age of volunteers in this study was 33.9 years (range 19-47 years). As a result, 14 subjects completed this study.

3.3 Pharmacokinetic Analysis

A total of 14 volunteers were available for pharmacokinetic evaluation. The mean donepezil concentrations versus time profile for both formulations are shown in Fig. 3. Descriptive statistics of the pharmacokinetic parameters for donepezil for test and reference products are summarized in Table 1, where the geometric mean values and the range for the AUC\(_{0-72h}\) and \( C_{\text{max}} \) values obtained for each formulation are shown. The pharmacokinetic characteristic \( t_{\text{max}} \) was presented as mean values. The mean obtained values for test and reference products were 23.80 ng/mL and 23.78 ng/mL for \( C_{\text{max}} \) and 685.13 ng.h/mL and 697.33 ng.h/mL for AUC\(_{0-72h}\). The median \( t_{\text{max}} \) for test and reference formulations were 3 h. The parametric 90% confidence intervals for ratio T/R ranged from 91.39%-107.32% (point estimate 99.03%) for \( C_{\text{max}} \) and 93.65%-104.16% (point estimate 98.76%) for AUC\(_{0-72h}\). The intra-subject variability for \( C_{\text{max}} \) and AUC\(_{0-72h}\) estimated from the coefficient of variables as determined by ANOVA were 11.92% and 7.87%, respectively.

![Fig. 2. Dissolution profiles of two donepezil ODT formulations in three media](image-url)
Fig. 3. Mean plasma concentration-time profiles of donepezil after oral administration of two different 10 mg donepezil ODT formulation

Table 1. Pharmacokinetic results of donepezil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test formulation</th>
<th>Reference formulation</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-72h&lt;/sub&gt; (ng.h/mL)</td>
<td>685.13</td>
<td>147.76</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>23.80</td>
<td>6.94</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>3.18</td>
<td>0.95</td>
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Table 2. Statistical results of donepezil

<table>
<thead>
<tr>
<th></th>
<th>Test formulation</th>
<th>Reference formulation</th>
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<tbody>
<tr>
<td>AUC&lt;sub&gt;0-72h&lt;/sub&gt; (%)</td>
<td>98.76</td>
<td>99.03</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (%)</td>
<td>93.65 to 104.16</td>
<td>91.39 to 107.32</td>
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<td>90% geometric CI</td>
<td>7.87</td>
<td>11.92</td>
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3.4 Safety and Tolerability

Both formulations of donepezil were well-tolerated at the administered dose and no significant adverse clinical events were observed. A total of 75 adverse events were experienced during the study and there were no serious adverse events. The list of adverse events is shown in Table 3. All the volunteers also reported the donepezil formulation as tasteless.

4. DISCUSSION

The originator product comprises a basic medicine (donepezil) having the unpleasant taste and an anionic polymer or acidic polysaccharide (κ-carrageenan) to prevent the basic medicine from dissolving in saliva [6].

In development of taste masking, the test formulation is based on ion exchange resin.

The addition of flavors or sweeteners is limited and not effective enough to mask the unpleasant taste of donepezil. Taste masking by drug resin complexation is achieved when an ionizable drug reacts with a suitable ion exchange resin to form a drug resinate complex. The complex should be sufficiently stable to prevent breakdown in salivary fluid and at the same time release the drug completely under the gastrointestinal environment.

The complexation process between drug and resin will be as follow:

\[ \text{Resin} - K^+ + \text{Drug}^+ \rightarrow \text{Resin} - \text{Drug}^+ \]

The drug release process will be as follow:

\[ \text{Resin} - \text{Drug}^+ + X^- \text{ (ions in GIT)} \rightarrow \text{Resin} - X^- + \text{Drug}^+ \]
According to the current Biopharmaceutics Classification System, donepezil is categorized to class 1 (high soluble-high permeability). A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1-7.5. In addition, FDA recommended a biowaiver for immediate release tablets containing donepezil [8,9].

<table>
<thead>
<tr>
<th>Table 3. Disposition of adverse events</th>
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<tr>
<td><strong>Test</strong></td>
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<tr>
<td>n (%)</td>
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<tr>
<td>Headache</td>
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<td>Nausea</td>
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<td>Drowsiness</td>
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<tr>
<td>Fatigue</td>
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<tr>
<td>Vomiting</td>
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<tr>
<td>Flatulence</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Dry throat</td>
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<tr>
<td>Weakness</td>
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<tr>
<td>Total</td>
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</tbody>
</table>

In vitro drug release study in medium with pH 1.2 has shown that the two donepezil orally disintegrating tablet formulations were dissolved more than 85% within 15 minutes. However, in another medium the drug releases from test product were less than 40% (in medium with pH 4.5) and less than 85% (in medium with pH 6.8) at the end of 45 minutes. It could be related to the stable taste masked resinate of donepezil. Drug release in pH 6.8 from resinate was around 30% at the end of 5 min which shows evidence of taste masking. The pharmacokinetic study was performed to examine the bioequivalence of ODT formulation with using different taste masking technique from the originator formulation.

Regarding pharmacokinetic parameters (C<sub>max</sub> and AUC<sub>0-72h</sub>), there was no significant difference for C<sub>max</sub> (23.80±6.94 ng/mL for the test and 23.78±6.01 for the reference) or AUC<sub>0-72h</sub> (685.13±147.76 ng.h/mL for the test and 697.33±169.39 ng.h/mL for the reference). In conclusion, this study demonstrated that the two formulations were bioequivalent.

5. CONCLUSION

In conclusion, the orally disintegrating tablet formulation of donepezil using ion exchange resin for taste masking was bioequivalent to the reference product.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES