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Effect of Gongjindan, a Traditional Korean Polyherbal Formula, on the Pharmacokinetics Profiles of Donepezil in Male SDRats (2)

— Single Oral Combination Treatment of Donepezil 10mg/kg with Gongjindan 100mg/kg, 1.5hr-intervals with 7-day Repeated Treatment —

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Abstract

Purpose : This study was aim to evaluate effects of pharmacodynamics and toxicity in combination therapy of donepezil with Gongjindan.The effects of Gongjindan co-administration on the pharmacokinetics (PK) of donepezil were observed after single and 7-day repeated oral co-administration with 1.5hr-intervals, to evaluate synergic pharmacodynamics and reduce toxicity of combination therapy of donepezil with Gongjindan.

Materials and Methods : After 10mg/kg of donepezil treatment, Gongjindan100mg/kg was administered with 1.5hr-intervals. The plasma were collected at 30min before administration, 30min, 1, 2, 3, 4, 6, 8 and 24hrs after end of first and last 7th donepezil treatment, and plasma concentrations of donepezil were analyzed using LC-MS/MS methods.

Results : Gongjindan markedly inhibited the absorption of donepezilregardless of sample time, from 30min to 8hrs after end of first 1.5hr-interval co-administration as compared with donepezil single treated rats. Especially the absorption of donepezilwas significantly decreased at 2, 4, 6 and 8hrs after co-administration as compared with donepezilsingle treated rats. Accordingly, the Cmax (-26.236%), AUC_{0-t} (-26.02%) and AUC_{0-inf} (-25.90%)of donepezil in 1.5hr-interval co-administered rats were dramatically

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decreased as compared with donepezilsingle treated rats, respectively. However, no meaningful changes on the plasma donepezil concentrations and pharmacokinetic parameters were detected after end of last 7th 1.5hr-interval co-administration as compared with donepezil single treated rats, except for non-significant slight increases of Tmax (16.67%) detected in co-administered rats as compared with donepezil single treated rats.

Conclusion : These findings are considered as direct evidences that Gongjindan also decreased oral bioavailability of donepezil as inhibited the absorptions, when they were co-administered with 1.5hr-intervals, but they may be adapted after 7 days continuous repeated 1.5hr-interval co-administration.

Key words : Gongjindan, Pharmacokinetics, Drug-drug interactions, Rat, Donepezil, Repeat oral dose

1. Introduction

Donepezil (Aricept^{TM}) is a centrally acting reversible acetylcholinesterase inhibitor, and main therapeutic use is in the palliative treatment of mild to moderate Alzheimer's disease[1]. Donepezil is indicated for symptomatic treatment of patients with mild, moderate and severe dementia of the Alzheimer's type[2, 3]. Donepezil has been tested in other cognitive disorders including Lewy body dementia[4] and vascular dementia[5]. Donepezil has also been found to improve sleep apnea in Alzheimer's patients[6], and recently the evidence that donepezil can improve speech in children with autism was also suggested[7, 8]. However, it also showed severe toxicity in preclinical studies, 50% lethal dose (LD_{50}) in rodents are located between 30~40mg/kg, and No Observed Effect Level (NOEL) of 13 weeks repeat dose toxicity test in rat and dog is 1mg/kg/day, respectively[9] and undesirable side effects in clinical use[10]. Common side effects include gastrointestinal upset; include bradycardia, nausea, diarrhea, anorexia, abdominal pain, vivid dreams, and mania[11-13].

As results of combination therapies with other drugs to improve the side effects of donepezil or to achieve synergic effects, various drug-drug interactions of donepezil have been evaluated ; Although, pharmacokinetic studies showed no significant effects of donepezil on the pharmacokinetic profiles of cimetidine[14], combinations containing any of the following medications, depending on the amount present, may also interact with anticholinergics - cholinesterase inhibitors such as donepezil have the potential to interfere with the activity of these medications, nonsteroidal anti-inflammatory drugs (NSAIDs) - donepezil may increase gastric acid secretion due to increased cholinergic activity patients should be monitored for symptoms of active or occult gastrointestinal bleeding and carbamazepine, dexamethasone, phenobarbital, phenytoin or rifampin - They may induce the isoenzymes CYP2D6 and CYP3A4, thus increasing the rate of elimination of donepezil[9]. In addition concurrent use of cholinergic agonists like, bethanechol or neuromuscular blockingagents metabolized by plasma

cholinesterase like succinylcholine and mivacurium with donepezil may be expected with a synergistic effect[9]. Ketoconazole also can be influenced on the pharmacokinetics and pharmacodynamics of donepezil through inhibition of the metabolism of donepezil[9, 15]. Interactions with herbal products have not been established except for some restricted single herb extracts or natural compounds; *Ginkgo biloba* extracts[16], digoxin[17], theophylline[18], or warfarin[19], did not influenced on the pharmacokinetics of donepezil.

Gongjindan, a traditional Korean polyherbal formula is one of the most famous tonic agents, and consisted of 4 herbs including *Angelicae gigas* radix, Ginseng steamed red, *Corni fructus* and *Rehmanniaeradix* preparata, and 2 animal resources - antler and musk. These 6 agents were plastered using honey, and coated by gold plates. The hypolipidemic and immune stimulatory effects of Gongjindan are relatively well documented[20, 21] with anti-oxidative effects[22], anti-gliosis effects on middle cerebral artery occlusion rats[23] and anti-dementia effects[24, 25]. In addition, single oral dose toxicity[26] and micronucleus test[27] of Gongjindan itself were already reported.

In the previous study[28], co-administration of Gongjindan with donepezil within 5min showed marked decreases of donepezil absorption and consequently, reduced the oral bioavailability of donepezil, therefore, the effects of Gongjindan co-administration on the pharmacokinetics of donepezil were observedafter single and 7-day repeated oral co-administration with 1.5hr-intervals (reasonable intervals considering the Tmax after donepezil single oral administration detected in the previous study[28]) as a process of the comprehensive and integrative medicine, combination therapy of donepezil with Gongjindan to achieve synergic pharmacodynamics and reduce toxicity in the present study. After 10mg/kg of donepezil treatment, Gongjindan 100mg/kg was administered with 1.5hr- intervals. The plasma were collected at 30min before administration, 30min, 1, 2, 3, 4, 6, 8 and 24hrs after end of first and last 7th donepezil treatment, and plasma concentrations of donepezil were analyzed using LC-MS/MS methods. PK parameters of donepezil (Tmax, Cmax, AUC, $t_{1/2}$ and MRT_{inf}) were analysis as compared with donepezil single administered rats.

2. Materials &Methods

2.1. Animals and husbandry

Total tenmale Sprague-Dawley (SD)rats (6-wk old upon receipt, SLC, Japan) were used after acclimatization for 9 days. Animals were allocated five per polycarbonate cage in a temperature (20-25℃) and humidity (40-45%) controlled room. Light : dark cycle was 12hr : 12hr and feed (Samyang, Korea) and water were supplied free to access. All animals were marked by picric acid, and overnight fasted (about 18 hrs; water was not restricted) before first and last treatment, and further fasted during 3 hrs after end of treatment.

2.2. Test articles and formulation

Gongjindan, prepared and purchase from Daegu Oriental Hospitals, Daegu Haany Uni-

Table 1. Composition of Gongjindan Used in This Study

Herbs	Scientific Names	Amounts (g/1 pill)
Antler (Cornus cervi parvum)	*Cervus elaphus* Linne	0.683
Angelicae gigantis radix	*Angelica gigas* Nakai	0.683
Ginseng steamed red	Panax ginseng CA Mey.	0.683
Corni fructus	*Cornus officinalis* Sieb. Et Zucc	0.683
Rehmanniae radix preparata	*Rehmannia glutinosa* (Gaertner) Liboschitz	0.683
Musk	*Moschus moschiferus* Linne	0.122
Honey	*Apis indica* Radoszkowski	2.506
Gold plate		0.006
Total	8 types	6.050

Gongjindan used in this study was purchased from Daegu Oriental Hospital of Daegu Hanny University (Daegu, Korea)

versity(Table 1; Daegu, Korea) and donepezil (Jeil Pharm., Co., Ltd, Youngin, Korea) was used as control drug as listed follows. Donepezil and Gongjindanwere stored in a refrigerator at 4℃ to protect from light and degeneration until use. Both drugs are well suspended or dissolved (upto 20 mg/ml suspensions in Gongjindan and upto 2mg/ml solutions in donepezil) in distilled water as vehicle, respectively.

2.3. Groupings and administration

Five rats per group (two groups) were used in this study as follows. The doses of test materials were selected based on theirtoxicity and pharmacodynamics - 10mg/kg of donepezil with 100mg/kg of Gongjindan. After 10mg/kg of donepezil treatment, Gongjindan100mg/kg was administered with 1.5hr-intervals. In donepezil single treated rats, 10mg/kg of donepezil was orally administered and 1.5hrs after treatment, only distilled water 5ml/kg was orally administered, instead of Gongjindan suspensions. Each donepezil or Gongjindanwas orally administered, in a volume of 5m/kg, dissolved in distilled water, once a day for 7 days.

2.4. Changes in body weights

Changes of body weight were daily measured from 1 day before initiation of co-administration to the last 7th co-administration day using an automatic electronic balance (Precisa Instrument, Switzland). At initiation and last 7th administration, all experimental animals were overnight fasted (water was not; about 12hr) to reduce the differences from feeding. In addition, body weight gains during co-administration days as body weights at last 7th treatment day - body weights at first treatment day.

2.5. Plasma collections

All rats were slightly anesthesia under ethyl ether (Duksan Pure Chemical, Seoul, Korea) and blood samples (0.5 ml) were collected into

50IU heparinized tubes via the orbital plexus at 30min before treatment (as a control), 30min, 1, 2, 3, 4, 6, 8 and 24hrs after end of first and last 7th oral administration. Blood samples were immediately centrifuged for 10 min at 13,000 rpm and about 0.3ml aliquots of plasma were stored in a -70℃ deep freezer until analysis of donepezil.

2.6. Sample preparation and calibrations

Primary stock solution, 1.0mg/ml of donepezilin acetonitrile (Sigma, MO, USA) and internal standard working solution, carbamazepine (Sigma, MO, USA) 500ng/ml in acetonitrile were prepared. Working standard solutions were prepared by dilution with acetonitrile. All standard solutions were stored at -20℃ in the dark when not in use, and calibrated the standard samples as 100μl of blank plasma, working standard solutions and internal standard working solution were mixed with 100μl of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000rpm for 10min at 4℃. The clear supernatants were transferred to injection vials and the aliquot was injected into the LC-MS/MS system. In addition, 100μl of sample plasma and internal standard working solution were mixed with 200μl of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000rpm for 10min at 4℃. Clear supernatants (3.5μl) were directly transferred to injection vials and the aliquot was injected into the LC-MS/MS system.

2.7. LC-MS/MS conditions

Concentrations of donepezil in the rat plasma samples were determined LC-MS/MS method. Chromatographic analysis was performed using an Agilent 1100 Series HPLC (Agilent Technologies, CA, USA) equipped with on-line degasser, binary pump, autosampler and column compartment. Separation of the analyte from potentially interfering material was achieved at ambient temperature using Waters Xterra MS C18columns (2.1×50mm, 3.5μm) (Waters Corp., MA, USA) at column oven 30℃. The mobile phase used for the chromatographic separation was composed of 2% acetonitrile/98% distilled water (0.1% formic acid) to 98% acetonitrile/2% distilled water (0.1% formic acid), and was delivered isocratically at a flow rate of 0.35ml/min. The column effluent was monitored using an API 2000 triple-quadruple mass-spectometric detector (Applied Biosystems, CA, USA). The instrument was equipped with an electrospray interface in positive ion mode, and controlled by the Analyst version 1.4.2 software (Applied Biosystems, CA, USA). Samples were introduced to the interface through a Turbo IonSpray with the temperature set at 400°C. A high positive voltage of 5.0 kV was applied to the ion spray. Nitrogen was used as the nebulizer gas, curtain gas, and collision gas with the settings of 12, 6, and 8, respectively. The multiple reaction monitoring (MRM) detection method was employed for the detection of donepezil the transitions monitored were carbamazepine (IS): m/z 237>194 (Retention time: 2.4 min), donepezil: 380>91 (Retention time: 2.3 min). Calibration curves of donepezil were linear over

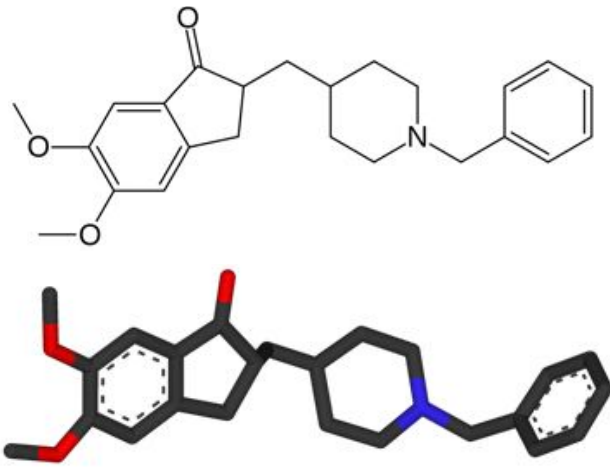

Fig 1. Chemical Structure of Donepezil Used in This Study

the ranges studied with r^2>0.999. The lower limit of quantification of the donepezil in the rat plasma was 1ng/ml.

2.8. Pharmacokinetic analysis

The plasma concentration data were analyzed using a noncompartmental method on commercial pharmacokinetics data analyzer programs (PK solutions 2.0; Summit, CO, USA)[29]. The eliminationrate constant (K_{el}) was calculated by the log-linear regressionof donepezilconcentration data during the elimination phase,and the terminal half-life ($t_{1/2}$) was calculated by 0.693/K_{el}. Thepeak concentration (Cmax) and time to reach the peak concentration(Tmax) of donepezilin the plasma were obtained by visualinspection of the data in the concentration-time curve. Thearea under the plasma concentration-time curve (AUC_{0-t}) fromtime zero to the time of the last measured concentration (C_{last})was calculated using the linear trapezoidal rule[30]. The AUCzero to infinity (AUC_{0-inf}) was obtained by adding AUC_{0-t} andthe extrapolated area was determined by C_{last}/K_{el}. The mean residencetime infinity (MRT_{inf}) was calculated by dividing the first moment of AUC($AUMC_{0-inf}$) by AUC_{0-inf}.

2.9. Statistical analyses

All the means are presented with their standard deviation of five rats (Mean ± SD of five rat plasma concentrations of donepezil). The pharmacokinetic parameters were compared using a non-parametric comparison test, Mann-Whitney U (MW) test, on the SPSS for Windows (Release 14.0K, SPSS Inc., USA). A p-value <0.05 was considered statistically significant. In addition, the percent changes between donepezil single treated rats and donepezil with Gongjindanco-administered rats were calculated to help the understanding of the effects of co-administration.

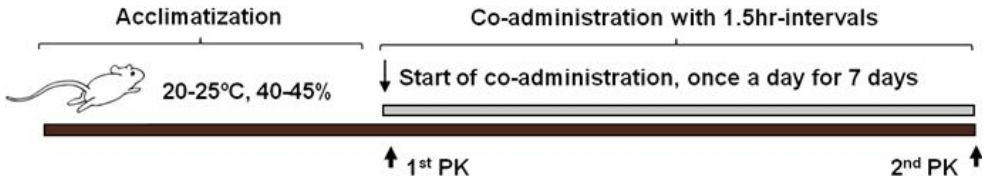

Fig 2. Experimental Designs Used in This Study

After 10mg/kg of donepezil treatment, Gongjindan 100mg/kg was administered with 1.5-hr intervals. In donepezil single treated rats, 10mg/kg of donepezil was orally administered and 1.5hrs after treatment, only distilled water 5ml/kg was orally administered, instead of Gongjindan suspensions. Each donepezil or Gongjindan was orally administered, in a volume of 5m/kg, dissolved in distilled water, once a day for 7 days, and bloods were sampled after first and last 7th donepezil administration, respectively.

3. Results

3.1. Changes on the plasma concentrations of donepezil

No meaningful changes on the body weight and gains were detected in Gongjindan and donepezil co-administered rats as compared with donepezil single treated rats throughout experimental periods, respectively (Table 2, Fig 3).

3.2. Changes on the plasma concentrations of donepezil

Donepezil was detected from 30min to 8hrs after end of administration in the both donepezil single and co-administered rats with Gongjindan after first and last 7th co-administration, respectively. Gongjindansignificantly ($p<0.01$ or $p<0.05$) inhibited the absorption of donepezilat 2, 4, 6 and 8hrs after first 1.5hr-interval co-administration of donepezil10mg/kg with Gongjindan100mg/kg as compared with donepezil single treated rats, and the absorption of donepezil were also non-significant but markedly decreased at 30min, 1 and 3hrs after 1.5hr-interval co-administration as compared with donepezil single treated rats, in the present study. However, no meaningful changes on the plasma donepezil concentrations were detected

Table 2. Body weight gains during Administration (from first to last treatment) of Donepezil with and without Gongjindan in Male Rats

Groups	Donepezil (10mg/kg)	
	Without Gongjindan co-administration (Distill water)	With Gongjindan co-administration (100mg/kg)
Body weights		
At first treatment [A]	223.80 ± 4.82	222.80 ± 6.06
At last treatment [B]	243.40 ± 8.85	242.60 ± 6.35
Body weight gains		
[B] - [A]	19.60 ± 6.39	19.80 ± 3.19

Values are expressed as mean ± SD of five rats

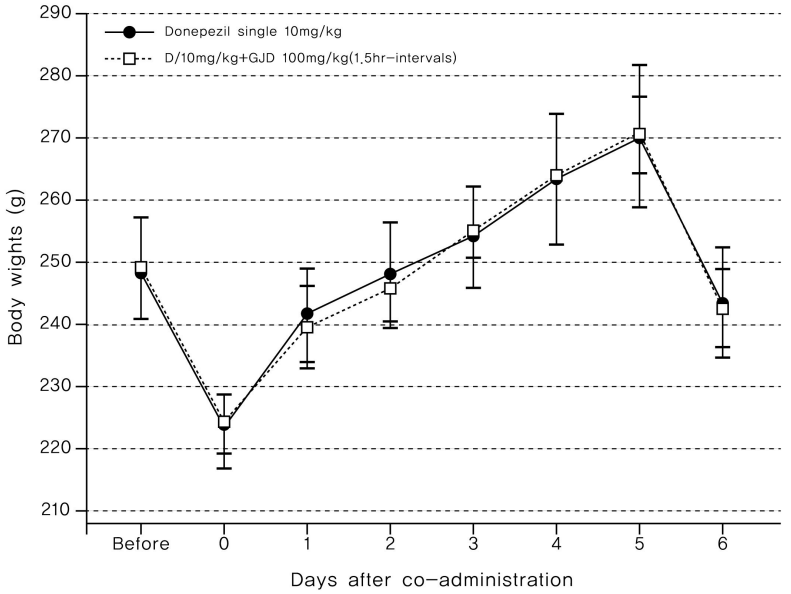

Fig 3. Changes on the Body Weights during 1.5hr-interval Co-administration of Donepezil with and without Gongjindan in Male Rats
No meaningful changes on the body weight and gains were detected in Gongjindan and donepezil co-administered rats as compared with donepezil single treated rats throughout experimental periods, in the present study. Values are expressed as mean ± SD of five rats. All animals were overnight fasted before first and last treatment. Before, 1 day before first treatment D, donepezil GJD, Gongjindan.

in Gongjindan co-administratedrats as compared with donepezil single treated rats after last 7th 1.5hr-interval co-administration, except for non-significant slight increases of plasma donepezil concentration detected in Gongjindan co-administrated rats as compared with donepezil single treated rats at 1 hr after last 7th1.5hr-interval co-administration, in the present study (Fig 4 and 5).

The plasma donepezil concentrations at 30min, 1, 2, 3, 4, 6 and 8hrs after end of first 1.5hr-interval co-administration were changed as -26.89, -18.17, -29.69, -29.53, -27.88, -26.78 and -25.71% in donepezil + Gongjindan treated rats as compared with donepezil single treated rats, respectively.

The plasma donepezil concentrations at 30min, 1, 2, 3, 4, 6 and 8hrs after end of last 7th 1.5hr-interval co-administration were changed as 9.97, 16.45, -7.99, -7.39, -2.89, -14.69 and -12.85% in donepezil + Gongjindan treated rats as compared with donepezil single treated rats, respectively.

3.3. Changes on the Tmax of donepezil

The Tmax of donepezil wasslightly and non-significantly decreased as 11.11% in co-administrated rats with donepezil10mg/kg and Gongjindan 100mg/kg (1.00±0.00hr) as compared

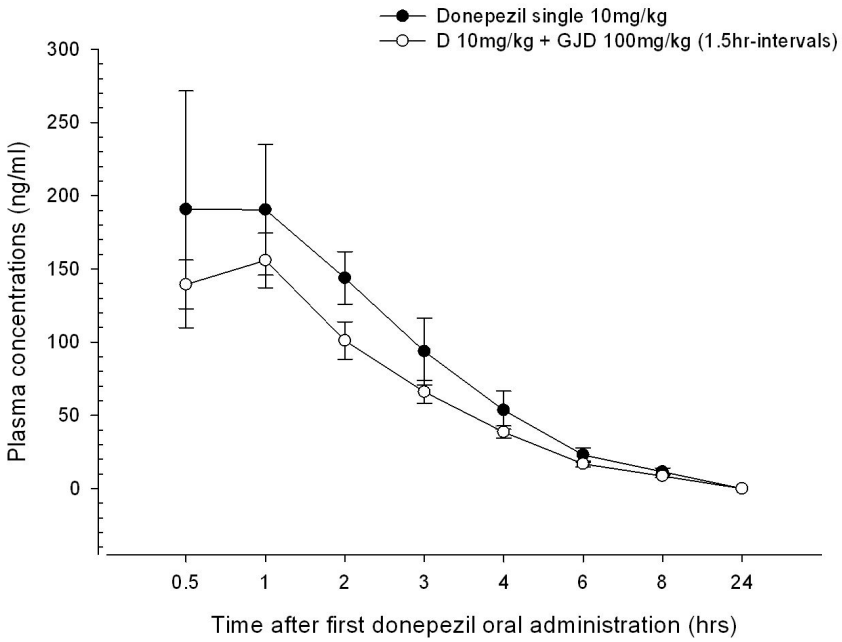

Fig 4. Plasma Concentrations of Donepezil with and without Gongjindan after First Co-administration with 1.5hr-intervals in Male Rats

Donepezil was detected from 30min to 8hrs after end of administration in the both donepezil single and co-administered rats with Gongjindan after first co-administration, respectively. Gongjindan significantly ($p<0.01$ or $p<0.05$) inhibited the absorption ofdonepezil at 2, 4, 6 and 8hrs after first 1.5hr-interval co-administration of donepezil 10mg/kg with Gongjindan 100mg/kg as compared with donepezil single treated rats, and the absorption of donepezil were also non-significant but markedly decreased at 30min, 1 and 3hrs after 1.5hr-interval co-administration as compared with donepezil single treated rats, in the present study.Values are expressed as mean ± SD of five rats. D, donepezil GJD, Gongjindan. $^{*}p<0.01$ and $^{**}p<0.05$ as compared with donepezil single treated rats by MW test

with donepezil single treated rats (00.90±0.65hr) after end of first 1.5hr-interval co-administration of donepezil 10mg/kg with Gongjindan 100mg/kg. However, the Tmax of donepezil was slightly and non-significantly increased as 16.67% in 1.5hr-intervals co-administrated rats with donepezil and Gongjindan (0.70±0.27hr) as compared with donepezil single treated rats (0.60±0.22hr) after end of last 7th 1.5hr-interval co-administration of donepezil 10mg/kg with Gongjindan 100mg/kg, in the present study (Table 3 and 5).

3.4. Changes on the Cmax of donepezil

The Cmax of donepezil was non-significantly but markedly decreased as -26.23% in co-

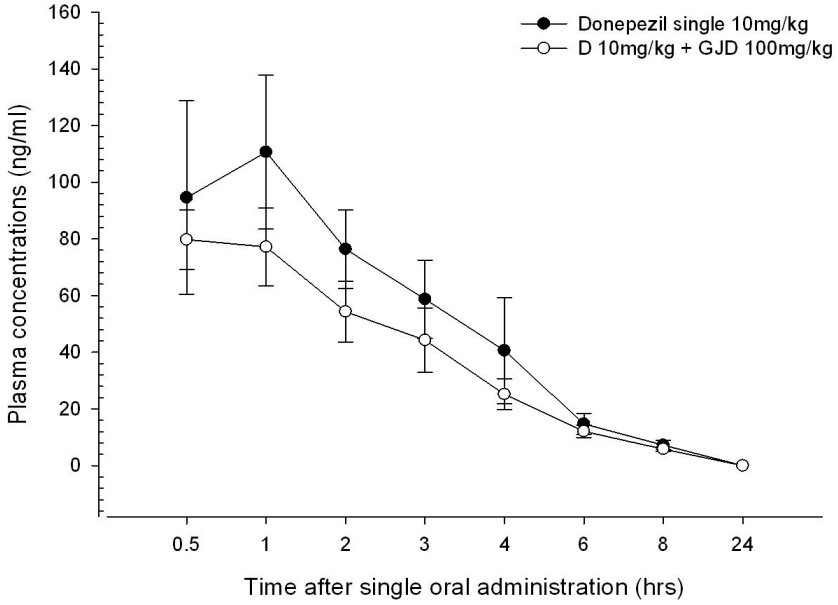

Fig 5. Plasma Concentrations of Donepezil with and without Gongjindan after Last 7th Co-administration with 1.5hr-intervals in Male Rats

Donepezil was detected from 30min to 8hrs after endof administration in the both donepezil single and co-administered rats with Gongjindan after last 7th co-administration, respectively. No meaningful changes on the plasma donepezil concentrations were detected in Gongjindan co-administrated rats as compared with donepezil single treated rats after last 7th 1.5hr-interval co-administration, except for non-significant slight increases of plasma donepezil concentration detected in Gongjindan co-administrated rats as compared with donepezil single treated rats at 1 hr after last 7th 1.5hr-interval co-administration, in the present study.Values are expressed as mean ± SD of five rats. D, donepezil GJD, Gongjindan.

administrated rats with donepezil 10mg/kg and Gongjindan 100mg/kg (155.80±18.74ng) as compared with donepezil single treated rats (211.20±54.79ng) after end of first 1.5hr-interval co-administration. However, the Cmax of donepezil was slightly and non-significantly increased as 10.94% in Gongjindan co-administrated rats (241.40±23.31ng) as compared with donepezil single treated rats (217.60±35.32ng) after end of last 7th 1.5hr-interval co-administration, in the present study (Table 3 and 5).

3.5. Changes on the AUC of donepezil

The AUC_{0-t} and AUC_{0-inf} of donepezil were significantly ($p<0.05$) decreased as -26.02 and -25.90% in co-administrated rats with donepezil 10mg/kg and Gongjindan 100mg/kg (453.90±47.40 and 476.33±50.82 hr · ng/ml) as compared with donepezil single treated rats (613.54±87.85 and 642.78±95.33hr · ng/ml) after end of first 1.5hr-interval co-administration. However, the

Table 3. Pharmacokinetic Parameters of Donepezil with and without Gongjindan after First Co-administration with 1.5hr-intervals in Male Rats

Parameters	Donepezil (10mg/kg)	
	Without Gongjindan co-administration (Distill water)	With Gongjindan co-administration (100mg/kg)
Tmax (hrs)	0.90 ± 0.65	1.00 ± 0.00
Cmax (ng/ml)	211.20 ± 54.79	155.80 ± 18.74
AUC_{0-t} (hr · ng/ml)	613.54 ± 87.85	453.90 ± 47.40*
AUC_{0-inf} (hr · ng/ml)	624.78 ± 95.33	476.33 ± 50.82*
$t_{1/2}$ (hr)	1.75 ± 0.21	1.80 ± 0.10
MRT_{inf} (hr)	2.73 ± 0.25	2.71 ± 0.07

Values are expressed as mean ± SD of five rats. Cmax: The peak plasma concentration, Tmax: Time to reach Cmax, AUC0-t: The total area under the plasma concentration-time curve from time zero to time measured, AUC0-inf: The total area under the plasma concentration-time curve from time zero to time infinity, t1/2: half life, MRTinf: mean residence to time infinity. * $p<0.05$ as compared with donepezil single treated rats by MW test

Table 4. Pharmacokinetic Parameters of Donepezil with and without Gongjindan after Last 7th Co-administration with 1.5hr-intervals in Male Rats

Parameters	Donepezil (10mg/kg)	
	Without Gongjindan co-administration (Distill water)	With Gongjindan co-administration (100mg/kg)
Tmax (hrs)	0.60 ± 0.22	0.70 ± 0.27
Cmax (ng/ml)	217.60 ± 35.32	241.40 ± 23.31
AUC_{0-t} (hr · ng/ml)	632.21 ± 45.74	656.79 ± 11.72
AUC_{0-inf} (hr · ng/ml)	672.35 ± 58.56	710.84 ± 49.11
$t_{1/2}$ (hr)	1.90 ± 0.25	2.15 ± 0.53
MRT_{inf} (hr)	2.94 ± 0.27	3.08 ± 0.68

Values are expressed as mean ± SD of five rats. Cmax: The peak plasma concentration, Tmax: Time to reach Cmax, AUC_{0-t}: The total area under the plasma concentration-time curve from time zero to time measured, AUC_{0-inf}: The total area under the plasma concentration-time curve from time zero to time infinity, $t_{1/2}$: half life, MRTinf: mean residence to time infinity

AUC_{0-t} and AUC_{0-inf} of donepezil were no meaningfully changed as 3.89 and 5.72% in co-administrated rats with donepezil and Gongjindan (656.79±11.72 and 710.84±49.11hr · ng/ml) as compared with donepezil single treated rats (632.21±45.74 and 672.35±58.56hr · ng/ml) after end of last 7th 1.5hr-interval co-administration, in the present study (Table 3 and 5).

3.6. Changes on the $t_{1/2}$ of donepezil

The $t_{1/2}$ of donepezil was slightly and non-significantly increased as 3.40% in co-administrated rats with donepezil 10mg/kg and Gongjindan 100mg/kg (1.80±0.1hr) as compared

with donepezil single treated rats (1.75±0.21hr) after end of first 1.5hr-interval co-administration. In addition, the $t_{1/2}$ of donepezil was also slightly and non-significantly increased as 13.16% in co-administrated rats with donepezil and Gongjindan (2.15±0.53hr) as compared with donepezil single treated rats (1.90±0.25hr) after end of last 7th 1.5hr-interval co-administration, in the present study (Table 3 and 5).

3.7. Changes on the MRT_{inf} of donepezil

The MRT_{inf} of donepezil was non-significantly changed as -0.77% in co-administrated rats with donepezil 10mg/kg and Gongjindan 100mg/kg (2.71±0.07hr) as compared with donepezil single treated rats (2.73± 0.25hr) after end of first 1.5hr-interval co-administration. In addition, the MRT_{inf} of donepezil was also non-significantly changed as 4.69% in co-administrated rats with donepezil and Gongjindan (3.08±0.68hr) as compared with donepezil single treated rats (2.94±0.27hr) after end of last 7th 1.5hr-interval co-administration, in the present study (Table 3 and 5).

4. Discussion

Although donepezil is a centrally acting reversible acetylcholinesterase inhibitor, frequently used for palliative treatment of mild to moderate Alzheimer's disease[1] and for symptomatic treatment of patients with mild, moderate and severe dementia of the Alzheimer's type[2, 3], it also showed severe toxicity in preclinical studies[9] and undesirable side effects in clinical use[10-13]. In addition, donepezil has been showed various drug-drug interactions[9, 15], but the interactions with herbal natural products have not been established except for some restricted single herb extracts or natural compounds[16-19]. Gongjindan, a traditional Korean polyherbal formula is one of the most famous tonic agents and the hypolipidemic and immune stimulatory[20, 21], anti-oxidative[22], anti-gliosis effects[23] and anti-dementia effects[24, 25] of Gongjindan are relatively well documented.

In the previous study[28], co-administration of Gongjindan with donepezil within 5min showed marked decreases of donepezil absorption and consequently, reduced the oral bioavailability of donepezil, therefore, the effects of Gongjindan co-administration on the pharmacokinetics of donepezil were observed after single and 7- day repeated oral co-administration with 1.5hr- intervals as a process of the comprehensive and integrative medicine, combination therapy of donepezil with Gongjindanto achieve synergic pharmacodynamics and reduce toxicity in the present study. After 10mg/kg of donepezil treatment, Gongjindan 100mg/kg was administered with 1.5hr-intervals. The plasma were collected at 30min before administration, 30min, 1, 2, 3, 4, 6, 8 and 24hrs after end of first and last 7th donepezil treatment, and plasma concentrations of donepezil were analyzed using LC-MS/MS methods. PK parameters of donepezil were analysis as compared with donepezil single administered rats using noncompartmental pharmacokinetics data analyzer programs.

Gongjindanmarkedly inhibited the absorption of donepezil regardless of sample time, from 30min to 8hrs after end of first 1.5hr-

interval co-administration as compared with donepezil single treated rats. Especially the absorption of donepezil were significantly ($p<0.05$) decreased at 2, 4, 6 and 8hrs after co-administration as compared with donepezil single treated rats. Accordingly, the Cmax (-26.236%), AUC_{0-t} (-26.02%) and AUC_{0-inf} (-25.90%) of donepezil in 1.5hr-interval co-administered rats were dramatically decreased as compared with donepezil single treated rats, respectively. However, no meaningful changes on the plasma donepezil concentrations and pharmacokinetic parameters were detected after end of last 7th 1.5hr-interval co-administration as compared with donepezil single treated rats, except for non-significant slight increases of Tmax (16.67%) detected in co-administered rats as compared with donepezil single treated rats. These findings are considered as direct evidences that Gongjindan also decreased oral bioavailability of donepezil as inhibited the absorptions, when they were co-administered with 1.5hr-intervals as previous 5min co-administration study[28], but they may be adapted after 7 days continuous repeated 1.5hr-interval co-administration.

All rats used in this study, showed normal body weight increases ranged in normal age-matched rats regardless of treatment in the present study[31, 32]. In addition, no meaningful changes on the body weight and gains were detected in Gongjindanand donepezil co-administered rats as compared with donepezil single treated rats throughout experimental periods, in the present study.

Donepezil was well absorbed, with a relative oral bioavailability of 100%[33]. The rate and extent of absorption are not influenced by food intake or the time of administration[34]. Donepezil showed very high protein bindings, it showed approximately 96% protein bindings[35], mainly to albumins (about 75%) and α1-acid glycoprotein (about 21%). Tmax of donepezil in human subject after oral administration is 3 to 4 hours and slowly eliminated through renal and biliary system[36], t1/2 of donepezil in healthy human volunteers is about 70 hours[2, 37]. In rats, Tmax of donepezil is 0.5～1 hr after single oral administration and t1/2 is about 2～3 hrs[38].

In the present study, Tmax of donepezil in donepezil single oral treated rats was detected as 0.90±0.65hr after first 1.5hr-interval co-administration, and Cmax, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} were detected as 211.20±54.79ng, 613.54±87.85hr・ng/ml, 642.78±95.33hr・ng/ml, 1.75±0.21hr and 2.73±0.25hr after first 1.5hr-interval co-administration, respectively. In donepezil with Gongjindan co-administered rats, Tmax, Cmax, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} of donepezil were detected as 1.00±0.00hr, 155.80±18.74ng, 453.90±47.40hr・ng/ml, 476.33±50.82 hr・ng/ml, 1.80±0.10hr and 2.71±0.07hr changed as 11.11, -26.23, -26.02, -25.90, 3.40 and -0.77% as compared with donepezil 10mg/kg single oral treated rats after first 1.5hr-interval co-administration, respectively. Especially, the AUC_{0-t} and AUC_{0-inf} of donepezil in donepezil with Gongjindan co-administered rats were significantly ($p<0.05$) decreased as compared with donepezil single treated rats after first 1.5hr-interval co-administration, respectively. In addition, Tmax of donepezil in donepezil single oral treated rats was detected as 0.60±0.22hr after last 7th 1.5hr-interval co-administration, and Cmax,

AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} were detected as 217.60±35.32ng, 632.21±45.74hr · ng/ml, 672.35±58.56hr · ng/ml, 1.90±0.25hr and 2.94±0.27hr after last 7th 1.5hr-interval co-administration, respectively. In donepezil with Gongjindan co-administered rats, Tmax, Cmax, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRTinf of donepezil were detected as 0.70±0.27hr, 241.40±23.31ng, 656.79±11.72 hr · ng/ml, 710.84±49.11hr · ng/ml, 2.15±0.53hr and 3.08±0.68hr changed as 16.67, 10.94, 3.89, 5.72, 13.16 and 4.69% as compared with donepezil 10mg/kg single oral treated rats after last 7th 1.5hr-interval co-administration as similar to those of donepezil single treated rats, respectively.

Donepezil undergoes first pass metabolism to four major metabolites, two of which are known to be active, and a number of minor metabolites[36]. Donepezil is metabolized by cytochrome P450 isoenzymes CYP2D6 and CYP3A4[36, 39] and, therefore, donepezil can be interacted with other drugs act on these isoenzymes[9, 15]. After first co-administration of Gongjindan with 1.5hr-intervals,the absorption of donepezil was also dramatically decreased, quite similar to those of co-administration within 5min[28] in the present study. It, therefore, considered that 1.5hr-interval co-administration with Gongjindanalso can be reduced the toxicity or side effects of donepezil[10-13] because toxicity or side effects of donepezil weredirectly related to the plasma concentrations. However, it also obvious evidences that the reduction of donepezil absorptions induced the decreases of pharmacodynamics. Although adaption trends were detected after 7-day repeated co-administration with 1.5hr-intervals - no meaningful changes on the plasma donepezil concentrations and pharmacokinetic parameters were detected after end of last 7th 1.5hr-interval co-administration with Gongjindan as compared with donepezil single treated rats in the present study, it also observed that Gongjindan also decreases the oral bioavailability of donepezil as inhibited the absorption after end of first 1.5hr-interval co-administration, quite similar as results of the previous 5min co-administration study[28]. Therefore, it seems to need co-administration pharmacokinetic studies with more prolong intervals than 1.5hrs, prior to the pharmacodynamics as a process of comprehensive and integrative medicine, combination therapy of donepezil with Gongjindan. More detail pharmacokinetic studies should be tested as the effects of Gongjindan on the pharmacokinetics of donepezil, when they were co-administered with more prolong intervals than MRT_{inf} of donepezil single oral administration (about 3hr-intervals) and after repeated co-administrations.

5. Conclusion

Although no meaningful changeson the plasma donepezil concentrations and pharmacokinetic parameters were detected after end of last 7th 1.5hr-interval co-administration with Gongjindanas compared with donepezil single treated rats, it also observed that Gongjindan also decreases theoral bioavailability of donepezil as inhibited the absorption after end of first 1.5hr-interval co-administration, quite similar as results of the previous 5min co-administration study[28], based on the results of the present study. Hence, it seems to need

co-administration pharmacokinetic studies with more prolong intervals than 1.5hrs, prior to the pharmacodynamics as a process of comprehensive and integrative medicine, combination therapy of donepezil with Gongjindan. I and my colleagues strongly suggested that pharmacokinetic studies should be tested like the effects of Gongjindanon the pharmacokinetics of donepezil, when they were co-administered with more prolong intervals than MRT_{inf} of donepezil single oral administration (about 3hr-intervals) and after repeated co-administrations

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