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# Effect of *Gongjindan-gamibang* on the Pharmacokinetics Profiles of Sorafenib in Male SD Rats (2)

## - Single Oral Combination Treatment of Sorafenib 50mg/kg with Gongjindan-gamibang 100 mg/kg, 3.5hr-intervals with 7-day Repeated Treatment -

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### Abstract

**Objective** : In the previous study, co-administration of Gongjindan-gamibang (GJD) with sorafenib increased oral bioavailability of sorafenib through augment the absorption, therefore, the effects of GJD co-administration on the pharmacokinetics of sorafenib were observed after single and 7-day repeated oral co-administration with 3.5 hr-intervals in the present study.

**Method** : After 50 mg/kg of sorafenib treatment, GJD 100 mg/kg was administered with 3.5 hr-intervals. The plasma were collected at 30 min before administration, 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of first and last 7th sorafenib treatment, and plasma concentrations of sorafenib were analyzed using LC-MS/MS methods. PK parameters of sorafenib ($T_{max}$, $C_{max}$, AUC, $t_{1/2}$ and $MRT_{inf}$) were analysis as compared with sorafenib single administered rats.

**Results** : GJD markedly inhibited the absorption of sorafenib, from 1 hr to 24 hrs after end of first 3.5 hr-interval co-administration, the $C_{max}$ (-43.27%), $AUC_{0-t}$ (-56.29%) and $AUC_{0-inf}$ (-66.70%) of sorafenib in co-administered rats were dramatically decreased as compared with sorafenib single treated rats. However, GJD significantly increased the absorption of sorafenib, from 4 hr to 8 hrs after end of last 7th 3.5 hr-interval co-administration, the $AUC_{0-t}$ (34.08%) and $AUC_{0-inf}$ (37.31%) of sorafenib in co-administered rats were dramatically increased as compared with sorafenib single treated rats.

**Conclusion** : Although GJD decreased the oral bioavailability of sorafenib through inhibition of gastrointestinal

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absorptions after end of first 3.5 hr-interval co-administration, it is observed that GJD increases the oral bioavailability of sorafenib as facilitated the absorption after end of last 7th repeated co-administration. Hence, the co-administration of GJD and sorafenib should be avoided in the combination therapy of sorafenib with GJD on anticancer therapy.

**Key words** : Gongjindan-gamibang, Pharmacokinetics, Drug-drug interactions, Rat, Sorafenib, Repeat oral dose

## I. Introduction

Sorafenib (Nexavar™) is an antineoplastic agent approved for the treatment of primary kidney cancer (advanced renal cell carcinoma) and advanced primary liver cancer (hepatocellular carcinoma)[1]. It act as antineoplastic agent through inhibition of several tyrosine protein kinases (VEGFR and PDGFR) and Raf kinases (more avidly C-Raf than B-Raf)[2]; Protein kinases are overactive in many of the molecular pathways that cause cells to become cancerous and these pathways include Raf kinase, PDGF (platelet-derived growth factor), VEGF receptor 2 and 3 kinases and c Kit the receptor for Stem cell factor[3]. Recently, the evidences that sorafenib also effective in non-responsive thyroid cancer[4], in some kinds of lung cancer with squamous-cell histology[5] and in recurrent glioblastoma[6] were suggested. However, various side effects were also arise from sorafenib treated patients, especially include skin rash, hand-foot skin reactions, diarrhea, hypertension, reversible posterior leukoencephalopathy syndrome and erythrocytosis[1,7], and also hypersensitivity to sorafenib or any ingredient in the formulation were also known[8].

Common adverse effects of sorafenib is hypophosphatemia, diarrhea, increased lipase concentrations, rash/desquamation, fatigue, hand-foot syndrome, increased amylase concentrations, alopecia, nausea, lymphopenia, pruritus, neutropenia, hypertension, anorexia, vomiting, constipation, hemorrhage (all sites, including gastrointestinal and respiratory tract), dyspnea, cough, sensory neuropathy, dry skin, pain (abdominal, joint, headache, mouth, bone, and tumor), weight loss, erythema, asthenia, leucopenia[1]. Sorafenib should be used with caution in children, pregnancy, lactation, elder, situations where a patient has a history of hypersensitivity, renal and hepatic impairment[1,8].

As results of combination therapies with other drugs to improve the side effects of sorafenib or to achieve synergic effects, various drug-drug interactions of sorafenib have been evaluated; Although sorafenib does not appear to affect pharmacokinetics of gemcitabine[9] and oxaliplatin[10], Sorafenib containing any of the following medications, depending on the amount present, may also interact with anticonvulsants (carbamazepine, phenobarbital, phenytoin) - possible decreased plasma sorafenib concentrations, dexamethasone - possible decreased plasma sorafenib concentrations, dextromethorphan, ketoconazole, midazolam, omeprazole - pharmacokinetic interaction unlikely, doxorubicin - possible increased AUC of doxorubicin, irinotecan - possible increased AUC of irinotecan and its active metabolite, SN-381, with rifampin - possible decreased plasma sorafenib concentrations[1,11,12]. Interactions with herbal products have not been established except for some restricted natural compounds, sorafenib increased risk of bleeding interact with warfarin[13] and single extracts, St. John's wort (*Hypericum perforatum*) possible decreased plasma sorafenib concentrations[1].

Gongjindan, a traditional Korean polyherbal formula is one of the most famous tonic agents, and is consisted of 4 herbs including Angelicae gigas radix, Corni fructus, and 2 animal resources - antler and musk. These 4 agents were plastered using honey, and coated by gold plates. The hypolipidemic and immune stimulatory effects of Gongjindan are relatively well documented[14] with anti-oxidative effects[15], anti-gliosis effects on middle cerebral artery occlusion rats[16] and anti-dementia effects[17]. In addition, single oral dose toxicity[18] and micronucleus test[19] of Gongjindan itself were already reported.

In the previous study[20], co-administration of Gongjindan-gamibang (GJD) with sorafenib within 5 min showed marked increases of sorafenib oral bioavailability (47.02%) through augment the absorption, therefore, the effects of GJD co-administration on the pharmacokinetics of sorafenib were observed after single and 7-day repeated oral co-administration with 3.5 hr-intervals (reasonable intervals considering the $T_{max}$ after sorafenib single oral administration detected in the previous study[20]) as a process of the comprehensive and integrative medicine, combination therapy of sorafenib with GJD to achieve synergic pharmacodynamics and reduce toxicity on hepatocellular cancers in the present study.

## II. Materials and Methods

### 1. Animals and husbandry

Total ten male Sprague-Dawley (SD) rats (6-wk old upon receipt, SLC, Japan) were used after acclimatization for 10 days. Animals were allocated five per polycarbonate cage in a temperature (20-25℃) and humidity (40-45%) controlled room. Light : dark cycle was 12 hr : 12 hr 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. Test articles and formulation

GJD was purchased from Daegu Oriental Hospitals, Daegu Haany University (Table 1; Daegu, Korea) and sorafenib (Jeil Pharm., Co., Ltd, Youngin, Korea) was used as control drug as listed follows. Sorafenib and GJD were stored in a refrigerator at 4℃ to protect from light and degeneration until use. Both drugs are well suspended or dissolved (up to 20 mg/ml suspensions

Table 1. Composition of GJD 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 |

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

in GJD and up to 10 mg/ml solutions in sorafenib) in distilled water as vehicle, respectively.

### 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 their toxicity and pharmacodynamics - 50 mg/kg of sorafenib with 100 mg/kg of GJD, and co-administration of 3.5 hr-intervals were selected based on the results of previous study[20]; co-administration of GJD and sorafenib within 5 min marked increased the absorption and oral bioavailability of sorafenib. After 50 mg/kg of sorafenib treatment, GJD 100 mg/kg was administered with 3.5 hr-intervals. In sorafenib single treated rats, 50 mg/kg of sorafenib was orally administered and 3.5 hrs after treatment, only distilled water 5 ml/kg was orally administered, instead of GJD suspensions. Each sorafenib or GJD was orally administered, in a volume of 5 ml/kg, dissolved in distilled water, once a day for 7 days.

### 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 12 hr) 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.

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

### 6. Sample preparation and calibrations

Primary stock solution, 1.0 mg/ml of sorafenib in 90% acetonitrile (Sigma, MO, USA) mixtures with distilled water and internal standard working solution, carbamazepine (Sigma, MO, USA) 500 ng/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 100 μl of blank plasma, working standard solutions and internal standard working solution were mixed with 100 μl of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000 rpm for 10 min at 4℃. The clear supernatants were transferred to injection vials and the aliquot was injected into the LC-MS/MS system. In addition, 100 μl of sample plasma and internal standard working solution were mixed with 200 μl of acetonitrile. The mixtures were mixed by vortex-mixing and centrifuged at 12,000 rpm for 10 min at 4℃. Clear supernatants (10.0 μl) were directly transferred to injection vials and the aliquot was injected into the LC-MS/MS system.

### 7. LC-MS/MS conditions

Concentrations of sorafenib 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 C18 columns (2.1×50 mm, 3.5 μm) (Waters Corp., MA, USA) at column oven 30℃. The mobile phase used for the chromatographic separation was composed of 5% acetonitrile/95% distilled water (0.1% formic acid) to 95% acetonitrile/5% distilled water (0.1% formic acid), and was delivered isocratically at a flow rate of 0.35 ml/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℃. 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 sorafenib; the transitions monitored were carbamazepine (IS): m/z 237〉194 (Retention time: 2.4 min), sorafenib: 465〉252 (Retention time: 2.7 min). Calibration curves of sorafenib were linear over the ranges studied with $r^2$>0.999. The lower limit of quantification of the sorafenib in the rat plasma was 1 ng/ml.

### 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)[21]. The elimination rate constant ($K_{el}$) was calculated by the log-linear regression of sorafenib concentration data during the elimination phase, and the terminal half-life ($t_{1/2}$) was calculated by 0.693/$K_{el}$. The peak concentration ($C_{max}$) and time to reach the peak concentration ($T_{max}$) of sorafenib in the plasma were obtained by visual inspection of the data in the concentration-time curve. The area under the plasma concentration-time curve ($AUC_{0-t}$) from time zero to the time of the last measured concentration ($C_{last}$) was calculated using the linear trapezoidal rule[22]. The AUC zero to infinity ($AUC_{0-inf}$) was obtained by adding $AUC_{0-t}$ and the extrapolated area was determined by $C_{last}/K_{el}$. The mean residence time infinity ($MRT_{inf}$) was calculated by dividing the first moment of AUC ($AUMC_{0-inf}$) by $AUC_{0-inf}$.

### 9. Statistical analyses

All the means are presented with their standard deviation of five rats (Mean ± SD of five or four rats). 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 sorafenib single treated rats and sorafenib with GJD co-administered rats were calculated to help the understanding of the effects of co-administration.

## III. Results

### 1. Changes on the body weight and gains

No significant changes on the body weight and gains were detected in GJD and sorafenib co-administered rats as compared with sorafenib single treated rats throughout experimental

periods, respectively (Table 2, Fig 1).

## 2. Changes on the plasma concentrations of sorafenib

Sorafenib was detected from 30 min to 24 hrs after end of administration in the both sorafenib single and co-administered rats with GJD after first and last 7th co-administration, respectively. GJD non-significantly inhibited the absorption of sorafenib from 1 hr to 24 hrs after first 3.5 hr-interval co-administration of sorafenib 50 mg/kg with GJD 100 mg/kg as compared with sorafenib single treated rats. However, GJD significantly ($p<0.05$) increased the absorption of sorafenib from 4 to 8 hrs after last 7th 3.5 hr-interval co-administration of sorafenib 50 mg/kg with GJD 100 mg/kg as compared with

Table 2. Body weight gains during administration (from first to last treatment) of Sorafenib with or without Gongjindan in male rats

| Groups | Sorafenib (50 mg/kg) | |
|---|---|---|
| | Without Gongjindan co-administration (Distilled water) | With Gongjindan co-administration (100 mg/kg) |
| Body weights | | |
| At first treatment [A] | 236.40 ± 7.40 | 235.60 ± 11.97 |
| At last treatment [B] | 256.50 ± 8.27 | 254.00 ± 16.09 |
| Body weight gains | | |
| [B] - [A] | 22.75 ± 6.24 | 18.40 ± 4.77 |

Values are expressed as mean ± SD of five or four rats

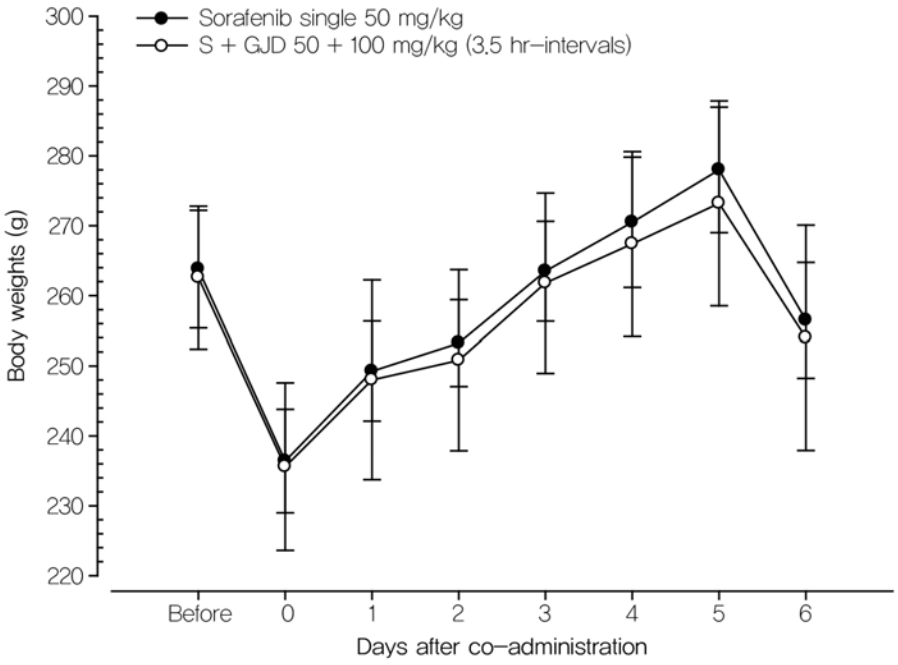


Fig 1. Changes on the body weights during 3.5 hr-interval co-administration of Sorafenib with and without GJD in male rats. No meaningful changes on the body weight and gains were detected in KJD and sorafenib co-administered rats as compared with sorafenib single treated rats throughout experimental periods, in the present study. Values are expressed as mean ± SD of five or four rats (g). All animals were overnight fasted before first and last treatment. Before means 1 day before first treatment. S; sorafenib, GJD; Gongjindan aqueous suspensions.

sorafenib single treated rats with non-significant but marked increased of plasma sorafenib concentrations at 1, 3 and 24 hrs after end of last 7th 3.5 hr-interval co-administration in GJD and sorafenib co-administered rats, in the present study (Fig 2 and 3). The plasma sorafenib concentrations at 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of first 3.5 hr-interval co-administration were changed as −7.09, −19.08, −39.13, −42.48, −51.00, −45.49, −43.85 and −28.59% in sorafenib + GJD treated rats as compared with sorafenib single treated rats, respectively. The plasma sorafenib concentrations at 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of last 7th 3.5 hr-interval co-administration were changed as 1.44, 29.65, 5.07, 27.93, 32.16, 44.61, 38.06 and 39.99% in sorafenib + GJD treated rats as compared with sorafenib single treated rats, respectively.

### 3. Changes on the $T_{max}$ of sorafenib

The $T_{max}$ of sorafenib was slightly and non-significantly decreased as 10.77% in co-administrated rats with sorafenib 50 mg/kg and GJD 100 mg/kg (3.60±1.34 hr) as compared with sorafenib single treated rats (3.25±0.50 hr) after end of first 3.5 hr-interval co-administration of sorafenib 50 mg/kg with GJD 100 mg/kg. However, the $T_{max}$ of sorafenib was slightly and non-significantly increased as 50.00% in 3.5 hr-intervals

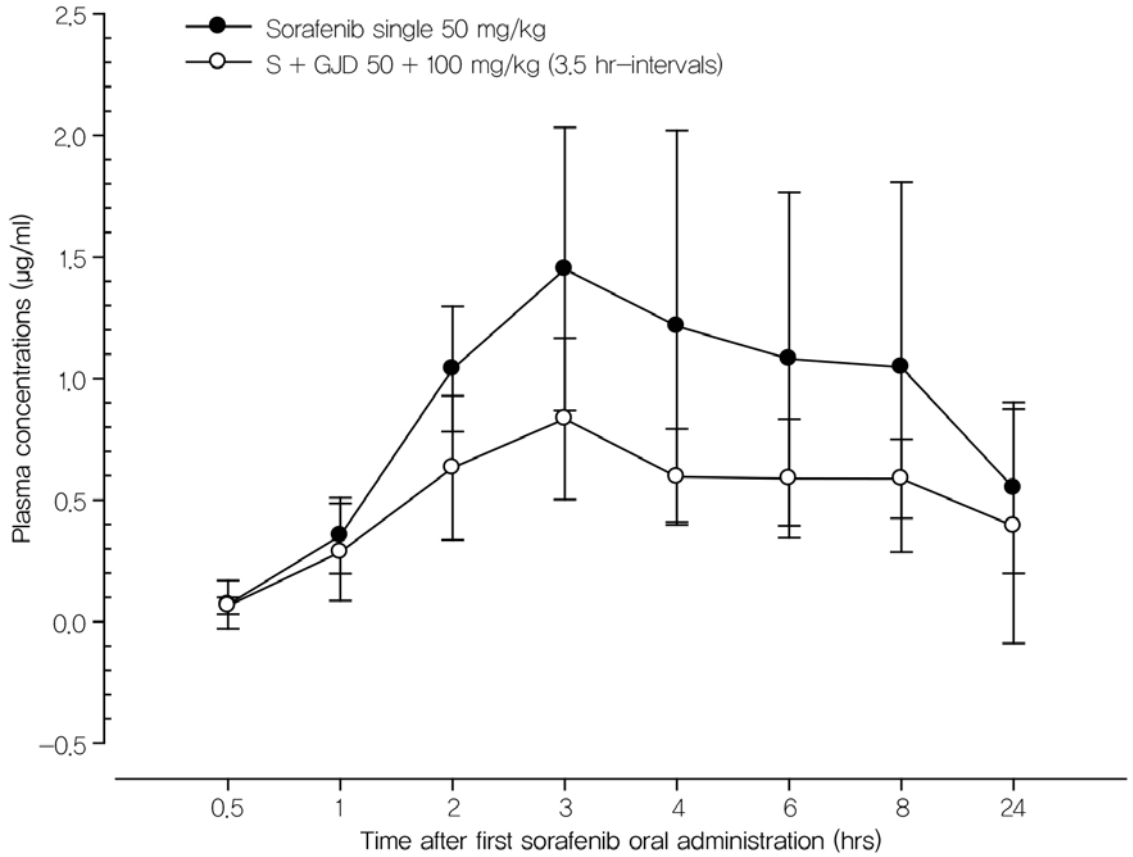


Fig 2. Plasma concentrations of Sorafenib with and without GJD after first co-administration with 3.5 hr-intervals in male rats. Sorafenib was detected from 30 min to 24 hrs after end of administration in the both sorafenib single and co-administered rats with GJD after first co-administration, respectively. GJD non-significantly inhibited the absorption of sorafenib from 1 hr to 24 hrs after first 3.5 hr-interval co-administration of sorafenib 50 mg/kg with GJD 100 mg/kg as compared with sorafenib single treated rats, in the present study. Values are expressed as mean ± SD of five or four rats (μg/ml). S; sorafenib, GJD; Gongjindan aqueous suspensions.

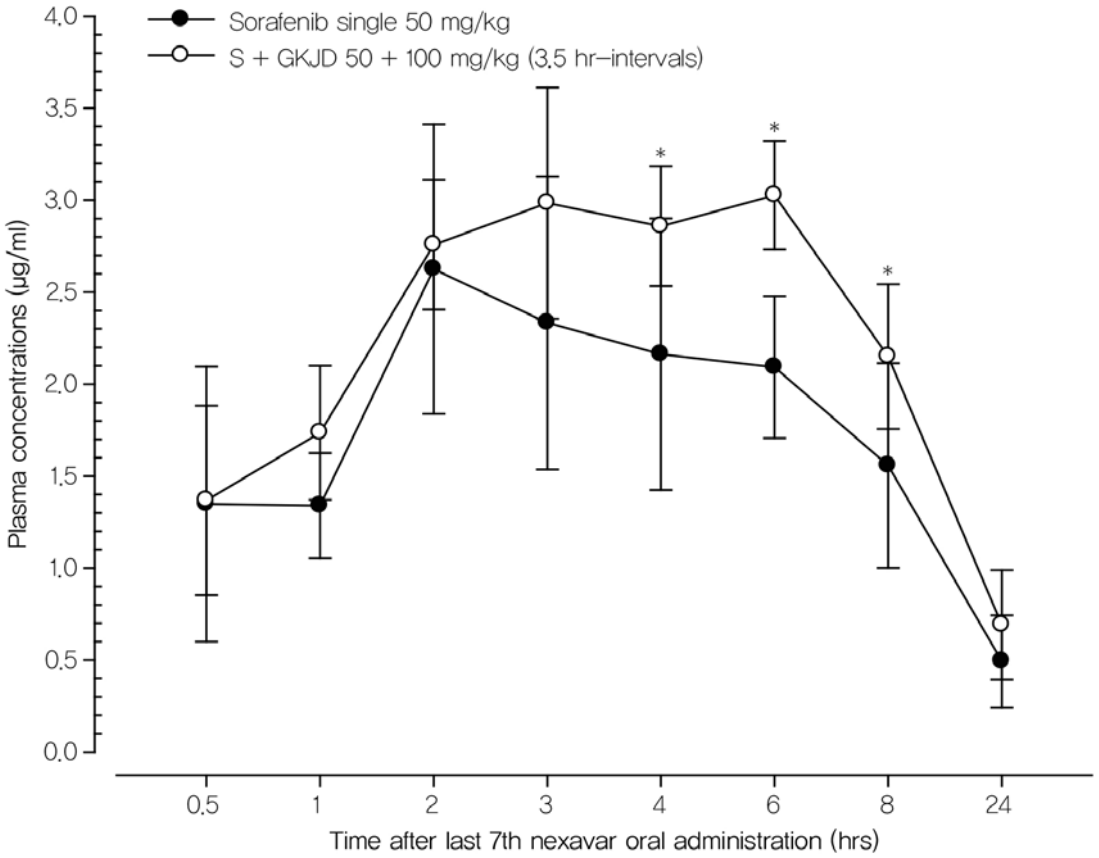


Fig 3. Plasma concentrations of Sorafenib with and without GJD after last 7th co-administration with 3.5 hr-intervals in male rats. Sorafenib was detected from 30 min to 24 hrs after end of administration in the both sorafenib single and co-administered rats with GJD after last 7th co-administration, respectively. GJD significantly ($p<0.05$) increased the absorption of sorafenib from 4 to 8 hrs after last 7th 3.5 hr-interval co-administration of sorafenib 50 mg/kg with GJD 100 mg/kg as compared with sorafenib single treated rats with non-significant but marked increased of plasma sorafenib concentrations at 1, 3 and 24 hrs after end of last 7th 3.5 hr-interval co-administration in GJD and sorafenib co-administered rats, in the present study. Values are expressed as mean ± SD of five or four rats (μg/ml). S; sorafenib, GJD; Gongjindan aqueous suspensions. * $p<0.05$ as compared with sorafenib single treated rats by MW test

co-administrated rats with sorafenib and GJD (3.00±0.71 hr) as compared with sorafenib single treated rats (2.00±0.00 hr) after end of last 7th 3.5 hr-interval co-administration of sorafenib 50 mg/kg with GJD 100 mg/kg, in the present study (Table 3 and 4).

### 4. Changes on the Cmax of sorafenib

The $C_{max}$ of sorafenib was non-significantly but markedly decreased as -43.27% in co-administrated rats with sorafenib 50 mg/kg and GJD 100 mg/kg (0.84±0.33 μg/ml) as compared with sorafenib single treated rats (1.48±0.63 μg/ml) after end of first 3.5 hr-interval co-administration. However, the $C_{max}$ of sorafenib was non-significantly increased as 21.83% in GJD co-administrated rats (3.20±0.32 μg/ml) as compared with sorafenib single treated rats (2.63±0.79 μg/ml) after end of last 7th 3.5 hr-interval co-administration, in the present study (Table 3 and 4).

**Table 3. Pharmacokinetic parameters of Sorafenib with or without Gongjindan after first co-administration with 3.5 hr-intervals in male rats**

| Parameters | Sorafenib (50 mg/kg) | |
|---|---|---|
| | Without Gongjindan co-administration (Distilled water) | With Gongjindan co-administration (100 mg/kg) |
| $T_{max}$(hrs) | 3.25 ± 0.50 | 3.60 ± 1.34 |
| $C_{max}$(μg/ml) | 1.48 ± 0.63 | 0.84 ± 0.33 |
| $AUC_{0-t}$(hr・μg/ml) | 20.59 ± 12.83 | 9.00 ± 2.90* |
| $AUC_{0-inf}$(hr・μg/ml) | 37.63 ± 21.25 | 12.53 ± 3.17 |
| $t_{1/2}$(hr) | 19.57 ± 11.86 | 10.39 ± 4.07 |
| $MRT_{inf}$(hr) | 29.42 ± 18.68 | 15.29 ± 4.93 |

Values are expressed as mean ± SD of five or four rats
$C_{max}$ : The peak plasma concentration
$T_{max}$ : 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
$MRT_{inf}$ : mean residence to time infinity
* $p<0.05$ as compared with sorafenib single treated rats by MW test

**Table 4. Pharmacokinetic parameters of Sorafenib with or without Gongjindan after last 7th co-administration with 3.5 hr-intervals in male rats**

| Parameters | Sorafenib (50 mg/kg) | |
|---|---|---|
| | Without Gongjindan co-administration (Distilled water) | With Gongjindan co-administration (100 mg/kg) |
| $T_{max}$(hrs) | 2.00 ± 0.00 | 3.00 ± 0.71 |
| $C_{max}$(μg/ml) | 2.63 ± 0.79 | 3.20 ± 0.32 |
| $AUC_{0-t}$(hr・μg/ml) | 32.03 ± 10.01 | 42.95 ± 6.60 |
| $AUC_{0-inf}$(hr・μg/ml) | 39.04 ± 14.92 | 53.61 ± 12.95 |
| $t_{1/2}$(hr) | 9.10 ± 2.07 | 9.66 ± 3.04 |
| $MRT_{inf}$(hr) | 12.86 ± 2.74 | 13.95 ± 4.47 |

Values are expressed as mean ± SD of five or four rats
$C_{max}$ : The peak plasma concentration
$T_{max}$ : 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
$MRT_{inf}$ : mean residence to time infinity

## 5. Changes on the AUC of sorafenib

The $AUC_{0-t}$ of sorafenib was significantly ($p<0.05$) decreased as −56.26% in co-administrated rats with sorafenib 50 mg/kg and GJD 100 mg/kg (9.00±2.90 hr・μg/ml) as compared with sorafenib single treated rats (20.59± 12.83 hr・μg/ml) after end of first 3.5 hr-interval co-administration, and the $AUC_{0-inf}$ of sorafenib was also non-significantly but markedly decreased as −66.70% in co-administrated rats with sorafenib 50 mg/kg and GJD 100 mg/kg (12.53±3.17 hr・μg/ml) as compared with sorafenib single treated rats (37.63±21.25 hr・μg/ml) after end of first 3.5

hr-interval co-administration. However, the $AUC_{0-t}$ and $AUC_{0-inf}$ of sorafenib were markedly increased as 34.08 and 37.31% in co-administrated rats with sorafenib and GJD (42.95±6.60 and 53.61 ±12.95 hr·μg/ml) as compared with sorafenib single treated rats (32.03±10.01 and 39.04±14.92 hr·μg/ml) after end of last 7th 3.5 hr-interval co-administration, in the present study (Table 3 and 4).

### 6. Changes on the $t_{1/2}$ of sorafenib

The $t_{1/2}$ of sorafenib was markedly but non-significantly increased as -46.89% in co-administrated rats with sorafenib 50 mg/kg and GJD 100 mg/kg (10.39±4.07 hr) as compared with sorafenib single treated rats (19.57±11.86 hr) after end of first 3.5 hr-interval co-administration. However, the $t_{1/2}$ of sorafenib was also slightly and non-significantly increased as 6.12% in co-administrated rats with sorafenib and GJD (9.66±3.04 hr) as compared with sorafenib single treated rats (9.10±2.07 hr) after end of last 7th 3.5 hr-interval co-administration, in the present study (Table 3 and 4).

### 7. Changes on the $MRT_{inf}$ of sorafenib

The $MRT_{inf}$ of sorafenib was non-significantly and dramatically decreased as -48.02% in co-administrated rats with sorafenib 50 mg/kg and GJD 100 mg/kg (15.29±4.93 hr) as compared with sorafenib single treated rats (29.42 ±18.68 hr) after end of first 3.5 hr-interval co-administration. However, the $MRT_{inf}$ of sorafenib was non-significantly changed as 8.51% in co-administrated rats with sorafenib and GJD (13.95± 4.47 hr) as compared with sorafenib single treated rats (12.86±2.74 hr) after end of last 7th 3.5 hr-interval co-administration, in the present study (Table 3 and 4).

## IV. Discussion

Although sorafenib is an antineoplastic agent approved for the treatment of primary kidney cancer (advanced renal cell carcinoma) and advanced primary liver cancer (hepatocellular carcinoma)[1] through inhibition of several tyrosine protein kinases and Raf kinases[2] and may offer a novel therapeutic strategy for non-responsive thyroid cancer[4,23], some kinds of lung cancer with squamous-cell histology[5,24] and recurrent glioblastoma[6,25], various side effects were also arise from sorafenib treated patients, especially include skin rash, hand-foot skin reactions, diarrhea, hypertension, reversible posterior leukoencephalopathy syndrome and erythrocytosis[1,7], and also hypersensitivity to sorafenib or any ingredient in the formulation were also known[8]. In addition, sorafenib also has been showed various drug-drug interactions with drugs affecting hepatic microsomal enzymes, metabolized by hepatic microsomal enzymes and uridine diphosphate-glucuronosyltransferase, like dexamethasone, ketoconazole, rifampin and doxorubicin[1,11,12]. Interactions with herbal products have not been established except for some restricted natural compounds, sorafenib increased risk of bleeding interact with warfarin[13] and single extracts, St. John's wort (*Hypericum perforatum*) possible decreased plasma sorafenib concentrations[1]. Gongjindan, a traditional Korean polyherbal formula is one of the most famous tonic agents and the hypolipidemic and immune stimulatory[14], anti-oxidative[15], anti-gliosis effects[16] and anti-dementia effects[17] of Gongjindan are relatively well documented. *Rehmanniae Radix Preparata* was traditionally used for nourishing Yin and tonifying the kidney and red ginseng was traditionally used for nourishing Qi. In recent study, *rehmanniae radix preparata* polysaccharides exerted the effect of anti-fatigue through the increase

of the storage of hepatic glycogen and the decrease of the accumulation of SUN and BLA[26], Korea red ginseng may have potential to protect liver damage[27]. Therefore GJD used in this study was added this 2 herbal medicines for the protection of liver damage against chemotherapy.

In the previous study[20], co-administration of GJD with sorafenib within 5 min showed marked increases of sorafenib oral bioavailability (47.02%) through augment the absorption, therefore, in the present study, the effects of GJD co-administration on the pharmacokinetics of sorafenib were observed after single and 7-day repeated oral co-administration with 3.5 hr-intervals (reasonable intervals considering the $T_{max}$ after sorafenib single oral administration detected in the previous study[20]) as a process of the comprehensive and integrative medicine, combination therapy of sorafenib with GJD to achieve synergic pharmacodynamics and reduce toxicity on hepatocellular cancers. After 50 mg/kg of sorafenib treatment, GJD 100 mg/kg was administered with 3.5 hr-intervals. The plasma were collected at 30 min before administration, 30 min, 1, 2, 3, 4, 6, 8 and 24 hrs after end of first and last 7th sorafenib treatment, and plasma concentrations of sorafenib were analyzed using LC-MS/MS methods. PK parameters of sorafenib ($T_{max}$, $C_{max}$, AUC, $t_{1/2}$ and $MRT_{inf}$) were analysis as compared with sorafenib single administered rats using noncompartmental pharmacokinetics data analyzer programs.

GJD markedly inhibited the absorption of sorafenib, from 1 hr to 24 hrs after end of first 3.5 hr-interval co-administration as compared with sorafenib single treated rats, and accordingly, the $C_{max}$ (-43.27%), $AUC_{0-t}$ (-56.29%) and $AUC_{0-inf}$ (-66.70%) of sorafenib in 3.5 hr-interval co-administered rats were dramatically decreased as compared with sorafenib single treated rats, contrary to the results of previous single co-administration study within 5 min[20]. However, GJD significantly ($p<0.05$) increased the absorption of sorafenib, from 4 hr to 8 hrs after end of last 7th 3.5 hr-interval co-administration as compared with sorafenib single treated rats, and accordingly, the $AUC_{0-t}$ (34.08%) and $AUC_{0-inf}$ (37.31%) of sorafenib in 3.5 hr-interval co-administered rats were dramatically increased as compared with sorafenib single treated rats, similar to the results of previous single co-administration study within 5 min[20]. These are mean GJD influenced on the pharmacokinetics of sorafenib, irregularly, and therefore, the co-administration of GJD and sorafenib should be avoided.

All rats used in this study, showed normal body weight increases ranged in normal age-matched rats regardless of treatment in the present study[20]. In addition, no meaningful changes on the body weight and gains were detected in GJD and sorafenib co-administered rats as compared with sorafenib single treated rats throughout experimental periods, in the present study.

Sorafenib was absorbed after oral administration with relative oral bioavailability of 38-49%[11]. High fat meals can be reduced bioavailability of sorafenib by about 29%[11]. Sorafenib showed relatively high 99.5% protein bindings[28]. $T_{max}$ of sorafenib after oral administration is approximately 3 hrs[29], and slowly eliminated by feces (77%) and urine (19%) with relatively long approximately 25-48 hr of $t_{1/2}$[30,31]. AUC in Japanese patients receiving sorafenib 400 mg twice daily reduced by 45% compared with data from phase 1 studies in Caucasian patients[32-34].

In the present study, $T_{max}$ of sorafenib in sorafenib single oral treated rats was detected as 3.25±0.50 hr after first 3.5 hr-interval co-administration, and $C_{max}$, $AUC_{0-t}$, $AUC_{0-inf}$, $t_{1/2}$ and $MRT_{inf}$ were detected as 1.48±0.63 μg/ml, 20.59±12.83 hr·μg/ml, 37.63±21.25 hr·μg/ml, 19.57±11.86 hr and 29.42±18.68 hr after first 3.5 hr-interval co-administration, respectively. In sorafenib with GJD co-administered rats, $T_{max}$,

$C_{max}$, $AUC_{0-t}$, $AUC_{0-inf}$, $t_{1/2}$ and $MRT_{inf}$ of sorafenib were detected as 3.60±1.34 hr, 0.84±0.33 μg/ml, 9.00±2.90 hr・μg/ml, 12.53±3.17 hr・μg/ml, 10.39 ±4.07 hr and 15.29± 4.93 hr; changed as 10.77, −43.27, −56.29, −66.70, −46.89 and −48.02% as compared with sorafenib 50 mg/kg single oral treated rats after first 3.5 hr−interval co−administration, respectively. Especially, the $AUC_{0-t}$ of sorafenib in sorafenib with GJD co−administered rats were significantly ($p<0.05$) decreased as compared with sorafenib single treated rats after first 3.5 hr−interval co−administration. In addition, $T_{max}$ of sorafenib in sorafenib single oral treated rats was detected as 2.00±0.00 hr after last 7th 3.5 hr−interval co−administration, and $C_{max}$, $AUC_{0-t}$, $AUC_{0-inf}$, $t_{1/2}$ and $MRT_{inf}$ were detected as 2.63 ±0.79 μg/ml, 32.03±10.01 hr・μg/ml, 39.04±14.92 hr・μg/ml, 9.10±2.07 hr and 12.86±2.74 hr after last 7th 3.5 hr−interval co−administration, respectively. In sorafenib with GJD co−administered rats, $T_{max}$, $C_{max}$, $AUC_{0-t}$, $AUC_{0-inf}$, $t_{1/2}$ and $MRT_{inf}$ of sorafenib were detected as 3.00±0.71 hr, 3.20 ±0.32 μg/ml, 42.95±6.60 hr・μg/ml, 53.61±12.95 hr・μg/ml, 9.66 ±3.04 hr and 13.95±4.47 hr; changed as 50.00, 21.83, 34.08, 37.31, 6.12 and 8.51% as compared with sorafenib 50 mg/kg single oral treated rats after last 7th 3.5 hr−interval co−administration. Increases of oral bioavailability of sorafenib were demonstrated after repeated oral co−administration of GJD and sorafenib with 3.5 hr−intervals, in the present study.

Sorafenib extensively metabolized mainly in the liver via oxidation by CYP3A4 and glucuronidation by UGT1A9, to 8 metabolites, including an active metabolite, a pyridine N−oxide derivative[35] and, therefore, sorafenib can be interacted with other drugs affecting hepatic microsomal enzymes, metabolized by hepatic microsomal enzymes and uridine diphosphate−glucuronosyltransferase, like dexamethasone, ketoconazole, rifampin and doxorubicin[1,11,12,36−38]. In addition, interactions with warfarin[13] and St. John's wort[1] were also already reported. Although GJD markedly inhibited the absorption of sorafenib, and decreased the $C_{max}$, $AUC_{0-t}$ and $AUC_{0-inf}$ of sorafenib after first 3.5 hr−interval co−administration, contrary to the results of previous single co−administration study within 5 min[20], GJD significantly ($p<0.05$) increased the absorption of sorafenib, and the $AUC_{0-t}$ and $AUC_{0-inf}$ of sorafenib after 7−day repeated 3.5 hr−interval co−administration, similar to the results of previous single co−administration study within 5min[20]. These are mean GJD influenced on the pharmacokinetics of sorafenib, irregularly, and therefore, the co−administration of GJD and sorafenib should be avoided because side effects of sorafenib including skin rash, hand−foot skin reactions, diarrhea, hypertension, reversible posterior leukoencephalopathy syndrome and erythrocytosis[1,7,39] and the incidence of the hypersensitivity to sorafenib[8,40,41] were directly related to the plasma concentrations. A limitation of this study is that the effects of GJD co−administration on the pharmacokinetics of sorafenib were observed in this study. However, GJD was added 2 more herbal medicines, Rehmanniae radix preparata and Korea red ginseng, in Gongjindan, therefore the pharmacokinetic profiles of sorafenib with GJD result from the present study shouldn't be generalized.

## V. Conclusions

Although GJD decreased the oral bioavailability of sorafenib through inhibition of gastrointestinal absorptions after end of first 3.5 hr−interval co−administration, it is observed that GJD increases the oral bioavailability of sorafenib as facilitated the absorption after end of last 7th repeated 3.5 hr−interval co−administration, quite similar as results of the previous 5 min co−administration study, based on the results of the present study. Hence, the co−administration of

GJD and sorafenib should be avoided in the comprehensive and integrative medicine, combination therapy of sorafenib with GJD on anticancer therapy. I and my colleagues strongly suggest that candidates for hepatic cancer patients in the comprehensive and integrative medicine should be changed from sorafenib or GJD.

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