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Effect of *Gongjindan*, a Polyherbal Formula on the Pharmacokinetics Profiles of Sorafenib in Male SD Rats (1)

- Single Oral Combination Treatment of Sorafenib 50mg/kg with *Gongjindan* 100mg/kg within 5min -

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Abstract

Objective : The co-administration effects of *Gongjindan* (GJD) on the pharmacokinetics (PK) of sorafenib were observed as a process of the comprehensive and integrative medicine.

Methods : After sorafenib treatment, GJD was administered within 5 min. The plasma were collected at 30min before administration, 30min, 1, 2, 3, 4, 6, 8 and 24hrs after end of GJD 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 : The absorption of sorafenib were significantly increased at 30min, 1, 6 and 6hrs after co-administration with GJD as compared with sorafenib single treated rats. Accordingly, the AUC_{0-t} (47.20%) of sorafenib was significantly increased but $t_{1/2}$ (-30.63%) and MRT_{inf} (-34.11%) in co-administered rats were non-significantly decreased. These findings are considered as direct evidences that GJD increased the oral bioavailability of sorafenib through increase of the absorption, when they co-administered within 5min.

Conclusion : Based on the results, co-administration of GJD increased the oral bioavailability of sorafenib through increase of the gastrointestinal absorption. It is considered that the more detail pharmacokinetic studies should be tested to conclude the effects of GJD on the pharmacokinetics of sorafenib, when they were co-administered, like the effects after co-administration with reasonable intervals considering the T_{max} of sorafenib (about 3.5hr-intervals) and after repeated co-administrations.Hence, concomitant uses of GJD with sorafenib may require close monitoring for potential drug interactions.

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Key words : Gongjindan, Pharmacokinetics, Drug-drug interactions, Rat, Sorafenib, Nexavar

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)[1,2]. It acts as antineoplastic agent through inhibition of several tyrosine protein kinases (VEGFR and PDGFR) and Raf kinases (more avidly C-Raf than B-Raf)[3,4]; Protein kinases are overactive in many of the molecular pathways that cause cells to become cancerous and these pathways include Raf kinase, platelet-derived growth factor (PDGF), Vascular endothelial growth factor(VEGF) receptor 2 and 3 kinases and c Kit the receptor for Stem cell factor[5-7]. Recently, the evidences that sorafenib also effective in non-responsive thyroid cancer[8,9], in some kinds of lung cancer with squamous-cell histology[10,11] and in recurrent glioblastoma[12,13] 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[1,14,15], and also hypersensitivity to sorafenib or any ingredient in the formulation were also known[16-18].

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[1,19,20]. 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, 16-18].

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[21,22] and oxaliplatin[23], 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, 24-28]. Interactions with herbal products have not been established except for some restricted natural compounds, sorafenib increased risk of bleeding interact with warfarin[29] 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 consisted of 4 herbs including *Angelicae gigas* radix, Ginseng steamed red, Corni fructus and Rehmanniae radix 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[30,31] with anti-oxidative effects[32], anti-gliosis effects on middle cerebral artery occlusion rats[33] and anti-dementia effects[34,35]. In addition, single oral dose toxicity[36] and micronucleus test[37] of Gongjindan itself were already reported.

In the present study, the effects of GJD co-administration on the pharmacokinetics of sorafenib were observed as a process of the comprehensive and integrative medicine.

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 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 treatment, and further fasted during 3 hrs after end of treatment. All laboratory animals were treated according to the national regulations of the usage and welfare of laboratory animals, and approved by the Institutional Animal Care and Use Committee in Daegu Haany University (Gyeongsan, Gyeongbuk, Korea) prior to animal experiment.

2. Test articles and formulation

GJD, prepared and purchase from Daegu Oriental Hospitals, Daegu Haany University (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 (upto 20mg/ml suspensions in GJD and up to 10mg/mlsolutions in sorafenib) in distilled water as vehicle, respectively.

3. Groupings and administration

Five rats per group (two groups; A: Sorafenib 50mg/kg single orally administered group, B: Sorafenib 50mg/kg and GJD 100mg/kg orally co-administered group) were used in this study as follows. The doses of test materials were selected based on their toxicity and pharmacodynamics. After 50mg/kg of sorafenib treatment, GJD 100mg/kg was administered, within 5min. In sorafenib single treated rats, 50mg/kg of sorafenib was orally administered and after 5 min later, only distilled water 5ml/kg was orally administered, instead of GJD suspensions. Each sorafenib or GJD was single orally administered, in a volume of 5ml/kg, dissolved in distilled water.

4. 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 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.

5. Sample preparation and calibrations

Primary stock solution, 1.0mg/ml of sorafenib in 90% acetonitrile (Sigma, MO, USA) mixtures with distilled water 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 (10μl) were directly transferred to injection vials and the aliquot was injected into the LC−MS/MS system.

6. 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 (2.1×50mm, 3.5μm) (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 0.35ml/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℃. 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: 2.4min), 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 1ng/ml.

7. Pharmacokinetic analysis

The plasma concentration data were analyzed using a noncompartmental method on commercial pharmacokinetics data analyzer programs (PK solutions2.0; Summit, CO, USA)[38,39]. The elimination rate constant (Kel) 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/Kel. 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[40]. The AUC zero to infinity (AUC_{0-inf}) was obtained by adding AUC_{0-t} and the extrapolated area was determined by C_{last}/Kel. The mean residence time infinity (MRT_{inf}) was calculated by dividing the first moment of AUC ($AUMC_{0-inf}$) by AUC_{0-inf}.

8. Statistical analyses

All the means are presented with their standard deviation of five rats (Mean ± SD of five rat plasma concentrations of sorafenib). 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 plasma concentrations of sorafenib

Sorafenib was detected from 30min to 24hrs after end of administration in the both sorafenib single and co-administered with GJD, respectively. GJD significantly ($p<0.01$ or $p<0.05$) increased the absorption of sorafenib at 30, 1, 6 and 8hrs after co-administration of sorafenib 50mg/kg and GJD 100mg/kg as compared with sorafenib single treated rats, and the absorption of sorafenib were also non-significant but markedly increased at 2, 3 and 4hrs after co-administration as compared with sorafenib single treated rats, in the present study(Fig 1).The plasma sorafenib concentrations at 30min, 1, 2, 3, 4, 6, 8 and 24hrs after end of treatment were changed as 423.38, 278.43, 53.04, 21.57, 21.30, 65.17, 67.18 and 3.11% in sorafenib + GJD treated rats as compared with sorafenib single treated rats, respectively.

2. Changes on the T_{max} of sorafenib

The T_{max} of sorafenib were non-significantly and slightly decreased as −6.67% in co-administrated with sorafenib 50mg/kg and GJD 100mg/kg

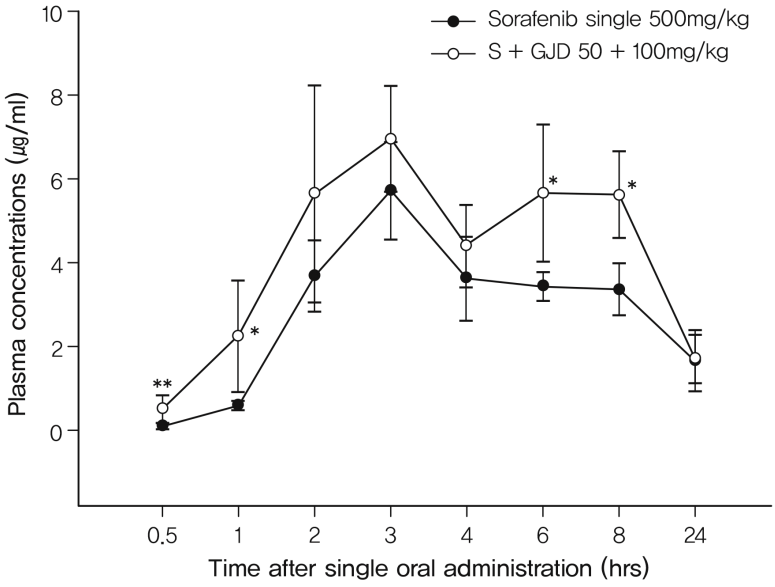

Figure 1. Plasma concentrations of sorafenib with and without GJD co-administration in male rats. Sorafenib was detected from 30min to 24hrs after end of administration in the both sorafenib singleand co-administered rats with GJD, respectively. GJD significantly ($p<0.01$ or $p<0.05$) increased the absorption of sorafenib at 30, 1, 6 and 8hrs after co-administration of sorafenib 50mg/kg and GJD 100mg/kg as compared with sorafenib single treated rats, and the absorption of sorafenib were also non-significant but markedly increased at 2, 4 and 6hrs after co-administration as compared with sorafenib single treated rats. Values are expressed as mean ±SD of five rats (μg/ml). S sorafenib, GJD Gongjindan aqueous suspensions (Test material). * $p<0.01$ and ** $p<0.05$ as compared with sorafenib single treated rats by MW test

Table 1. Pharmacokinetic parameters of sorafenib with and without GJD co-administration in male rats

Parameters	Sorafenib (50mg/kg)	
	Without GJD co-administration (Distill water)	With GJD co-administration (100mg/kg)
T_{max} (hrs)	3.00 ± 0.00	2.80 ± 0.45
C_{max} (μg/ml)	5.72 ± 1.17	7.21 ± 1.68
AUC_{0-t} (hr・μg/ml)	65.70 ± 10.78	96.71 ± 21.82*
AUC_{0-inf} (hr・μg/ml)	111.14 ± 38.43	126.72 ± 32.77
$t_{1/2}$ (hr)	16.85 ± 7.03	11.69 ± 2.88
MRT_{inf} (hr)	24.94 ± 11.17	16.43 ± 4.14

Values are expressed as mean ± SD of five rats. GJD Gongjindan aqueous suspensions (Test material), C_{max} The peak plasma concentration, T_{max} Time to reach C_{max}, 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

(2.80±0.45hr) as compared with sorafenib single treated (3.00±0.00hr) (Table 1).

3. Changes on the C_{max} of sorafenib

The C_{max} of sorafenib were non-significantly increased as 25.97% in co-administrated with sorafenib 50mg/kg and GJD 100mg/kg (7.21±1.68μg/ml) as compared with sorafenib single treated rats (5.72±1.17μg/ml), in the present study(Table 1).

4. Changes on the AUC of sorafenib

The AUC0-t of sorafenib were significantly ($p<0.05$) increased as 47.02% in co-administrated rats with sorafenib 50mg/kg and GJD 100mg/kg (96.71±21.82hr・μg/ml) as compared with sorafenib single treated rats (65.70±10.78hr・μg/ml). In addition, AUC_{0-inf} of sorafenib were also non-significantly increased as 14.02% in co-administrated rats with sorafenib and GJD (126.72±32.77hr・μg/ml) as compared with sorafenib single treated rats (111.14±38.43hr・μg/ml), in the present study(Table 1).

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

The $t_{1/2}$ of sorafenib were markedly but non-significantly decreased as −30.63% in co-administrated rats with sorafenib 50mg/kg and GJD 100mg/kg (11.69±2.88hr) as compared with sorafenib single treated rats (16.85±7.03hr), in the present study(Table 1).

6. Changes on the MRT_{inf} of sorafenib

The MRT_{inf} of sorafenib were markedly but non-significantly increased as −34.11% in co-administrated rats with sorafenib 50mg/kg and GJD 100mg/kg (16.43±4.14hr) as compared with sorafenib single treated rats (24.94±11.17hr), in the present study(Table 1).

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)[1,2] through inhibition of several tyrosine protein kinases and Raf kinases[3,4] and may

offer a novel therapeutic strategy for non-responsive thyroid cancer[8,9], some kinds of lung cancer with squamous-cell histology [10,11] and recurrent glioblastoma [12,13], various side effects were also arise from sorafenib treated patients, especially include skin rash, hand-foot skin reactions, diarrhea, hypertension, reversible posterior leukoencephalo-pathy syndrome and erythrocytosis[1,14,15], and also hypersensitivity to sorafenib or any ingredient in the formulation were also known[16-18]. 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, 24-28]. Interactions with herbal products have not been established except for some restricted natural compounds, sorafenib increased risk of bleeding interact with warfarin[29] and single extracts, St. John's wort (*Hypericum perforatum*) possible decreased plasma sorafenib concentrations[1]. GJD, 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 Rehmanniae radix preparata, and 2 animal resources - antler and musk. These 6 agents were plastered using honey, and coated by gold plates. In the present study, the effects of GJD co-administration on the pharmacokinetics of sorafenib were observed as a process of the comprehensive and integrative medicine, combination therapy of sorafenib with GJD to achieve synergic pharmacodynamics and reduce toxicity. After 50mg/kg of sorafenib treatment, GJD 100mg/kg was administered within 5min. The plasma were collected at 30min before administration, 30min, 1, 2, 3, 4, 6, 8 and 24hrs after end of GJD 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.

GJD markedly increased the absorption of sorafenib (from 0.5hr after co-administration of sorafenib 50mg/kg and GJD 100mg/kg) as compared with sorafenib single treated rats, the absorption of sorafenib were significantly ($p<0.01$ or $p<0.05$) increased at 30min, 1, 6 and 8hrs after co-administration as compared with sorafenib single treated rats. Accordingly, the AUC_{0-t} (47.20%) of sorafenib was significantly ($p<0.05$) increased but $t_{1/2}$ (-30.63%) and MRT_{inf} (-34.11%) in co-administered rats were non-significantly decreased as compared with sorafenib single treated rats, respectively. These findings are considered as direct evidences that GJD increased the oral bioavailability of sorafenib through increase of the absorption, when they co-administered within 5min.

Sorafenib was absorbed after oral administration with relative oral bioavailability of 38-49%[26,41]. High fat meals can be reduced bioavailability of sorafenib by about 29%[26]. Sorafenib showed relatively high 99.5% protein bindings[42,43]. Tmax of sorafenib after oral administration is approximately 3hrs[44,45], and slowly eliminated by feces (77%) and urine (19%) with relatively long approximately 25-48hr of $t_{1/2}$[46,47]. AUC in Japanese patients receiving sorafenib 400mg twice daily reduced by 45% compared with data from phase 1 studies in Caucasian patients [48-50].

In the present study, T_{max} of sorafenib in sorafenib single oral treated rats was detected as 3.00±0.00hr, and C_{max}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and MRT_{inf} were detected as 5.72±1.17μg, 65.70± 10.78hr·μg/ml, 111.14±38.43hr·μg/ml, 16.85±7.03hr and 24.94±11.17hr, 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 2.80±0.45hr, 7.21±1.68μg, 96.71±21.82hr·μg/ml, 126.72±32.77hr·

μg/ml, 11.69±2.88hr and 16.43±4.14hr; changed as −6.67, 25.97, 47.20, 14.02, −30.63 and −34.11% as compared with sorafenib 50mg/kg single oral treated rats, respectively. Especially, AUC_{0-t} of sorafenib in co-administered rats were significantly ($p<0.05$) increased as compared with sorafenib single treated rats.

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[51] 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, 24-28]. In addition, interactions with wafarin[29] and St. John's wort[1] were also already reported. In the present study, co-administration of GJD with sorafenib within 5min induced marked increases of oral bioavailability of sorafenib through the increases of absorption. It, therefore, considered that co-administration with GJD can be increased the pharmacodynamics of sorafenib because toxicity or pharmacodynamics of sorafenib were directly related to the plasma concentrations. However, it also obvious evidences that the toxicity or side effects of sorafenib, including skin rash, hand-foot skin reactions, diarrhea, hypertension, reversible posterior leukoencephalopathy syndrome and erythrocytosis[1,14,15] with the incidence of the hypersensitivity to sorafenib[16-18] may be increased. More detail pharmacokinetic studies should be tested to select optimal dosing regimens and to observe the possibilities that can be used as comprehensive and integrative therapy with GJD and sorafenib for hepatic cancer, when they were co-administered, like the effects after co-administration with reasonable intervals considering the T_{max} of sorafenib (about 3.5hr-intervals, in my opinion) and after repeated co-administrations.

V. Conclusions

Based on the results, co-administration of GJD increased the oral bioavailability of sorafenib through increase of the gastrointestinal absorption. Hence, concomitant uses of GJD with sorafenib may require close monitoring for potential drug interactions. Co-administration of GJD significantly increased AUC_{0-t}, but marked decreased $t_{1/2}$ and MRT_{inf} of sorafenib as compared with sorafenib single oral treatment in rats. It, therefore, is considered that the more detail pharmacokinetic studies should be tested to conclude the effects of GJD on the pharmacokinetics of sorafenib, when they were co-administered, like the effects after co-administration with reasonable intervals considering the T_{max} of sorafenib (about 3.5hr-intervals) and after repeated co-administrations.

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