

Effects of Yukmijihwang-tang, a Polyherb, on the Pharmacokinetics of Metformin

Seok-Bong Kang^{1#}, Ho-Sang Shon^{2#}, Soo-Jin Park^{3#}, Young Joon Lee⁴,
Sae-Kwang Ku^{3*} and Chang-Hyun Song^{3*}

¹Department of Nephrology, College of Korean Medicine, Daegu Haany University, Gyeongsan, Republic of Korea

²Department of Internal Medicine, Catholic University of Daegu, School of Medicine, Daegu, Republic of Korea

³Department of Anatomy and Histology, College of Korean Medicine, Daegu Haany University, Gyeongsan, Republic of Korea

⁴Department of Preventive Medicine, College of Korean Medicine, Daegu Haany University, Gyeongsan, Republic of Korea

Abstract: Metformin is one of the most common medicines for the treatment of type 2 diabetes, however, recent studies suggest that concomitant antihyperglycemic agents should be administered for better efficacy. Yukmijihwang-tang (YMJHT) is a nephroprotective polyherb prescribed for renal disorders or diabetic mellitus in traditional Korean medicine. Therefore, the pharmacokinetics between metformin and YMJHT were examined for their coadministration. Rats were orally coadministered with metformin and YMJHT as a combination group or metformin and distilled water as the corresponding control. Then, the metformin concentration in plasma and its pharmacokinetic parameters including maximum concentration (C_{max}) and area under the plasma concentration time curve (AUC) were analyzed. There were no interactions between metformin and YMJHT in the single coadministration at intervals within 5 min. However, pretreatments with YMJHT for 6 days increased the metformin concentration and its C_{max} and AUC ($p < 0.05$). The repeated coadministration for 8 days increased the C_{max} of metformin ($p < 0.05$). Conversely, when the combination was coadministered at 2h-intervals, there were no interactions between metformin and YMJHT after a single dosing or repeated dosing of coadministration for 7 days. These results of the present study will help structure proper dosing regimens for the concomitant therapy of metformin and YMJHT.

Keywords: Interactions, metformin, pharmacokinetics, polyherb, Yukmijihwang-tang.

INTRODUCTION

Type 2 diabetes mellitus is a complex metabolic disorder characterized by elevated blood glucose levels, altered secretion of insulin and insulin resistance (DeFronzo, 2009). Metformin is a first-line oral medicine for the treatment of type 2 diabetes, particularly in obese people (Nasri and Rafieian-Kopaei, 2014). Although it has the risk of increasing lactic acidosis and some gastrointestinal adverse effects (Pasquel *et al.*, 2015; Tahrani *et al.*, 2011), the drug is widely prescribed as an antidiabetic medicine (Rhee *et al.*, 2017). Furthermore, the clinical applications are increasing more since it has been prescribed to treat polycystic ovary syndrome, nonalcoholic fatty liver disease and cancer as well (Nasri and Rafieian-Kopaei, 2014; Rouabhia *et al.*, 2014).

The pharmacological efficacy of metformin is related to improve hyperglycemia by suppressing glucose production in the liver and its absorption in the gastrointestinal tract and enhancing peripheral glucose uptake and insulin sensitivity (Bailey and Turner, 1996; Towler and Hardie, 2007). However, owing to the progressive functional loss of pancreatic β -cells caused by the insulin-independent mechanism of metformin, concomitant therapy with another antihyperglycemic

agent is required (Haak, 2015). Based on their different glucose-lowering mechanisms, many compounds have been reported as potential candidates, however, most treatments have shown adverse effects such as hypoglycemia (e.g., sulfonylureas, amylin analogue, and insulin), weight gains (e.g., sulfonylureas, insulin, and thiazolidinediones), gastrointestinal disturbances (e.g., α -glucosidase inhibitors), genital and urinary tract infections (e.g., sodium/glucose cotransporter 2: SGLT2 inhibitors), and peripheral edema and potential cardiovascular effects (e.g., thiazolidinediones) (Tahrani *et al.*, 2011). In addition, the beneficial effects of metformin on glycemia are not sustainable in the long term because the function of β -cell is progressively declining (Del Prato *et al.*, 2007). This suggests that targeting a single defect may be insufficient to provide better glycemic control or slow the disease progression. Indeed, there have been studies to improve the overall efficacy in combination therapies of metformin with dipeptidyl peptidase-4 inhibitors (eg. linagliptin) (Forst *et al.*, 2010) or SGLT2 inhibitors (e.g. henagliflozin) (Wang *et al.*, 2015).

Yukmijihwang-tang (YMJHT; Liuweidihuangtang in Chinese and Rokumijio-to in Japanese) is a traditional Korean medicine composed of 6 herbs (table 1) (Xie *et al.*,

*Corresponding authors e-mails: dvmsong@hotmail.com, gucci200@hanmail.net

#Contributed equally

2008), and it has been prescribed to treat diabetes, glomerulonephritis and retarded mental and physical development in children (Chen *et al.*, 1997). The YMJHT has been reported to have therapeutic potentials in experimental animal models of renal ischemia/reperfusion (Kang *et al.*, 2006a) and patients with diabetes (Song *et al.*, 2004; Pu *et al.*, 2013). It has shown various pharmacological effects involved in inhibiting inflammation, oxidative stresses, and adiponectin (Perry *et al.*, 2014; Lee *et al.*, 2005). It also has shown to improve memory (Kang *et al.*, 2006b), inhibit bone loss (Shim *et al.*, 2011) and protect against prostate hyperplasia (Shin *et al.*, 2012). We hypothesized that the nephroprotective and anti-diabetic effects of YMJHT may enhance the pharmacological properties of metformin as a comprehensive and integrative medicine, while reducing the side effects. For the combination therapy, the safety pharmacology or pharmacokinetic herb-drug interactions should be sufficiently monitored, but there are currently no studies on the pharmacokinetic interactions between metformin and YMJHT. Therefore, we performed comprehensive pharmacokinetic analyses between YMJHT and metformin.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (6-week-old, SLC Inc., Japan) were randomly allocated to polycarbonate cages (n=5 each) in a room controlled at 20-25°C temperature and humidity of 40-45%, with a 12h-light and 12h-dark cycle. Feed and water were provided ad libitum. All animal experiments were carried out according to the Guidelines for Care and Use of Laboratory Animals of the Daegu Haany University (Gyeongsan, Korea).

Reagents

Commercial YMJHT (Lot No.: 12003) was purchased from Korea INS Pharm. (Hwasoon, Korea). The contents of YMJHT are shown in table 1. Metformin was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Both drugs were dissolved in distilled water as a vehicle.

Experimental design

Rats were acclimatized for 2 weeks and divided into 2 groups of control and combination (n=5 per group), based on body weight. The combination group was orally coadministered with metformin and YMJHT, while the control group was coadministered with metformin and distilled water. The pharmacokinetic interactions were analyzed in 4 different protocols as follows: Single coadministration within 5 min between metformin and YMJHT or distilled water (Study I); the repeated coadministration for 8 days after either pretreatment with YMJHT in the combination group for 6 days or pretreatment with distilled water in the control (Study II); Single (Study III) or repeated (Study IV) dosing of the

coadministration for 7 days at 2 h-intervals between both administration. The doses of metformin and YMJHT were determined at 500mg/kg and 100mg/kg, respectively, by their toxicity and pharmacodynamics. The drugs were coadministered once a day in a volume of 5ml/kg. The body weight was measured at every administration. To avoid any influence of the diet, animals were fasted overnight prior to the pretreatment or the initial and last coadministration of the repeated dosing.

Blood collections

Blood samples for pharmacokinetic analyses were collected, as described previously (Kang *et al.*, 2014; Kim *et al.*, 2015). In brief, rats were slightly anesthetized with diethyl ether (Duksan Pure Chemical, Seoul, Korea), and blood via the orbital plexus was sampled at 0.5h before the coadministration (-0.5h) and 0.5, 1, 2, 3, 4, 6, 8 and 24h after the coadministration. The samples were collected into heparin (50 IU) containing tubes, and centrifuged for 10 min at 11,400g. The resultant clear plasma was stored at -80°C until the pharmacokinetic analyses.

Sample preparation and calibrations

Standard metformin (Sigma, St. Louis, MO, USA) was prepared at 1.0mg/ml in acetonitrile, and it was dose-dependently diluted for working standard solutions. Carbamazepine (Sigma) was used at 500ng/ml in acetonitrile as an internal standard (IS) solution. Metformin was calibrated with the dose-dependent working standard solutions and IS solution. Plasma sample was added with IS solution and centrifuged at 9,700g for 10 min at 4°C. The clear supernatants were subjected to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system.

LC-MS/MS conditions

Concentration of metformin was determined using an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of an on-line degasser, a binary pump, an autosampler and column compartment. The chromatographic separation was conducted using a Shiseido CAPCELL PAKTM C18 MGII (5µm, 2.0×150 mm) (Shiseido, Tokyo, Japan), at a mobile phase flow rate of 0.20ml/min. The mobile phase consisted of 60% acetonitrile and 40% distilled water containing 10mM NH₄OAc and 0.1% formic acid. The analytes were detected by an API 2000 triple-quadrupole mass-spectrometric detector (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray interface in the positive ion mode. The instrument was operated with 5.0 kV ion spray voltage, and nitrogen was used as 12 psi for nebulization, 6 psi for curtain and 8 psi for collision. The gas temperature was set at 375°C. The fragmentation transitions for the multiple-reaction monitoring were 130>71 (retention time: 1.3 min) for metformin and m/z 237>194 (retention time: 2.5 min) for the IS. It was

Table 1: Composition of herbs for Yukmijihwang-tang aqueous extracts.

Herbs	Scientific Names	Amounts
Rehmanniae Radix Preparat	<i>Rehmannia glutinosa</i> Liboschitz var. <i>purpurea</i> Makino	2g
Dioscoreae Rhizoma	<i>Dioscorea batatas</i> Decaisne	1g
Corni Fructus	<i>Cornus officinalis</i> Siebold et Zuccarini	1g
Hoelen	<i>Poria cocos</i> Wolf	1g
Moutan Cortex	<i>Paeonia suffruticosa</i> Andrews	1g
Alismatis Rhizoma	<i>Alisma orientale</i> Juzepczuk	1g
Total	6 types	7g

The commercial Yukmijihwang-tang was composed of 6 types of polyherbs (Korea INS Pharm., Hwasoon, Korea).

Table 2: Metformin pharmacokinetics in single coadministration at intervals within 5 min (Study I)

Parameters	Control (Metformin + distilled water)	Combination (Metformin + YMJHT)
T_{max} (h)	1.10±0.55	0.90±0.22
C_{max} (µg/ml)	25.82±3.06	23.24±3.83
AUC_{0-t} (µg h/ml)	147.63±24.37	156.57±27.06
AUC_{0-inf} (µg h/ml)	177.06±35.17	200.00±29.04
$t_{1/2}$ (h)	9.23±3.23	10.56±1.73
MRT_{inf} (h)	11.35±5.18	14.44±2.65

Values are expressed as means ± SD in 5 sample sizes.

Table 3: Metformin pharmacokinetics in 8 day-repeated coadministration at intervals within 5 min after 6 day-pretreatments (Study II).

Parameters	Control (Metformin+distilled water)	Combination (Metformin+YMJHT)
<i>After first coadministration</i>		
T_{max} (h)	0.90±0.22	0.90±0.22
C_{max} (µg/ml)	20.20±3.42	34.80±9.04*
AUC_{0-t} (µg h/ml)	126.22±24.71	175.50±18.37*
AUC_{0-inf} (µg h/ml)	159.94±26.47	213.16±32.84*
$t_{1/2}$ (h)	10.72±3.37	9.97±1.28
MRT_{inf} (h)	13.99±4.80	11.71±2.80
<i>After last coadministration</i>		
T_{max} (h)	3.00±1.00	2.38±2.50
C_{max} (µg/ml)	42.20±10.57	66.75±30.74*
AUC_{0-t} (µg h/ml)	425.14±225.60	531.99±181.63
AUC_{0-inf} (µg h/ml)	499.36±211.07	612.11±155.90
$t_{1/2}$ (h)	9.63±4.95	8.59±4.09
MRT_{inf} (h)	12.61±5.86	11.44±6.02

Values are expressed as means ± SD in 5 sample sizes. * $p < 0.05$, by MW tests vs control.

Rats received repeated oral coadministration of control (metformin + distilled water, DW) or combination (metformin + Yukmijihwang-tang, YMJHT). (A) Combination group was pretreated with YMJHT for 6 days, followed by repeated coadministration of combination for 8 days at intervals within 5 min, while the corresponding control group was pretreated with DW, followed by repeated coadministration of control (Study II). (B) The coadministration was repeated for 7 days at 2 h-intervals (Study IV). Values are expressed as means ± SD (g) of five rats.

controlled by the Analyst version 1.4.1 software (Applied Biosystems). The calibration curve for metformin was linear over the ranges studied with an $r^2 > 0.999$ and the lower quantification limit was 100ng/ml.

Pharmacokinetic analysis

Pharmacokinetic analysis from the metformin concentration data was performed using PK solution 2.0 analyzer programs (Summit, CO, USA). Elimination rate constant (K_e) was calculated by the linear regression of the metformin concentration in the elimination phase, and the half-life ($t_{1/2}$) was calculated as $0.693/K_e$. The

maximum plasma concentration (C_{max}) of metformin and time of the maximal concentration (T_{max}) were estimated by visual inspection of the concentration data. Area under the plasma concentration-time curve from 0h to the time of the last quantifiable concentration (C_{last}) was calculated as AUC_{0-t} using the trapezoidal rule. The AUC from 0h to infinity (AUC_{0-inf}) was derived by adding the AUC_{0-t} and then extrapolating the area determined by the C_{last}/K_e . The mean residence time extrapolated to infinity (MRT_{inf}) was estimated by dividing area under the first moment curve of the AUC by the AUC_{0-inf} .

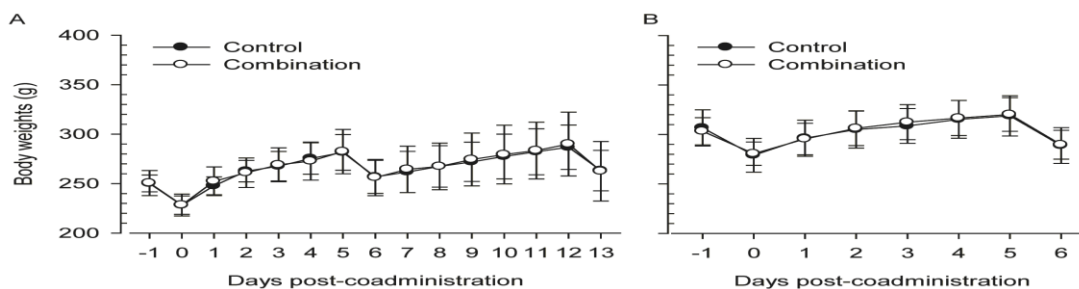


Fig. 1: Body weight changes in repeated coadministration of metformin with YMJHT.

Table 4: Metformin pharmacokinetics in single and 7 day-repeated coadministration at 2 h-intervals (Studies III and IV)

Parameters	Control (Metformin + distilled water)	Combination (Metformin + YMJHT)
<i>After single coadministration</i>		
T _{max} (h)	1.40±0.97	1.20±0.71
C _{max} (µg/ml)	24.90±3.70	25.30±7.63
AUC _{0-t} (µg h/ml)	169.41±53.54	182.01±75.71
AUC _{0-inf} (µg h/ml)	206.79±88.71	224.95±109.24
t _{1/2} (h)	9.61±3.58	9.76±3.59
MRT _{inf} (h)	10.89±5.14	11.56±5.47
<i>After repeated coadministration</i>		
T _{max} (h)	2.00±0.94	2.00±1.37
C _{max} (µg/ml)	30.20±3.11	27.80±3.27
AUC _{0-t} (µg h/ml)	190.47±32.44	199.24±61.78
AUC _{0-inf} (µg h/ml)	211.43±36.92	221.75±70.49
t _{1/2} (h)	8.30±2.27	8.09±2.14
MRT _{inf} (h)	8.68±2.66	8.81±2.62

Values are expressed as means ± SD in 5 sample sizes.

STATISTICAL ANALYSIS

Values are expressed as means ± the standard deviation (SD) in 5 sample sizes per group. The data for the plasma concentration of metformin and body weights were analyzed using a two-way analyses of variance (ANOVA) with main effects of the group. The time of blood samples or day when the body weight was measured were treated as repeated measurements. The pharmacokinetic parameters were examined by non-parametric comparison tests, followed by Mann-Whitney U (MW) tests. The statistical significance was defined as p<0.05.

RESULTS

Body weight changes

In the studies II and IV, no significant differences were found in the body weights between the control and combination (data not shown). Further, there were no meaningful changes in body weight gains after the pretreatment with YMJHT and the repeated administration of the combination at intervals within 5 min (Study II) or at 2 h-intervals (Study IV) (fig. 1).

Effect of YMJHT on pharmacokinetic profiles of metformin

Study I. Single dosing of coadministration at intervals within 5 min

Metformin was detected in plasma at 0.5 to 24 h after the coadministration in both the control and combination groups, but there were no significant changes in the plasma concentration of metformin (fig. 2A). In addition, the pharmacokinetic parameters between both groups showed no significant differences (table 2).

Study II. Repeated dosing of coadministration for 8 days at intervals within 5 min after pretreatment with YMJHT for 6 days.

The pharmacokinetics of metformin in combination with YMJHT was analyzed in 2 phases: after repeated pretreatments and after repeated coadministration. In the first coadministration after the pretreatments, the two-way ANOVA for the plasma concentration of metformin showed significant main effects for the group (p<0.01) and time (p<0.01) (fig. 2B). There were also significant interactions between both main effects of group and time (p<0.05). The post hoc revealed increases in the concentration of metformin at 0.5 to 3h post-coadministration (p<0.05). After repeated coadministration, metformin was found at -0.5 to 24h post-coadministration (fig. 2C). There were significant main effects of time (F=17.6; p<0.01), representing natural time-dependent pharmacokinetics, but no main effects for the group. There were no significant interactions between the group and time, although metformin concentration seemed to be increased in the

combination compared with the control. The pharmacokinetic parameters of metformin, the C_{max} and AUC, significantly increased in the first combination after pretreatments with YMJHT, compared with the control ($p < 0.05$) (table 3). Furthermore, the C_{max} was significantly increased after repeated coadministration ($p < 0.05$).

Study III. Single dosing of coadministration at 2 h-intervals

Because pharmacokinetic interactions between metformin and YMJHT were observed in Study II, the YMJHT was administered 2h after metformin. The two-way ANOVA indicated no significant differences in the metformin concentration in plasma between the control and combination groups (fig. 3A). There were no significant differences in the pharmacokinetic parameters of metformin between both groups (table 4).

Study IV. Repeated dosing of coadministration for 7 days at 2 h-intervals

The pharmacokinetic interactions were further analyzed by repeated coadministration of the combination at 2 h-intervals. No significant differences were found in the plasma concentration of metformin and its pharmacokinetic parameters between the control and combination (fig. 3B and table 4).

DISCUSSION

Combination therapies using herbal medicines with synthetic drugs have been studied for the beneficial effects on certain diseases, however, some combination therapies can cause adverse reactions or interactions between the herb and drug can influence bioavailability, leading to adverse effects (Yang *et al.*, 2006). In the current study, no interactions between metformin and YMJHT were noticed when they were coadministered within 5 min of each other. However, at the intervals within 5 min, the coadministration of combination after pretreatments with YMJHT for 6 days increased the plasma concentration of metformin and the pharmacokinetic parameters (C_{max} and AUC), and the repeated coadministration of combination for 8 days also increased C_{max} . This suggests that YMJHT should be avoided prior to treatment with metformin or when coadministered with metformin at short intervals (within 5 min). When the coadministration interval was modified to 2h, the coadministration showed no interactions between metformin and YMJHT, after the single and even after the repeated coadministration of combination.

Metformin is absorbed slowly in the body, and clinical pharmacokinetic analyses showed the C_{max} within 1-3h and 50-60% oral bioavailability in fasting conditions (Gong *et al.*, 2012). It has negligible plasma protein binding, and it is easily excreted by tubular secretion, with very low metabolism in the body (Gong *et al.*, 2012; Scheen, 1996). Based on this context, the increased C_{max}

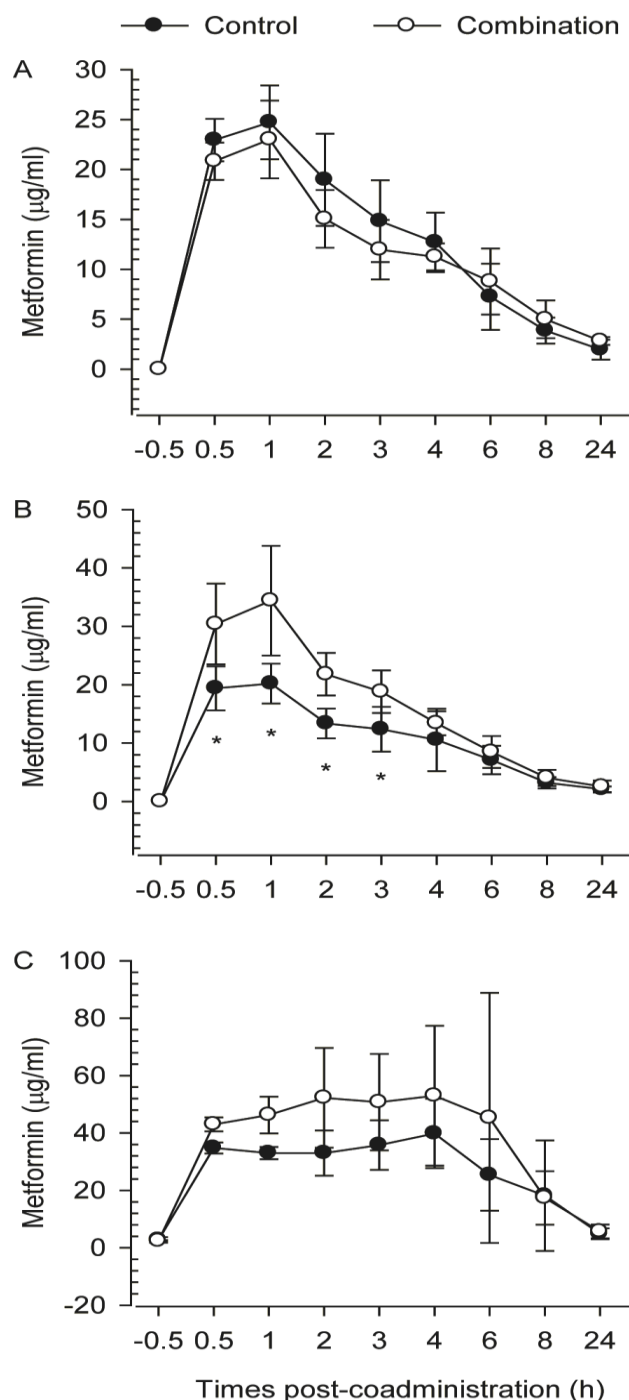


Fig. 2: Plasma concentrations of metformin in coadministration with YMJHT at intervals within 5 min.

Rats received oral coadministration of control (metformin + distilled water, DW) or combination (metformin + Yukmijihwang-tang, YMJHT) and the coadministration was performed at intervals within 5 min. The plasma concentration of metformin was analyzed after the single coadministration (Study I) (A), and the first (B) and last (C) coadministration of repeated treatments for 8 days (Study II). Values are expressed as means \pm SD ($\mu\text{g/ml}$) of five sample sizes. *: $p < 0.05$.

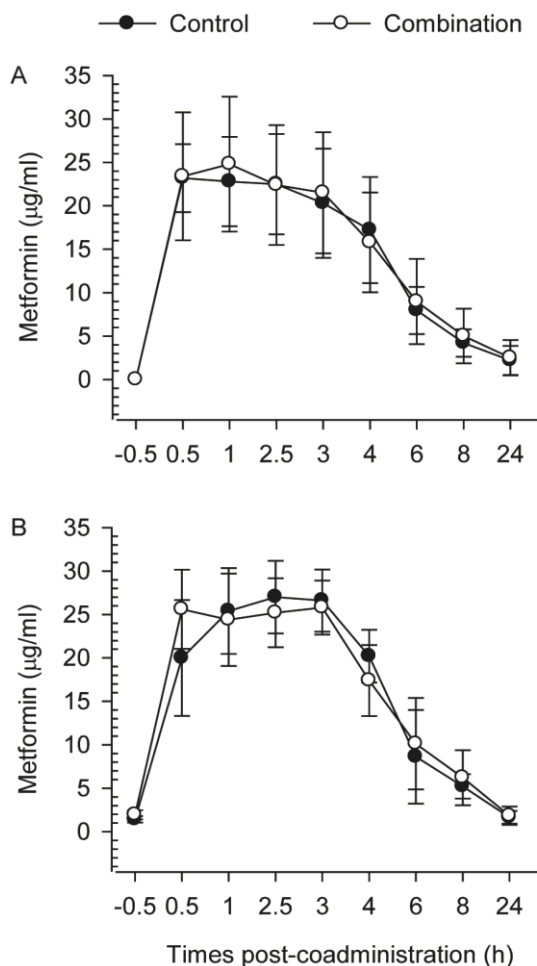


Fig. 3: Plasma concentrations of metformin in coadministration with YMJHT at 2 h-intervals.

Rats received oral coadministration of control (metformin + distilled water, DW) or combination (metformin + Yukmijihwang-tang, YMJHT), and the coadministration was performed at 2 h-intervals. The plasma concentration of metformin was analyzed after the single coadministration (Study III) (A), and repeated coadministration for 7 days (Study IV) (B). Values are expressed as means \pm SD ($\mu\text{g/ml}$) of five sample sizes.

of metformin or its AUC, observed in combination with YMJHT in Study II, represents the increased systemic exposure and bioavailability of metformin, which might be involved in the decreased elimination of metformin by delayed renal clearance through the organic cationic (positively charged) transporters (Graham *et al.*, 2011), rather than the influences in the absorption or metabolism. Similarly, there are various drug-drug interactions when metformin is coadministered with other drugs. For example, cimetidine, one of H_2 -receptor antagonists, increases the metformin concentration in plasma by inhibiting its renal clearance (Somogyi *et al.*, 1987). It is considered that the cationic form of cimetidine may interfere with the clearance of metformin by competing

with the same cationic transport mechanism for tubular secretion. Cefalexin is also known to increase metformin concentrations by inhibiting the clearance mechanism (Jayasagar *et al.*, 2002). Furthermore, increases in C_{max} or AUC have been reported in combination of metformin with itraconazole (Choi *et al.*, 2010), pyrimethamine (Kusuhara *et al.*, 2011) or telithromycin (Lee *et al.*, 2010), accompanying with reduced renal elimination or hepatic metabolism. This suggests that the coadministration of metformin with YMJHT at 2 h-intervals has a limited impact on the renal clearance; therefore, there are minimal pharmacokinetic interactions between metformin and YMJHT.

In long-term treatment of diabetes, proper dosing regimens for novel combination therapies require sufficient evidence from pharmacokinetic analyses before they can be considered safe. A life threatening condition of lactic acidosis is a well-recognized complication of biguanide therapy (Pasquel *et al.*, 2015). In the present study, the altered bioavailability of metformin after pretreatments with YMJHT or its repeated combination may cause unexpected adverse reactions as well as development of lactic acidosis. However, YMJHT is not considered to highly influence the excretion of metformin when coadministered at 2 h-intervals. These provide important information for further pharmacokinetic studies on proper dosing regimen for a comprehensive and integrative therapy in diabetes.

REFERENCES

- Bailey CJ and Turner RC (1996). Metformin. *N. Engl. J. Med.*, **334**: 574-579.
- Chen Y, Wei L, Ma M, Wu G, Zhang G and Wei Z (1997). Effect of jiawei shenqi dihuang tang on the content of urinary protein in patients with diabetic nephropathy. *J. Tradit. Chin. Med.*, **17**(3): 184-186.
- Choi YH, Lee U, Lee BK and Lee MG (2010). Pharmacokinetic interaction between itraconazole and metformin in rats: competitive inhibition of metabolism of each drug by each other via hepatic and intestinal CYP3A1/2. *Br. J. Pharmacol.*, **161**(4): 815-829.
- DeFronzo RA (2009). Banting Lecture. From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes*, **58**: 773-795.
- Del Prato S, Bianchi C and Marchetti P (2007). Beta-cell function and anti-diabetic pharmacotherapy. *Diabetes Metab. Res. Rev.*, **23**(7): 518-527.
- Forst T, Uhlig-Laske B, Ring A, Graefe-Mody U, Friedrich C, Herbach K, Woerle HJ and Dugi KA (2010). Linagliptin (BI 1356), a potent and selective DPP-4 inhibitor, is safe and efficacious in combination with metformin in patients with inadequately controlled Type 2 diabetes. *Diabet. Med.*, **27**(12):

- 1409-1419.
- Gong L, Goswami S, Giacomini KM, Altman RB and Klein TE (2012). Metformin pathways: pharmacokinetics and pharmacodynamics. *Pharmacogenet. Genomics*, **22**(11): 820-827.
- Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P and Williams KM (2011). Clinical pharmacokinetics of metformin. *Clin. Pharmacokinet.*, **50**(2): 81-98.
- Haak T (2015). Combination of linagliptin and metformin for the treatment of patients with type 2 diabetes. *Clin. Med. Insights Endocrinol. Diabetes*, **8**: 1-6.
- Jayasagar G, Krishna Kumar M, Chandrasekhar K, Madhusudan Rao C and Madhusudan Rao Y (2002). Effect of cephalixin on the pharmacokinetics of metformin in healthy human volunteers. *Drug Metabol. Drug Interact.*, **19**(1): 41-48.
- Kang DG, Sohn EJ, Moon MK, Mun YJ, Woo WH, Kim MK and Lee HS (2006a). Yukmijihwang-tang ameliorates ischemia/reperfusion-induced renal injury in rats. *J. Ethnopharmacol.*, **104**(1-2): 47-53.
- Kang M, Kim JH, Cho C, Lee KY, Shin M, Hong M, Shim I and Bae H (2006b). Effects of Yukmijihwang-tang derivatives (YMJd) on ibotenic acid-induced amnesia in the rat. *Biol. Pharm. Bull.*, **29**(7): 1431-1435.
- Kang SB, Shon HS, Park SJ, Song CH and Ku SK (2014). Effects of Chungsinoryungsan, a polyherbal complex, on the pharmacokinetic profiles of perindopril in rats. *Biomed. Rep.*, **2**(6): 855-860.
- Kim DJ, Ryu HM, Park SI, Park SJ, Song CH and Ku SK (2015). Pharmacokinetic properties of ondansetron in combination with Ijintanggamibang, polyherbal complex in rats. *Int. J. Pharmacol.*, **11**(4): 351-358.
- Kusuhara H, Ito S, Kumagai Y, Jiang M, Shiroshita T, Moriyama Y, Inoue K, Yuasa H and Sugiyama Y (2011). Effects of a MATE protein inhibitor, pyrimethamine, on the renal elimination of metformin at oral microdose and at therapeutic dose in healthy subjects. *Clin. Pharmacol. Ther.*, **89**(6): 837-844.
- Lee JH, Kang HE and Lee MG (2010). Pharmacokinetic interaction between telithromycin and metformin in diabetes mellitus rats. *Xenobiotica*, **40**(3): 217-224.
- Lee KS, Lim BV, Chang HK, Yang HY, Bahn GH, Paik EK, Park JH and Kim CJ (2005). Liuweidihuang-tang improves spatial memory function and increases neurogenesis in the dentate gyrus in rats. *Fitoterapia*, **76**(6): 514-519.
- Nasri H and Rafieian-Kopaei M (2014). Metformin: Current knowledge. *J. Res. Med. Sci.*, **19**(7): 658-664.
- Pasquel FJ, Klein R, Adigweme A, Hinedi Z, Coralli R, Pimentel JL and Umpierrez GE (2015). Metformin-associated lactic acidosis. *Am. J. Med. Sci.*, **349**(3): 263-267.
- Perry B, Zhang J, Saleh T and Wang Y (2014). Liuwei Dihuang, a traditional Chinese herbal formula, suppresses chronic inflammation and oxidative stress in obese rats. *J. Integr. Med.*, **12**(5): 447-454.
- Pu R, Geng XN, Yu F, Liang HG and Shi LW (2013). Liuwei dihuang pills enhance the effect of Western medicine in treating type 2 diabetes: A meta-analysis of randomized controlled trials. *Chin. J. Integr. Med.*, **19**(10): 783-791.
- Rhee SY, Kim HJ, Ko SH, Hur KY, Kim NH, Moon MK, Park SO, Lee BW, Choi KM, Kim JH and Committee of Clinical Practice Guideline of Korean Diabetes A (2017). Monotherapy in patients with type 2 diabetes mellitus. *Diabetes Metab. J.*, **41**(5): 349-356.
- Rouabhia S, Milic N and Abenavoli L (2014). Metformin in the treatment of non-alcoholic fatty liver disease: safety, efficacy and mechanism. *Expert. Rev. Gastroenterol. Hepatol.*, **8**(4): 343-349.
- Scheen AJ (1996). Clinical pharmacokinetics of metformin. *Clin. Pharmacokinet.*, **30**(5): 359-371.
- Shim KS, Ma CJ, Kim DS and Ma JY (2011). Yukmijihwang-tang inhibits receptor activator for nuclear Factor-kappaB ligand-induced osteoclast differentiation. *J. Med. Food*, **14**(11): 1439-1447.
- Shin IS, Lee MY, Ha HK, Seo CS and Shin HK (2012). Inhibitory effect of Yukmijihwang-tang, a traditional herbal formula against testosterone-induced benign prostatic hyperplasia in rats. *BMC Complement. Altern. Med.*, **12**: 48.
- Somogyi A, Stockley C, Keal J, Rolan P and Bochner F (1987). Reduction of metformin renal tubular secretion by cimetidine in man. *Br. J. Clin. Pharmacol.*, **23**(5): 545-551.
- Song XY, Chen Q and Qi XY (2004). Effect of liuwei dihuang pill on erythrocyte aldose reductase activity in early diabetic nephropathy patients. *Zhongguo Zhong Xi Yi Jie He Za Zhi*, **24**(12): 1087-1090.
- Tahrani AA, Bailey CJ, Del Prato S and Barnett AH (2011). Management of type 2 diabetes: New and future developments in treatment. *Lancet*, **378**(9786): 182-197.
- Towler MC and Hardie DG (2007). AMP-activated protein kinase in metabolic control and insulin signaling. *Circ. Res.*, **100**(3): 328-341.
- Wang L, Wu C, Shen L, Liu H, Chen Y, Liu F, Wang Y and Yang J (2015). Evaluation of drug-drug interaction between henagliflozin, a novel sodium-glucose co-transporter 2 inhibitor and metformin in healthy Chinese males. *Xenobiotica*, **46**(8): 703-708.
- Xie B, Gong T, Tang M, Mi D, Zhang X, Liu J and Zhang Z (2008). An approach based on HPLC-fingerprint and chemometrics to quality consistency evaluation of Liuwei Dihuang Pills produced by different manufacturers. *J. Pharm. Biomed. Anal.*, **48**(4): 1261-1266.
- Yang XX, Hu ZP, Duan W, Zhu YZ and Zhou SF (2006). Drug-herb interactions: Eliminating toxicity with hard drug design. *Curr. Pharm. Des.*, **12**(35): 4649-4664.