의학박사 학위논문

약동학 연구를 위한 통상적인 채혈법과 Volumetric Absorptive Microsampling (VAMS) device를 이용한 채혈법의 비교

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2020 년 7 월

서울대학교 대학원

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Abstract

Comparison of Conventional Blood Sampling and Sampling by Volumetric Absorptive Microsampling (VAMS) Device for Pharmacokinetic Analysis

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Introduction: A volumetric absorptive microsampling (VAMS) device was developed to improve patient-centricity in healthcare services and clinical trials that require various laboratory tests. By absorbing a specific amount of blood in its hydrophilic porous tip, this device can be used for therapeutic drug monitoring and pharmacokinetic studies. The objective of this study was to compare the metformin concentration collected using the VAMS device to that collected using conventional venous sampling. In addition, the pharmacokinetic parameters acquired from the metformin concentrations in the respective samples were compared.

Methods: An open-label, single-dose study was conducted in healthy subjects. Subjects orally received one tablet of 500 mg metformin once and serial blood samples were collected up to 10 hours after dose. At each sampling time, three samples were collected as follows: A) plasma samples were collected by conventional venous sampling and centrifuged, B) venous blood samples were collected using the VAMS device from the whole blood acquired in A), and C) capillary blood samples were collected by VAMS. The plasma (A), venous (B), and capillary (C) blood concentrations of metformin were measured by high-performance liquid chromatography/tandem mass spectrometry, and pharmacokinetic parameters were determined by non-compartmental analysis. In addition, the blood-to-plasma ratio of metformin was calculated from the results of an experiment that assessed the whole blood and plasma metformin concentrations, and

the ratio was used to adjust the plasma metformin concentration obtained in the clinical trial.

Results: A total of 20 subjects completed the study. The geometric mean ratios and 90% confidence intervals of the area under the concentration-time curve (AUC) and the maximum concentration (C_{max}) of B compared to A were 0.8929 (0.8221 - 0.9698) and 0.7966 (0.7328 - 0.8660), respectively. The corresponding values of C compared to A were 0.8936 (0.8249 - 0.9680) and 0.7819 (0.7227 - 0.8459), respectively. The intraclass correlation coefficients were 0.778 for B/A and 0.781 for C/A. Blood-to-plasma ratio of metformin without incubation time was 0.66 - 0.80, and the increment of adjusted plasma metformin concentration was comparable to the increment of capillary concentration collected by VAMS.

Conclusion: There was a difference between the plasma and whole blood (venous and capillary blood) concentrations of metformin collected by conventional sampling and VAMS; however, this difference was due to the characteristics of metformin such as low intrinsic blood-to-plasma ratio, slow blood cell distribution and relatively fast plasma clearance, and not the difference in the sampling methods.

keywords : Volumetric absorptive microsamping (VAMS), Pharmacokinetics, Metformin, Patient-Centricity

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List of Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ANOVA	analysis of variance
AUC	area under the plasma or blood drug concentration-time profiles
BMI	body mass index
CI	confidence intervals
CL/F	Apparent plasma clearance
C_{max}	maximum drug concentration
DBS	dried blood spot
ECG	12-lead electrocardiogram
GMR	geometric mean ratio
ICC	intraclass correlation coefficient
LC-MS/MS	liquid chromatography/tandem mass spectrometry
LLOQ	lower limit of quantification
MRM	multiple reaction monitoring

PCORI	Patient-Centered Outcomes Research Institute
SD	standard deviation
TDM	therapeutic drug monitoring
T_{max}	time to C_{max}
$T_{1/2}$	terminal elimination half-life
VAMS	Volumetric Absorptive Microsampling

INTRODUCTION

In the early 2010s, the term "patient-centricity" ("patient-centered" or, "patient-centric") came to the attention of researchers in the United States[1] after the enactment of the *Patient Protection and Affordable Care Act (2010)* - also known as Obamacare - and the creation of the Patient-Centered Outcomes Research Institute (PCORI). The mission of the Act and the PCORI was to "promote the inclusion of the patient's perspective and patient-oriented outcomes in clinical and health services research."[2] This concept of patient-centricity had already been presented in the 2000s to a certain extent, as contrary to the disease-centered healthcare and studies.[3,4] The Institute of Medicine had presented the six aims for the healthcare system in the States in 2001, which suggested providing patient-centered care that was respectful of, and responsive to, individual patients.[4]

Even though this concept had been presented for almost 20 years, the actual healthcare system and related research had not changed. Especially within clinical research, there are not enough published data that quantify patient-centric activities.[5] To date, a harmonized definition of patient-centric drug development or design has not been established.[6] Although researchers agree that clinical studies, including clinical trials, should have a paradigm shift toward patient-centricity, an agreement on how to initiate such a shift is lacking.

Clinical trials are important because they produce pivotal data that are essential to the development of new drugs or interventions. From the early phase clinical trials that recruit healthy volunteers to assess the safety and pharmacokinetics of a new drug to the late phase clinical trials that evaluate the actual effectiveness and superiority of the new drug or intervention, data are accumulated to enable the legal authorities to decide whether the new intervention can be marketed.

However, conducting clinical trials is not an easy task. Clinical trials traditionally require a large number of patients and healthy volunteers. The number starts small in Phase I clinical trials, with less than a hundred healthy volunteers; however, the number becomes larger in Phases II and III clinical trials that are conducted with hundreds or thousands of patients. Subsequently, recruiting participants is an obvious challenge for conducting a successful clinical trial. In addition, recruiting healthy volunteers who have to donate 200 to 300 mL of blood for pharmacokinetic analysis and might need additional interventions is also a challenge. Healthy volunteers and patients are required to visit the hospital often for hospitalization and outpatient visits, which is again an obstacle by itself. These are a few of the reasons why potential volunteers are reluctant to participate in clinical trials.

Conventionally, a clinical trial is conducted by investigators and pharmaceutical companies whose primary interest is acquiring complete data. Safety is one of their main concerns; however, in

many cases, the participants must undergo extensive blood sampling and endure inconvenience to have their safety checked. Safety tests listed under blood and urine laboratory tests, such as hematology, chemistry, and urinalysis, require the participants to visit the hospital and have samples taken by healthcare professionals. In this conventional way of conducting clinical trials, the research is not centered on patients; rather, it is centered on the interests of the researchers. Therefore, healthy volunteers and patients are reluctant to participate in clinical trials and are less likely to be compliant with treatments.

Several ways of promoting patient participation in clinical trials and therapy have been discussed previously.[7,8] This is particularly important in those countries where the access to healthcare is limited and far between. In countries such as the United States, methods for specimen sampling at home are gaining popularity. However, there are several limitations that must be overcome if patient-centric clinical practice or clinical trials are to be conducted. The specimen sampling technique must be simple and easy to perform. Patients and healthy volunteers are usually not healthcare professionals, i.e., they cannot perform conventional blood sampling such as venous puncture and drawing venous blood in a syringe. Hence, there is a need for a simplified blood collection system to be developed.

Another important aspect is the stability of the sample. In hospital settings, the sample that has been taken from the patient is

delivered to the department of laboratory medicine within one or two hours and most results can be reported within the day. Even if the tests require the shipping of samples to other commercial laboratories, the samples are stored in a stable environment (such as centrifuging the sample and storing only the plasma or serum in a −70 ℃ refrigerator) and shipped according to the laboratory manual to ensure the stability of the sample.

Even if there is no problem associated with aforementioned conditions, it would not be adequate if the results are different between blood sampling techniques. This is especially true if the results are those of drug concentrations, as required in therapeutic drug monitoring (TDM) and clinical trials that investigate the pharmacokinetics of drugs. Therefore, there is an unmet need for an easy way to sample blood to measure the concentration of drugs or other compounds in the blood, with adequate sample stability and accuracy.

In light of such unmet needs, a novel device called a volumetric absorptive microsampling (VAMS) device was developed in the early 2010s and gained attention in certain areas.[9] This is a device that can collect a specific volume of blood in a hydrophilic porous tip, with the usage being similar to that of using blood glucose test strips. The fingertip of the patient is pricked with a lancet and the porous tip is placed in direct contact with the blood droplet. After 2 seconds, the tip collects an exact volume of blood and this sample can be dried and shipped for subsequent analysis.

Because this process can be performed at home and the patients or healthy volunteers do not need to go to the hospital, this device is important for future TDM and clinical trials.

If VAMS is to be used in a pharmacokinetic study, the drug concentrations that are measured in samples collected by VAMS must be comparable to those measured in samples collected by conventional methods. Each drug concentration should be comparable, at all timepoints, and the final pharmacokinetic parameters calculated using the drug concentrations in the VAMS samples must be comparable to those parameters calculated from the samples collected by the conventional blood sampling. If there is a difference in concentrations between samples collected by the different sampling methods, further studies to find out whether there is a way to adjust for such difference should be performed.

In the present study, metformin, a widely used antidiabetic drug, was selected to assess the pharmacokinetics of samples collected by VAMS.

Objectives

The main objective of this study was to compare the metformin concentration in whole blood and capillary blood samples collected by VAMS to the plasma metformin concentration collected by conventional venous sampling, as well as to compare the

pharmacokinetic parameters acquired from metformin concentrations in each of the samples. A clinical trial and an additional experiment were performed to obtain the plasma, whole blood, and capillary blood metformin concentration values.

MATERIALS AND METHODS

1. Study Subjects

Healthy volunteers, defined as individuals with no clinically significant abnormalities in medical history, physical examination, 12-lead electrocardiogram (ECG), and clinical laboratory tests, were recruited after obtaining written informed consent. The eligible age of the subjects was at least 19 years old, with a body mass index (BMI) $\geq$ 17.5 kg/m^2 and $<$ 30.5 kg/m^2. Specific exclusion criteria included history or current evidence of acute or chronic illness, including hypersensitivity to metformin or any other biguanide drugs. Individuals with clinically significant abnormalities in blood chemistry, hematology, serology, and urinalysis were excluded. Subjects who participated in other clinical trials within 3 months of screening, donated whole blood within 2 months of screening, or who had received blood transfusion or donated blood components within 1 month of screening were excluded. Those subjects with liver function test values of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) $>$ 2 times the upper limit of the normal range were excluded.

No prescription or over-the-counter drugs were allowed during the study, as well as grapefruit consumption. Alcohol consumption was restricted 24 hours before hospitalization until the end of the pharmacokinetic sampling, and smoking and caffeine

consumption were restricted during hospitalization.

Descriptive statistics were used to analyze the demographic characteristics of the subjects who had administered the investigational product.

2. Study Design

A single-dose, open-label Phase I study was conducted. The subjects were asked to fast overnight and were hospitalized in the morning of the investigational product administration, and discharged in the evening after the last pharmacokinetic blood sampling was completed. The subjects received one tablet of 500 mg metformin with 150 mL of water once at approximately 8 a.m. Serial blood samples were collected at the following timepoints: 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 h (post-dose).

The pharmacokinetic samples were collected using three different methods. The blood samples were first collected by conventional venous blood sampling and were centrifuged at 1882 *g* for 10 minutes at 4 ℃ to obtain plasma aliquots (A, Plasma). The plasma samples were stored at -70 ℃ until further analysis. Second, the hydrophilic tip of the VAMS device was placed in direct contact with the whole blood collected in (A) before centrifugation. The samples collected by VAMS were dried at room temperature (B, Venous) for one hour and stored at -70 ℃ until further analysis. In

venous (B) samples, the metformin concentration in whole blood collected by VAMS was analyzed. Lastly, to imitate the actual usage of VAMS (such as at home), the fingertip of the subject was pricked first using a lancet and, without squeezing the fingertip, the hydrophilic tip of the VAMS device was placed in direct contact with the blood droplet formed at the fingertip. After 2 seconds of direct contact, the tip of the VAMS device was removed and dried at room temperature for one hour and stored at -70 ℃ until further analysis. The metformin concentration in capillary blood collected by VAMS was analyzed (C, Capillary).

Figure 1. Three different pharmacokinetic sampling method. Plasma, A) Conventional sampling of venous blood, then centrifuged to get plasma. Venous, B) The tip of VAMS device was in direct contact with venous blood collected in A. Capillary, C) VAMS was used to collect capillary blood from the subject's fingertip directly.

3. Ethical Consideration

The study protocol was approved by the Institutional Review Board of Jeonbuk National University Hospital (Jeonju-si, South Korea) and conducted in accordance with the principles of the Declaration of Helsinki and Korean Good Clinical Practice. All subjects provided written informed consent before screening for eligibility.

4. Quantification of Metformin Concentrations

Metformin concentrations in plasma and VAMS samples were measured using a validated high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS, Agilent Technology 1100 Series and AB SCIEX 4000 QTRAP, USA) using carbamazepine as the internal standard.

Briefly, the plasma samples were thawed at room temperature, added to 800 μL of methanol and 25 μL of internal standard (carbamazepine, 1 μg/mL), and mixed thoroughly by vortexing and sonication. The supernatant (850 μL) was transferred to a new tube and vacuum evaporated at 45 ℃ for 75 minutes. Then, 500 μL of 50% methanol was added to the tube, mixed thoroughly and centrifuged at 16,100 *g* for 10 minutes at 4 ℃, and then 10 μL of the supernatant was injected into HPLC-MS/MS. Chromatographic

separation was performed using Kinetex C18 column (2.1 mm ID × 50 mm L, 2.6 μm, Phenomenex, USA) at a flow rate of 0.2 mL/min. Two mobile phases were used: mobile phase A, 10 mM ammonium acetate and B, acetonitrile in the ratio of 40:60. Electrospray ionization in positive ion mode was used for detection and quantification. The multiple reaction monitoring (MRM) transition was m/z 130.23 -> 71.10 for metformin and 237.10 -> 194.10 for the internal standard. A calibration curve covering the range of 10 to 2000 ng/mL was constructed and was linear over the concentration range ($r^2 \geq 0.9990$).

Validation of metformin concentration analysis in whole blood (venous and capillary blood) samples collected by VAMS was also conducted. Briefly, 10 μL of whole blood was absorbed into the VAMS device and dried for 1 hour at room temperature. The VAMS tip that absorbed blood was separated from the device and, after the addition of 200 μL of methanol and 2.5 μL of internal standard (carbamazepine, 1 μg/mL), was mixed thoroughly by vortexing and sonication. The supernatant (190 μL) was transferred to a new tube and vacuum evaporated at 45 ℃ for 20 minutes. Then, 50 μL of 50% methanol was added to the tube, mixed thoroughly and centrifuged at 16,100 *g* for 10 minutes at 4 ℃, and then 10 μL of the supernatant was injected into HPLC-MS/MS. The chromatographic separation was conducted under identical conditions as that for the plasma samples.

Metformin concentrations in the sample were measured by calculating the peak area ratio of the analyte to each internal

standard using the previously prepared calibration curve. Five batches, each consisting of replicates of quality control samples, were used to assess the precision and accuracy of the assay. The validation of the metformin concentration analysis in plasma samples showed that the intra-day accuracies ranged from 92.5 - 105.0% and precisions varied within 0.6 - 7.3%, whereas the inter-day accuracies ranged from 92.0 - 97.2%, and precisions varied within 1.3 - 5.0%. At the lower limit of quantification (LLOQ, 10 ng/mL), the intra- and inter-day accuracies were 90.4% and 80.8%, respectively, and the precisions were 3.7% and 8.0%, respectively. The validation of metformin concentration analysis in the VAMS samples showed that the intra-day accuracies ranged from 95.5 - 105.4% and precisions varied within 1.4 - 7.6%, whereas the inter-day accuracies ranged from 97.1 - 105.0% and precisions varied within 0.9 - 6.2%. At LLOQ, the intra- and inter-day accuracies were 104.0% and 101.6%, respectively, and the precisions were 8.8% and 8.2%, respectively.

The intra- and inter-day accuracies were within 85 - 115% and the precisions varied within <15%, which were within the acceptable limits. The stability of the samples was tested at three different concentrations of quality control samples, including freeze and thaw stability (3 cycles) and long term stability (125 days). All the assays were validated according to the Guideline on Bioanalytical Method Validation published by the Korean Ministry of Food and Drug Safety (2013).

5. Pharmacokinetic Analysis

The pharmacokinetic analysis included all the subjects who had completed the pharmacokinetic blood sampling according to the protocol. The pharmacokinetic parameters were assessed using the non-compartmental method provided by Phoenix WinNonlin software (version 6.3, Pharsight, Mountain View, CA, USA). The maximum plasma or blood drug concentration (C_{max}) and time to C_{max} (T_{max}) were obtained directly from the plasma or blood concentration-time profiles. The terminal elimination half-life ($T_{1/2}$) was calculated as ln 2/λz, where λz is the slope of the apparent elimination phase of the natural logarithmic (ln) transformation of the drug concentration-time profiles. The area under the plasma or blood drug concentration-time profiles (AUC) was calculated according to the linear trapezoidal method. The apparent plasma clearance (CL/F) was calculated as Dose/AUC.

6. Statistical Analysis

Metformin concentrations from the samples collected by different means were compared using different analysis methods. First, the pharmacokinetic parameters calculated from the plasma samples were compared to the parameters calculated from the VAMS

samples to assess whether the pharmacokinetic assessment was feasible. The log-transformed AUC and C_{max} were analyzed using a mixed-effects analysis of variance (ANOVA) with the sampling method as a fixed effect and subject within sequence as a random effect in SAS (version 9.3, SAS Institute, Inc., Cary, NC, USA). The results for AUC and C_{max} were reported as 90% confidence intervals (CIs) surrounding the geometric mean ratios (GMRs) of the pharmacokinetic parameters. As stated by pharmacokinetic equivalence criteria, if the 90% CIs for the pharmacokinetic parameters were within the range of 80-125%, then the pharmacokinetic parameters calculated from the samples collected by different means were considered as comparable.

To assess whether the metformin concentrations at each timepoint were comparable between the different sampling methods, the intraclass correlation coefficient (ICC) was calculated using SAS (version 9.3, SAS Institute, Inc.). The ICC values were calculated using the PROC MIXED procedure between metformin concentrations sampled in the venous versus plasma, and the capillary versus plasma samples. The %INTRACC macro available from SAS homepage[10] was used to calculate 6 ICC values at the same time, between 1) plasma versus venous concentrations, 2) plasma versus capillary concentrations, and 3) concentrations in plasma, venous and capillary samples.

7. Safety Analysis

The safety analysis included all subjects who received the investigational product. Safety measurements included physical examination, clinical laboratory test results (including hematology, serum chemistry, and urinalysis), vital signs 10 hours after the dose administration, and assessment of adverse events. Descriptive statistics were used to summarize any clinically significant findings.

8. Evaluation of Metformin Blood-to-Plasma Ratio in an Additional Experiment

The blood-to-plasma ratio of metformin was evaluated by conducting an additional experiment as follows: 10 μL of standard metformin solution was added to 90 μL of whole blood to make triplicate samples at four different concentrations of metformin. The amounts of metformin in each sample were 5, 20, 80, and 150 ng, respectively. Aliquots (10 μL) of triplicate samples were used to quantify the metformin concentration in whole blood as stated in Section 4 of Materials and Methods. Another 10 μL aliquots of whole blood were absorbed to the tip of the VAMS device, dried for one hour at room temperature, and the metformin concentration in the VAMS tip was analyzed as stated in Section 4. The remaining whole blood samples were centrifuged at 1882 *g* for 10 minutes at 4 ℃ to

obtain plasma samples, and 10 μL aliquots of plasma samples were used to quantify the metformin concentration in plasma, as stated in Section 4.

The concentration in whole blood (not absorbed by VAMS) was compared to that of plasma to assess the blood-to-plasma ratio of metformin.

9. Comparison of Capillary Metformin Concentration and Adjusted Plasma Metformin Concentration using Blood-to-Plasma Ratio

The plasma metformin concentration from the clinical trial was adjusted using the metformin blood-to-plasma ratio acquired from the experimental evaluation stated in Section 8 of Materials and Methods. The adjusted plasma metformin concentrations were calculated using the lowest and highest blood-to-plasma ratio values from the experiment and were plotted against the capillary metformin concentration from the clinical trial. Linear regression analysis was performed to assess the linear relationship between capillary and adjusted plasma concentrations.

10. Comparison of Capillary Metformin Concentration and Adjusted Plasma Metformin

Concentration using Individual Hematocrit

The plasma metformin concentration from the clinical trial was adjusted using the individual hematocrit values of the subjects collected at the time of screening. The adjusted plasma metformin concentrations were calculated as follows:

$$
\begin{aligned}
&Concentration_{blood} \\
&= Concentration_{plasma} \times \frac{Volume_{plasma}}{Volume_{blood}} + Concentration_{erythrocyte} \times \frac{Volume_{erythrocyte}}{Volume_{blood}} \\
&= Concentration_{plasma} \times (1 - Hematocrit) \\
&\quad + Concentration_{plasma} \times Ratio_{e/p} \times Hematocrit
\end{aligned}
$$

where $Ratio_{e/p}$=Ratio of concentration in erythrocyte versus plasma. Because the metformin concentration in erythrocyte was not obtained in the clinical trial or in the additional experiment, the value of $Ratio_{e/p}$ was obtained from the literature to be 0.18.[11]

RESULTS

1. Subject Disposition and Demographics

A total of 20 subjects were enrolled in the study and received one dose of metformin 500 mg tablet. There were no drop-out subjects after drug administration. All 20 subjects completed the pharmacokinetic blood sampling and were included in the pharmacokinetic analysis. Ten male and ten female subjects were enrolled in the study. The mean ± standard deviation (SD) of the subjects' age, height, weight, and BMI was 24.95 ± 5.02 years, 168.14 ± 7.47 cm, 66.43 ± 7.74 kg, and 23.46 ± 2.36 kg/m^2, respectively. No significant deviations in histories of smoking, alcohol and caffeine consumption were reported, and the enrolled subjects had no clinically significant medical histories.

2. Pharmacokinetics of Metformin in Plasma and VAMS Samples

The mean plasma concentration-time profile of metformin in samples collected by conventional blood sampling is shown as a black line in Figure 1. In the same figure, the mean blood concentration-time profile of metformin in venous samples collected by VAMS is shown as a red line, and the mean blood

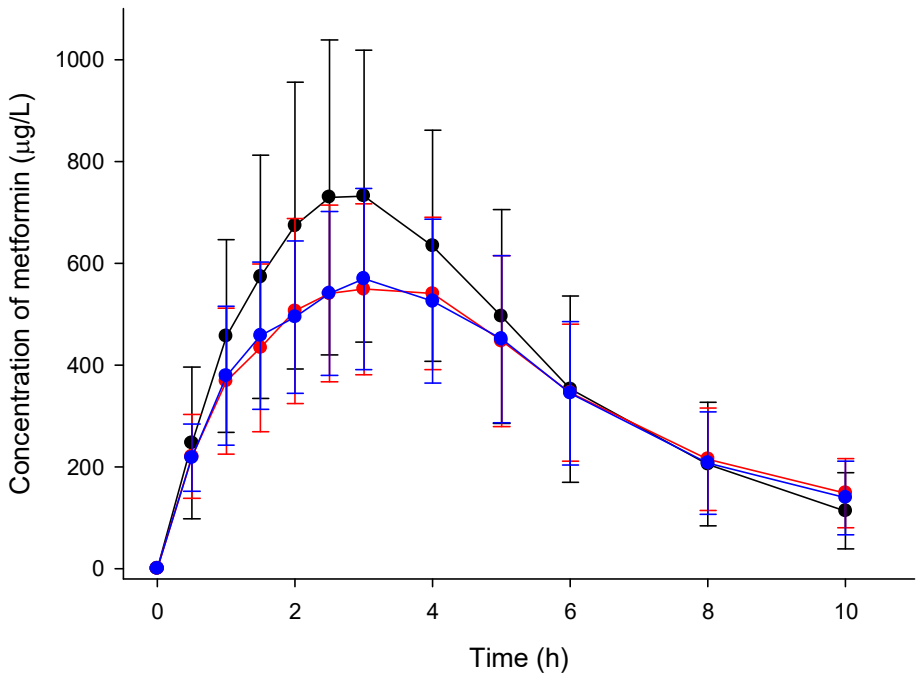
concentration-time profile of metformin in capillary samples directly collected by VAMS is shown as a blue line. The plasma concentration of metformin was higher than the venous blood or capillary blood concentration of metformin collected by VAMS for the first 5 hours after administration; however, higher whole blood concentrations (venous blood and capillary blood concentrations collected by VAMS) than that of plasma concentrations were reported 10 hours after administration. For all sampling timepoints, the capillary metformin concentration values did not differ from the venous metformin concentration values.

The pharmacokinetic parameters assessed from concentrations in respective samples are summarized in Table 1.

3. Comparison of Pharmacokinetic Parameters between Three Different Sampling Methods

The pharmacokinetic equivalence criteria were used to compare the AUC and C_{max} calculated from the metformin concentrations in samples collected by different sampling methods, and the results are summarized in Table 2. The 90% CIs did not fall in the pharmacokinetic equivalent criteria of 0.8 - 1.25 for C_{max} when pharmacokinetic parameters calculated from venous or capillary samples were compared to those from plasma samples; however, the 90% CIs were within the pharmacokinetic equivalent limit when the

AUC was compared between the different sampling methods.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600
400
200
0
0
2
4
6
8
10
Time (h)

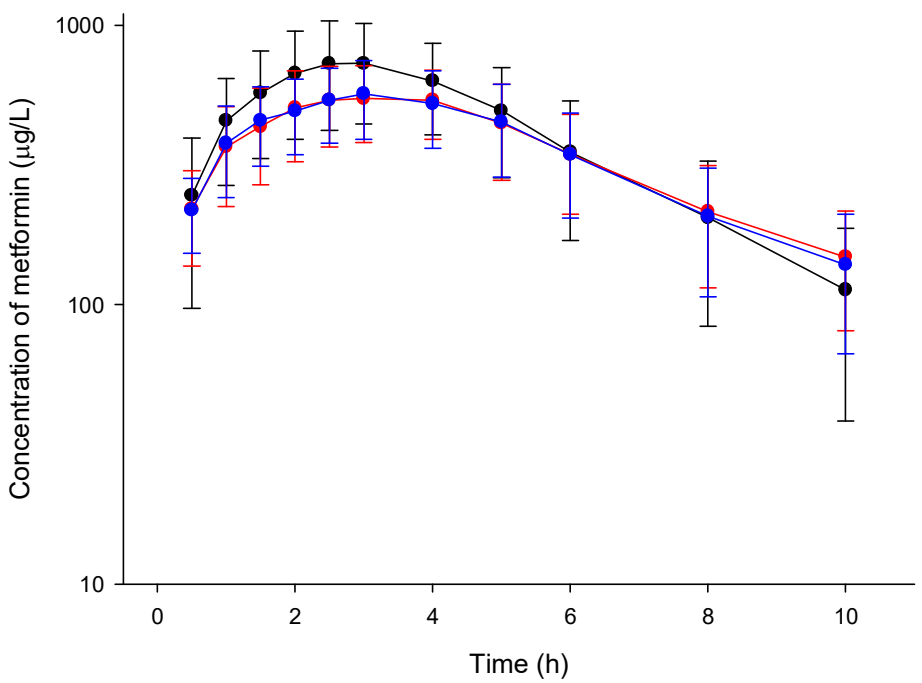
Concentration of metformin (μg/L)
1000
100
10
0
2
4
6
8
10
Time (h)

Figure 2. The (plasma or blood) mean concentration-time profiles of metformin in plasma samples collected by conventional venous sampling (black lines) and in blood samples collected by VAMS (red lines, venous; blue lines, capillary) in linear (upper) and semi-logarithmic scale (lower). Each timepoint represents arithmetic mean ± standard deviation (SD) of metformin concentration.

Table 1. Summary of pharmacokinetic parameters of metformin analyzed from plasma, venous and capillary samples.

	Statistics					
Pharmacokinetic Parameters (unit)	N	Arithmetic mean	Arithmetic SD	Median	Min	Max
Plasma						
AUC(h*ng/mL)	20	4104.93	1593.95	4202.41	1690.62	8390.97
C_{max}(ng/mL)	20	805.15	301.78	828.50	334.00	1420.00
AUC_{inf}(h*ng/mL)	20	4549.34	1857.20	4475.13	1890.42	10160.22
T_{max}(h)	20	2.65	0.67	2.50	1.50	4.00
$T_{1/2}$(h)	20	2.60	0.72	2.48	1.46	4.80
CL/F (L/h)	20	127.83	53.55	111.80	49.21	264.49
Vd/F (L)	20	488.84	281.78	403.00	222.11	1190.21
Venous						
AUC(h*ng/mL)	20	3560.64	1054.62	3514.87	1788.39	5764.77
C_{max}(ng/mL)	20	623.02	177.79	647.50	299.50	843.75
AUC_{inf}(h*ng/mL)	20	4316.98	1452.41	4321.06	2128.02	7641.43
T_{max}(h)	20	3.25	0.94	3.00	2.00	5.03
$T_{1/2}$(h)	20	3.30	0.96	3.34	2.00	6.76
CL/F (L/h)	20	130.22	48.61	115.73	65.43	234.96
Vd/F (L)	20	598.06	219.58	549.79	316.80	1157.50
Capillary						
AUC(h*ng/mL)	20	3554.74	1062.72	3572.28	1992.05	6206.52
C_{max}(ng/mL)	20	610.82	172.02	649.25	297.30	865.00
AUC_{inf}(h*ng/mL)	20	4214.48	1491.82	4015.70	2153.15	8932.58
T_{max}(h)	20	2.98	0.94	3.00	1.52	5.07
$T_{1/2}$(h)	20	3.04	0.78	2.79	1.92	4.70
CL/F (L/h)	20	131.61	43.25	124.54	55.97	232.22
Vd/F (L)	20	564.85	201.84	516.35	255.36	1064.36

*T_{max}, time to reach maximum concentration at steady state; $T_{1/2}$,

terminal elimination half-life; C_{max}, maximum plasma or blood concentration; AUC, area under the plasma or blood concentration-time curve; AUC_{inf}, area under the plasma or blood concentration-time curve from time 0 to infinity; CL/F, apparent total clearance; and V/F, apparent volume of distribution.

Table 2. Comparison of pharmacokinetic parameters (AUC, C_{max}) of metformin between samples (Venous/Plasma, Capillary/Plasma, Capillary/Venous), represented as geometric mean ratio (GMR) and 90% confidence intervals (CIs).

Pharmacokinetic parameters (unit)	Point Estimate of Geometric Mean Ratio (GMR)	90% Confidence Interval (CIs)
Venous/Plasma		
AUC(h*ng/mL)	0.8929	0.8221 - 0.9698
C_{max}(ng/mL)	0.7966	0.7328 - 0.8660
Capillary/Plasma		
AUC(h*ng/mL)	0.8936	0.8249 - 0.9680
C_{max}(ng/mL)	0.7819	0.7227 - 0.8459
Venous/Capillary		
AUC(h*ng/mL)	1.0007	0.9521 - 1.0519
C_{max}(ng/mL)	0.9816	0.9337 - 1.0319

4. Comparison of Metformin Concentrations at Each Timepoint between Three Different Sampling Methods

The ICC was calculated to compare the measurements made on the same subject, at the same time, by different sampling methods. The ICC was 0.777 when metformin concentrations in venous samples were compared those in plasma samples using PROC MIXED procedure, and the Winer reliability and Shrout-Fleiss reliability for single score were calculated as 0.778 when the %INTRACC macro was used. The ICC was 0.780 when metformin concentrations in capillary samples were compared to those in plasma samples using PROC MIXED procedure, and the Winer reliability and Shrout-Fleiss reliability for single score were calculated to be 0.781 when the %INTRACC macro was used.

When the %INTRACC macro was used to calculate the ICC between the metformin concentrations in plasma, venous, and capillary samples together, both Winer reliability and Shrout-Fleiss reliability for single score were calculated to be 0.813 (Table 3).

Table 3. The intraclass correlation coefficient (ICC) values calculated with two raters (plasma and venous concentrations, plasma and capillary concentrations) and with three raters.

Intraclass correlation coefficient (ICC)	Plasma vs Venous	Plasma vs Capillary	Plasma vs Venous vs Capillary
Winer reliability: single score	0.778	0.781	0.813
Winer reliability: mean of k scores	0.875	0.877	0.929
Winer reliability: mean of 10 scores	0.972	0.973	0.978
Shrout-Fleiss reliability: single score	0.778	0.781	0.813
Shrout-Fleiss reliability: random set	0.783	0.786	0.816
Shrout-Fleiss reliability: fixed set	0.823	0.825	0.847
Shrout-Fleiss reliability: mean k scores	0.875	0.877	0.929
Shrout-Fleiss reliability: random set, mean k scores	0.878	0.880	0.930
Shrout-Fleiss reliability: fixed set, mean k scores	0.903	0.904	0.943

5. Safety

No clinically significant changes in vital signs and physical examination were reported. None of the subjects reported any adverse events after the administration of metformin.

6. Adjustment of Plasma Metformin Concentration using Blood-to-Plasma Ratio and Hematocrit

The arithmetic mean ± SD values of metformin blood-to-plasma ratio calculated at each metformin concentration are reported in Table 4. The minimum value of the blood-to-plasma ratio was 0.66 and the maximum value was 0.80. These two values were used to adjust the plasma metformin concentration acquired in the clinical trial and plot the adjusted plasma concentration values against capillary concentration values (Figure 3). While the original plasma metformin concentration versus capillary concentration plot gave a linear relationship of $y = 1.33x - 43.5$, the adjusted plasma metformin concentration versus capillary concentration plots reported the following linear relationships: $y = 0.87x - 28.7$ (when adjusted by lowest blood-to-plasma ratio) and $y = 1.06x - 34.9$ (when adjusted by highest blood-to-plasma ratio).

The original plasma metformin concentration acquired from the clinical trial was also adjusted by the hematocrit of the individual

subject, and the adjusted plasma metformin concentration versus capillary metformin concentration plots are shown in Figure 4. The linear regression analysis yielded the following relationship: y = 0.90x - 45.0.

Table 4. Evaluation of blood-to-plasma ratio of metformin.

Amount of Metformin Added	Metformin Concentration (Plasma) (μg/L)	Metformin Concentration (Whole blood) (μg/L)	Metformin Concentration (VAMS) (μg/L)	Blood-to-plasma ratio
5 ng	66.2 ± 1.9	47.1 ± 1.9	51.0 ± 1.9	0.71 ± 0.02
20 ng	294.7 ± 9.0	199.7 ± 2.3	200.3 ± 7.6	0.68 ± 0.02
80 ng	1106.7 ± 51.3	825.3 ± 11.9	779.0 ± 19.7	0.75 ± 0.02
150 ng	1992.0 ± 56.0	1543.3 ± 45.1	1496.7 ± 25.2	0.78 ± 0.04

*Blood-to-plasma ratio of metformin was calculated as the ratio of metformin concentration in whole blood samples to metformin concentration in plasma samples.

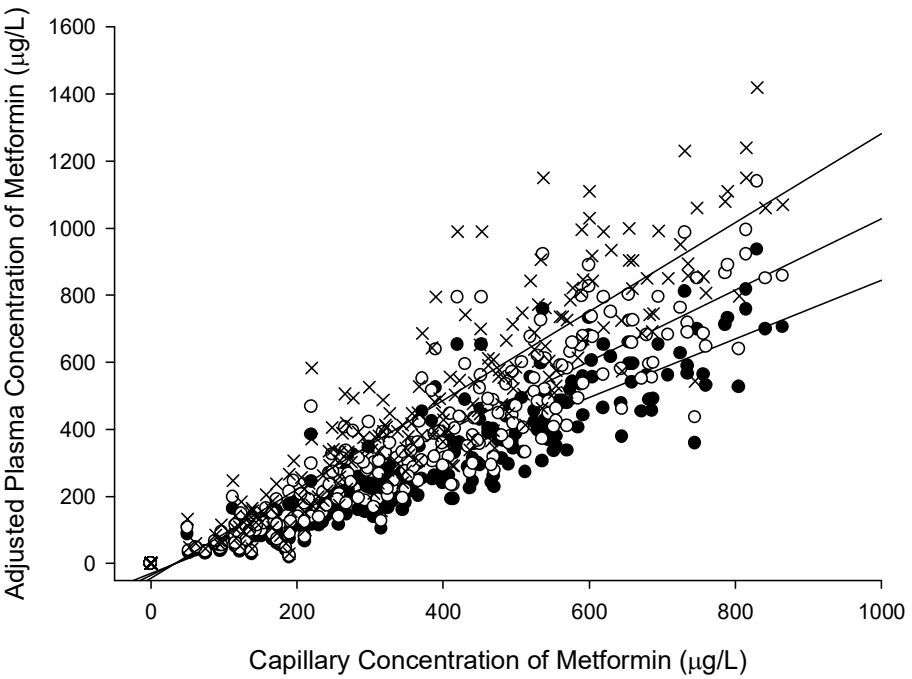

Figure 3. The scatter plot of plasma concentration of metformin adjusted by blood-to-plasma ratio, against capillary concentration of metformin. The original plasma concentrations (marked as x), adjusted plasma concentrations by lowest blood-to-plasma ratio (closed circle), and adjusted plasma concentrations by highest blood-to-plasma ratio (open circle) were plotted against capillary concentration of metformin.

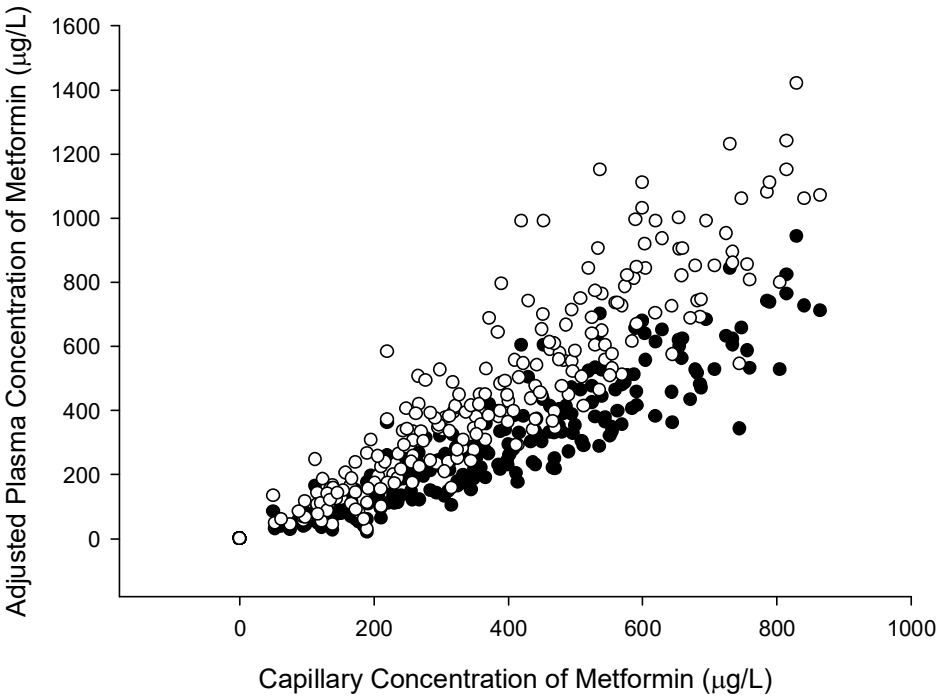

Figure 4. The scatter plot of plasma concentration of metformin adjusted by individual subject's hematocrit, against capillary concentration of metformin. The original plasma concentrations (open circle) and adjusted plasma concentrations by hematocrit (closed circle) were plotted against capillary concentration of metformin.

DISCUSSION

The pharmacokinetic assessment using metformin concentrations from plasma and VAMS samples showed that there was a difference between pharmacokinetic parameters, especially in C_{max} values, calculated from metformin concentrations collected by different means. However, the ICC evaluation showed that the reliability between different sampling methods was >0.75, and the plasma metformin concentration adjusted by experimentally acquired blood-to-plasma ratio of metformin showed a linear relationship with capillary metformin concentration.

VAMS is not the first device to be used for the quantification of drugs or compounds; the dried blood spot (DBS) method has been available for several years.[12,13] The DBS method is considered as an attractive alternative for quantification of drug concentration in clinical trials and its possible usage in TDM and individualization of drug treatment has been assessed for some time. However, one major limitation exists with the DBS method, i.e., there is a possible source of variability in drug quantification due to possible blood spot inhomogeneity and variability in blood spot volumes or hematocrit values.[14] Because the DBS method requires the blood droplet of the patient to be blotted and dried on a piece of filter paper, this inhomogeneity is expected to be common. Therefore, the DBS method may be adequate for qualitative purposes such as detecting DNA,[15] but it has a limited value in pharmacokinetic studies. Therefore,

VAMS, a device that absorbs a fixed volume of blood and can process a homogeneous sample, gained attention when it was first introduced.[16]

Conventionally, pharmacokinetic analysis mostly uses plasma or serum samples to analyze drug concentrations. Some exceptions such as tacrolimus and cyclosporine exist;[17,18] however, these are rare in number. When whole blood samples are centrifuged, cells such as erythrocytes and platelets become sequestered to hematocrit (which consists of approximately 45% of blood) and other cell compounds such as leukocytes are sequestered in the buffy coat, which leaves the actual drug-containing plasma to be extracted and analyzed for drug concentration. It was mainly because of this difference between whole blood and plasma that the results of the present study showed lower metformin concentration in the VAMS samples. That is, because VAMS devices collect whole blood samples and the sample is not centrifuged to extract the plasma, metformin concentrations in venous samples and capillary blood samples are lower than those of plasma samples, at least for the first few hours after dose administration. Some of the previous publications have shown good agreement between drug concentrations sampled by different methods;[19,20] however, the drugs were not metformin. This implies that the difference between plasma and VAMS samples is likely because of the characteristics of metformin not found in other drugs. These characteristics of metformin, such as slow partitioning into erythrocytes and relatively rapid plasma clearance, were searched for

in the literature as follows.

One main limitation of the present study is that a direct comparison between whole blood metformin concentration collected by conventional venous sampling and whole blood metformin concentration collected by VAMS was not performed. To complement this limitation, an additional experiment was performed to acquire the whole blood concentration of metformin and assess the blood-to-plasma ratio of metformin. The plasma metformin concentration acquired in the clinical trial was adjusted by the blood-to-plasma ratio or individual hematocrit value. As a result, it was found that the whole blood concentration of metformin in samples not collected by VAMS was similar to the whole blood concentration in samples collected by VAMS, which was further evidence that the collection of blood samples by VAMS did not alter the whole blood concentration of metformin. Moreover, the blood-to-plasma ratio of metformin was found to be 0.66 - 0.80 without incubation time, which was consistent with previous studies,[11,21,22] and adjusting the plasma metformin concentration using the blood-to-plasma ratio showed the slope of the linear regression approaching 1, against capillary blood concentration values of metformin acquired in the clinical trial.

The results of this additional experiment suggest that VAMS may be useful in pharmacokinetic studies that use whole blood drug concentration as a standard. Aforementioned immunosuppressants such as tacrolimus and cyclosporine are some examples of such

drugs and because these drugs are also candidates for TDM, there is a possibility that VAMS could be more useful in pharmacokinetic studies or TDM of these immunosuppressants compared to other drugs that use plasma drug concentration as a standard.

In the present study, metformin concentrations in whole blood (venous or capillary blood collected by VAMS) were lower than those in plasma for the first few hours after administration; however, by the end of the pharmacokinetic sampling, the concentrations were either similar to those in plasma or higher (Figure 2). This is consistent with previous studies,[21,22] which also reported an increasing tendency in the blood-to-plasma ratio of metformin from the initial value of 0.6 - 0.7 to 0.8 - 1.4 after incubation. This change in drug partitioning is likely due to slow blood cell distribution relative to rapid plasma clearance.[22] The present study also showed that when the plasma metformin concentration was adjusted by the blood-to-plasma ratio, the increment of adjusted plasma concentrations was comparable to the increment of capillary metformin concentrations collected by VAMS (slope=1.06). Therefore, if there is a method to adequately adjust the plasma concentration to whole blood concentration, VAMS could be useful in pharmacokinetic studies of drugs that use plasma concentration profiles as the standard. The blood-to-plasma ratio could be a candidate for such a method for metformin. Adjustment of plasma concentration by individual hematocrit values also showed comparable increment of adjusted plasma concentrations to the increment of capillary

concentrations (Figure 4, slope=0.90). However, because the ratio of erythrocyte and plasma concentration was not obtained from the experiment, there is a limitation that the partitioning of metformin into erythrocyte was not estimated per individual.

Further studies with other drugs will need to have the comparison performed between whole blood and plasma samples collected by conventional blood sampling after incubation time, to assess the drug partitioning to blood and plasma as time passes. In addition, the comparison using the ICC values or comparison of pharmacokinetic parameters using GMR and 90% CIs have limited clinical implications. The present study reported the ICC values of more than 0.75, which in many cases are a sign of good correlation between raters (in the present study, between different sampling methods).[23] However, the pharmacokinetic assessment indicated the opposite for C_{max} values, which was likely because the C_{max} values were read directly from the graph. Thus, the discrepancy between plasma and whole blood metformin concentrations was large at first, but became smaller as time passed, which affected the ICC values. Because the venous metformin concentrations and capillary metformin concentrations did not differ greatly, the ICC values calculated using all three methods (plasma versus venous versus capillary) showed increased reliability scores.

Although there was a difference between the metformin concentrations in samples collected by different means, the clinical implications of the VAMS sampling method can be summarized as

follows. One, it can be used in TDM of drugs that use whole blood drug concentration as the standard; however, the target drug concentration range in capillary blood must be studied in further research. Two, although with limitations, it can be used in clinical trials that assess pharmacokinetics. Limitations include early phase clinical trials require extensive pharmacokinetic sampling in one day with frequent blood sampling, which means that frequent pricking of fingers is essential. VAMS enables the reduction of the required volume of blood for pharmacokinetic analysis; however, the disadvantage of frequent lancet uses and the related pain to the trial participants should be addressed. Another disadvantage is that it would be difficult to prepare back-up samples using VAMS. In most pharmacokinetic studies, back-up samples are prepared in case of accidents, such as loss of main samples or the need for re-analysis. However, a blood droplet from the fingertip is only enough to be absorbed by one VAMS tip and is not enough for preparing a back-up sample. This difficulty in preparing back-up samples may not be troublesome in TDM, but it should be overcome when designing clinical trials.

In the present study, metformin was selected because it is a drug commonly used in diabetic patients who require regular blood glucose check, which utilizes lancet finger pricks similar to VAMS. In addition, antidiabetic therapies are mostly individualized nowadays,[24] and because hypoglycemia is a serious complication and patients must be treated quickly, metformin can be a candidate for TDM to achieve

adequate glycemic control. However, as mentioned earlier, drugs that use whole blood concentrations as the standard could be better candidates for pharmacokinetic studies or TDM utilizing VAMS.

Commercial companies advertise the advantage of specimen sampling at home as being convenient and inexpensive because there is no need to visit the clinic or meet the physician in person. The samples are taken at home and sent to commercial laboratories for testing via mail, and the results are returned within days or weeks. This specimen sampling at home is not only limited to clinical practice, but it also provides advantages for clinical trial participants, who do not have to visit the hospital to participate in a clinical trial. For clinical practice such as TDM, drug concentration results could be reported to the physician who would contact the patient later. For clinical trial participants, it can be used to measure steady state drug concentration after multiple oral administrations or to assess pharmacokinetic and pharmacodynamic parameters in late phase clinical trials. This device could also be used in early phase clinical trials where the exact timing of blood sampling is important to evaluate pharmacokinetic parameters; however, the primary advantage of VAMS is not because this device can be used at home, but mainly because of the decreased volume of blood sampling that is required.

In conclusion, there was a difference between the metformin concentrations in venous and capillary blood samples collected by VAMS and those in plasma samples collected by conventional venous

sampling within hours of T_{max}. This difference is most likely due to concentration differences in plasma and whole blood, and not due to the VAMS sampling method. An additional experiment found that the blood-to-plasma ratio of metformin was initially low. The adjustment of the plasma metformin concentration using this blood-to-plasma ratio showed comparable adjusted plasma metformin concentration against capillary concentration of metformin collected by VAMS. VAMS may be more useful in studies and TDM of drugs that utilize whole blood drug concentrations.

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국문초록

서론: Volumetric absorptive microsampling (VAMS) 기기는 환자 진료와 여러 다양한 검사가 필요한 임상시험에서 환자 친화도를 향상시키기 위해 개발되었으며, 극소량의 혈액을 기기의 다공성 tip에 흡수하는 것으로 치료약물농도감시(therapeutic drug monitoring, TDM) 또는 약동학 연구에 사용될 수 있다. 본 연구에서는 VAMS로 채혈한 검체에서의 메트포르민 농도와 기존 정맥 채혈법을 이용해 채혈한 검체에서의 메트포르민 농도를 비교하고 채혈법 차이에 따른 메트포르민 농도를 이용하여 약동학 파라미터를 비교하였다.

방법: 건강인 자원자에서 공개, 단회투여 임상시험을 수행하였다. 건강 자원자는 메트포르민 500 mg 1정을 1회 구강으로 투여받았으며, 투약 후 10시간까지 약동학 분석을 위한 채혈을 진행하였다. 각 채혈 시점마다 다음과 같은 3개의 검체를 채취하였다: A) 기존의 정맥 채혈법으로 채혈한 정맥혈을 원심분리한 혈장 검체, B) A에서 채혈한 정맥혈을 VAMS에 채취한 정맥혈 검체, 그리고 C) 건강 자원자의 손가락 끝에서 VAMS를 이용하여 직접적으로 채취한 말초혈액 검체였다. 혈장(A) 검체, 정맥혈(B) 그리고 말초혈액(C) 검체에서의 메트포르민 농도는 liquid chromatography/tandem mass spectrometry를 이용하여 분석하였고, 약동학 파라미터를 non-compartmental analysis를 이용하여 계산하였다. 추가적으로 메트포르민의 전혈-혈장 농도 비율을 실험적으로 계산하기 위하여 전혈과 혈장에서의 메트포르민 농도를 분석하였으며, 이 비율을 이용하여 임상시험에서 얻은 혈장 메트포르민 농도를 보정하여 말초혈액 메트포르민 농도와 비교하였다.

결과: 총 20명의 건강 자원자가 임상시험을 완료하였다. Area under concentration-time curve (AUC)와 maximum concentration (C_{max}) 의 geometric mean ratios 와 90% confidence intervals 은 정맥혈(B)을 혈장(A)과 비교하였을 때 각각 0.8929 (0.8221 - 0.9698) 와 0.7966 (0.7328 - 0.8660) 이었다. 말초혈액(C)과 혈장(A)을 비교하였을 때는 0.8936 (0.8249 - 0.9680) 와 0.7819 (0.7227 - 0.8459) 이었다. 급내상관계수 (intraclass correlation coefficient)는 정맥혈(B)을 혈장(A)과 비교하였을 때 0.778, 말초혈액(C)과 혈장(A)을 비교하였을 때는 0.781이었다. 메트포르민의 전혈-혈장 농도 비율은 0.66 - 0.80 내로 계산되었으며, 이 비율을 이용하여 보정한 혈장 메트포르민 농도의 증가폭은 VAMS로 채혈한 말초혈액 메트포르민 농도의 증가폭과 비슷하였다.

결론: 기존의 정맥 채혈법으로 얻어진 혈장 내 메트포르민 농도는 VAMS로 채혈한 전혈 (정맥혈과 말초혈액) 내 메트포르민 농도와 차이를 보였다. 그러나 이 차이는 메트포르민의 특성(메트포르민 자체의 낮은 전혈-혈장 농도 비율, 혈구로의 느린 재분포 및 상대적으로 빠른 혈장 청소율 등)에 의한 것이며, 채혈법에 의한 차이가 아닌 것으로 판단된다.

주요어 : Volumetric absorptive microsamping (VAMS), 약동학, 메트포르민, 환자 친화적 임상시험
학 번 : 2013-21772