Metabolism and excretion of GCC-4401C, a factor Xa inhibitor, in humans

Aim: This study investigated the pharmacokinetics, excretion and metabolism of [^{14}C]-GCC-4401C, a factor Xa inhibitor, using a microtracer approach. **Results/methodology:** Total [^{14}C] in excreta and plasma, and radiochromatographic profiles were determined using accelerator MS and liquid scintillation counting. LC/MS was used to assess pharmacokinetics and metabolism. The average plasma exposure for [^{14}C] was higher than for GCC-4401C, indicating that metabolites with a longer half-life than GCC-4401C were formed. Mean total recovery of [^{14}C] in urine and feces was 87.3%. GCC-4401C was the primary contributor (58%) to total radioactivity in plasma. A total of 12 metabolites were identified; M11 and M12 were the major circulating metabolites. The major metabolic routes of GCC-4401C in humans were oxidation and dehydrogenation. **Discussion/conclusion:** The study successfully assessed the elimination and metabolism of GCC-4401C.

First draft submitted: 2 August 2016; Accepted for publication: 7 December 2016; Published online: 23 February 2017

Keywords: accelerator mass spectrometry (AMS) • clinical trial • excretion • factor Xa inhibitor • mass balance • metabolism • metabolite structure • microtracer • radiochromatography

GCC-4401C is a new orally active factor Xa inhibitor being developed for venous thromboembolic disease. Venous thromboembolic disease including deep vein thrombosis and pulmonary or cerebral embolism causes a significant health risk. It can occur as a complication after surgery or trauma and is the third most common cardiovascular illness after acute coronary syndrome and stroke. Deep vein thrombosis may release parts of a thrombus in the circulation, which can block narrow arteries in the lung. This may be fatal if untreated. Thromboprophylaxis and anticoagulants are recommended for prevention of venous thrombosis. Current anticoagulant therapies include vitamin K antagonists, heparin and low-MW heparins. These treatments have some disadvantages; vitamin K antagonists need continuous monitoring for optimal effect and heparin has a short half-life and can only be given parenterally.

To counter these disadvantages, new groups of anticoagulants, the oral factor Xa inhibitors and the direct thrombin inhibitors have been developed. These molecules do not need continuous monitoring. Rivaroxaban (Xarelto®; marketed by Bayer Pharma AG, Berlin, Germany) is the first compound in the class of factor Xa inhibitors registered for the treatment of venous thromboembolic disease and to prevent deep vein thrombosis [1].

Factor Xa inhibitors prevent the formation of a thrombus by inhibiting the conversion of prothrombin to thrombin, thereby blocking the common pathway of the blood coagulation cascade. A disadvantage of factor Xa inhibitors is that there is no antidote in case of a spontaneous bleeding or if acute surgery is necessary. This may be overcome by the development of andexanet alfa, which was recently submitted to the authorities [2].

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Figure 1. Molecular structure of GCC-4401C methanesulfonate salt, (S)-5-chloro-N-((3-(4-(5,6-dihydro-1,2,4-triazin-1(4H)-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)thiophene-2-carboxamide methanesulfonate.

One direct comparison has been made as yet between the new anticoagulants. In a head-to-head investigation into safety and effectiveness in atrial fibrillation, dabigatran, rivaroxaban and apixaban demonstrated similar effectiveness in preventing stroke, but apixaban may be associated with a lower risk of major bleeding in clinical practice [3].

GCC-4401C molecular structure differs from rivaroxaban in that it has a unique 5,6-dihydro-1,2,4-triazin-1(4H)-yl group which makes the molecule amenable to salt formation and which offers a pharmaceutical advantage of increased physicochemical stability [4]. GCC-4401C is presented as the methanesulfonate salt form in **Figure 1**.

In the first-time-in-human (FTIH) study, single doses of 2.5, 5, 10, 20, 40 and 80 mg were generally well-tolerated and a maximum tolerated dose was not reached. Maximum plasma concentrations were reached approximately 3 h after drug administration, and apparent plasma elimination half-life was approximately 7.5 h. GCC-4401C peak concentrations (C_{max}) and total exposure (AUC_{last} , AUC_{0-inf}) were generally dose-proportional across the dose range of 2.5 to 80 mg. Less than 8% of the administered dose was excreted unchanged in urine.

Rivaroxaban is metabolized by cytochrome P450 (CYP) enzymes CYP3A4/5, CYP2J2 and hydrolysis, and 36% is eliminated by the renal route [5]. Apixaban is metabolized through CYP3A4 (major route) and through CYP1A2, 2C8, 2C19, 2J2 (all minor routes), and 27% is removed by the kidneys [6].

In vitro metabolism studies showed that GCC-4401C was potentially metabolized by multiple enzymes including CYP isoenzymes 2D6 and A4, and that it inhibits CYP2D6, CYP2C19 and CYP2B6 at high concentrations (>20 μ M) only. This suggests that GCC-4401C has a very low potential for affecting the metabolism of co-administered drugs.

The objectives of the present study were to investigate the safety, the pharmacokinetics, the routes and rates of excretion in human, and to identify metabolites of GCC-4401C after a single dose administration

of 40 mg containing a microtracer amount (37 kBq) of the [14 C]-labeled compound. Detection of radiolabeled drug material in blood, excreta and liquid chromatographic (LC) fractions was performed by Accelerator MS (AMS) at Eckert & Ziegler Vitalea Science (EZVS, CA, USA) with support from liquid scintillation counting [7–9]. Metabolite structure elucidation and GCC-4401C plasma concentrations (parent pharmacokinetics) were performed at BioCore (Seoul, South Korea). Clinical testing was conducted at PRA Health Sciences (at that time located in Zuidlaren, The Netherlands; currently in Groningen, The Netherlands). The use of a microtracer dose expedited timelines by reducing the human radiation burden to exempted levels that did not require human dosimetry estimates based upon animal tissue distribution data [10,11].

Materials & methods

Study drug preparation

GCC-4401C was manufactured by Dr. Reddy's Laboratories Ltd, India, with a chemical purity of $\geq 97\%$.

[14 C]-GCC-4401C was manufactured with one [14 C] positioned as indicated in **Figure 1** by Curachem (Gimpo, Gyeonggi-do, Korea), at a specific activity of 56.1 mCi/mmol and a radiochemical purity of $\geq 98\%$. Radiosynthesis used the same synthetic route as published for unlabeled GCC-4401C [4], with the use of [14 C]-CDI (1,1'-carbonyldiimidazole [carbonyl- 14 C]) to introduce the [14 C]-label in Step 7.

The capsules for this study were prepared at the PRA Health Sciences Pharmacy under Good Manufacturing Practice (GMP) conditions; 40 mg GCC-4401C was added in an opaque white size 00 hard gelatin Capsugel® capsule (sourced from Capsugel, Bornem, Belgium). [14 C]-GCC-4401C was dissolved in absolute ethanol at 37 kBq per approximately 50 μ l and added to the GCC-4401C in the capsule. This resulted in a capsule dose of 40 mg GCC-4401C with 37 kBq [14 C]-radioactivity, with a final specific activity of 2.49×10^{-5} Ci/g (salt form). For this low radioactivity dose, the radiation burden was estimated to be lower than 0.1 mSv and was considered a 'trivial dose' in the classification of the International Commission on Radiological Protection [12]. Risks related to radiation exposure were thus negligible in this study. To obtain reliable results with this low dose of radioactive label, the analyses were done by using AMS where necessary and liquid scintillation counting where possible [13].

Study design

This was an explorative, open label, Phase I trial with six healthy male subjects receiving a single dose of 40 mg GCC-4401C, with a [14 C]-tracer dose of approximately 37 kBq (1 μ Ci). Blood samples were

Table 1. Summary of demographic characteristics.			
Parameter	Category	Statistic	Overall (n = 6)
Age (years)	–	Mean (SD)	40 (19)
		Median (min-max)	42 (21–58)
Weight (kg)	–	Mean (SD)	75.0 (9.9)
		Median (min-max)	72.0 (64.7–89.4)
Height (cm)	–	Mean (SD)	176 (2)
		Median (min-max)	176 (173–179)
BMI (kg/m ²)	–	Mean (SD)	24.3 (3.3)
		Median (min-max)	23.0 (20.7–28.5)
Sex	Male	n (%)	6 (100%)
Ethnicity	Not Hispanic or Latino	n (%)	6 (100%)
Race	White	n (%)	6 (100%)
Max: Maximum; Min: Minimum; n: Number of subjects.			

taken for the analysis of GCC-4401C (8 ml) and total [¹⁴C] radioactivity (3 ml) and for metabolite profiling/identification (3 ml). The blood samples were taken via an indwelling intravenous catheter or by direct venipuncture into K3 EDTA-containing tubes.

Blood sampling was done predose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24 and 48 h postdose. Urine was collected within 12 h before dosing and for intervals 0–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h postdose. Feces were collected within 48 h before dosing and for intervals 0–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h postdose. Healthy

nonsmoking male volunteers were included in the study based on the following inclusion criteria: aged 18–65 years, inclusive, with at least 25% of them aged between 55 and 65 years; weight ≥ 50 kg; BMI 18.0–30.0 kg/m²; medical screening including electrocardiogram (ECG), blood pressure, clinical chemistry and hematology showing no clinically relevant changes from normal. Exclusion criteria further guaranteed the enrolment of healthy volunteers, and this study especially excluded a history of bleeding risk (easy bruising, gingival bleeding, prolonged bleeding after trauma or dental extraction), recent trauma or surgery, or GI, urinary or gum bleeding in the last 3 months.

Figure 2. Mean concentration-time profiles of GCC-4401C and [¹⁴C]-radioactivity in plasma. The difference in AUC between GCC-4401C and total radioactivity provides an assessment of the total metabolite burden. Total radioactivity concentration kinetics were determined using a 25 μL aliquot of plasma with Accelerator MS analysis for the [¹⁴C] concentration above the natural baseline. Parent GCC-4401C was quantified by LC/MS.

For safety and tolerability evaluation, adverse events, clinical laboratory, vital signs, 12-lead ECG and physical examination assessments were performed at regular intervals. The study was conducted in compliance with US FDA guidelines [14] and under International Conference on Harmonization (ICH) guidelines [15] as incorporated in the European Clinical Trial Directive and the Dutch law on medical research involving human subjects, and complied with the principles of the Declaration of Helsinki. The Clinical Trial Application was approved by the nationally accredited Ethics Committee of BEBO (Beoordeling Ethiek Biomedisch Onderzoek) Foundation, Assen, The Netherlands. Written informed consent was obtained from all participants.

Analysis of GCC-4401C in plasma

The analysis of GCC-4401C in plasma samples was performed at BioCore Co., Ltd, Seoul, South Korea, using a validated LC–MS/MS method. Briefly, GCC-4401C was extracted from human plasma using protein precipitation. Samples were then analyzed using LC with tandem mass spectrometric detection (MS/MS) in the positive mode. There were no endogenous interference peaks at the same retention time as GCC-4401C or the internal standard (rivaroxaban), and the S/N at the LLOQ of 1 ng/ml was greater than 5. The coefficient of determination (r^2) for the calibration curve was better than 0.9900 ($r \geq 0.9950$).

Figure 3. Recovery of [14C]-radioactivity in urine and feces. Urine was analyzed directly or after carbon dilution (early time points) by accelerator MS (AMS). Feces was analyzed exclusively by AMS after carbon dilution. Carbon dilution is enlisted to avoid excessively high [14C] signal in the AMS instrument, thus extending the dynamic range of the assay in practice.

Table 2. Plasma PK parameters of GCC-4401C and total radioactivity.		
PK Parameter	GCC-4401C	[14C]- radioactivity
T _{max}	1.5 h	3.0 h
T _{1/2}	6.91 h	11.7 h
C _{max}	505 ng/ml	872 ng-eq/ml
AUC _{0-inf}	2836 ng.h/ml	8000 ng-eq.h/ml
LLOQ	1 ng/ml	1.78 ng eq/ml

Analysis of total radioactivity in plasma, urine & feces

To define the routes and rates of elimination and mass balance of [^{14}C]-labeled GCC-4401C, total radioactivity (TRA) was determined by AMS (BioMICADAS AMS; Swiss Federal Institute of Technology, Zurich, Switzerland) at EZVS. Samples were analyzed for total [^{14}C] content after conversion to solid graphite using standardized procedures that have been previously described [16]. Neat plasma, urine and fecal homogenates (1:1 slurry in 50% methanol/water) were analyzed to an LLOQ of 1.78 ng-eq/ml, 0.44 and 10.6 ng-eq/ml for plasma, urine (neat analysis) and feces (homogenate), respectively. The LLOQ was based upon an S/N of six times the standard deviation of no drug predose samples [17].

Analysis of metabolites

Radiochromatographic profiling in plasma, urine and feces was performed across all six subjects on selected pooled plasma, urine and feces samples based on the TRA data. Plasma samples were pooled by equal volume across subjects and AUC pooling across the time points from 0 up to 48 h after drug intake [18]. Urine (0–48 h) and fecal homogenate samples (0–96 h) were pooled as equal percentages of collected volume or weights across the subjects for each collection time interval. Pooled samples were extracted with acetonitrile for the plasma pool and a mixture of methanol:water:ethyl acetate (6:3:1, v:v:v) for the fecal homogenate pool. Urine was UPLC-fractionated after dilution with mobile phase (1:1 v:v).

Metabolite radioprofiling for each matrix sample was performed by reverse-phase UPLC fractionation (Waters Acquity connected to a Waters Fraction Collector III fitted with a Phenomenex Luna C18 150 mm $\times$ 4.6 mm, 5- μm column), followed by AMS analysis of collected fractions after conversion to solid graphite using 1 μl of tributyrin as the carbon diluent [19]. The pooled plasma, urine and feces from EZVS were shipped to BioCore for metabolite identification. Full positive TOF scanning followed by information-dependent acquisition was used for metabolite identification. Metabolites were identified based on accurate masses of protonated molecular ions and their collision-induced dissociation fragmentation.

Results

Demographics & safety

Six healthy male subjects were enrolled in the study and completed all study assessments. Their demographic characteristics are summarized in **Table 1**. The subjects were between 21 and 58 years of age and had a BMI between 20.7 and 28.5 kg/m². Three males

were relatively young (21, 23 and 26 years old) and three males were older (57, 57 and 58 years old). All baseline renal and liver function tests were within normal ranges, except protein in urine (trace found in one subject), serum phosphate (slightly below normal in one subject) and serum urea (slightly above normal in one subject); these were all judged as not clinically significant by the principal investigator.

A single dose of 40 mg GCC-4401C, with a [^{14}C]-tracer dose of approximately 37 kBq, was well tolerated and there were no findings of clinical relevance with respect to clinical laboratory, vital signs, ECG or physical examination. The only adverse event reported was mild skin irritation on the arm of one subject, which was considered to be unlikely related to the study medication and had resolved the next day.

GCC-4401C concentrations in plasma

The mean concentration-time profile of GCC-4401C is shown in **Figure 2**. Mean plasma concentrations of GCC-4401C increased rapidly, with initial individual maximum concentrations being reached between 0.5 and 2 h postdose. GCC-4401C mean concentrations showed biphasic elimination, declining rapidly up to

Figure 4. Radiochromatogram of GCC-4401C in human pooled plasma (0–48 h). The dominant [^{14}C] peak appears at a retention time that coincided with the retention time of GCC-4401C, and contained 63.0% of the on-column radioactivity. Metabolite M5 co-eluted just prior to the parent compound whereas M9 was partially resolved at the tail of the parent peak. A distinct metabolite, M12, contained 14.6% of the injected radioactivity though LC/MS analysis suggested this peak could contain both M12 and M11. This radiochromatogram was generated using 15-s temporal eluate fraction collections. Higher temporal resolution could have been obtained using smaller time fractions, but this need was not anticipated prior to study initiation. High-resolution LC/MS was used to assign peak identity.

Figure 5. Representative TOF-MS scan chromatogram of GCC-4401C in human pooled plasma (0–48 h).

12 h after administration and at a slower rate thereafter. At approximately 4 h postdose, a second peak was seen for subjects 003–006 (and even a third peak for subject 003 at 3 h postdose), and a shoulder was seen for subjects 001 and 002. For some subjects, the second peak was higher in concentration than the first peak.

Radioactivity in plasma, urine & feces

After drug administration, the mean concentration-time profiles of GCC-4401C and [^{14}C]-radioactivity in plasma changed in parallel, but with overall higher concentrations for [^{14}C]-radioactivity (Figure 2). Similarly as for GCC-4401C mean plasma concentrations of [^{14}C]-radioactivity increased rapidly with individual maximum concentrations being reached between 0.5 and 2 h postdose. The second peak was seen for subjects 003–006 as for GCC-4401C but the third peak for subject 003 was seen at 5 h postdose for [^{14}C]-radioactivity. Following the secondary peak, [^{14}C] radioactivity mean concentrations declined rapidly up to 12 h after administration, after which they declined at a slower rate, suggesting biphasic elimination.

With a mean of 87.3% of the administered 37 kBq (1 μCi) tracer dose, recovery of [^{14}C]-radioactivity in urine and feces was apparently complete by 168 h postdose (Day 8, which was the end of the last collection interval) (Figure 3). Most of the administered [^{14}C]-radioactivity was recovered within 96 h postdose (average of 86.2%). A slightly higher percentage of [^{14}C]-radioactivity was recovered in feces (average of 45.4%) than in urine (average of 41.8%). Individual [^{14}C]-radioactivity recovery ranged between subjects from 36.0 to 53.9% in feces and from 37.6 to 46.8% in urine. Together with the 8% of the administered dose which was excreted unchanged in urine in the FTIH study, the plasma concentrations in the current study indicate that the largest part of the [^{14}C]-radioactivity excreted in urine originates from one or more GCC-4401C metabolites with a longer plasma elimination half-life (Table 2).

Metabolite profiles in plasma, urine & feces

GCC-4401C was identified by radiochromatographic profiles in all matrices. GCC-4401C was the predominant circulating compound, accounting for approximately 58.4% of the UPLC radioactivity in the collected fractions. The remaining [^{14}C] activity was observed in two other fraction regions, representing 7.71 and 14.6% of the radioactivity, respectively (Figure 4). In urine, GCC-4401C accounted for 15.7% of the radioactivity or less if other metabolite(s) had co-eluted at the same retention time. The remaining [^{14}C] activity was observed in 13 other fraction regions. Two of these accounted for 33.2 and 12.1% of

the radioactivity, respectively; in all other regions each represented less than 10% of the radioactivity. Finally, the predominant component in feces was likely GCC-4401C, representing 23.3% of radioactivity or less if other metabolite(s) had co-eluted at the same retention time. The remaining [^{14}C] activity was observed in nine other fraction regions. Two of these accounted for 14.8 and 13.6% of the radioactivity, respectively; in all other regions each represented less than 10% of the radioactivity.

In summary, GCC-4401C was metabolized by humans after oral administration of 40 mg/37 kBq [^{14}C]-tracer GCC-4401C to six healthy human subjects. Radioprofiling of the plasma pools indicated that parent GCC-4401C was the primary contributor (58.4%) to the TRA in the sample. Many metabolites were identified in plasma, urine and feces (up to 12 unique metabolites in each matrix). M11 and M12 were the major circulating metabolites (Figures 4, 5 and 6), accounting for 14.6% of the total plasma radioactivity. The proposed major metabolic routes of GCC-4401C in humans are oxidation and dehydrogenation, followed by further metabolic reaction. Glucuronidation was the major Phase II metabolic route. The metabolic pathways of GCC-4401C in humans are proposed in Figure 8.

Discussion

In this study, the safety, pharmacokinetics, routes and rates of excretion, the mass balance and the metabolites of GCC-4401C were successfully assessed in healthy male volunteers receiving a single dose of GCC-4401C labeled with [^{14}C]. The study drug was found to be safe and well tolerated. Exposure to TRA in plasma (which represents parent compound plus any circulating metabolites) was much higher than to parent GCC-4401C already during the first 4 h after oral drug intake. This shows that metabolism of parent GCC-4401C started soon after intake, which may well represent first pass metabolism. The shoulder, seen in the plasma concentration curve for GCC-4401C, can be attributed to a dissolution or absorption effect; enterohepatic recirculation was ruled out based on the fact that the shoulder was observed in the majority of the subjects before the time when lunch was provided.

Mean total recovery of [^{14}C]-radioactivity in urine and feces was apparently complete by 168 h post dose, with excretion almost equally divided between the renal and GI tract.

The parent GCC-4401C was the primary contributor to the plasma as indicated by radioprofiling. A total of 12 unique metabolites were identified. M11 (hydroxylated, dehydrogenated) and M12 (dehydrogenated) were the major circulating metabolites. It is at present unknown whether these metabolites possess any rel-

Figure 6. Representative TOF-MS scan chromatogram of GCC-4401C in human pooled urine (0–48 h).

Figure 7. Representative TOF-MS scan chromatogram of GCC-4401C in human pooled feces (0–96 h).

evant pharmacological activity. The proposed major metabolic routes of GCC-4401C in humans are oxidation and dehydrogenation, followed by further metabolic reactions. Glucuronidation was the major Phase II metabolic route.

The results as obtained for GCC-4401C in the current human ADME study showed some differences as compared with rivaroxaban [20]. Thus, parent GCC-4401C represented a lower fraction of total drug-related material in plasma when compared with rivaroxaban (58 vs 89%), indicating a higher plasma exposure to circulating metabolites. Furthermore, GCC-4401C drug material was eliminated to a higher extent in feces (45 vs 28%), and consequently to a lower extent in urine, which may have been due to either a lower level of absorption after oral administration and/or intrinsic differences in the excretion pathways of parent drug and metabolites. However, for both GCC-4401C as well as for rivaroxaban, despite differences in chemical structure, excretion was in large part in the form of parent compound plus oxidative metabolites.

Based on the results of the current study, further investigations into the potential for drug–drug interactions and/or the consequences of renal and liver failure on the pharmacokinetics of GCC-4401C seem warranted, as have indeed also been conducted for rivaroxaban [21,22]. A major part of the metabolites as excreted in urine and feces were oxidative metabolites, which may have been formed via CYP450 iso-enzymes 2D6, 3A4 and 2C19, which were found in *in vitro* metabolism experiments to metabolize GCC-4401C. Interactions or polymorphisms at the level of these iso-enzymes may result in relevant changes in the pharmacokinetics of GCC-4401C. Also, the consequences of decreased liver function on the pharmacokinetics of GCC-4401C should be considered. Since the excretion of unchanged compound in urine was relatively limited, both in this study as well as in the FTIH study, it seems that decreased renal function does not represent a major risk when it comes to the pharmacokinetics of GCC-4401C. However, the elimination of metabolites in urine may warrant further investigations, especially in case one or more of these metabolites would possess pharmacological activity. Whether or not the two major circulating metabolites in plasma (M11 and M12, together representing ~15% of the radioactivity in plasma, i.e., ~25% of parent exposure) require further safety testing as per FDA Guidance [14] or ICH M3 [15] will need to be investigated further, since metabolism has not been assessed in animals until now.

The extent of absorption of GCC-4401C in human may be estimated in this study as well, though it was not part of the study objectives. Thus, the 42% of the radioactive dose that was recovered from urine after

Figure 8. Proposed metabolic pathways of GCC-4401C in humans.

oral intake of the medication represents drug material that was indeed absorbed from the GI tract and distributed throughout the body. Moreover, in feces, 45% of the amount of radioactivity as administered orally was found, which may represent unabsorbed parent drug and/or systemic metabolites. Since maximally 23% of the radioactivity in feces was found to be parent drug, which was potentially not absorbed into the body, the remaining portion of radioactivity in feces was apparently represented by metabolites of GCC-4401C. From these data, we conclude that approximately 77% of the dose was absorbed into the body ($42\% \text{ plus } 0.77 \times 45\%$).

Conclusion

The results of this study show that orally administered GCC-4401C is metabolized in human and rapidly eliminated via renal and fecal routes. Though several metabolites were identified, any clinical relevance of these metabolites has yet to be clarified. These results support the further clinical development of GCC-

4401C as a factor Xa inhibitor. In the development, further studies to assess the consequences of drug–drug interactions and/or decreased function at the level of the liver and/or kidneys seem warranted.

Authors' contributions

SP van Marle and AF Roffel have equally contributed to the writing of this manuscript as co-first authors. S Choi conceived the study. RG Tiessen performed the study. SR Dueker oversaw aspects of radio-analysis and assisted with writing sections of the manuscript. HJ Lee oversaw the conduct of metabolite identification. S Choi, HJ Lee, SR Dueker and RG Tiessen critically reviewed the manuscript.

Financial & competing interests disclosure

This investigation was funded by Green Cross Corporation Korea and executed by PRA Health Sciences Early Development Services The Netherlands, BioCore Company Ltd Korea and Eckert & Ziegler Vitalea Science USA. Jan Hartstra, Senior Biostatistician at PRA Health Sciences, provided the analyses shown in **Figures 2** and **3** and **Tables 1** and **2**. SP van Marle,

Executive summary

- GCC-4401C is an orally active factor Xa inhibitor being developed for prevention of venous thromboembolic disease.
- In the present study, the safety, pharmacokinetics, excretion, mass balance and metabolism were investigated following a single oral dose of 40 mg GCC-4401C labeled with a microtracer amount of [^{14}C] in six healthy male volunteers.
- Total [^{14}C] in excreta and plasma, and radio-chromatographic profiles were determined using a combination of accelerator MS and liquid scintillation counting. LC/MS techniques were used to assess parent pharmacokinetics and metabolite identification.
- The average plasma exposure of [^{14}C] radioactivity was higher compared with the values for GCC-4401C, indicating that one or more metabolites with a longer $T_{1/2}$ than parent GCC-4401C were formed.
- Recovery of [^{14}C]-radioactivity in urine and feces was apparently complete by the end of excreta collection (168 h postdose), at 87.3%.
- Approximately equal portions of [^{14}C] administered were recovered in feces (45.4%) and urine (41.8%), indicating that both renal and hepatic excretion are of importance. Parent compound was found in both urine and feces.
- The fraction absorbed into the body was estimated to be approximately 77% of the dose, calculated as excretion of drug material in urine and metabolites in feces.
- GCC-4401C was the primary contributor (58%) to the total radioactivity in plasma.
- A total of 12 metabolites were identified in plasma, urine and feces.
- M11 (hydroxylated, dehydrogenated) and M12 (dehydrogenated) were the major circulating metabolites, but it is unknown whether these metabolites possess any relevant pharmacological activity.
- Metabolism of GCC-4401C started soon after intake, which may well represent first pass metabolism.
- The proposed major metabolic routes of GCC-4401C in humans are oxidation and dehydrogenation, followed by further metabolic reactions.
- Glucuronidation was the major Phase II metabolic route.
- A single oral dose of 40 mg GCC-4401C was found to be safe and well-tolerated in healthy male volunteers.
- The results of this study show that orally administered GCC-4401C is metabolized in humans and rapidly eliminated via renal and fecal routes.
- Based on the results of the current study, further investigations into the potential for drug–drug interactions and/or the consequences of renal and liver failure on the pharmacokinetics of GCC-4401C seem warranted.
- The low radioactivity microtracer approach shortened the time to study completion through easing the requirements for quantitative whole body autoradiography data for an estimation of the expected radiation burden in humans.

AF Roffel and RG Tiessen are employees of PRA Health Sciences. HJ Lee and SR Dueker are current employees of BioCore and SR Dueker is a paid consultant for Accium Biosciences. SR Dueker was previously employed at Eckert & Ziegler Vitalea Science. S Choi was an employee of Green Cross Corporation at the time this study was conducted. All authors were on their respective company's payrolls, and conducted their roles in the study as part of their regular job assignments. No conflicts of interest are reported. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The study was conducted compliant with FDA guidelines [14] and under ICH guidelines [15] as incorporated in the European Clinical Trial Directive and the Dutch law on medical research involving human subjects, and complied with the principles of the Declaration of Helsinki. The Clinical Trial Application was approved by the nationally accredited Ethics Committee of BEBO Foundation, Assen, The Netherlands. Written informed consent was obtained from all participants.

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