Citation: Clin Transl Sci (2021) 14, 231–238; doi:10.1111/cts.12848

ARTICLE

An Investigation of the Metabolism and Excretion of KD101 and Its Interindividual Differences: A Microtracing Mass Balance Study in Humans

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The absorption, metabolism, and excretion (AME) profiles of KD101, currently under clinical development to treat obesity, were assessed in humans using accelerator mass spectrometry (AMS) after a single oral administration of KD101 at 400 mg and a microdose of ^{14}C-KD101 at ~ 35.2 µg with a total radioactivity of 6.81 kBq. The mean total recovery of administered radioactivity was 85.2% with predominant excretion in the urine (78.0%). The radio-chromatographic metabolite profiling showed that most of the total radioactivity in the plasma and the urine was ascribable to metabolites. The UDP-glucuronosyltransferase (UGT), including UGT1A1, UGT1A3, and UGT2B7, might have contributed to the interindividual variability in the metabolism and excretion of KD101. The microtracing approach using AMS is a useful tool to evaluate the AME of a drug under development without risk for high radiation exposure to humans.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✔ The results of a mass balance study conducted in animals are not reliably translated to humans.

WHAT QUESTION DID THIS STUDY ADDRESS?

✔ What did the absorption, metabolism, and excretion (AME) profiles of KD101 and its interindividual variability look like in humans, and how different were they from those in rats?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

✔ A human mass balance study using the microtracing approach was useful to assess the differences in the AME profiles of KD101 between humans and rats. KD101 was extensively metabolized and excreted mainly in the urine in humans, whereas the feces was the major excretory route of KD101 in rats.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

✔ The microtracing AME study using accelerator mass spectrometry is a useful tool to evaluate the AME of a drug under development without risk for high radiation exposure in humans. Every drug development should pay due attention to inter-species difference in the AME of a drug between animals and humans.

Early identification of the absorption, distribution, metabolism, and excretion (ADME) properties of a candidate molecule is critical for successful new drug development. Various *in silico*, *in vitro*, and *in vivo* technologies are available to predict the ADME properties of a candidate molecule in humans. Of these options, the mass balance study using a radiolabeled candidate drug has been widely used to determine the extent of absorption and proportional exposure to the metabolites, to investigate the route(s) of elimination, and to identify the circulatory and excretory metabolites.[1]

Usually, the mass balance study is performed first in rats.[2] However, the results from rats are not easily translated to humans because drug metabolism is different, more complicated, and more variable in humans than in highly in-bred rats.[3] For example, rats have different regulation of bile acid synthesis, anatomy (i.e., absence of gallbladder), extent of bile excretion, and gut microbiome.[3,4] Therefore, drugs that are predominantly excreted into the bile have different pharmacokinetic (PK) characteristics between rats and humans.[3–5] Furthermore, the mass balance study in animals uses high radioactivity, such as 1–2 MBq for rats or

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Received: May 1, 2020; accepted: June 24, 2020. doi:10.1111/cts.12848

5–10 MBq for dogs,[6] whereas this amount of radioactivity cannot be administered to humans unless supported by safety data from the quantitative whole-body autoradiography, followed by estimates of human dosimetry. This is, of course, burdensome, expensive, and time-consuming. Thus, the microtracing mass balance study has become more popular in humans because it uses much smaller radioactivity, typically < 1,000 nCi or 0.0037 MBq, whereas a pharmacologically active dose is co-administered to ensure relevant PKs. The subsequent radioactivity levels in collected specimens can only reliably be detected by accelerator mass spectrometry (AMS).

KD101, a single isomer of an essential oil [(–)-α-cedrene)] extracted from the *Juniperus virginiana* (cedar tree), is under clinical development to treat obesity. KD101 promoted energy metabolism without inhibition of appetite, and improved obesity-related biomarkers in the preclinical studies.[7] KD101 is very lipophilic (log P = 6.38) and does not partition into pure water. The absolute bioavailability of KD101 in rats was ~ 78.2% after oral administration at 100 mg/kg. KD101 was extensively metabolized and mainly excreted into the feces (data on file).

The objectives of this study were to evaluate the absorption, metabolism, and excretion (AME) of KD101 and to profile its metabolites in humans using the microtracing approach after a single oral administration of KD101 at 400 mg and a microdose of ^{14}C-KD101 at ~ 35.2 μg (~ 6.81 kBq or 184 nCi). In addition, the effect of pharmacogenomic influence on the human AME of KD101 was explored.

METHODS

Study drug

Curachem (Cheongju-si, Korea) produced ^{14}C-KD101 by labeling a carbon in the cyclopentane ring of KD101 ((-) alpha-cedrene) with ^{14}C (**Figure S1**). The radiochemical purity and specific activity (SA) of ^{14}C-KD101 was 96.6% and 1.9 MBq/mg (52.2 μCi/mg), respectively. ^{14}C-KD101 at 35.2 μg was dissolved in medium-chain triglyceride to make 1.4-mL oral solution containing a radioactivity of 6.81 kBq or 184 nCi. The whole manufacturing processes of ^{14}C-KD101 strictly conformed to the current Good Manufacturing Practice requirements. On the other hand, unlabeled KD101 was provided by Kwangdong Pharmaceutical (Seoul, Korea) in the 200 mg soft capsule formulation.

Clinical study

Healthy male subjects 24–45 years of age with a body mass index ranging from 27–34 kg/m^2 with no history of clinically significant diseases or abnormal clinical laboratory test results at the time of screening were eligible for the clinical study. Six eligible subjects were admitted to the Clinical Trials Center at Seoul National University Hospital a day before study drug administration. On day 1, subjects orally received KD101 at 400 mg and ^{14}C-KD101 at ~ 35.2 μg after a standard meal. Serial blood, urine, and fecal samples were collected until 288 hours postdose. Safety monitoring was carried out throughout the entire study by physical examination, vital signs, laboratory tests, including 12-lead electrocardiography, and adverse event recordings. This study was approved by the Institutional Review Board of Seoul National University Hospital. All of the subjects gave written informed consent after they were fully explained about the study, and before any study procedure was performed. The clinical study was conducted in full accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use and other applicable regulations (clinicaltrials.gov registration number: NCT02819934).

Bioanalysis of unlabeled KD101

The plasma and urine concentrations of unlabeled KD101 were determined by modification of the previous gas chromatography-tandem mass spectrometric (GC-MS/MS) method.[8] The 100 μL of human plasma samples were mixed with 10 μL of 4-*tert*-amyl-phenol in ethyl acetate (internal standard) and 200 μL of ethyl acetate. The mixtures were vortex-mixed for 3 minutes and centrifuged at 13,000 g at 4 °C for 8 minutes. The aqueous layer was once more extracted with ethyl acetate. The organic layer was transferred to an amber glass vial. The aliquot (0.5 μL) was injected at 270°C in splitless mode into the GC-MS/MS system consisting of a Trace Ultra GC coupled with a Triplus auto-sampler and a TSQ 8000 Evo triple quadruple mass spectrometer (Thermo Scientific, San Jose, CA). Analytes were achieved on a fused silica capillary column, TG-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness; Thermo Scientific) using the ultra-high purity helium as a carrier gas at a flow rate of 1.2 mL/min. The gas chromatography oven temperature was at 50°C for 3 minutes after injection and then programmed to 150°C at 20°C/min, and at 30°C/min to 300°C, which was held for 5 minutes. The mass spectrometer was operated in a positive electron impact ionization mode (70 eV) with collision energy of 8 eV for KD101 and 3 eV for 4-*tert*-amyl-phenol. The selected reaction monitoring mode was used for the quantification of KD101 at m/z 204.2 → 119.0 and 4-*tert*-amyl-phenol at m/z 164.2 → 135.0. The Xcalibur program (version 2.10; Thermo Scientific) was used for GC-MS/MS system control and data processing. The calibration curves for KD101 in human plasma and urine were 2–800 ng/mL and 50–8000 ng/mL, respectively. Coefficient of variation values were within 6.7% and accuracy values were 89.0–104.8% at 3 quality control plasma sample levels, and coefficient of variation values were within 5.3% and accuracy values were 99.4–107.3% at 3 quality control urine samples.

Bioanalysis of total radioactivity and metabolite profiling

A liquid scintillation counter (Hidex, Finland) was used to screen samples that might require dilution before AMS processing. From this screening, it was evident that several early timepoint urine samples had a sufficient radioactivity enough to be quantified by the liquid scintillation counter. For those samples, 1 to 3 mL of urine was placed in a 20 mL scintillation vial, mixed with cocktail, and radioactivity

was counted. Accuracy and precision were assessed independently with the NIST traceable Quench Standards. Results were expressed in disintegration per minute (dpm) according to the following Eq. (1):

$$\text{Concentration} = \frac{\text{dpm from LSC output (total void volume)}}{\text{Vol}_{\text{sol}}}, \quad (1)$$

where Vol_{sol} was the volume of the matrix taken for analysis.

AMS was used to determine the total radioactivity (TRA) of the samples (plasma, urine, whole blood, and homogenated feces) with tributyrin added as a trapping agent because of the volatility of KD101. In general, 400 µL of a 200 mg/mL tributyrin carbon diluent in methanol was added to 40 µL of the well-mixed samples. After vortex mixing, a 10 µL aliquot was transferred into a quartz insert for graphitization. The aliquots were dried for ~ 10 minutes under vacuum concentration to remove methanol and water, which kept the loss of intact KD101 to a minimum (~ 90% recovery based on method development testing). Then, the aliquots were converted to solid graphite by undergoing combustion and reduction reactions. Graphite samples were prepared and analyzed for ^{14}C contents using an AMS instrument (6 MV Tandetron; High Voltage Engineering Europa B.V., Amersfoort, Netherland) at the Korean Institute of Science and Technology. Each batch was tracked with a set of oxalic acid standards for batch acceptance criteria. For any batch, > 67% of the standards had to be within ± 15% of the nominal value to be accepted.

Results of the AMS analysis were expressed as mass units for whole blood and plasma, and dpm for excreta (urine and feces) was calculated using the following Eq. (2) and based on the SA of ^{14}C-KD101. The carbon contribution from whole blood and plasma were not included in the equation, which would create a mixed carbon calculation. Rather, the carbon from tributyrin was used as the sole carbon source in the calculations and added in excess to dominate the carbon content. This may slightly underestimate the final concentration determinations, but, as stated above, tributyrin addition was a necessary tradeoff to prevent losses of KD101 during vacuum concentration. Fecal homogenates and urine had low native carbon values so using tributyrin as the sole source of carbon was correct:

$$\text{DPM/mL or g} = \frac{(R_{\text{meas}} - R_{\text{bkg}})\left(0.01356\frac{\text{dpm}}{\text{mg}}\text{C}\right)(\text{C}_{\text{trib}})}{\text{Vol}_{\text{samp}}}, \quad (2)$$

where mass units are obtained by dividing the DPM/mL or g concentration by the SA when expressed at DPM/ng, R_{meas} was the measured $^{14}C/C$ ratio in units of Modern, R_{bkg} was calculated from the average of the AMS data resulting from the predose samples and was calculated individually for each matrix, and C_{trib} was the concentration of C in the tributyrin solution (carbon diluent).

Plasma samples at peak concentration (C_{max}) and urine samples obtained over 0–12 hours postdose were pooled and prepared for further bioanalyses at the BioCore ADME Laboratory (Seoul, Korea). Aliquots of pooled plasma and urine samples were processed for direct ultra-performance liquid chromatography injection after protein precipitation or dilution, respectively. Timed fractions comprising a profile were collected and analyzed based on elution times.

Pharmacokinetic analysis

A noncompartmental method in the Phoenix WinNolin (version 6.3; Certara, St. Louis, MO) was used to derive the PK parameters of KD101 and TRA. For C_{max} and time to reach at C_{max} (T_{max}) of KD101 and TRA, the observed values were used. The area under the plasma concentration–time curve from zero to the last measurable concentration (AUC_{last}) was calculated using the linear trapezoidal rule. AUC_{inf} was the sum of AUC_{last} and the last measurable concentration divided by the slope of the regression line between the logarithmically transformed concentrations and time. The urine and fecal recovery of radioactivity at each collection time was calculated using the following Eq. (3):

$$\text{Recovery of radioactivity } (\%) = \frac{\text{dpm per collection}}{\text{Total dpm contained in administered dose}} \times 100 \quad (3)$$

Cumulative recovery was calculated by summing the percentages of recovery at each collection time.

Genotyping and association analysis

DNA processing and genotyping were conducted by DNA Link (Seoul, Korea) using the DMET platform (Affymetrix, Santa Clara, CA). Each genotype was reported as major homozygotes, heterozygotes, or minor homozygotes. To explore genetic variants associated with the PK interindividual variability of KD101, the analysis of variance test, Mann–Whitney *U* test, and LASSO regression analysis was performed. The SAS software (version 9.3; SAS Institute, Cary, NC) was used for statistical analyses and the R package *glmnet* implemented in the R software (version 3.0.1; R Development Core Team, Vienna, Austria) particularly for LASSO regression. A P value < 0.05 was considered statistically significant.

RESULTS

Clinical study

Six healthy male subjects completed the study as planned, and the study drug was well-tolerated. No adverse events, clinically significant findings, or laboratory test abnormalities were observed during the entire study.

Pharmacokinetics of KD101 and total radioactivity in humans

KD101 was rather slowly absorbed after a single oral administration (median T_{max}: 5.0 hours postdose; **Table 1**), and eliminated according to a multi-exponential pattern

Table 1 Pharmacokinetic parameters of total radioactivity and KD101 in six healthy male subjects after a single oral administration of ^{14}C-KD101 (6.81 kBq) and 400 mg KD101

	Total radioactivity	KD101	Ratio of total radioactivity-to-KD101
C_{max}, μg or μg-eq/mL	7.6 ± 1.1	1.5 ± 0.7	6.1 ± 3.6
T_{max}, hour	6.0 (5.0–6.0)	5.0 (3.0–8.0)	–
AUC_{last}, μg*h or μg-eq/mL	201.8 ± 39.1	6.7 ± 2.9	–
AUC_{inf}, μg*h or μg-eq/mL	321.6 ± 80.1	7.2 ± 3.3	51.8 ± 25.4
$t_{1/2}$, hour	152.8 ± 48.2	45.5 ± 15.0	–
Whole blood-to-plasma ratio of TRA	0.42 (0.28–0.61)		

Results are presented as mean ± SD except T_{max} and whole blood-to-plasma ratio of TRA, for which median (range) is shown.
AUC_{extra}, percentage of AUC_{inf} due to extrapolation from time of last observed concentration to infinity; AUC_{inf}, area under the plasma concentration-time curve from time 0 hour to infinity; AUC_{last}, area under the plasma concentration-time curve from time 0 hour to the last measurable concentration; C_{max}, maximum plasma concentration; $t_{1/2}$, terminal half-life; T_{max}, time to maximum plasma concentration after oral administration; TRA, total radioactivity.

(**Figure 1**). In contrast, plasma TRA of KD101 was gradually eliminated (mean terminal half-life: 152.8 hours; **Table 1**); TRA was still detected even at 288 hours postdose, the last sampling point, in all of the subjects (**Figure 1**). The discrepancy in the plasma PKs between TRA and KD101 resulted in a huge ratio between them: 6.1 ± 3.6 (mean ± SD) and 51.8 ± 25.4 for C_{max} and AUC_{inf}, respectively (**Table 1**). The median whole blood-to-plasma ratio of TRA at 4 hours postdose was 0.42 (range 0.28–0.61; **Table 1**). The ratios decreased over time (data not shown).

Mass balance and metabolite profiling in humans

The mean recovery of radioactivity up to 288 hours postdose was 85.2% (range 75.4–99.0%) of the administered dose (**Table 2**). The administered radioactivity was recovered predominantly in the urine: 78.0% (range 68.3–92.3%) and 7.3% (range 5.9–8.5%) in the urine and feces, respectively (**Table 2**). Most of the radioactivity in the urine was excreted within 48 hours, whereas the radioactivity slowly excreted into the feces and reached the plateau after ~ 120 hours in most subjects (**Figure 2a**).

A peak at 8.995 minutes in the plasma radiochromatogram was overlapped with the peak of KD101 in the UV chromatogram, which accounted for only 10.7% of the total on-column radioactivity (**Figure 3a**). The remaining radioactivity was widely spread over the entire chromatogram, with 33.5% eluting in the unretained fraction (**Figure 3a**). In contrast, no peak was associated with KD101 at 8.995 minutes in the urine radiochromatogram (**Figure 3b**).

Interindividual variability in the pharmacokinetic parameters of KD101 and associated genetic factors in humans

The PKs of KD101 and TRA showed relatively high interindividual variability, particularly for the exposure to KD101 and ratios of TRA to KD101 in C_{max} and AUC_{inf} (**Table**

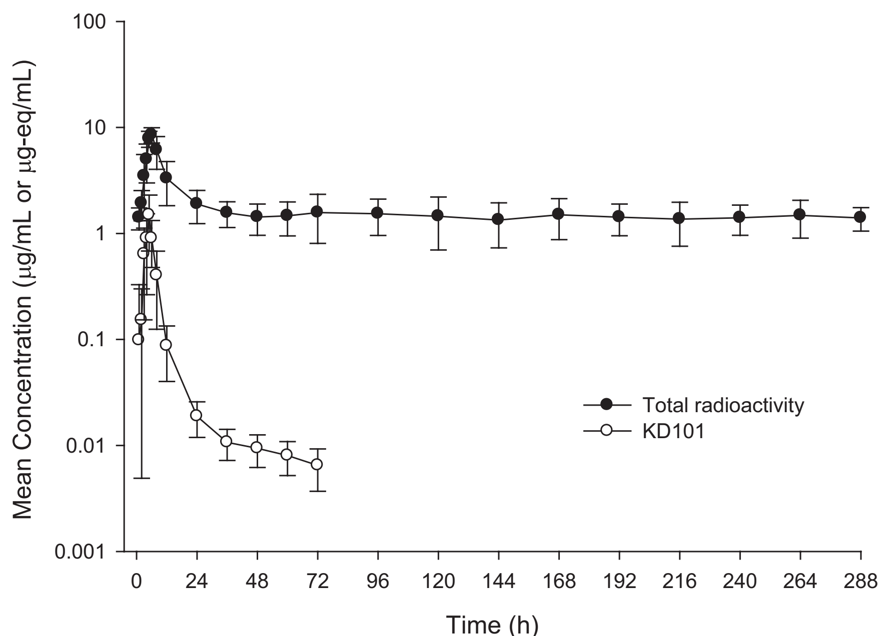

Figure 1 Mean plasma concentration-time profiles of total radioactivity (•) and KD101 (○) after a single oral administration at 6.81 kBq of ^{14}C-KD101 and 400 mg KD101, respectively. Concentrations are shown in the logarithmic scale. The error bars denote the SDs.

Table 2 Results of mass balance studies in humans and rats after oral administration of ^{14}C-KD101

	Human (n = 6)	Rat (n = 3)[16]
Sex, age at dosing, body weight	Male, 24–45 years, 88.1–114 kg	Male, 7–8 weeks, 193.0–272.9 g
Dose and radioactivity of ^{14}C-KD101	400.0352 mg (6.81 kBq) after a standard meal	100 mg/kg[a] (37,000 kBq/kg) at fasted state
Sampling timepoints, hours, postdose	(Plasma) predose, 1, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 192, 216, 240, 264, and 288 (Urine) 0–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168, 168–192, 192–216, 216–240, 240–264, and 264–288 (Feces) every 24 hours until 288	(Plasma) predose, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 48, 72, 96, 120, 144 and 168 (Urine) 0–6, 6–12, 12–24, 48–72, 72–96, 96–120, 120–144, and 144–168 (Feces) 0–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168
Total recovery, %	85.2 ± 7.8	97.6 ± 2.2 (including radioactivity of carcass)
Urine recovery, %	78.0 ± 7.9	28.7 ± 2.4
Feces recovery, %	7.3 ± 0.9	66.4 ± 1.5
Radioactivity excretion rates in expired air and bile, at 24 hours postdose, %	Not measured	0 (expired air) and 27.5 (bile)
Relative distribution (percent of dose) of KD101in pooled samples	(Plasma) 10.7% at T_{max}, (urine) not detected, (feces) not measured	(Plasma) 41.9% at 4 hours postdose, (urine) not detected, (feces) 9% during 0–48 hours postdose

T_{max}, time to maximum plasma concentration after oral administration. [a]Human equivalence dose = 16.1 mg/kg.

S1). Several phase II metabolizing enzymes, such as the UDP-glucuronosyltransferase (UGT) 1A1, 1A3, and 2B7, and transporters were likely associated with these PK parameters (**Table S2**). Subject 3 having the wild type of single-nucleotide polymorphisms (SNPs) in UGT2B7 (i.e., *rs7668258*, *rs7438284*, and *rs7439366*) showed the highest exposure to KD101 as expressed in AUC_{inf}, whereas subject 1, who carried the variant type in all of those SNPs, had less than one third exposure to KD101 (**Figure S2a**). As a result, the AUC_{inf} ratio of TRA to KD101, which represents the metabolic burden, revealed a strong negative correlation with the wild-type SNP combinations in UGT1A1, UGT1A3, and UGT2B7 (**Figure S2b**). For example, subject 1 with all variant types in UGT1A1, UGT1A3, and UGT2B7 showed the highest metabolic burden, whereas subject 3 carrying the wild type in those SNPs showed the lowest metabolic burden (**Figure S2a**). In addition, subject 1 exhibited the highest urinary recovery rate of > 90% in TRA (**Figure S2c**).

DISCUSSION

We showed that the microtracing approach is a viable alternative to the conventional high-radioactivity study to evaluate the AME of a drug in humans. Although ^{14}C-KD101 was given at an extremely low radioactivity of ~ 6.81 kBq or 184 nCi in this study, we were able to recover the mean total radioactivity up to 85%. This is acceptable given that the mean recovery rate of radioactivity in 36 human mass balance studies was 87%.[2] The conventional high-radioactivity mass balance approach is limited due to the concern for considerable radiation exposure and long-term, unpredictable

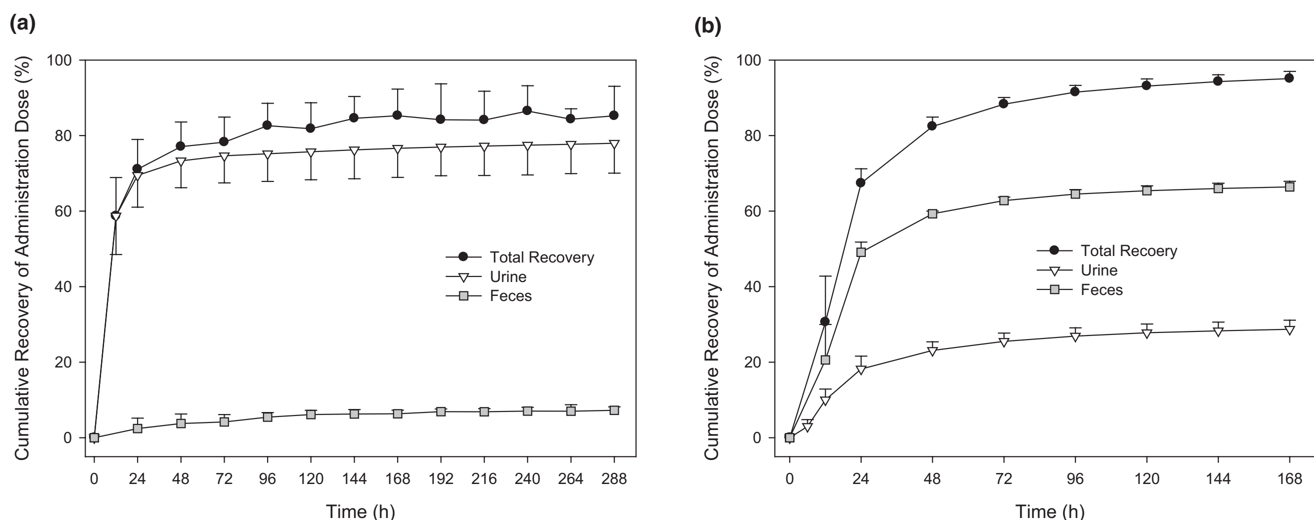

Figure 2 Mean cumulative recovery of total radioactivity in the urine and feces after a single oral administration of ^{14}C-KD101 to humans at 6.81 kBq (**a**) and rats at 37,000 kBq/kg (**b**).[16] The error bars denote the SDs.

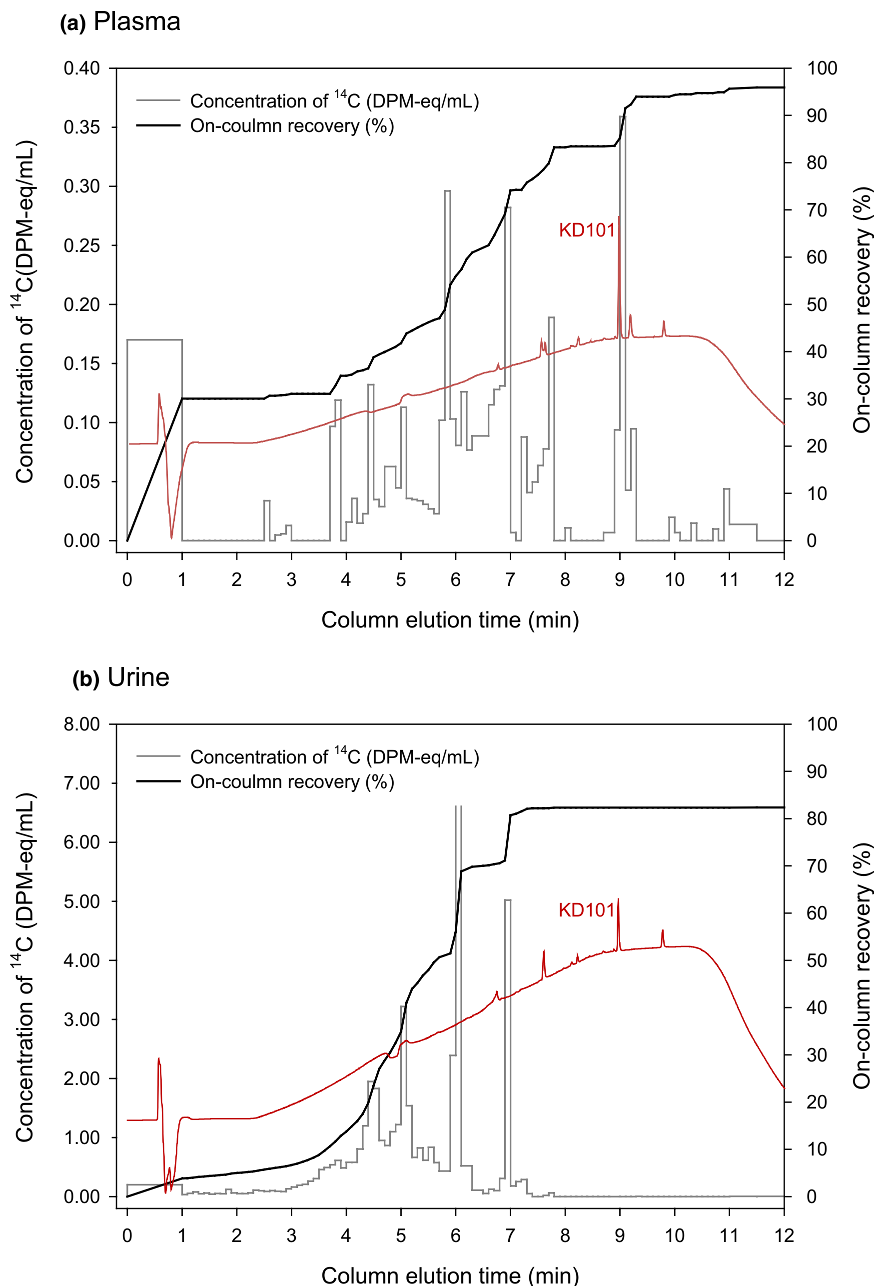

Figure 3 Radiochromatograms of metabolites after a single oral administration of ^{14}C-KD101 to healthy subjects. (**a**) Pooled plasma samples at peak plasma concentration (C_{max}); (**b**) pooled urine samples during 0–12 hours post-dose. UV/Vis trace at 220 nM (red line) is overlaid on the ^{14}C fraction contents and KD101 elutes of 8.995 minutes.

follow-up of human subjects. However, the mass balance study using a microtracer dose < 1,000 nCi is without the radiation risk, thereby alleviating the regulatory requirements for the quantitative whole-body autoradiography study and complex dosimetry calculation. AMS, a highly sensitive analytical tool, has enabled for the human AME study to be performed with a trace radioactive dose.[9] As the cost of AMS-based bioanalysis has continuously decreased,[10,11] many drug development studies have actively adopted the AMS technology.[12–14] This may facilitate faster and earlier decision making during drug development, which leads to shortened overall drug development timeline and increased efficiency.[15]

It is noteworthy that our results in humans were quite opposite to those reported in the mass balance study in rats with KD101.[16] After oral administration of ^{14}C-KD101 to 3 rats, the mean total recovery of radioactivity, obtained from the feces, urine, and carcass, was 97.6% of the dose by 168 hours post-dose. However, radioactivity in the rats was recovered mainly in the feces (66.4%), which was nine times greater than that in humans (7.3%; **Table 2**). The bile-duct cannulated rats showed that the cumulative excretion rates of radioactivity

until 48 hours to the injected radioactivity were 54.7% (feces), 27.5% (bile), and 2.3% (urine). In addition, 94% of the injected radioactivity was reabsorbed in the intestine, suggesting enterohepatic recirculation in rats.[16] Furthermore, the AME study in rats showed that KD101 was transformed to various metabolites during absorption and enterohepatic recirculation.[16]

In *in vitro* experiments, KD101 was extensively metabolized by phase I and II metabolisms and a considerable portion of metabolites was converted to varied glucuronide metabolites (data on file). Rats are a good biliary excreter (threshold: 325 Da), whereas humans poorly excrete xenobiotics and their metabolites through the biliary route (threshold: 500 Da).[17,18] Therefore, we hypothesize glucuronide compounds are more readily diffused into the blood flow in humans than in rats, thereby easily excreted in the urine (**Figure 4**). In contrast, glucuronide metabolites in rats would rather be excreted through the bile, followed by enterohepatic recirculation. To support this hypothesis, previous studies reported that glucuronide metabolites were excreted mainly in the bile in rats, but in the urine in humans.[19,20] Furthermore, the time for cumulative excretion through the main excretory route to reach plateau was much longer in rats (72 hours, feces) than in humans (24 hours, urine; **Figure 2**, **Table S1**). This finding could also support our hypothesis. As we showed in this study, the best model for humans is human. For this reason, Obach *et al*. proposed a strategy for human-only mass balance study.[21] Although that is a controversial proposal, a consensus has been reached on the necessities for the human mass balance study, particularly at an early stage of drug development.[22,23]

Several genotypes were associated with the exposure to KD101, its metabolic burden and excretory recovery rate (**Figure S2**, **Table S2**). Subject 1 with deficiency in the UGT1A1 enzyme showed less glucuronidation, resulting in a total bilirubin greater than two-times the upper limit and a much larger quantity of KD101 metabolites. The association between UGT1A1 variants and greater metabolic burden and the distinctly high urine recovery rate indicated that KD101 bypassing UGT1A1 glucuronidation might be transformed to relatively smaller or more hydrophilic metabolites, which are easily excreted in the urine. Furthermore, UGT2B7 appears to have an important role in the glucuronidation of KD101. The wild type of UGT2B7 such as *rs7668258* and *rs7438284* were associated with a higher exposure to valproic acid[24,25] or a lower clearance or higher plasma concentrations of lamotrigine,[26,27] consistent with our findings.

This study had several limitations. First, we did not identify the metabolites of KD101 in humans mainly because it is highly lipophilic and hard to handle, thereby not evaluating the qualitative and quantitative differences in metabolites between rats and humans. Therefore, the safety of the circulating metabolites of KD101 in humans remains unanswered. Second, the mean total recovery of radioactivity was 85%, which may not be large enough, particularly by the criteria proposed previously: 85–95% or above[28] or 90%.[29] Nevertheless, the confinement period in our study (14 days) was sufficiently long

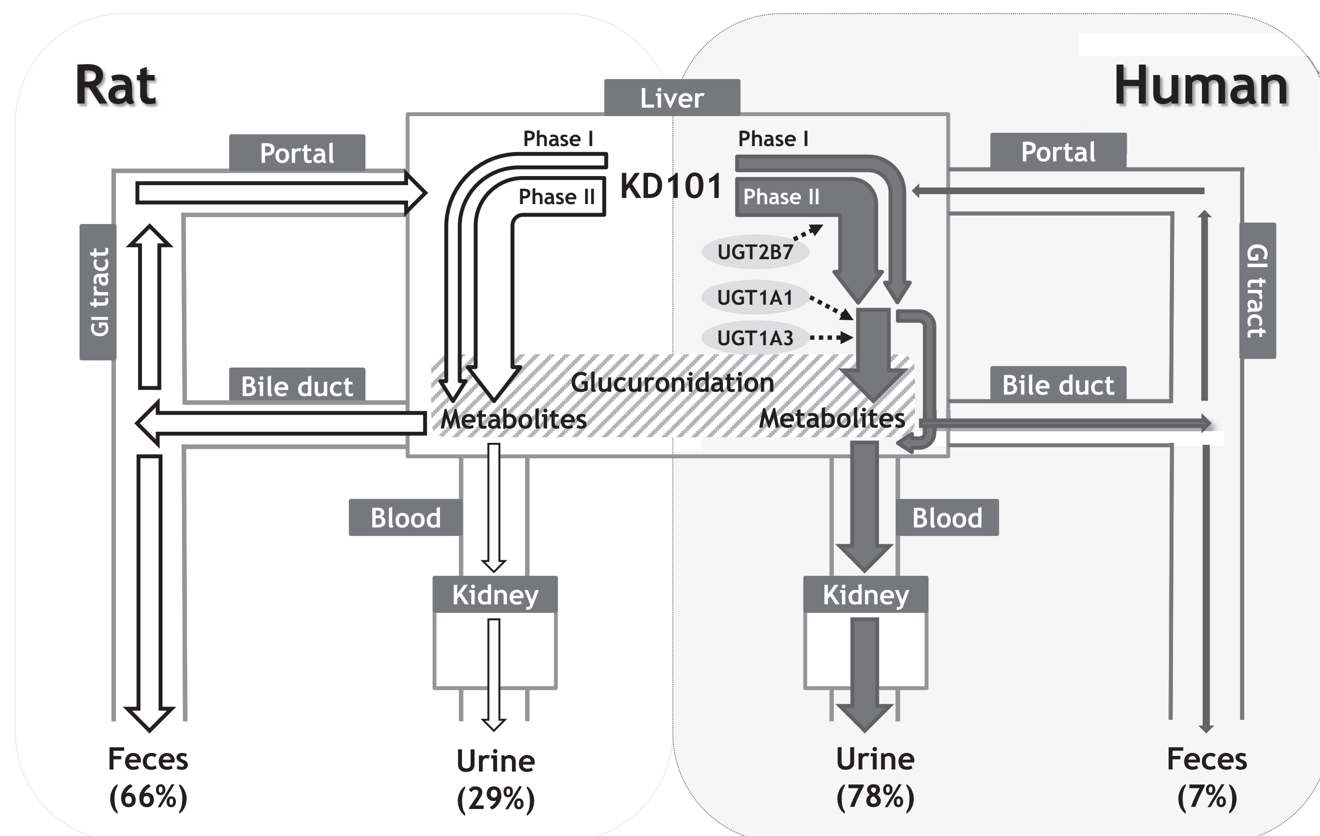

Figure 4 Absorption, metabolism, and excretion of KD101 in rats and humans. GI, gastrointestinal.

enough in terms of scientific and practical reasons. Third, although we found some associations among the polymorphic drug metabolizing enzymes, particularly UGTs and the PK of KD101, it was mainly exploratory due to the small number of subjects. Therefore, future studies with a larger sample size are warranted to confirm our findings.[30,31]

In conclusion, the human mass balance study using the microtracing approach was indispensable for elucidating the differences in the AME profiles of KD101 between humans and rats. KD101 was extensively metabolized and excreted mainly in the urine in humans, whereas the feces were the major excretory route of KD101 in rats.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Acknowledgments. The authors thank Kwangdong Pharmaceutical Co. Ltd. for providing the study drug, Curachem for re-purifying ^{14}C-KD101, Dr. Boeun Lee, RPh, for compounding ^{14}C-KD101, and BioCore Co., Ltd. and the Korea Institute of Science and Technology for bioanalysis of TRA. Special thanks also go to the Clinical Trial Center and the Radiation Safety Lab at Seoul National University Hospital.

Funding. This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Korea (grant numbers: HI17C0927 and HI14C2770), and by the BK21 Plus Program of the National Research Foundation of Korea (NRF; 10Z20130000017).

Conflict of Interest. The authors declared no competing interests for this work.

Author Contributions. A.K., S.R.D., J.G.H., J.Y., S.-W. L., H.S.L., B.-Y.Y., K-S.Y., and H.L. wrote the manuscript. A.K., J.G.H., J.Y., S.-W.L., K.-S.Y., and H.L. designed the research. A.K., J.G.H., J.Y., S.-W.L., K.-S.Y., and H.L. performed the research. H.S.L., B.-Y.Y., S.R.D., A.K., J.G.H., S.-W.L., and H.L. analyzed the data.

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