A double-tracer technique to characterize absorption, distribution, metabolism and excretion (ADME) of [14C]-basimglurant and absolute bioavailability after oral administration and concomitant intravenous microdose administration of [13C6]-labeled basimglurant in humans

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Abstract

1. The emerging technique of employing intravenous microdose administration of an isotope tracer concomitantly with an [14C]-labeled oral dose was used to characterize the disposition and absolute bioavailability of a novel metabotropic glutamate 5 (mGlu5) receptor antagonist under clinical development for major depressive disorder (MDD).

2. Six healthy volunteers received a single 1 mg [12C/14C]-basimglurant (2.22 MBq) oral dose and a concomitant i.v. tracer dose of 100 μg of [13C6]-basimglurant. Concentrations of [12C]-basimglurant and the stable isotope [13C6]-basimglurant were determined in plasma by a specific LC/MS-MS method. Total [14C] radioactivity was determined in whole blood, plasma, urine and feces by liquid scintillation counting. Metabolic profiling was conducted in plasma, urine, blood cell pellet and feces samples.

3. The mean absolute bioavailability after oral administration (F) of basimglurant was ~67% (range 45.7–77.7%). The major route of [14C]-radioactivity excretion, primarily in form of metabolites, was in urine (mean recovery 73.4%), with the remainder excreted in feces (mean recovery 26.5%). The median tmax for [12C]-basimglurant after the oral administration was 0.71 h (range 0.58–1.00) and the mean terminal half-life was 77.2 ± 38.5 h. Terminal half-life for the [14C]-basimglurant was 178 h indicating presence of metabolites with a longer terminal half-life. Five metabolites were identified with M1-Glucuronide as major and the others in trace amounts. There was minimal binding of drug to RBCs. IV pharmacokinetics was characterized with a mean ± SD CL of 11.8 ± 7.4 mL/h and a Vss of 677 ± 229 L.

4. The double-tracer technique used in this study allowed to simultaneously characterize the absolute bioavailability and disposition characteristics of the new oral molecular entity in a single study.

Keywords
Basimglurant, bioavailability, intravenous, microtracer, oral, pharmacokinetic

Introduction
Basimglurant is a metabotropic glutamate (mGlu) 5 receptor antagonist being developed by F. Hoffmann-La Roche Ltd (Roche, Basel, Switzerland) for the adjunctive treatment of major depressive disorder (MDD). MDD remains an area of considerable medical need despite many agents having been approved for treatment of this illness. Response rates (reduction in symptoms of at least 50% from baseline) for initial treatment are estimated to be about 50%, while remission (the virtual absence of symptoms), considered to be the goal of treatment, ranges from only 15 up to 40% (Nemeroff, 2007).

New treatments addressing inadequate response to antidepressant therapy in MDD would fulfill an important medical need. During the last decade, evidence has accumulated indicating the pathophysiological role of deregulated cortical glutamatergic pathways in major depression, including the demonstration of abnormal levels of glutamate and altered expression of glutamate receptors in depressed patients (Connolly & Thase, 2012; Duman et al., 2012; Licznerski & Duman, 2013; Manji et al., 2003; Zarate et al., 2006b; Zarate & Tohen, 2004).

Supportive evidence for the antidepressant effects of anti-glutamatergic drugs stems from pilot clinical trials with ketamine, an N-methyl-D-aspartate (NMDA) channel blocking agent, shown to have a fast acting antidepressant effect in
treatment-resistant patients (Berman et al., 2000; Zarate et al., 2006a). Given the concerns regarding the clinical use of ketamine including psychotogenic effects and potential for addiction (Krystal et al., 2013), mGlu5 negative allosteric modulators offer an attractive target for the development of novel antidepressants (Chaki et al., 2013).

In order to understand the disposition of basimglurant, it was important to characterize the metabolic profile and the rate and routes of elimination of the study drug.

The concept of simultaneous dosing with a labeled intravenous dose and a non-labeled oral dose to determine absolute oral bioavailability is well established using a stable nonradioactive isotope such as $^{13}$C or radioactive $^{14}$C. This technique allows the fate of the i.v. dose to be distinguished from the oral dose by means of the isotopic tracer (Lappin et al., 2006; Sarapa et al., 2005). In this study, an oral dose of $^{[14]}$C- and $^{[12]}$C-basimglurant and an intravenous microdose of $^{[13]}$C$_6$-basimglurant (stable isotope, not radioactive) was administered virtually simultaneously to healthy volunteers to determine absolute oral bioavailability of basimglurant. Plasma concentrations were determined by LC-MS/MS and the $^{[13]}$C$_6$-labeled drug administered intravenously was distinguished from the $^{[12]}$C/$^{14}$C-labeled drug administered orally by virtue of their different molecular masses, using mass spectrometry detection. Dose-normalized AUCs of $^{[12]}$C-labeled drug were compared to $^{[13]}$C$_6$-labeled drug to determine absolute oral bioavailability. The principle advantage of the isotopic method is that the plasma drug concentration relating to the intravenous and oral doses are measured in the same plasma samples, thereby eliminating inter-occasional variability and, in theory, eliminating any concentration-dependent clearance. This technique exploits the basic principle of isotopic dilution, by which non-labeled drug and isotopically labeled drug mix systemically (Rubin et al., 1987). It has been demonstrated that the estimation of absolute oral bioavailability is more accurate when oral and i.v. doses are given simultaneously than in a crossover design. The estimation is further improved by delaying i.v. administration of the tracer for a time equal to 50% of the oral dose peak time or by administering the tracer dose by constant-rate infusion from the time of oral dosing to the peak time (Rubin et al., 1987). As there is virtually no kinetic isotope effect, the i.v. $^{[13]}$C$_6$-tracer is considered to follow the same clearance time course as the unlabeled drug from the oral dose (Lappin et al., 2006). In this study, 0.1 mg of $^{[13]}$C$_6$-basimglurant, the i.v. tracer, was administered 30 min after the oral dose of 1 mg basimglurant as a 30 min infusion. In a previous single dose administration study in healthy volunteers, the median $t_{max}$ was 1 h after oral administration. Administering i.v. dose 30 min after oral dose allowed sufficient concentrations to be attained in the body and potentially reduced errors in bioavailability estimation due to concentration dependent clearance, if present, in this dose range.

**Material and methods**

**Study design and population**

The study was conducted according to the provisions of the Declaration of Helsinki, and written informed consent was obtained from each study participant prior to conducting any protocol-related procedures. The study was approved by the local independent ethics committee, health authority and Administration of Radioactive Substances Advisory Committee.

This was a single center, open-label study, investigating the excretion balance, PK and metabolism following a single oral dose of $^{[14]}$C-labeled basimglurant under fasting conditions in six healthy male volunteers.

In addition, the subjects were administered an intravenous (i.v.) tracer dose of $^{[14]}$C$_6$-labeled basimglurant 30 min after the oral drug administration to determine the absolute oral bioavailability and further characterize the PK of basimglurant. The study was conducted at PRA in Zuidlaren (The Netherlands) from March to May 2012.

Six male nonsmoker healthy subjects were enrolled into this study according to inclusion/exclusion criteria based on medical history, physical examination (including body weight), clinical laboratory (blood chemistry, hematology, coagulation and urinalysis), vital signs (systolic and diastolic blood pressure and pulse rate), 12-lead electrocardiogram (ECG) and previous and concomitant medication. They agreed to use two effective methods of contraception with their female partners up to 3 months after dosing.

Subjects received a single 1 mg $^{[12]}$C/$^{14}$C-basimglurant dose (consisting of 0.76 mg $^{12}$C and 0.24 mg $^{14}$C) as an oral capsule providing a maximum target dose of 2.22 MBq ($^{14}$C)-radiolabeled basimglurant followed by an i.v. tracer dose of 100 μg of $^{[13]}$C$_6$-labeled basimglurant (30 min constant infusion) 30 min after the oral administration.

The oral and i.v. basimglurant solution were prepared on-site by the PRA pharmacist.

Because of the relatively long apparent $t_{1/2}$ of basimglurant, subjects collected their excreta quantitatively over a 13–17 day in-house period after dosing (at minimum up to Day 14 and at maximum up to Day 18). Thereafter, they were discharged and they returned at weekly intervals for 24 h in-house urine and feces collections until less than 1% of the administered dose was excreted within 24 h in two consecutive collection intervals based on quick count results. Mass balance studies in rats with $^{[14]}$C-basimglurant indicate complete excretion within 120 h of administration; total drug radioactivity of 20% and 80% was recovered in urine and feces, respectively.

In the single dose escalation study, using unlabeled basimglurant in healthy volunteers, no unchanged parent compound was detected in the urine. Therefore, in this study basimglurant and its metabolites ($^{[12]}$C entities) were not analyzed by LC/MS-MS assay in the urine samples. Detailed timepoints collected for this study are listed below.

A venous blood sample was taken for PK of basimglurant in plasma, $^{[13]}$C$_6$-labeled basimglurant in plasma and total $^{[14]}$C radioactivity in whole blood and plasma: at pre-dose and at 30, 35, 40, 45, 50 and 55 min post-dose, at 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 168, 216, 264 and 312 h (up to Day 14) post-dose. Subsequently, sampling continued in 24-h intervals for the next 4 days until Day 18 post dose.
For determination of total $^{14}$C radioactivity and metabolite profiling in urine and feces, urine samples were collected at pre-dose and at 0–12 and 12–24 h post-dose. From Day 2, urine collection continued in 24-h intervals until Day 18. Feces were collected at pre-dose and in 24-h intervals up to Day 18.

If, by Day 18 the release criterion had not been met, samples (blood, urine and feces) were taken at the 24-h intervals until it was met.

Additional plasma samples for metabolite profiling were collected at pre-dose and at 2, 4, 8 and 24 h post-dose.

Safety was monitored throughout the study in form of vital signs and ECGs (daily while in clinic and at subsequent 24 h admissions and at follow up), laboratory tests (at screening, Day 1, Day 10 and at follow up) continuous cardiac monitoring (Day 1, pre and postdose) and AEs (from screening to follow up).

Chemicals and isotopes

$^{[12C/14C]}$-basimglurant and $^{[13C_6]}$-basimglurant (Figure 1) were synthesized by the Isotope Synthesis Group and by the Highly Active Substance Laboratory of Process Research and Synthesis at F. Hoffmann-La Roche Ltd, Basel, Switzerland, in compliance with current good manufacturing practice (GMP) regulations and supplied by Roche Clinical Trial Supply, Kaiseraugst, Switzerland, as solid powder bulk material. The radiochemical purity of $^{[12C/14C]}$-basimglurant was >99% and the specific activity was 2.22 MBq/mg. The chemical purity of $^{[13C_6]}$-basimglurant was >99% and the isotopic enrichment was >98% $^{13}$C$_6$ (13C$_0$<0.1%). The internal standard for the assay of $^{[12C]}$-basimglurant and $^{[13C_6]}$-basimglurant was provided by Process Research and Synthesis at F. Hoffmann-La Roche Ltd, Basel, Switzerland, while for the $^{[14C]}$-basimglurant it was provided by Perkin Elmer, Boston, MA. All reagent and solvents were obtained from commercial sources.

Sample analysis

Concentrations of $^{[12C]}$-basimglurant and the stable isotope $^{[13C_6]}$-labeled basimglurant were determined in plasma by a specific LC-MS/MS method. Total $^{[14C]}$-radioactivity was determined in whole blood, plasma, urine and feces by liquid scintillation. Metabolic profiling was conducted in plasma, urine and feces samples.

Measurement of total radioactivity for mass balance determination

$^{[14C]}$-radioactivity levels were determined in plasma, whole blood, urine and feces with a Perkin Elmer Tri-Carb™ 3100 TR liquid scintillation (LS) analyzer (Waltham, MA) equipped with low level counting mode (LLCM) and normal counting mode (NCM). For plasma and urine no sample preparation was needed; whole blood was analyzed after a solubilization and decolorizing steps while feces were homogenized and combusted prior to analysis.

[1-methyl-$^{14}$C]-caffeine was used as reference standard to prepare quality control samples for LS analysis.

Bioanalytical assay for unlabeled basimglurant and $^{[13C_6]}$-basimglurant

Unlabeled and $^{[13C_6]}$-basimglurant concentrations were determined in human plasma samples using a validated turbo ion spray mass spectrometry procedure. Human plasma samples (50 µL) were fortified with 200 µL of 1 ng/mL internal standard working solution ($^{[C_{10}]}$-basimglurant). Following the addition of 200 µL of reagent alcohol (a blend of 90% Ethyl Alcohol, 5% Methanol and 5% Isopropyl Alcohol) to control blank samples, samples were vortexed, centrifuged, and the supernatant transferred to a 96-well plate. The final extracts were analyzed via LC-MS/MS using a Fluorophase PFP analytical column (2.1 x 50 mm, 5-µm particle size, Thermo Fisher Scientific Inc., Waltham, MA) with gradient elution. The mobile phases consisted of 0.1% formic acid in water and 950:49:1 methanol/water/formic acid. Detection was performed using an AB SCIEX API 5000 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA) operated in positive ion mode using SRM. The selected transitions (m/z) were 326.1 → 249.1 for unlabeled basimglurant, 332.1 → 101.1 for $^{[13C_6]}$-basimglurant and 336.1 → 259.1 for $^{[13C_{10}]}$-basimglurant (internal standard).

The calibration standards were prepared in human K$_3$EDTA plasma. The calibration range was 0.0500 ng/mL to 25.0 ng/mL for unlabeled basimglurant and 0.0100 ng/mL to 10.0 ng/mL for $^{[13C_6]}$-basimglurant. The calibration curve was established by weighted (1/X^2) linear regression from peak area ratios (peak area of analyte/peak area of internal standard) versus nominal concentrations. The assay was validated and performed as per FDA
requirements. The performance of sample analysis was monitored by quality control (QC) samples in human plasma spiked with four different concentrations of basimglurant. Quality control samples were analyzed along with the samples from the study. Results were within FDA specifications.

Metabolic pattern analysis of plasma and excreta samples

Plasma, urine and feces extracts were analyzed by HPLC combined with fraction collection into 384-well LumaPlates for solid scintillation counting (TopCount™ Microplate Scintillation Counter). A detailed description of the HPLC methods used can be found in Supplementary material. The reconstructed radiochromatograms were used to determine the exposure of each metabolite in plasma and the percentage of the dose for urine and feces. Identification and structural elucidation of metabolites was conducted by HPLC/SSC combined with accurate mass spectrometry. For details see Supplementary material.

Metabolites were quantified on the basis of the relative amount of radioactivity assigned to a peak in relation to the total amount of radioactivity present in the sample. Parent drug and metabolites were expressed as percentage of total radioactivity in plasma or as percentage of the dose in excreta.

Product ion spectra of metabolites were acquired with the QTRAP (AB Sciex, Framingham, MA), Synapt G2 (Waters, Milford, MA) or Orbitrap (Thermo Fisher Scientific, Waltham, MA) mass spectrometer. Metabolite identification was based on matching of retention times and product ion spectra with that of reference compounds, if available (basimglurant, M1, M2 and M1-glucuronide). In addition, the product ion spectra of metabolites were interpreted to elucidate or narrow down their structures for which no reference compounds were available. For elucidation of the glucuronidated metabolites, 0.5 mL of a 0.2 M sodium-acetate buffer pH 4.8 or pH 6.0, respectively and 200 µL of betaglucuronidase Type VII-A from E. coli as a 1 mg/mL solution in water (corresponding to about 1000 u) was added to 60 µL isolated fractions from human urine each. The incubation was performed at 37 °C for 3 h. LC-MS analytics of the cleavage products was performed as described above.

Pharmacokinetic analysis

Calculation of PK parameters of basimglurant was performed according to standard non compartmental methods using WinNonlin version 5.2 (Pharsight, Mountain View, CA).

PK parameters calculated included, maximum observed plasma concentration, (Cmax), Time to maximum observed plasma concentration (tmax), Apparent terminal elimination half-life (t1/2), Area under the plasma concentration-time curve from time zero to the last measurable plasma concentration time point (AUCt), Area under the plasma concentration-time curve form time zero extrapolated to infinity (AUCinf).

For parent compound: absolute bioavailability (F), apparent clearance (CL/F), apparent volume of distribution (Vz/F) and apparent volume of distribution at steady state (Vss) were also reported.

Results

Demographic and disposition

Six male healthy subjects were enrolled in this study. All six subjects completed the study. The subjects were between 36 and 61 years old (mean 52.2 years) with a body mass index between 22.9 and 29.8 kg/m² (mean 26.37 kg/m²) and body weight between 71.0 and 95.8 kg (mean 81.27 kg). All subjects were of Caucasian origin apart from one of Asian origin.

Excretion of [14C]-radioactivity in urine and feces

The recovery of [14C]-radioactivity in urine and feces was almost complete; at 408 h after the oral dosing the total mean [14C]-radioactivity recovered was 99.9% of the orally administered dose. Individual total recovery of [14C]-radioactivity ranged between 97.8% and 104%.

The major route of [14C]-radioactivity excretion was urine (mean recovery 73.4%), with the remainder excreted in feces (mean recovery 26.5%). Individual [14C]-radioactivity recovery ranged between subjects from 67.2% to 77.0% in urine and 23.1% to 30.6% in feces.

The cumulative excretion data for urine and feces were calculated by carrying forward the last value for each subject up to 408 h post-dose. The mean cumulative [14C]-radioactivity excreted in urine, feces and combined is displayed in Figure 2 and is summarized in Table 1.

The release criterion for the study was that the cumulative total [14C]-radioactivity excreted in urine and feces had to be less than 1% of the administered dose in two consecutive collection intervals, based on quick counts. All subjects apart from one met release criteria between Days 14 and 18. One subject met the criteria at Day 19.

[14C]-radioactivity in plasma

After administration of a single oral dose of 1 mg [12C/14C]-basimglurant (consisting of 0.76 mg 12C and 0.24 mg 14C) followed 30 min later by a 30 min i.v. infusion of 100 µg [13C6]-labeled basimglurant, the median tmax for [14C]-radioactivity was 0.75 h after the oral administration. The

Figure 2. Mean cumulative [14C] radioactivity recovery-time profile for urine, feces and total.
mean Cmax was 16.3 ng eq/mL. The mean terminal half-life was 178 h. Quantifiable [14C]-radioactivity plasma concentrations were detectable for one subject up to 576 h after dosing.

One subject (1002) was noted in the plasma PK parameters for [14C] radioactivity showing a half-life much shorter compared to the other subjects as displayed in Table 2. This subject also had more rapid excretion of [14C] radioactivity as shown in the section above.

Summary statistics of [14C]-radioactivity plasma PK parameters are displayed in Table 2.

Whole blood to plasma ratio of [14C]-radioactivity
The whole blood to plasma ratio of [14C]-radioactivity was calculated per subject at each time point where data were available. Ratios generally ranged between 0.7 and 0.8 for all subjects and there was little change over time in the ratios. Mean concentration-time profiles of [14C] radioactivity in whole blood and plasma are shown in Figure 3.

Pharmacokinetics of [12C]-basimglurant in plasma
After administration of a single oral dose of 1 mg [12C/14C]-basimglurant (consisting of 0.76 mg 12C and 0.24 mg 14C) followed 30 min later by a 30 min i.v. infusion of 100 µg [13C6]-labeled basimglurant, the median tmax for [12C]-basimglurant was 0.71 h after the oral administration. The mean Cmax was 7.97 ng/mL. After the initial peak, the concentration declined rapidly up to 12 h after administration, after which concentrations declined at a slower rate. The mean terminal half-life was 77.2 h. Quantifiable [12C]-basimglurant plasma concentrations were detectable for the same subject as noted in the 14C section above up to 264 h after dosing.

There was little variation in the plasma PK parameters for [12C]-basimglurant across the individual subjects, with the exception of Subject 1002. For this subject, half-life was notably shorter compared to the other subjects, and AUClast and AUCinf were notably lower. His CL/F of [12C]-basimglurant was similarly very high (58.6 L/hr) compared with the other subjects (10.9–13.7 L/h). Summary statistics of [12C]-basimglurant plasma PK parameters are presented in Table 3.

Pharmacokinetics of [13C6]-basimglurant in plasma
After administration of a single oral dose of 1 mg [12C/14C]-basimglurant (consisting of 0.76 mg 12C and 0.24 mg 14C) followed 30 min later by a 30 min i.v. infusion of 100 µg [13C6]-labeled basimglurant, the mean tmax for [13C6]-basimglurant was 0.5 h after the start of the i.v. infusion (end of infusion time). The mean Cmax was 1.73 ng/mL, which occurred at a median of 0.5 h after the start of the infusion, after which concentrations declined at a slower rate. The mean half-life was 78.9 h. Quantifiable [13C6]-basimglurant plasma concentrations were detectable for Subject 1002 at 263.5 h after the start of the i.v. infusion. The subject’s CL of [13C6]-basimglurant was very high (26.8 L/hr) compared with the other subjects (8.1–10.1 L/h). Summary statistics of [13C6]-basimglurant plasma PK parameters are presented in Table 4.

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**Table 2. Summary statistics of [14C]-radioactivity plasma PK parameters.**

| Subject, statistic | Tmax (h) | Cmax (ng eq/mL) | Half-life lambda Z (h) | AUClast (ng eq*h/mL) | AUCinf (ng eq*h/mL) |
|--------------------|----------|-----------------|------------------------|----------------------|---------------------|
| 1001               | 0.67     | 15.3            | 195                    | 334                  | NR                  |
| 1002               | 0.75     | 16.9            | 43.3                   | 129                  | 144                 |
| 1003               | 1.00     | 10.5            | 351                    | 308                  | NR                  |
| 1004               | 0.58     | 20.6            | 141                    | 311                  | 388                 |
| 1005               | 0.75     | 14.6            | 166                    | 287                  | 357                 |
| 1006               | 0.75     | 20.1            | 169                    | 292                  | NR                  |
| N                  | 6        | 6               | 6                      | 6                    | 3                   |
| Mean               | 0.75     | 16.3            | 178                    | 277                  | 296                 |
| SD                 | 0.14     | 3.76            | 109                    | 74.4                 | 132                 |
| Min                | 0.58     | 10.5            | 43.3                   | 129                  | 144                 |
| Median             | 0.75     | 16.1            | 168                    | 300                  | 357                 |
| Max                | 1.00     | 20.6            | 351                    | 334                  | 388                 |
| CV%                | 18.6     | 23              | 56.4                   | 26.8                 | 44.7                |
| Geometric Mean     | 0.74     | 15.9            | 151                    | 265                  | 271                 |

NR: Not reported. For subjects 1001, 1003 and 1006 AUCinf could not be estimated accurately. AUClast was reported instead.

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**Table 1. Summary of the cumulative recovery of [14C]-radioactivity in urine, feces and total by 408 h after oral administration.**

| Subject, statistic | [14C]-Radioactivity urine (%) | [14C]-Radioactivity feces (%) | [14C]-Radioactivity total (%) |
|--------------------|-------------------------------|-------------------------------|-------------------------------|
| 1001               | 74.0                          | 24.9                          | 98.9                          |
| 1002               | 75.1                          | 28.6                          | 104                           |
| 1003               | 67.2                          | 30.6                          | 97.8                          |
| 1004               | 77.0                          | 23.1                          | 100                           |
| 1005               | 71.7                          | 26.9                          | 98.6                          |
| 1006               | 75.8                          | 24.7                          | 100                           |
| N                  | 6                             | 6                             | 6                             |
| Mean               | 73.4                          | 26.5                          | 99.9                          |
| SD                 | 3.58                          | 2.77                          | 2.09                          |
| Min                | 67.2                          | 23.1                          | 97.8                          |
| Median             | 74.5                          | 25.9                          | 99.5                          |
| Max                | 77.0                          | 30.6                          | 104                           |
| CV%                | 4.90                          | 10.5                          | 2.10                          |
| Geometric Mean     | 73.4                          | 26.4                          | 99.9                          |

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**Figure 3. Mean (±SD) concentration-time profiles of [14C]-radioactivity in plasma and whole blood.**
Absolute bioavailability of basimglurant

The mean absolute bioavailability of basimglurant after oral administration (F) was ~67%. The F was similar for most subjects (~68–78%), whereas the Subject 1002 with the higher CL had an F of 45.8%. Excluding this subject, the mean F was 72%. The mean absolute bioavailability after oral administration (F) is summarized in Table 3.

Metabolite identification

The metabolism of basimglurant has previously been characterized in multiple in vitro and animal in vivo studies (unpublished data). In vitro metabolism studies in liver microsomes and hepatocytes of human, rat, dog, cynomolgus monkey and mouse indicated that basimglurant was mainly metabolized to the hydroxylated metabolite M1 and its glucuronide as well as to some glutathione and cysteine adducts. The excretion balance study in naive and bile-duct cannulated rats showed 79.9–90.7% excretion within 48 h, mainly via biliary excretion into feces (55.3–57.4% in bile duct cannulated rats and 63.7–75.7% of the dose in naive rats). Overall, the metabolism in rat involved hydroxylations of basimglurant to M1, M2 and M6 followed by glucuronidation. In cynomolgus monkeys, unchanged parent, M1 and its glucuronide were the only drug-related peaks detected in plasma, and the drug was eliminated after metabolism to M1 and M1-glucuronide into urine and feces.

In this study after oral administration of 1 mg [12C/14C]-labeled basimglurant, radioactivity was mainly excreted via urine (mean recovery 73.4%), with the remainder excreted in feces (mean recovery 26.5%). For metabolite identification, urine was pooled across all subjects up to 240 h (70.1% of dose) and feces were pooled across subjects up to 288 h (24.1% of dose) to avoid dilution of the drug-related material. Overall, the metabolism in rat involved hydroxylations of basimglurant to M1, M2 and M6 followed by glucuronidation. In cynomolgus monkeys, unchanged parent, M1 and its glucuronide were the only drug-related peaks detected in plasma, and the drug was eliminated after metabolism to M1 and M1-glucuronide into urine and feces.

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Drug related material in plasma

After a single oral dose of $^{[12C/14C]}$-basimglurant, mainly the parent compound and the M1-glucuronide were detected in plasma samples at 2 h, 4 h and 8 h after administration. Parent compound accounted for 76, 80 and 82% at 2, 4 and 8 h, respectively. M1-Glucuronide was a major metabolite (21, 16 and 14% of total drug-related material). In addition, M1 and M2 were identified as minor metabolites accounting for 2% and 1–2% of circulating drug-related material, respectively, and the M2- and the M6-glucuronide were detected as trace metabolites by MS only. For Subject 1002, likewise, mainly parent compound (basimglurant, 38% of total) and the M1-glucuronide (50% of total) were detected in plasma 2 h post administration. In addition, the hydroxylated metabolites M1 (2%) and M6 (3%) as well as the M2-glucuronide (3%) and an additional glucuronide (parent + 2O + glucuronic acid) (5%) were detected as minor metabolites, and M2 and the M6-Glucuronide were detected as trace metabolite by MS only.

Metabolite pattern in urine

The main metabolite in urine was the M1-glucuronide, accounting for about 56% of the dose. In addition, the M2-glucuronide accounted for ~12% of the dose and two minor unknown metabolites (<2% of dose) were identified. In addition, traces of M6-glucuronide were found. For Subject 1002, the metabolite pattern was qualitatively comparable (64% of the dose M1-glucuronide, 9% M2-glucuronide, two minor unknown metabolites at <2% of the dose and traces of M6-glucuronide). No unchanged parent compound was detected in urine.

Metabolite pattern in feces

The metabolite pattern in feces showed mainly M1 accounting for about 20% of the dose. In addition, the hydroxylated metabolite M2 and an unknown metabolite accounted for ~2% of dose each. For Subject 1002, the metabolite pattern was the same (21% of the dose M1, 2% M2 and 3% of the unknown metabolite). No unchanged parent compound was detected in feces, indicating complete absorption.

Structure elucidation of glucuronide metabolites

The identity of M1-glucuronide was confirmed using the respective reference compound. For the other glucuronide metabolites, structure assignment was done based on isolation of the respective peaks from urine, followed by digestion with β-glucuronidase to yield the respective aglycons that could be clearly assigned based on their specific retention time and MS/MS spectra.

Safety and tolerability

A single oral dose of 1 mg $^{[12C/14C]}$-basimglurant followed 30 min later by a 30-min i.v. infusion of 100 μg $^{[13C_6]}$-labeled basimglurant in six healthy male subjects was well tolerated. All subjects completed the study as per protocol. There were no SAEs or withdrawals due to AEs during the study. The most frequently reported AEs were disturbance in attention, dizziness and somnolence. The majority of the AEs experienced during the study were considered probably related to study medication. There were no findings of clinical relevance with respect to laboratory parameters, vital signs, 12-lead ECG, continuous cardiac monitoring or physical examinations.
Table 5. Metabolites identified in human plasma, urine and feces.

| Compound                      | Proposed structure | RT method 1 | Key fragments used for identification |
|-------------------------------|--------------------|-------------|---------------------------------------|
| basimglurant*                 |                    | 13.3        | 326.08548                             |
| m/z 326.08548                 |                    | 13.3        | 136.05570                             |
|                               |                    |             | 311.06200                             |
| M1*                           |                    | 12.8        | 342.08039                             |
| m/z 342.08039                 |                    | 12.8        | 324.06983                             |
|                               |                    |             | 162.07135                             |
|                               |                    |             | 121.04514                             |
| M2*                           |                    | 12.3        | 342.08039                             |
| m/z 342.08039                 |                    | 11.3        | 324.07010                             |
|                               |                    |             | 312.0688                              |
|                               |                    |             | 283.0421                              |
|                               |                    |             | 236.0738                              |
|                               |                    |             | 136.05544                             |
|                               |                    |             | 122.04049                             |
|                               |                    |             | 121.04515                             |
| M6                            |                    | –           | 358.1                                 |
| m/z 358.07531                 |                    | 10.5        | 322.0                                 |
|                               |                    |             | 286.0                                 |
|                               |                    |             | 260.0                                 |
|                               |                    |             | 133.0                                 |
|                               |                    |             | 120.1                                 |
| M1-Glucuronide*               |                    | 11.3        | 518.11249                             |
| m/z 518.11249                 |                    | 10.5        | 342.08039                             |
|                               |                    |             | 324.07132                             |
|                               |                    |             | 297.0574                              |
|                               |                    |             | 256.0317                              |
|                               |                    |             | 162.0709                              |
|                               |                    |             | 121.04541                             |
| M2-Glucuronide                 |                    | 10.4        | 518.1182                              |
| m/z 518.11249                 |                    | 9.5         | 342.0798                              |
|                               |                    |             | 324.0691                              |
|                               |                    |             | 312.0700                              |
|                               |                    |             | 283.0434                              |
| M6-Glucuronide                 |                    | –           | 534.1074                              |
| m/z 534.10740                 |                    | 9.4         | 358.1856                              |
|                               |                    |             | 340.2177                              |
|                               |                    |             | 322.0607                              |

*Reference compounds were available for basimglurant, M1, M2 and M1-glucuronide, showing identical retention times and MS fragments.
**Discussion**

The objectives of this study were to characterize the disposition and absolute bioavailability of a novel metabolic glutamate 5 (mGlu5) receptor antagonists under clinical development for major depressive disorder (MDD). Concomitant administration of a double tracer [14C]-basimglurant given orally and as an i.v. microdose of [13C6]-basimglurant allowed this characterization in a single study.

A [12C/14C]-radiolabelled single dose was administered orally in order to determine the mass balance and the routes of elimination of basimglurant, to identify and quantify the circulatory and excretory metabolites, to determine the basimglurant clearance mechanism and to determine the exposure of basimglurant and its metabolites. These are typical objectives of an ADME study (Penner et al., 2009; Roffey et al., 2007). The concomitant i.v. administration of [13C6] additionally allowed the accurate estimation of absolute bioavailability, total clearance and volume of distribution at steady state, avoiding a two-way crossover design, day to day PK variability and shortened the overall study. The majority of microdose studies have used accelerator mass spectrometry (AMS) analysis, but improvements in MS technology due to development of more sophisticated and sensitive techniques, LC-MS/MS can be used (Garner, 2010; Ings, 2009; Lappin et al., 2009; Maeda & Sugiyama, 2011). Furthermore, the synthesis of a stable isotope requires careful thinking as the position of the [13C6] has to be metabolically stable.

In this study, six healthy male subjects received a single oral dose of 1 mg [12C/[14C]-basimglurant (consisting of 0.76 mg 12C and 0.24 mg 14C) followed 30 min later by a 30 min i.v. infusion of 100 μg [13C6]-labeled basimglurant the time corresponding to 50% of the oral dose peak time. The excretion balance demonstrated near complete recovery (total mean 99.9%) over the 408 h collection interval with most of the administered radioactivity recovered in urine and feces in the first 192 h post-dose (90.5%). The major route of [14C]-radioactivity excretion was urine (mean recovery 73.4%), with the remainder excreted in feces (mean recovery 26.5%). Lower concentrations of radioactivity were found in blood compared to plasma with a blood to plasma ratio of 0.7–0.8 with minimal changes over time. This indicates that there was some association of [14C]-radioactivity with RBCs, but the [14C]-radioactivity did not become concentrated within the RBCs (as the ratio did not increase over time).

The total [14C]-radioactivity and parent compound [12C]-basimglurant correlated well, with the plots for mean plasma concentrations showing parallel curves. The AUC results were higher for total [14C]-radioactivity than [12C]-basimglurant, indicating that one or more metabolites of basimglurant were formed.

Following i.v. infusion of 100 μg [13C6]-labeled basimglurant the CL was relatively low, 11.8 L/h, while the volume of distribution at steady state was 677 L, suggesting that basimglurant is extensively distributed in tissues. Vss was lower than Vz (1080 L), which is generally the case for compounds for which disposition is described by a multi-compartmental model. Vss in this case is a better estimate of the volume of distribution as in the case of drugs exhibiting bi-exponential decay (2-compartmental model) or tri-exponential decay (3-compartmental model) (Gobburu & Holford, 2001). A NONMEM based population PK analysis of the pooled plasma concentration data from multiple clinical trials confirmed that the PK of basimglurant was best described by a two-compartment disposition model with first-order elimination and a transit compartment model (publication in progress). Absolute bioavailability was 67%.

One subject, 1002, had notably different values for several PK parameters. A box plot of the PK parameters showed that for this subject apart for Cmax all PK parameters were at least 1.5 interquartile ranges below or above the first or third quartile, hence this subject was considered as an outlier. However, the pattern and identity of the metabolites was similar to other subjects. Basimglurant undergoes significant metabolism by CYP450 enzymes. The two enzymes primarily responsible for its metabolism at the doses employed in this study are CYP1A2 and CYP3A4, with CYPs 1A1, 2C19 and 3A5 also known to be capable of basimglurant metabolism. It seems likely that the expression of one or more of these enzymes was substantially higher in subject 1002 compared with the other study subjects. The pharmacokinetics of the only subject of Asian origin was in line with the Caucasian subjects.

The elimination of basimglurant was mediated by biotransformation, followed by renal and fecal excretion. Negligible parent drug is found in urine. Basimglurant is predominantly cleared by cytochrome CYP P450-mediated hydroxylation to generate M1 and M2. The major drug-related compound in plasma was by far basimglurant. The only major circulating metabolite was the M1-glucuronide (14–21%), which was also present in rat and cynomolgus plasma.

A comparison of the mean terminal half-life for [14C]-basimglurant (178 h) with the half-life of [12C]-basimglurant (77.2 h) indicated possible presence of metabolites with a longer terminal half-life than that of the parent compound. While a terminal half-life of 77.2 h was estimated for [12C]-basimglurant since this compound has a large volume of distribution and multi-compartmental disposition characteristics effective half-life may be a better measure to estimate time to steady state. Using popPK analysis an effective half-life of 57.8 h was estimated (publication in progress).

In urine, the main component was the M1-Glucuronide, accounting for about 56% of the dose. The M2-Glucuronide accounted for ~11.5% of the dose. Two minor unknown metabolites (~2% of dose) and traces of M6-glucuronide were also identified. In feces, the metabolite pattern showed mainly M1 accounting for about 20% of the dose. In addition, the hydroxylated metabolite M2 and an unknown metabolite accounted for ~2% of dose each. Only trace amounts of the parent moiety were identified in both urine and feces.

**Conclusions**

The objectives of this study were to characterize the mass balance, metabolism routes and rates of elimination of
basimglurant including characterization of its major circulating metabolites, characterization of the i.v. kinetics and absolute bioavailability. All these objectives were met with this double-tracer technique in a single study. The recovery of $[^{14}\text{C}]$-radioactivity in urine and feces was almost complete; at 408 h after the oral dosing, the total mean $[^{14}\text{C}]$-radioactivity recovered was 99.9%. The major route of $[^{14}\text{C}]$-radioactivity excretion was urine (mean recovery 73.4%), with the remainder excreted in feces (mean recovery 26.5%). The higher area under the curve of $[^{14}\text{C}]$-radioactivity compared to $[^{12}\text{C}]$-basimglurant, indicated that one or more metabolites of basimglurant were formed. Terminal half-life for the $[^{14}\text{C}]$-basimglurant was 178 h versus a mean terminal half-life of 77.2 h for $[^{12}\text{C}]$-basimglurant also indicating presence of metabolites with a longer terminal half-life. The mean absolute bioavailability after oral administration (F) of basimglurant was $\approx 67\%$ (72% excluding one outlier).

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Supplementary material available online