Qualification and application of liquid chromatography-quadrupole time-of-flight mass spectrometric method for the determination of carisbamate in rat plasma and prediction of its human pharmacokinetics using physiologically based pharmacokinetic modeling

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ABSTRACT

Carisbamate is an antiepileptic drug and it also has broad neuroprotective activity and anticonvulsant reaction. In this study, a liquid chromatography-quadrupole time-of-flight mass spectrometric (LC-qTOF-MS) method was developed and applied for the determination of carisbamate in rat plasma to support in vitro and in vivo studies. A quadratic regression (weighted 1/concentration^2), with an equation y = ax^2 + bx + c, was used to fit calibration curves over the concentration range from 9.05 to 6,600 ng/mL for carisbamate in rat plasma. Preclinical in vitro and in vivo studies of carisbamate have been studied through the developed bioanalytical method. Based on these study results, human pharmacokinetic (PK) profile has been predicted using physiologically based pharmacokinetic (PBPK) modeling. The PBPK model was optimized and validated by using the in vitro and in vivo data. The human PK of carisbamate after oral dosing of 750 mg was simulated by using this validated PBPK model. The human PK parameters and profiles predicted from the validated PBPK model were similar to the clinical data. This PBPK model developed from the preclinical data for carisbamate would be useful for predicting the PK of carisbamate in various clinical settings.

Keywords: Qualification; LC-MS; Carisbamate; PBPK Modeling

INTRODUCTION

Carisbamate (S-2-O-carbamoyl-1-o-chlorophenyl-ethanol) is an investigational neuromodulatory agent, initially developed from SK Biopharmaceuticals (Seongnam, Korea) for antiepileptic treatment [1-6]. Although the exact mechanism of action for carisbamate is not known, carisbamate has been studied for the treatment of epilepsy, essential tremor and migraine [4,5]. In phase I clinical trials to healthy subjects, carisbamate has shown...
linear pharmacokinetics at doses of 100 to 1,500 mg, high oral bioavailability (F) of > 95% and a low oral clearance (CL/F) of 3.4–4.2 L/h, equalling < 5% of liver blood flow [1,2,7]. The drug also has shown efficacy and tolerability in a phase II clinical trial for epilepsy patients, and three phase III clinical trials have been completed for treatment in partial seizures [8-10]. Among them, only one phase III clinical trial (randomized double-blind) study showed significant efficacy results in adults with refractory partial seizures receiving a dose of 400 mg/day [11,12]. In 2008, carisbamate was provisionally approved by the Food and Drug Administration for adjunctive treatment for patients aged 16 years or over with partial onset seizures (brand name: compass), but failed to receive marketing approval in 2009. Carisbamate is currently undergoing clinical trials for Lennox Gastaut syndrome as an indication by SK Biopharmaceuticals.

Recently, physiologically based pharmacokinetic (PBPK) model has been increasingly used as a powerful tool to predict clinical pharmacokinetic (PK) parameters and profiles by using preclinical Absorption, Distribution, Metabolism, and Excretion (ADME)/PK data [13-20]. PBPK modeling is a very complex mathematical model that reflects systematically specific physiological properties and drug’s physicochemical properties [21]. The PBPK model has been applied in the pharmaceutical industry to predict ADME/PK properties of drugs by using in vitro and in vivo properties as an input data [22-24].

In this study, a simple and robust liquid chromatography-quadrupole time-of-flight mass spectrometric (LC-qTOF-MS) method for carisbamate was developed and successfully applied to in vitro and in vivo PK studies in rat. Based on these results, a PBPK model was built, optimized and validated for predicting the PK of carisbamate in humans. After establishing the PBPK model, the human PK of carisbamate was predicted and compared with the reference clinical PK data associated with food effect PK studies [7]. To the authors’ best knowledge, this is the first approach for human PK prediction of carisbamate through PBPK model. The information in this study will help designing the studies related to various clinical studies of carisbamate.

**METHODS**

**Chemicals and reagents**
Carisbamate was purchased from Toronto Research Chemical (Ontario, Canada). Verapamil was purchased from Sigma-Aldrich (St, Louis, MO, USA). Formic acid was purchased from Daejung Chemical (Siheung, Korea). Male Sprague-Dawley (SD) rat and human liver microsomes were purchased from Corning Incorporated (Corning, NY, USA). Acetonitrile (ACN) of HPLC grade was purchased from Honeywell Burdick & Jackson (Ulsan, Korea). Distilled water (DW) of HPLC grade was purchased from Samchun Chemical (Pyeongtaek, Korea). All other chemicals reagents were of analytical grade and purchased from commercial sources.

**Preparation of stocks, standard (STD) and quality control (QC) Samples**
Stock solution of carisbamate (1 mg/mL) was prepared in dimethyl sulfoxide (DMSO). The sub-stock solution (0.2 mg/mL) was made by diluting the stock solution with DMSO. Seven calibration standard concentrations of carisbamate were prepared by serial dilution of the sub-stock solution with DMSO. The calibration samples were prepared by spiking the diluted calibration standard solutions into blank rat plasma to obtain final plasma concentrations of 9.05, 27.2, 81.3, 244, 733, 2,200 and 6,600 ng/mL. QC samples with
concentrations of 15.04 (low QC), 165.46 (medium QC) and 1,820 (high QC) ng/mL were prepared in the same manner.

The stock solution (1 mg/mL) for the internal standard (ISTD, verapamil) was prepared in DMSO and stored in the refrigerator at −20°C until use. The ISTD was diluted to prepare a final spiking concentration of 20 ng/mL in ACN prior to sample preparation.

Sample preparation
Fourty microliters of the blank rat plasma samples were placed in cluster tubes, and 4 µL of the standard or QC samples was added to each cluster tube, whereas 4 µL of make-up solution (DMSO) was added to each study samples to prepare the same matrix conditions.

The 150 µL of ACN containing ISTD was added to the standard, QC and study samples for protein precipitation. Samples were centrifuged at 10,000 rpm for 5 minutes and then supernatants were evaporated to dryness under vacuum in a Savant SpeedVac™ (Thermo Scientific, Rockford, IL, USA). The dried residues were reconstituted using ACN/DW (1:1), vortexed, and then centrifuged at 10,000 rpm for 5 minutes. The resulting supernatants were transferred to liquid chromatography (LC) vial for LC-qTOF-MS analysis.

LC-qTOF-MS conditions
The liquid chromatography-high resolution mass spectrometric system consisted of Shimadzu CBM-20A HPLC pump controller (Shimadzu Corporation, Columbia, MD, USA), 2 Shimadzu LC-20AD pumps, CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC, USA), and quadrupole time-of-flight (qTOF) TripleTOF™ 5600 mass spectrometer (Sciex, Foster City, CA, USA). The HPLC analytical column used was a C18 column 2.1 × 50 mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of: mobile phase A, distilled and deionized water containing 0.1% formic acid; and mobile phase B, acetonitrile containing 0.1% formic acid. The gradient was as follows: from 0 to 0.5 minutes, 10% B; from 0.5 to 0.9 minutes by a linear gradient from 10% B to 95% B; 95% B was maintained for 0.6 minutes; from 1.5 to 1.6 minutes by a linear gradient from 95% B to 10% B, and then 10% B was maintained for 1.4 minutes for column re-equilibrium. The gradient was delivered at a flow rate of 0.4 mL/min and the injection volume was 10 µL.

The TOF-MS scan mass spectra and the product ion scan mass spectra were recorded in the positive ion mode. The scan range was m/z 100–600 for both TOF-MS scan and product ion scan. For the quantification, [M + H]⁺ ion of carisbamate and ISTD (m/z 216.0 and 455.3, respectively) were selected and their product ions at m/z 155.0 and 165.1 were used for quantitative analysis, respectively. The source temperature was set at 500°C with a curtain gas flow of 33 L/min. The ion spray voltage was set at 5,500 V. For Carisbamate and ISTD, declustering potential was 80 and 125 V, and the collision energy was 17 and 30 V, respectively.

Method qualification
The method qualification was performed with a ‘fit-for-purpose’ approach. The qualification run contained duplicate standards at seven concentrations and QCs at three concentrations. The acceptance criteria for standards and QCs in the qualification run were within ± 25% of the precision and accuracy values. A quadratic regression with an equation y = ax² + bx + c was used to fit calibration curves over the concentration range for carisbamate. The accuracy and precision were calculated at each QC level concentration.
Extraction efficiency of carisbamate was also assessed using the same QC samples. The ratio of the mean concentration of pre-extracted spiked plasma to that of the blank plasma extracts spiked after extraction at the same concentration was used to calculate the extraction efficiency.

Preliminary stability assessments were performed to evaluate the different stability conditions; stock solution, short-term, long-term and freeze-thaw using low, medium and high QC samples. For stock solution stability assessment, prepared stock solution was stored at −20°C for 28 days. The short-term stability samples were stored at room temperature for 6 hours and long-term stability samples were kept frozen at −20°C for 28 days. For the freeze-thaw stability assessment, the samples were subjected to three freeze and thaw cycles at −20°C. The acceptance criteria for all stability tests were within ±25% of the precision and accuracy values.

**In vitro experiments**

**Plasma protein binding**

The plasma protein binding assessment was carried out by equilibrium dialysis method in pooled SD rat and human plasma, respectively. The Thermo Scientific™ Rapid Equilibrium Dialysis device system (Thermo Scientific) was used for the equilibrium dialysis method. For the equilibrium dialysis, 300 µL of plasma samples containing carisbamate (1 µg/mL) were spiked into one insert and 500 µL of phosphate-buffered saline (PBS) was spike into the other insert, followed by equilibrium dialysis reaction for 4 hours at 37°C. After 4 hours, 100 µL of plasma samples was transferred to cluster tubes, and the same volume of PBS was added. In the same way, 100 µL of PBS samples was transferred to cluster tubes, and the same volume of blank plasma was added to make the same matrix conditions. Then, all samples were treated with the protein precipitation followed by the LC-qTOF-MS analysis. The unbound fraction of carisbamate in plasma (F_{up}) was obtained by the following formula:

\[
F_{up} = \left( \frac{C_u}{C_t} \right) \times 100
\]

In the above formula, Cu is the post-dialysis drug concentration in plasma, Ct is the post-dialysis drug concentration in PBS, which means the final concentration of unbound.

**Microsomal metabolic stability**

The microsomal metabolic stability in rat and human liver microsomes was investigated under the following final incubation conditions: carisbamate (0.2 µg/mL), rat and human liver microsomes (0.5 mg/mL) with β-nicotinamide adenine dinucleotide hydrate (NADPH) regenerating system solution (solution A is comprised of NADP+ and glucose-6-phosphate [Glc-6-PO_4], while solution B is comprised of glucose-6-phosphate dehydrogenase [G6PDH]). First, a 5 minutes pre-incubation (37°C) was conducted by adding cofactor solutions containing NADPH regenerating system solution A & B to the liver microsomes. After pre-incubation, carisbamate was added to the liver microsomes suspension. All incubations were quenched with ACN containing ISTD at 0, 30, 60, and 120 minutes after incubation. Then, all samples were centrifuged and the each supernatant was analyzed by the LC-qTOF-MS method.

The in vitro intrinsic clearance, CL_{int, in vitro} (mL/min/mg), was calculated by using the slope (k) of the log-linear regression analysis of the remaining % (the ratio of sample peak area/
ISTD peak area) versus time profiles, and the in vivo intrinsic clearance, CL_{int} (mL/min/kg), was calculated by scaling the in vitro values to the in vivo ones for rats and human by using the following equation.

\[
CL_{int, \text{in vitro}} = (\text{slope } k) \times \left( \frac{\text{mL microsomal volume}}{\text{mg microsomal protein}} \right)
\]

\[
CL_{int} = CL_{int, \text{in vitro}} \times \left( \frac{\text{mg microsomal protein}}{g \text{ liver}} \right) \times \left( \frac{g \text{ liver}}{kg \text{ body weight}} \right)
\]

Where the liver microsomal protein concentrations were 44.8 and 48.8 mg microsomal protein/g liver in rats and humans, respectively, and the liver concentrations were 40 and 25.7 g liver/kg body weight in rats and humans, respectively [25]. The hepatic clearance (CL_{H}) extrapolated from CL_{int} using “well stirred” model is expressed as shown in equation below [26,27]:

\[
CL_{H} = \left( \frac{Q_h \times CL_{int}}{Q_h + CL_{int}} \right)
\]

Where Q_h values, the hepatic blood flow, were 55.2 and 20.7 mL/min/kg in rats and humans, respectively.

Through “well-stirred” model, it was assumed that the liver was a well-stirred compartment and distribution equilibrium was achieved so quickly that the amount in the blood and the unbound amount in the liver were in equilibrium.

**Application for a PK study in rat**

All animal studies were performed in accordance with the “Guidelines in Use of Animal” established by the Chugnam National University Institutional Animal Care and Use Committee (Daejeon, Korea). This study was approved by the Chugnam National University Institutional Animal Care and Use Committee (No. CNU-01104). PK studies were conducted in SD rats (300 ± 10 g). All rats were fasted 12 hours prior to carisbamate administration. And then, carisbamate was administered to rats via intravenous bolus (IV) injection (1 and 2 mg/kg) or oral (PO) dose (1 and 5 mg/kg). The blood sampling time were 0, 2, 5, 10, 30, 60, 90, 120, 240, 360, and 1,440 min for IV PK and 0, 5, 15, 30, 60, 90, 120, 240, 360, and 1,440 minutes for PO PK in collection tube containing sodium heparin as anti-coagulant. Blood samples were centrifuged at 10,000 rpm for 5 minutes and supernatant plasma samples were stored at −20°C until analysis.

PK parameter estimates from IV or PO plasma concentration-time data were acquired using WinNonlin® version 8.0.0 (Certara, Princeton, NJ, USA). The nominal dose administered to each group was used to calculate PK parameters. PK parameters were calculated by non-compartmental analysis (NCA).

**Prediction of PK profiles using the PBPK model**

GastroPlus™ (version 9.7; Simulations Plus, Inc., Lancaster, CA, USA) PBPK model was used to predict the plasma concentration-time profile of rats and humans. In this study, the PBPK model was adopted as a perfusion-limited tissue model, which the kinetics of drug to tissue were determined by the Kp values (distribution coefficient).
In vitro data (unbound fraction in plasma and in vitro intrinsic clearance) were used as input parameters to build the PBPK model. GastroPlus™ ADMET predictor module was used for the prediction of physicochemical and ADME properties such as pKa, logP, permeability, solubility and blood/plasma ratio based on the structure of the carisbamate. The input parameters for PBPK simulation are summarized in Table 1. In addition to the in vitro data, PK parameters obtained by IV administration at a dose of 1 mg/kg in rats were used to optimize the PBPK model. The GastroPlus™ optimization module was used to optimize the PBPK model. The PBPK model was then validated using other in vivo PK data in rats and scaled to the PBPK model for human physiology. Then, human PK of carisbamate was simulated by population simulator (n = 12) using a scaled PBPK model, and the predicted PK results were compared with the observed baseline clinical data associated with food effect PK studies [7].

**RESULTS**

**Method qualification**

The calibration curves were selected based on the analysis of the data with weighted quadratic regression (1/concentration*). The mean correlation coefficient (r) value was > 0.99 for the linearity of calibration curve ranged from 9.05 to 6,600 ng/mL. Fig. 1 shows the calibration curve of carisbamate. Representative chromatograms of carisbamate (lower limit of quantification [LLOQ], 9.05 ng/mL) and verapamil (ISTD) samples are also shown in Fig. 2. The intra and inter-run accuracy and precision at 3 QC samples met the acceptance criteria of ±25% and the results are shown in Table 2. The extraction efficiency of carisbamate was found to be 33.44% ± 2.47%. The protein precipitation method for sample preparation showed the robust recovery.

**Table 1. Input parameters used in GastroPlus™ for PBPK model simulation of carisbamate**

| Parameters                        | Values                  |
|-----------------------------------|-------------------------|
| Molecular weight (g/mol)          | 215.64                  |
| pKa*                              | 11.98                   |
| LogP*                             | 1.05                    |
| Permeability (cm²/s)*             | 2.52                    |
| Solubility at pH 7 (mg/mL)*       | 6.66                    |
| Rbp in rat and human*             | 0.91                    |
| Unbound fraction (F_up) in rat and human (%)† | 47.55 and 51.29         |
| CL int, in vitro in rat and human (mL/min/kg)† | 0.0009 and 0.0006       |

PBPK, physiologically based pharmacokinetic; Rbp, blood/plasma ratio. All input parameters were calculated by GastroPlus™; *predicted values, †measured values.

**Figure 1.** A calibration curve (r = 0.99156, range = 9.05–6,600 ng/mL) for carisbamate in rat plasma. *Area ratio = Sample Peak Area/Internal Standard Peak Area.
LC-qTOF-MS determination and PBPK modeling of carisbamate

Figure 2. Typical chromatograms of carisbamate and verapamil. (A) LLOQ, 9.05 ng/mL of carisbamate, (B) verapamil (ISTD) with extracted blank matrix. ISTD, internal standard; LLOQ, lower limit of quantification.

Table 2. QC results and statistics from the quantification run for carisbamate in rat plasma

| Run No. | Statistics | Low QC (15.04 ng/mL) | Medium QC (165.46 ng/mL) | High QC (1,820 ng/mL) |
|---------|------------|----------------------|--------------------------|-----------------------|
| 1       | Mean       | 16.02                | 152.78                   | 1,685.67              |
|         | % Acc      | 106.54               | 92.34                    | 92.62                 |
|         | % CV       | 12.03                | 0.90                     | 6.29                  |
|         | No. of samples | 3            | 3                       | 3                     |
| 2       | Mean       | 15.75                | 153.41                   | 1,692.13              |
|         | % Acc      | 104.74               | 92.72                    | 92.97                 |
|         | % CV       | 8.35                 | 3.07                     | 5.35                  |
|         | No. of samples | 3            | 3                       | 3                     |
| 3       | Mean       | 16.94                | 200.62                   | 2,170.98              |
|         | % Acc      | 112.66               | 121.25                   | 119.28                |
|         | % CV       | 9.77                 | 3.73                     | 3.77                  |
|         | No. of samples | 3            | 3                       | 3                     |
|         | Inter-run  | Mean                 | 16.24                    | 168.94                | 1,849.59              |
|         | % Acc      | 107.98               | 102.10                   | 101.63                |
|         | % CV       | 9.42                 | 14.31                    | 13.75                 |
|         | No. of samples | 9            | 9                       | 9                     |

QC, quality control.
Statistics calculate as follows: % Acc = accuracy % = (observed mean value/theoretical value) * 100; % CV = coefficient of variation = (standard deviation/mean) * 100.

Table 3. Preliminary stability test results for carisbamate in rat plasma

| Assessment        | Statistics | Low QC (15.04 ng/mL) | Medium QC (165.46 ng/mL) | High QC (1,820 ng/mL) |
|-------------------|------------|----------------------|--------------------------|-----------------------|
| Short-term (RT, 6 hours) | Mean       | 14.71                | 170.28                   | 1,756.61              |
|                   | % Acc      | 97.78                | 102.91                   | 96.52                 |
|                   | % CV       | 18.57                | 6.46                     | 5.52                  |
|                   | No. of samples | 3            | 3                       | 3                     |
| Long-term (−20°C, 28 days) | Mean | 17.32                | 182.35                   | 1,907.17              |
|                   | % Acc      | 115.16               | 110.09                   | 104.79                |
|                   | % CV       | 4.87                 | 5.53                     | 1.84                  |
|                   | No. of samples | 3            | 3                       | 3                     |
| Freeze-thaw (−20°C, 3 cycles) | Mean | 14.87                | 150.62                   | 1,692.21              |
|                   | % Acc      | 98.87                | 91.03                    | 92.98                 |
|                   | % CV       | 17.66                | 3.30                     | 2.25                  |
|                   | No. of samples | 3            | 3                       | 3                     |
| Stock (−20°C, 28 days) | Mean       | 18.18                | 172.78                   | 1,890.50              |
|                   | % Acc      | 120.86               | 104.43                   | 103.87                |
|                   | % CV       | 12.53                | 4.87                     | 3.75                  |
|                   | No. of samples | 3            | 3                       | 3                     |

QC, quality control; RT, room temperature.
Statistics calculate as follows: % Acc = accuracy % = (observed mean value/theoretical value) * 100; % CV = coefficient of variation = (standard deviation/mean) * 100.
All the results of preliminary stability studies are shown in Table 3. As a result, the results indicated that carisbamate in rat plasma was stable for 6 hours at room temperature, which is sufficient for the sample preparation process, and stable for 28 days at −20°C, and also stable for 3 cycles of freeze-thaw process at −20°C. Also, carisbamate in stock solution was stable for 28 days at −20°C.

Generally, an LC-MS/MS method was applied to quantify small molecule drugs in bioanalytical samples because of sensitivity. However, due to the recent development of MS application technology, a LC-qTOF-MS also shows sufficient sensitivity to analyze bioanalytical samples, and many applications are applied in the references \[28-31\]. In addition, high resolution-full scan analysis using LC-qTOF-MS also can give us better picture of the metabolite profiles of the target compound. Therefore, the LC-qTOF-MS method was sufficient to develop and qualify for the bioanalysis of carisbamate in rat plasma.

**In vitro experiments**

As results of plasma protein binding test for carisbamate, $F_{up}$ values were 47.55% and 51.29% in rat and human plasma, respectively. And the results of the microsomal metabolic stability in rat and human liver microsomes are shown in Table 4. $\text{CL}_{\text{int, in vitro}}$ Values were 0.0009 and 0.0006 mL/min/mg microsomal protein in rat and human liver microsomes, respectively. The scaled $\text{CL}_{\text{int}}$ values were 1.70 and 0.72 mL/min/kg, and the extrapolated hepatic clearance values were 1.65 and 0.70 mL/min/kg in rat and human, respectively. These $F_{up}$ and $\text{CL}_{\text{int, in vitro}}$ values were used as input parameters for each species in Gastroplus™ (Table 1).

**Application for a PK study in rat**

The qualified LC-qTOF-MS method was successfully applied to a pharmacokinetic study of carisbamate in rats. Fig. 3 shows the pharmacokinetic profiles of carisbamate after IV and PO administration in rats. PK parameters were calculated by NCA using Phoenix WinNonlin® and shown in Table 5. The results show that maximum plasma concentration ($C_{\text{max}}$) and area under the curve (AUC) was dose proportional, while clearance (CL) and volume of distribution (Vd) are dose-independent in both IV and PO at dose range of 1 to 5 mg/kg, so that carisbamate has a linear PK in this dose range. The average value of CL was about 3.98 mL/min/kg, indicating that the drug was very stable \textit{in vivo} metabolic condition. The average of bioavailability (BA) was about 100%. It is assumed that the there is less concern of first-pass effect (FPE) or drug absorption for carisbamate in rats.

| Administration route | Dose (mg/kg) | No. of samples | $T_{1/2}$ (min) | $T_{\text{max}}$ (min) | $C_{\text{max}}$ (ng/mL) | AUC$_{\text{last}}$ (ng·min/mL) | AUC$_{\text{INF}}$ (ng·min/mL) | CL (mL/min/kg) | Vd (L/kg) |
|----------------------|-------------|----------------|----------------|-----------------|----------------------|--------------------------|--------------------------|----------------|-----------|
| IV                   | 1           | 3              | 315.22 ± 141.6 | 2.0 ± 0         | 1,262.02 ± 130.34   | 238,526.86 ± 51,457.51  | 252,189.38 ± 69,377.79  | 4.16 ± 1.07     | 1.56 ± 0.18 |
|                      | 2           | 3              | 265.75 ± 58.90 | 4.7 ± 4.62      | 2,091.42 ± 549.22   | 570,764.08 ± 235,907.17 | 587,742.97 ± 256,854.93 | 3.8 ± 1.37     | 1.24 ± 0.21 |
| PO                   | 1           | 4              | 336.74 ± 88.43 | 22.5 ± 20.31    | 580.42 ± 20.31      | 320,885.36 ± 30,274.45  | 338,124.98 ± 18,991.18  | 3.8 ± 1.37     | 1.24 ± 0.21 |
|                      | 2           | 3              | 276.81 ± 59.26 | 4.0 ± 17.32     | 3,660.12 ± 681.66   | 1,636,689.10 ± 183,116.71 | 1,681,244.93 ± 197,661.89 | 3.8 ± 1.37     | 1.24 ± 0.21 |

IV, intravenous bolus; PO, oral; $T_{1/2}$, half-life; $T_{\text{max}}$, time to maximum plasma concentration; $C_{\text{max}}$, maximum plasma concentration; AUC$_{\text{last}}$, area under the curve from 0 to last measurable time; AUC$_{\text{INF}}$, area under the curve from 0 to infinity; CL, clearance; Vd, volume of distribution.
Prediction of PK profiles using the PBPK model

The predicted and observed time-concentration profiles of carisbamate after a single IV administration at a dose of 1 mg/kg in rats are presented in Fig. 4. The first simulation was conducted using the input parameters only in Table 1 and it showed an over-estimated result at the initial time point and under-estimated result at the later time point compared to the observed in vivo data (Fig. 4A). It was expected that the discrepancy between the predicted and the measured PK profile was due to the difference between the predicted and the measured Vd values. The liver and kidney are mainly considered to be organs that determine the disposition of small molecule drug [19,20]. In case of carisbamate, the previous studies show little metabolic disposition in the kidney [24,32,33]. Therefore, we assumed that carisbamate is primarily distributed and metabolized to liver. Based on this information, we optimized the liver Kp value to correct the difference between the predicted and the observed Vd value. GastroPlus™ optimization module was conducted for this optimization process. The predicted PK profiles in the PBPK model optimized for the Vd values were more similar to the observed PK profiles (Fig. 4B). The optimized PBPK model was then validated with other in vivo PK data and the results are shown in Fig. 5. Fig. 5 shows that the optimized PBPK model was more accurate in predicting the PK profiles than the initial PBPK model.

Figure 4. Predicted and observed time-concentration profiles of carisbamate after a single intravenous administration at a dose of 1 mg/kg in rats. (A) PK profile predicted only using the input parameters in Table 1, (B) PK profile predicted after optimizing the Kp value of liver. PK, pharmacokinetic.
The PBPK model was well fitted when comparing the predicted PK profile with the observed PK profile at a different dose (2 mg/kg) or a different route of administration (oral administration). And then, the validated PBPK model by observed data in rats was scaled to human species. The human PK data orally administered at dose of 750 mg under physiologically fasted and fed condition were simulated by population simulator (n = 12) using scaled human PBPK model and compared to reference clinical data. The results are shown in Fig. 6 and Table 6.

As a result, prediction fold error value was within 2 folds between the predicted and the reference clinical data in both fasted and fed conditions, suggesting that the predicted human PK profile and parameters are considerably acceptable from the industrial perspective [16,19,20,34].
DISCUSSION

In this study, an LC-qTOF-MS method was developed and qualified for the quantification of carisbamate in rat plasma. The calibration curve was acceptable over the concentration range from 9.05 to 6,600 ng/mL for carisbamate using quadratic regression with 1/concentration$^2$ weighting. This LC-qTOF-MS method was sensitive, selective, accurate and reproducible for the determination of carisbamate concentration and has been successfully applied to various in vitro and in vivo PK studies in rats. Several in vitro experiments and in vivo PK studies from preclinical species were used to successfully built a PBPK model and applied to predict the PK of carisbamate in humans. Overall, The optimized and validated PBPK model reasonably predicted the PK profile and the parameters of carisbamate in clinical PK study.

In conclusion, this study might indicate that the human PK prediction of carisbamate through PBPK modeling using in vitro data and in vivo data from preclinical species is expected to be a useful way to reduce time and cost associated with food effect studies and other various human clinical trials.

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