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ORIGINAL ARTICLE

Mass balance, pharmacokinetics and pharmacodynamics of intravenous HSK3486, a novel anaesthetic, administered to healthy subjects

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Aims: This trial (NCT03751956) investigated the mass balance, pharmacokinetics and pharmacodynamics of HSK3486, a novel anaesthetic, in healthy subjects.

Methods: A single dose of 0.4 mg/kg [^{14}C]HSK3486 was administered to six healthy subjects. Blood, urine and faecal samples were collected, analysed for radioactivity, unchanged HSK3486 and profiled for metabolites. The Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale and vital signs were closely monitored during the study.

Results: The mean recovery of total radioactivity in excreta was 87.3% in 240 h, including 84.6% in urine and 2.65% in faeces. The exposure ($AUC_{0\text{-}t}$) of total radioactivity was much higher than that of unchanged HSK3486 in plasma, indicating there were circulating metabolites in plasma. The glucuronide conjugate of HSK3486 (M4) was found as the only major circulating metabolite in plasma (79.3%), while unchanged HSK3486 accounted for only 3.97% of the total radiation exposure. M4 also resulted in a longer estimated elimination half-life ($t_{1/2}$) of total radioactivity than that of unchanged HSK3486 in plasma. Fortunately, the metabolite was detected to be not specific to red blood cells and was suggested to be nonhypnotic and nontoxic. All the subjects were quickly anaesthetized (2 min) after drug administration and woke up smoothly after a short time (5.5–14.1 min) with few residual effects. The only adverse event in the study was mild (grade 1) and consisted of hypotension.

Conclusion: HSK3486 is a promising anaesthetic candidate with rapid onset of action and clear absorption, distribution, metabolism, excretion (ADME) processes. HSK3486 showed favourable pharmacokinetic characteristics, pharmacodynamic responses and safety at the study dose.

KEYWORDS

anaesthetic, HSK3486, major circulating metabolites, mass balance, pharmacokinetics

Chenrong Huang and Liyan Miao contributed equally to the work.

PI statement: The authors confirm that the Principal Investigator for this paper is Liyan Miao and that she had direct clinical responsibility for subjects.

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1 | INTRODUCTION

HSK3486, a novel 2,6-disubstituted phenol derivative, is a new candidate intravenous drug for the induction and maintenance of anaesthesia in clinical practice.[1,2] Like the most commonly used intravenous anaesthetic, propofol, HSK3486 is a γ-aminobutyric acid (GABA) receptor potentiator. HSK3486 has completed several clinical trials in China and Australia, and has shown good efficiency and safety. In the studies performed to date (NCT04054063, NCT04037657, NCT04033939, NCT03773835, NCT03698617, NCT03709056, NCT03773042, NCT03808844, NCT03674008), more than 400 subjects in total were enrolled in the HSK3486 groups, including healthy subjects, as well as subjects undergoing colonoscopy, gastroscopy or elective surgery.

Previous studies showed that HSK3486 possessed the favourable anaesthetic properties of rapid onset and rapid return of consciousness with only minor residual effects after a single dose. HSK3486 had good anaesthetic efficacy at a dose of 0.4 mg/kg, and 0.6 mg/kg HSK3486 had similar anaesthetic potency to 2.5 mg/kg propofol, which indicated that HSK3486 had the equivalent potency of propofol at one quarter to fifth of the dosage.[2] The adverse events associated with HSK3486 are generally similar to those of propofol, mainly affecting the cardiovascular and respiratory systems. HSK3486 may have less effect on respiratory function than propofol, and may have a lower risk of injection pain than propofol due to the lower concentration in the aqueous phase of the emulsion.[1]

HSK3486 is a promising intravenous anaesthetic candidate. However, the mass balance, pharmacokinetics and pharmacodynamics of HSK3486 have not been reported. Human radiolabelled mass balance study is a gold standard to obtain information about the metabolic disposition, elimination route and recovery of a new drug after a single radiolabelled dose.[3,4] In addition, due to the special pharmacological properties and clinical uses of HSK3486, the anaesthetic effects of the drug can be observed after a single dose. Therefore, the clinical effects of HSK3486 can also be investigated in mass balance study.

To fully understand the metabolic disposition and mass balance of HSK3486, and the impact of HSK3486-related metabolites on pharmacokinetics and pharmacology, we conducted a study in healthy Chinese subjects to clarify the distribution, metabolism and elimination, as well as anaesthetic effects and safety of HSK3486 after a single ^{14}C-labelled intravenous dose.

What is already known about this subject

- HSK3486, a novel 2,6-disubstituted phenol derivative, is a promising intravenous anaesthetic candidate undergoing clinical development.
- HSK3486 has completed several clinical trials from Phase I to Phase III in China and Australia, and has shown equivalent anaesthetic efficacy to propofol at one quarter to one fifth of the dosage. However, the mass balance, pharmacokinetics and pharmacodynamics of HSK3486 have not been reported.

What this study adds

- The mass balance and primary excretion routes of HSK3486 were investigated after a single ^{14}C-labeled intravenous dose in six healthy subjects.
- Twenty metabolites and the main metabolic pathways were identified. The major circulating metabolites in plasma were detected and quantified.
- The pharmacodynamics of HSK3486 was investigated, and the potential pharmacological effects of the major metabolites were evaluated.

2 | METHODS

2.1 | Study design

This was a Phase I, open-label, single-centre study conducted at the First Affiliated Hospital of Soochow University (Suzhou, China).

After a screening period of up to 13 days (day -14 to day -2), six healthy Chinese subjects were included in this study and stayed at the study centre until 240 hours after dosing. The subjects received a single intravenous dose of 0.4 mg/kg [^{14}C]HSK3486 in boluses within 1 minute ± 5 seconds on day 1 after fasting for at least 10 hours.

The blood samples, and all the urine and faeces were collected at specified time points or intervals 10 days after the administration of [^{14}C]HSK3486. The subjects were discharged from the study site when all the following criteria were met: (a) the cumulative excreted radioactivity from each subject exceeded 80% of the administered dose; (b) the excreted radioactivity in urine and faeces was less than 1% of the administered dose over a 24 hour period on two consecutive days; (c) the radioactivity in plasma was less than two times the radioactive background over two continuous time points.[5]

The study was conducted in accordance with the ICH GCP guidelines. The protocol was approved by Medical Ethics Committee of the First Affiliated Hospital of Soochow University. All subjects provided written informed consent.

2.2 | Subjects

Healthy male subjects aged 18–49 years with body mass index (BMI) of 19–26 kg/m^2 were eligible. Subjects were excluded if they met any of the following exclusion criteria: a history of drug or alcohol abuse or participation in any other clinical trials in the past 3 months; the use of prescribed or nonprescribed concomitant medications within 14 days of the study; hypersensitivity to propofol or other emulsion

injections; airway assessment of modified Mallampati score IV, significant clinical laboratory abnormalities; history of cardiovascular, respiratory, renal, hepatic, gastrointestinal disease or major surgery; or smoking addiction (≥5 cigarettes daily).

2.3 | Study medication

Radiolabelled HSK3486 was synthesized by Shanghai Qizhen Environmental Technology Co., Ltd. The radiochemical purity of [^{14}C]HSK3486 was >99% and the specific activity was 1.95 GBq/mmol. [^{14}C]HSK3486 was purified by Value Pharmaceutical Services Co., Ltd and mixed with blank emulsion (supplied by Haisco Pharmaceutical Group Co., Ltd). The formulation containing [^{14}C]HSK3486 was autoclaved and sealed in glass vials and diluted with sterile cold HSK3486 emulsion (supplied by Haisco Pharmaceutical Group Co., Ltd) to a concentration of 7.14×10^4 Bq/mg before use.

The dosimetry of subjects following administration of a single dose of 0.4 mg/kg [^{14}C]HSK3486 was calculated based on the data from a quantitative whole body autoradiography (QWBA) study in Long–Evans (LE) rats and a mass balance study in Sprague Dawley (SD) rats. The overall whole-body dose for male subjects (e.g., 60 kg) was estimated to be approximately 0.24 mSv (24 mrem). The doses for the whole body, active blood-forming organs, lens of the eye, gonads and other organs were all far below FDA constraints.[6–9]

2.4 | Sample collection

Blood samples (~4 mL) were collected in K_2-EDTA collection tubes at the following time points: 0 h (before dosing), 0.0167 h, 0.0333 h, 0.05 h, 0.0833 h, 0.133 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 120 h and 168 h after dosing. Blood samples were taken via an indwelling intravenous catheter or by direct vein puncture into K_2-EDTA collection tubes and immediately cooled with iced water. The blood samples were centrifuged at 3000 ± 10*g* for 10 minutes at 4°C, and supernatants were collected for plasma samples for radioactivity measurement and liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis. Extra blood samples (~10 mL) were collected at 0 h (before dosing), 0.0833 h, 0.5 h, 4 h and 24 h after dosing. Whole blood aliquots were taken for radioactivity measurement, and then the tube was centrifuged (3,000 ± 10*g*, 10 min, 4°C) to produce plasma for metabolite identification. All samples were stored at −20°C or below until analysis.

Urine and faeces were collected for mass balance study and metabolite profiling. Urine samples were collected at time 0 h (before dosing), 0–4 h, 4–8 h, 8–12 h, 12–24 h, 24–48 h, 48–72 h, 72–96 h, 96–120 h, 120–144 h and 144–168 h in polyethylene containers and stored at 4°C during the collection period. Urine samples were mixed thoroughly and weighed after each collection period, and then kept at −20°C or below until analysis. Faecal samples were collected at time 0 (before dosing), 0–24 h, 24–48 h, 48–72 h, 72–96 h, 96–120 h, 120–144 h and 144–168 h and stored at −20°C or below until analysis.

2.5 | Bioanalytical analysis

2.5.1 | Radioactivity analysis

Blood, plasma, urine and faecal samples were analysed for total radioactivity (TRA) using a liquid scintillation counter Tri-Carb Model 4910TR (PerkinElmer). Plasma (0.2–0.5 g) and urine (1 g) samples were directly analysed by liquid scintillation counting (LSC). Faecal samples were first homogenized with isopropanol/water (50/50, v/v). Blood (0.3 g) and faecal homogenate (0.3 g) samples were combusted using a Harvey OX-501 Biological Oxidizer before LSC.

2.5.2 | Quantification of HSK3486 in plasma

Plasma samples were analysed using a validated LC–MS/MS method to determine the concentration of HSK3486. The plasma samples were deproteinated with acetonitrile after addition of stable isotope labelled HSK3486 as internal standard and separated on a column of Phenomenex Gemini C18 (5 μm, 150 mm × 2.0 mm) at 40°C. The mobile phase was 0.05% ammonium hydroxide in 80% acetonitrile at the flow rate of 0.5 mL/min. The detection was performed on a Triple Quad 5500 tandem mass spectrometer coupled with electrospray ionization (ESI) source in negative mode. Quantification was conducted by multiple reaction monitoring (MRM) of the transitions of m/z 203.2 → 175.0 for HSK3486 and m/z 209.2 → 181.0 for IS, respectively. The calibration ranges for HSK3486 in human plasma were 5–5000 ng/mL (see Supplementary Material 1 for the accuracy and quality control of HSK3486 quantification).

2.5.3 | Metabolite profiling and identification

A plasma sample (0–120 h) was obtained by pooling plasma at each time point of a volume proportional to the time interval used for calculating the area under the concentration–time curve (AUC) ($AUC_{0-120\ h}$ pool) for each subject. Moreover, three plasma samples at time periods 0–0.0833 h, 0.133–2 h and 4–120 h were obtained by pooling equal volumes of plasma across individuals at the same time point.[10–12] Urine samples (0–168 h) were pooled at an equal percentage of collection volume to obtain one pooled sample for each subject. Moreover, the samples of urine were pooled across individuals at an equal volume to obtain one pooled sample per time period of 0–4 h, 4–12 h and 12–168 h. Faecal samples (0–96 h) were pooled at the same weight percentages across collection intervals for each subject. Moreover, faecal samples were pooled across individuals at an equal weight to obtain one pooled sample per time period of 0–48 h, 48–72 h and 72–96 h. All plasma and faecal homogenate samples

were extracted with acetonitrile. The supernatants were concentrated prior to analysis. Urine samples were centrifuged prior to analysis.

The samples were injected into an HPLC system Shimadzu LC-2030C with *v*ARC Radio-Assay for fractionation and online detection. Separation was performed on a column of ACE C18-AR (3.0 μm, 150 mm × 4.6 mm) at 25°C. The mobile phase consisted of 5mM ammonium acetate with 0.001% ammonium (A) and acetonitrile (B). The flow rate was 0.7 mL/min. The gradient elution is shown in Supplementary Material 2. The fractionation was collected at intervals of 15 s per well into Deepwell LumaPlate-96 microplates. The radioactivity in each fraction was measured by LSC or solid scintillation counting using a Packard TopCount NXT Microplate Counter. The data were reconstructed using ARC Convert and Evaluation software.

Metabolite identification was performed on an LTQ OrbiTrap XL LC–MS/MS System. Mass spectrometry was conducted in electrospray positive ion mode, and the instrument parameters were optimized for maximal sensitivity of the analytes of interest.

2.6 | Pharmacokinetics assessment

The data of plasma TRA and the plasma concentration of HSK3486 were used to determine the pharmacokinetic parameters, including the maximum concentration (C_{max}), time to reach C_{max} (T_{max}), AUC from time zero to one hour (AUC_{0-1h}), AUC from time zero to the time of the last quantifiable concentration (AUC_{0-t}), AUC form time zero to infinity ($AUC_{0-\infty}$), the elimination half-life time ($t_{1/2}$), the apparent volume of distribution (V_z) and the apparent clearance (CL). One gramme of plasma was equivalent to 1 mL in the calculation of C_{max} and AUC of TRA.

2.7 | Pharmacodynamics and safety assessment

The anaesthetic effects and safety of HSK3486 were evaluated during the study. The Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scale was used to assess the sedation levels of subjects (Table 1)[13] and was recorded every 2 minutes (2 min ± 30 s) after dosing. Cardiogram monitors were used to observe the vital signs, electrocardiogram and other indicators after drug administration until the subjects were fully awake (MOAA/S score was 5 three consecutive times).

TABLE 1 Responsiveness scores of the Modified Observer's Assessment of Alertness/Sedation scale

Score	Response
5	Responds readily to name spoken in normal tone
4	Lethargic response to name spoken in normal tone
3	Responds only after name is called loudly or repeatedly
2	Responds only after mild prodding or shaking
1	Responds only after painful trapezius squeeze
0	Does not respond to painful trapezius squeeze

The adverse events (AEs) were described according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) grading system (version 5.0). Clinical laboratory tests (haematology, serum chemistry, urinalysis and stool analysis), 12-lead electrocardiograms, physical examinations and vital signs were also part of the safety assessment. The injection pain was observed through the subjects' response to the drug injection, including facial expressions, complaints or arm retraction.

2.8 | Statistical analysis

Statistical analyses were performed on all subjects who received a dose of the study drug and completed the study. Pharmacokinetics outcomes were estimated using Phoenix WinNonlin (Version 7.0, Pharsight Corp.). Non-compartmental analysis was performed to obtain C_{max}, T_{max}, AUC_{0-1h}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, V_z and CL of total radioactivity (detected by LSC) and HSK3486 (detected by LC–MS/MS), respectively. Summary pharmacokinetics parameters were described as the mean and standard error of the mean. Safety outcomes were presented using descriptive statistics.

2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[14] and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16.[15]

3 | RESULTS

3.1 | Subjects and dose administration

Six healthy male Chinese subjects were enrolled in the study, with a median age of 29.5 years (range 27–33 years), a mean weight of 67.3 kg (62.5–73.0 kg) and a mean BMI of 22.9 kg/m^2 (range 21.1–24.2 kg/m^2). All the subjects completed the study. Baseline demographics are presented in Table 2.

The administered doses ranged from 24.5 to 29.5 mg (1.75–2.11 MBq) of HSK3486. The actual doses were 0.394 mg/kg on average, which is close to the target dose of 0.4 mg/kg. Doses were calculated by subtracting the residual radioactivity in the syringes and indwelling needles from the radioactivity of [^{14}C]HSK3486 pipetted into the syringes. The data are summarized in Table 3.

3.2 | Mass balance

The percentages of radioactivity recovered from urine and faeces are presented in Table 4, and the cumulative percentage recovery is

TABLE 2 Baseline demographics

Characteristics	Value
Sex, N (%)	
Male	6 (100)
Age (years)	
Mean (SD)	29.8 (2.32)
Median	29.5
Min, max	27, 33
Race, N (%)	
Han	6 (100)
Height (cm)	
Mean (SD)	171.7 (3.76)
Median	172.3
Min, max	165.0, 176.5
Weight (kg)	
Mean (SD)	67.3 (3.68)
Median	67.0
Min, max	62.5, 73.0
BMI (kg/m^2)	
Mean (SD)	22.9 (1.18)
Median	23.4
Min, max	21.1, 24.2

TABLE 3 The actual doses administered to six healthy subjects

	HSK3486		Radioactivity
Subject	(mg)	(mg/kg)	(MBq)
01	24.9	0.389	1.78
02	25.1	0.377	1.79
03	26.7	0.408	1.91
04	24.5	0.389	1.75
05	26.7	0.396	1.91
06	29.5	0.407	2.11
Mean ± SD	26.2 ± 1.85	0.394 ± 0.0119	1.88 ± 0.134

TABLE 4 Recovered radioactivity in excreta after a single intravenous injection of 0.4 mg/kg [^{14}C]HSK3486 in healthy subjects

	Recovery radioactivity		
Subject	Urine	Faeces	Total
01	84.9	2.53	87.4
02	84.6	3.18	87.8
03	84.7	3.38	88.1
04	82.9	2.05	85.0
05	86.3	3.00	89.3
06	84.2	1.77	86.0
Mean ± SD	84.6 ± 1.10	2.65 ± 0.646	87.3 ± 1.56

shown in Figure 1. The total recovery of radioactivity in urine and faeces was 87.3 ± 1.56% over 240 hours after dosing, ranging from 85.0% to 89.3%. Most of the radioactivity was excreted through urine, accounting for 84.6 ± 1.10% of the dose. Most of the administered radioactivity was excreted in the first 168 hours after dosing, with an average of approximately 85.3% of the administered dose and 97.8% of the total excretion. The daily radioactivity excretion in urine and faeces was less than 1% after 168 hours.

3.3 | Pharmacokinetics and blood-to-plasma ratio

The pharmacokinetics of total radioactivity and unchanged HSK3486 in plasma after a single intravenous dose of [^{14}C]HSK3486 are presented in Table 5, and the concentration–time curves are shown in Figure 2. The values of exposure to total radioactivity and HSK3486 (AUC_{0-t}) were 6716.7 ± 1106.6 h*ng Eq./mL and 266.3 ± 71.8 h*ng/mL, respectively. The exposure of unchanged HSK3486 accounted for only 3.97% of the total radiation, and the estimated elimination half-life ($t_{1/2}$) of total radioactivity in plasma (61.2 ± 7.82 h) was much longer than that of unchanged HSK3486 (1.50 ± 0.39 h).

The total radioactivity of whole blood samples was analysed at 0.0833 h, 0.5 h, 4 h and 24 h. The blood-to-plasma ratios of total radioactivity at 0.0833 h, 0.5 h and 4 h were 59.3%, 59.1% and 57.0%, respectively, while the radioactivity in whole blood at 24 h was below the lower limit of quantification (64.6 ng Eq./g).

3.4 | Metabolite profiles and identification

Representative radiochromatograms of HSK3486 and metabolites in pooled plasma, urine and faeces are shown in Figure 3. Twenty metabolites were identified in plasma, urine and faeces. The metabolites are methyl-oxidation and mono-oxidation products (M2-1, M2-2, M2-3 and M2-4), hydroxylation and sulfation products (M3), glucuronidation products (M4), monohydroxylation and glucuronidation products (M5-1, M5-2 and M5-3), dihydroxylation and glucuronidation products (M6-1, M6-2, M6-3, M6-4 and M6-5), monohydroxylation products (M7), methyl oxidation, mono-oxidation and glucuronidation products (M8-1 and M8-2), tri-hydroxylation and glucuronidation products (M9), and monohydroxylation, dehydrogenation and glucuronidation products (M10-1 and M10-2). HSK3486 and its metabolites in total plasma radioactive exposure (% AUC) or in urine and faeces as a percentage of dose (% dose) are shown in Table 6. The proposed fragmentation pathway is shown in Supplementary Material 3.

The main identified metabolites in plasma were M4, M2-4 and M5-2, which accounted for 79.3%, 4.36% and 3.93%, respectively of total plasma radioactivity. Trace amounts of M7 accounting for 0.18% of total plasma radioactive exposure was detected, while some radiochromatogram peaks accounting for 0.03% to 2.69% of the total radioactive exposure were not identified. In addition, the

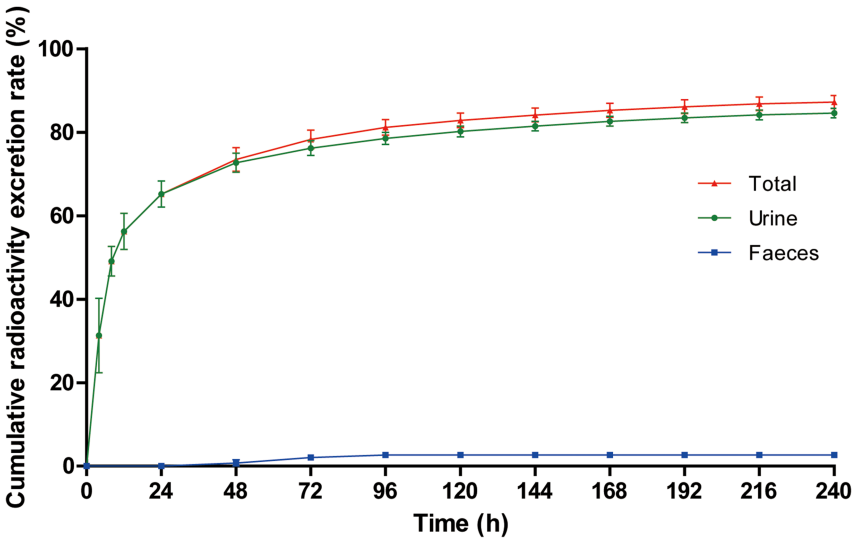

FIGURE 1 The cumulative radioactivity excretion after a single dose of 0.4 mg/kg [^{14}C] HSK3486 to healthy subjects (n = 6 subjects)

TABLE 5 Summary of pharmacokinetic parameters of total radioactivity and unchanged HSK3486 in plasma (n = 6 subjects)

Parameter	Total radioactivity mean ± SD	HSK3486 concentration mean ± SD
C_{max} (ng Eq./mL or ng/mL)	1753.2 ± 1267.2	1676.8 ± 1327.3
T_{max}(h)[a]	0.0333	0.0333
AUC_{0-1h} (h*ng Eq./mL or h*ng/mL)	788.3 ± 134.6	171.3 ± 54.3
AUC_{0-t} (h*ng Eq./mL or h*ng/mL)	6716.7 ± 1106.6	266.3 ± 71.8
$AUC_{0-\infty}$ (h*ng Eq./mL or h*ng/mL)	7665.0 ± 1148.3	289.2 ± 68.8
$t_{1/2}$ (h)	61.2 ± 7.82	1.50 ± 0.39
V_z (mL/kg)	4,610 ± 807	2,960 ± 370
CL (mL/h/kg)	52.3 ± 6.6	1418.2 ± 273.6

[a]T_{max} presented as median.

C_{max}, maximum concentration; T_{max}, time to reach C_{max}; AUC_{0-1h}, area under the concentration–time curve (AUC) from time zero to one hour; AUC_{0-t}, AUC from time zero to the time of the last quantifiable concentration; $AUC_{0-\infty}$, AUC form time zero to infinity; $t_{1/2}$, the elimination half-life time; V_z, apparent volume of distribution; CL, apparent clearance.

metabolites of M5-3, M5-1 and M3 were detected only by mass spectrometry and could not be quantified by radiochromatography. The parent drug was only detected in the 0–0.0833 h pooled plasma sample by LSC, accounting for only 1.16% of the total plasma radioactive exposure ($AUC_{0-120\ h}$ pool). According to the AUC_{0-t} calculated by measuring the concentration of HSK3486 by LC–MS/MS, unchanged HSK3486 accounted for only 3.97% of the total radioactivity exposure (AUC_{0-t}) in plasma. The main metabolites detected in urine were M4, M5-1, M5-3 and M3, which accounted for 51.6%, 19.3%, 6.87% and 2.28% of the dose, respectively. Trace amounts of M5-2, M2-4, M6-1, M6-2, M6-3, M6-4, M6-5, M9, M8-2, M10-1 and M10-2, accounting for 0.02–0.43% of the dose, were detected, while some radioactive peaks accounting for 0.03–0.69% of the dose were not identified. The metabolites of M2-1, M2-2, M2-3 and M8-1 were detected only by mass spectrometry, and could not be quantified by radiochromatography. The total radioactivity excreted in faeces accounted for only 2.65% of the dose, and the original drug accounted for 0.08% of the dose. Metabolites M3 and M7 detected in faeces accounted for 0.05–0.20% of the dose, while some radioactive peaks accounting for 0.01–0.79% of the dose were not identified.

The proposed metabolic pathways of HSK3486 in humans are shown in Figure 4. The main metabolic pathways of HSK3486 were oxidation, glucuronidation and sulfation.

3.5 | Pharmacodynamics and safety

HSK3486 was well tolerated. All the subjects were quickly anaesthetized and woke up smoothly after a short time.

The observed MOAA/S score–time curve for six subjects is shown in Figure 5. All the subjects had an MOAA/S score of 5 at baseline (before dosing). The scores decreased to a minimum of 0 or 1 rapidly 2 minutes after dosing and then gradually recovered. Half of the subjects recovered from MOAA/S score ≤ 1 and 5 in 6 and 10 minutes after dosing, respectively. The MOAA/S scores of all subjects returned to 5 at 14 minutes after administration. The average and median wake-up times of the six subjects were 10.6 and 11.2 minutes, respectively. The minimum and maximum wake-up times were 5.5 and 14.1 minutes, respectively.

The vital signs of systolic blood pressure and diastolic blood pressure decreased slightly and then gradually returned to baseline (fluctuation range within 12%) within 18 minutes after drug administration. The heart rate/pulse increased transiently and then gradually returned to the baseline level within 18 minutes after drug administration (fluctuation range of 16.6%). The temperature, respiratory frequency and pulse oximetry showed no significant changes after drug administration (Figure 6).

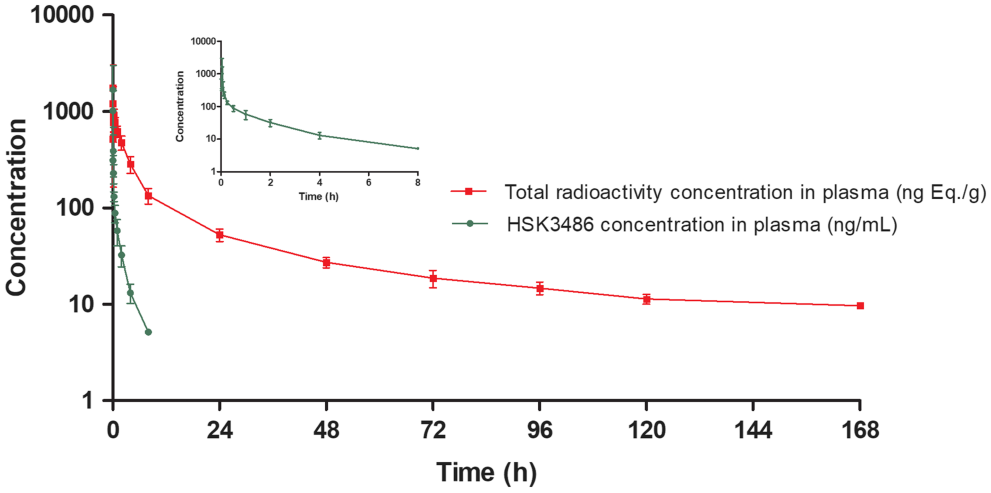

FIGURE 2 Mean (± SD) log-linear concentration–time curve of total radioactivity (HSK3486-related compounds) in whole blood and plasma after a single dose of 0.4 mg/kg [^{14}C] HSK3486 to healthy subjects (n = 6 subjects)

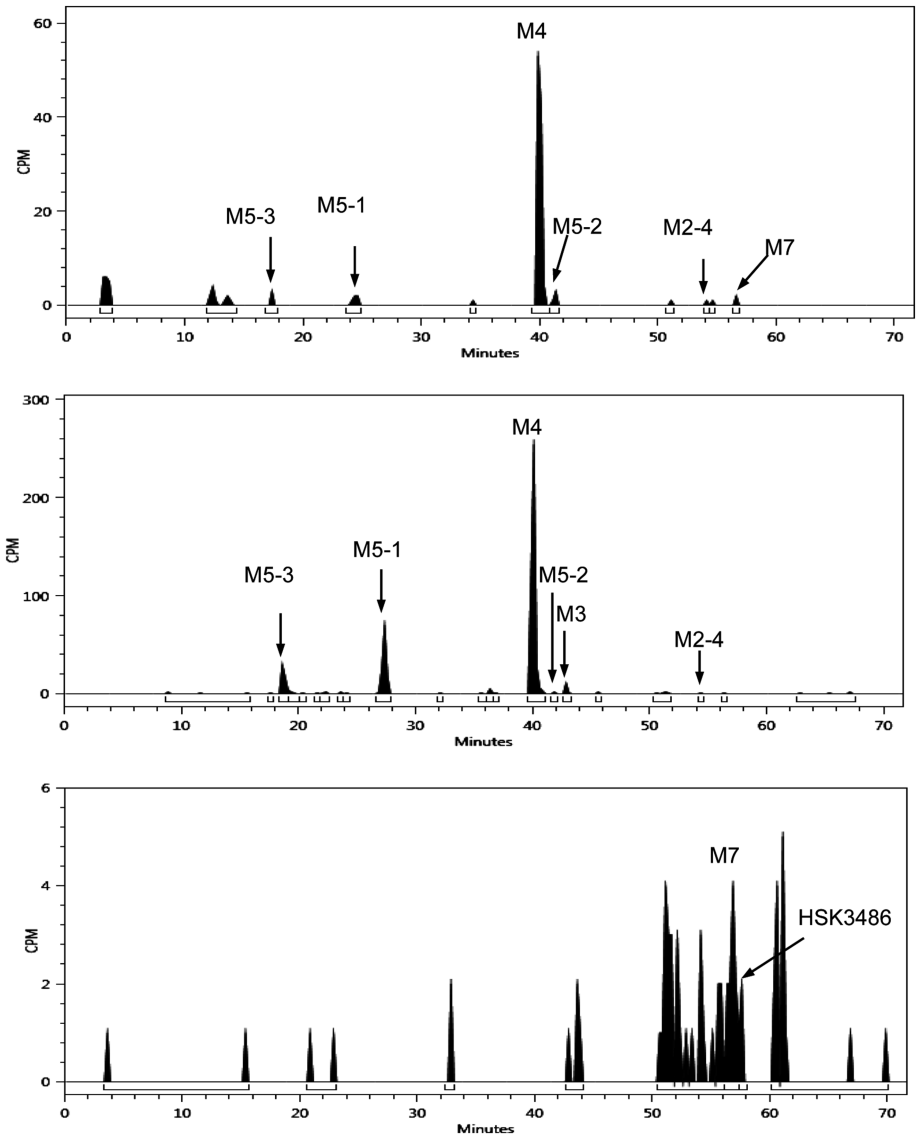

FIGURE 3 Representative radiochromatograms of HSK3486 and metabolites in pooled plasma (0–120 h), urine (0–168 h) and faeces (0–96 h)

The only adverse event in the study was mild (grade 1) and consisted of hypotension. Subject 03 experienced low blood pressure at 8 minutes after drug administration. The AE lasted 6 minutes and was completely relieved with no treatment. No injection pain was observed in the trial. No severe AEs or other significant abnormalities were observed in the study.

TABLE 6 Summary of [^{14}C]HSK3486 and its metabolites in total plasma radioactive exposure (% AUC) and in urine and faeces as a percentage of dose (% dose)

Metabolites	Structural modification	Plasma[a](0–120 h) % AUC	Urine(0–240 h) % dose	Faeces(0–240 h) % dose
HSK3486	Parent drug	1.16	ND	0.08
M2-1	Methyl oxidation, mono-oxidation	ND	+	ND
M2-2	Methyl oxidation, mono-oxidation	ND	+	ND
M2-3	Methyl oxidation, mono-oxidation	ND	+	ND
M2-4	Methyl oxidation, mono-oxidation	4.36	0.10	ND
M3	Hydroxylation, sulfation	+	2.28	0.05
M4	Glucuronidation	79.3	51.6	ND
M5-1	Monohydroxylation, glucuronidation	+	19.3	ND
M5-2	Monohydroxylation, glucuronidation	3.93	0.33	ND
M5-3	Monohydroxylation, glucuronidation	+	6.87	ND
M6-1	Dihydroxylation, glucuronidation	ND	0.42	ND
M6-2	Dihydroxylation, glucuronidation	ND	0.07	ND
M6-3	Dihydroxylation, glucuronidation	ND	0.04	ND
M6-4	Dihydroxylation, glucuronidation	ND	0.15	ND
M6-5	Dihydroxylation, glucuronidation	ND	0.02	ND
M7	Monohydroxylation	0.18	ND	0.20
M8-1	Methyl oxidation, mono-oxidation, glucuronidation	ND	+	ND
M8-2	Methyl oxidation, mono-oxidation, glucuronidation	ND	0.25	ND
M9	Tri-hydroxylation, glucuronidation	ND	0.27	ND
M10-1	Monohydroxylaion, dehydrogenation, glucuronidation	ND	0.43	ND
M10-2	Monohydroxylaion, dehydrogenation, glucuronidation	ND	0.07	ND
Unidentified peaks		11.0	2.36	2.34

ND: Not detected; +: Only mass spectrum detected.
[a]Data are derived from the sum of the %AUC of parent drug and metabolites in segmented plasma sample from 0 to 120 h.

4 | DISCUSSION

HSK3486 is a promising intravenous anaesthetic candidate undergoing clinical development. In this study, we characterized the mass balance, pharmacokinetics and pharmacodynamics of HSK3486 after a single ^{14}C-labelled intravenous dose of 0.4 mg/kg in healthy Chinese subjects.

The chemical dose of 0.4 mg/kg administered to subjects in the study was an expected clinical dose. The radiation dose administered to subjects in the study was carefully calculated to ensure the safety of subjects. The overall whole-body dose for a male subject (e.g., 60 kg) was estimated to be approximately 0.24 mSv (24 mrem), and the risk of individual exposure was minor according to the International Commission on Radiological Protection (ICRP).[7] A total of 33 subjects were screened, six of whom were successfully enrolled in this study. All six subjects had good medication compliance and completed the sample collection and safety evaluation according to the protocol. HSK3486-related compounds (parent drug and its metabolites with ^{14}C-labelled) were detected and quantified by radioactivity and mass spectrometry detection in the study.

In the mass balance study, the overall recovery was 87.3 ± 1.56% within 240 hours, which generally reached a mass balance. Urine was the main excretory route of HSK3486, accounting for 84.6 ± 1.10% of the administered dose, and faecal elimination accounted for only 2.65 ± 0.646%. It is suggested that the excretion characteristics of HSK3486 are similar to those of propofol, approximately 88% of which is eliminated in urine within 5 days and less than 2% in faeces.[16] In addition, an extremely small amount (on the order of one billionth) of propofol was suggested to be excreted through exhalation,[17] suggesting that HSK3486 may also be excreted through the same route. However, the quantitation of expired HSK3486 remains challenging and it was not performed in this study.[18]

In the pharmacokinetics study, HSK3486 was absorbed intravenously and quickly eliminated from plasma. The concentration–time curves of HSK3486 had similar characteristics to those of propofol. The plasma concentration of HSK3486 declined in a multiphasic manner after intravenous administration. The terminal elimination half-lives of HSK3486 and propofol were 90 and 97 minutes, respectively.[16] The exposure (AUC_{0-t}) of total radioactivity was much higher than that of unchanged HSK3486 in plasma, and the estimated

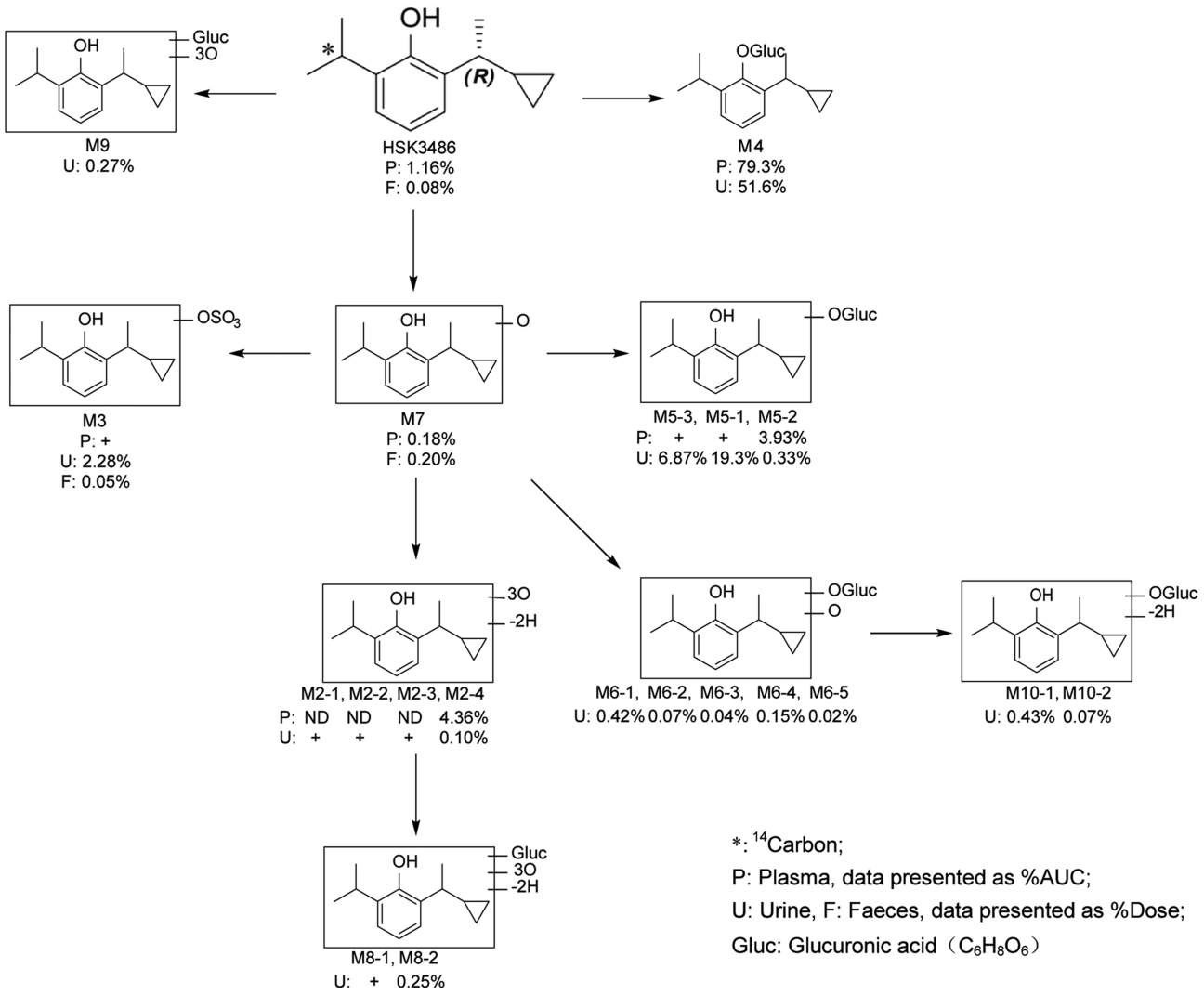

FIGURE 4 Proposed metabolic pathways of HSK3486 in humans

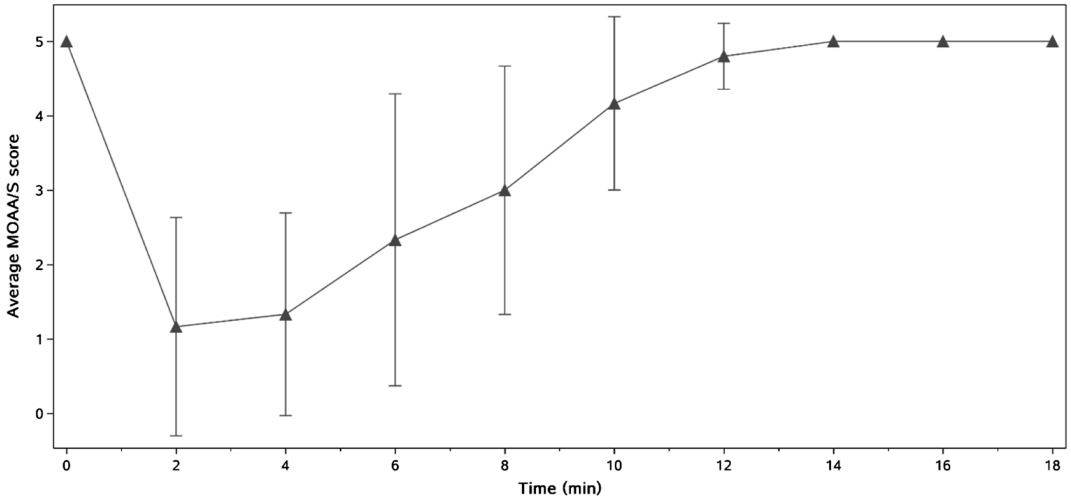

FIGURE 5 The observed MOAA/S score–time curve (n = 6 subjects)

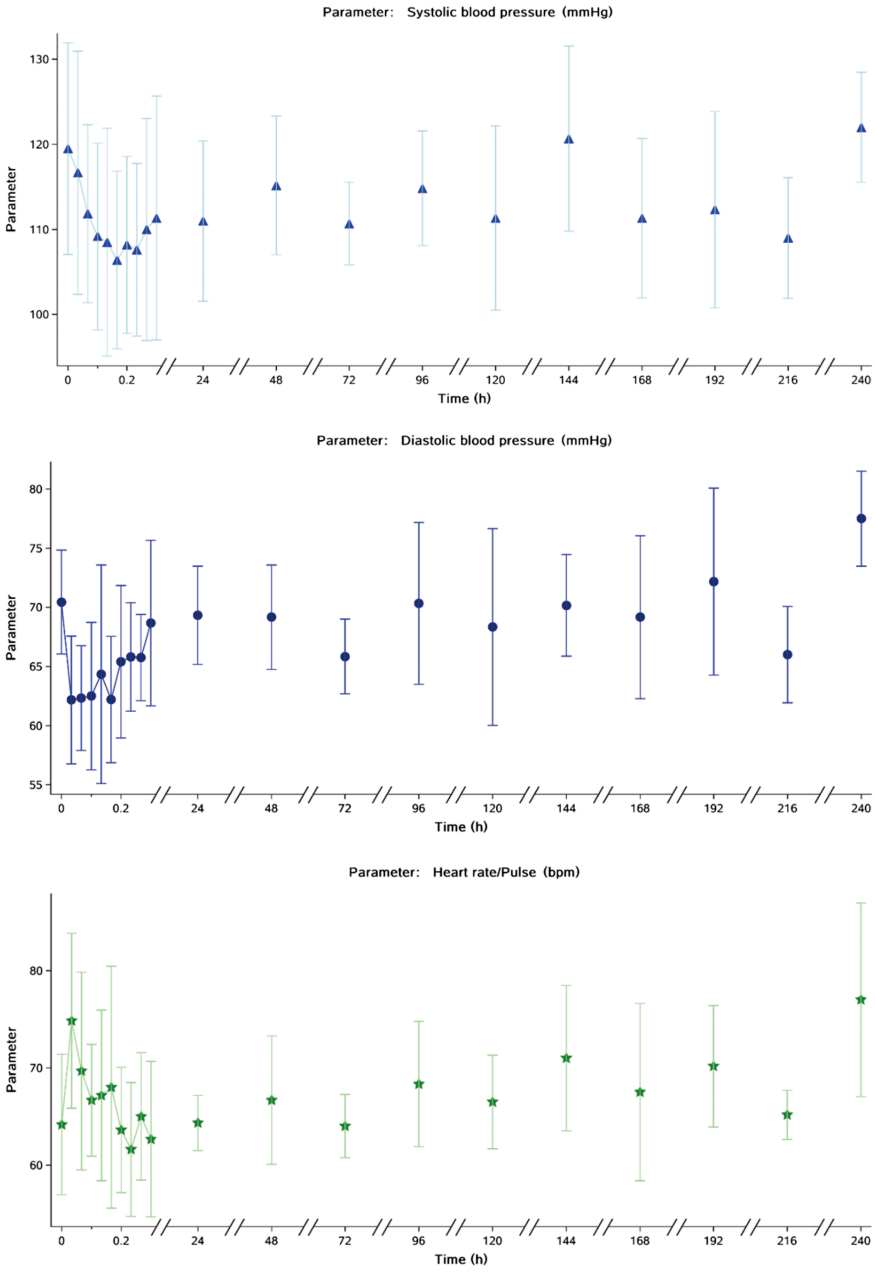

FIGURE 6 The vital sign–time curves

elimination half-life ($t_{1/2}$) of total radioactivity was much longer than that of unchanged HSK3486, indicating that there were circulating metabolites in plasma. The blood-to-plasma ratios of total radioactivity were all less than 1 at different time points, indicating that there was no significant binding between total radioactivity (HSK3486-related compounds) and blood cells. The radioactivity in whole blood at 24 hours was below the lower limit of quantification (64.6 ng Eq./g), while the total radioactivity concentration in plasma samples could be detected 120 hours after administration. This finding is not only because of the reduced concentration of total radiation in whole blood, but also because of the reduced lower limit of quantification of plasma radioactivity (9.80 ng Eq./g).

The main identified metabolite M4 (glucuronide conjugate) was found to be the only major circulating metabolite in plasma, accounting for 79.3% of the total plasma radioactivity, and it was also the main identified metabolite in urine (approximately 51.6%). It shows that glucuronidation, as well as oxidation and sulfation, are the main metabolic pathways of HSK3486, which are consistent with propofol, and the metabolite of glucuronidation is generally considered to be nonhypnotic and nontoxic.[17]

HSK3486 showed favourable anaesthetic properties with little residual effects in the study. The MOAA/S score showed that all the subjects were anaesthetized 2 minutes after dosing and then gradually recovered in 5 to 14 minutes, which showed that HSK3486 had a

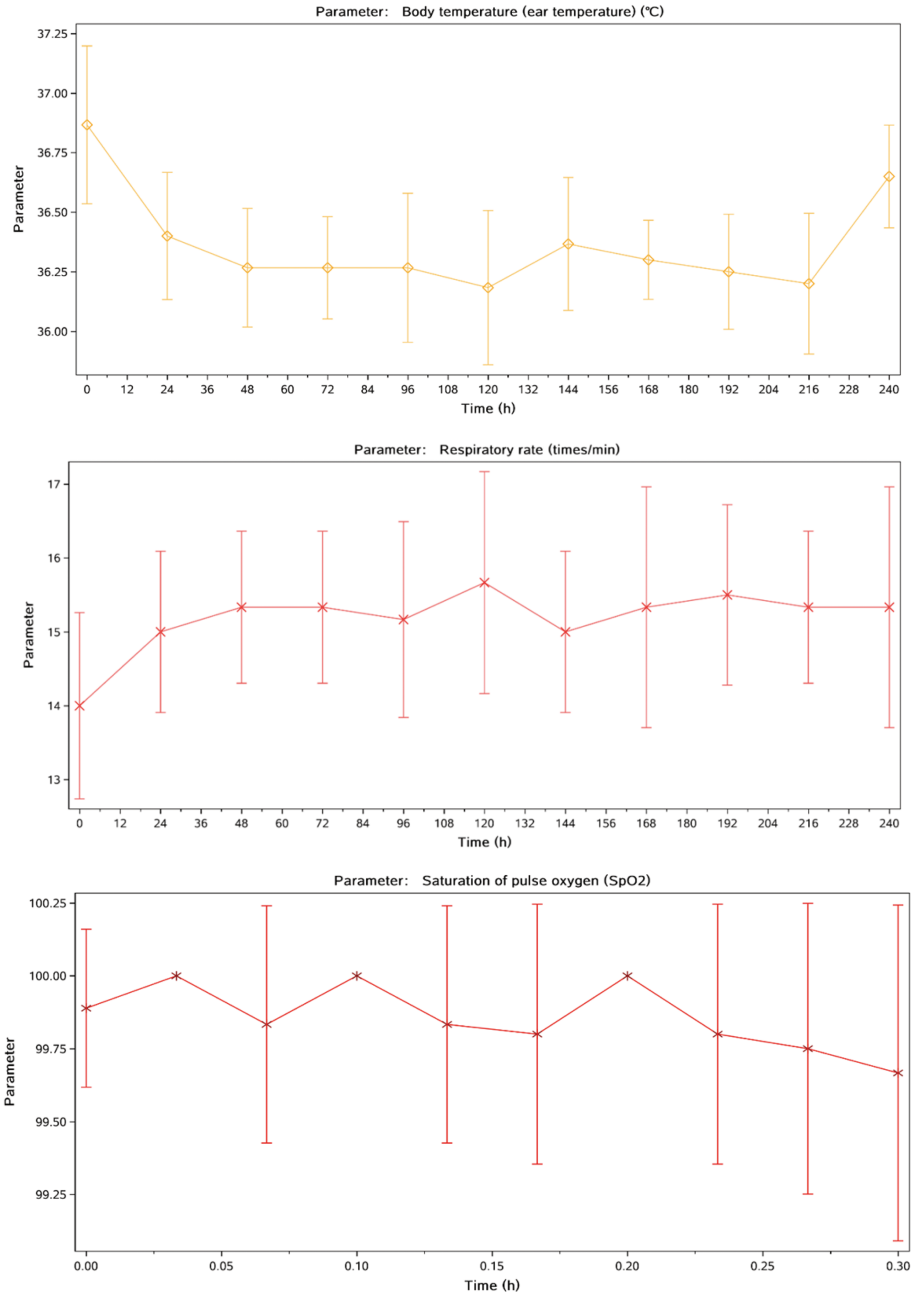

FIGURE 6 (Continued)

rapid onset of action, and subjects woke up smoothly and quickly. These results also indicated that the metabolites with longer elimination half-lives had no anaesthetic efficacy. The estimated plasma HSK3486 concentration was 335.3 ± 134.6 ng/mL and 200.8 ± 37.5 ng/mL, respectively, when half of the subjects (3 subjects) recovered from MOAA/S scores ≤1 (6 min) and fully awakened (10 min). However, an accurate EC_{50} was not obtained in the study.

Particular attention to was paid to apnoea, hypoxaemia, bradycardia, hypotension and injection pain as potential side effects of HSK3486 due to the special pharmacological effects of anaesthetics in the study. The results showed that HSK3486 was well tolerated and had almost no injection pain. HSK3486 may have less impact on breathing than propofol, and the side effects on blood pressure and heart rate were not inferior to propofol. Only one mild adverse event of hypotension occurred in the study, which is believed to be a common adverse event of sedative/anaesthetic drugs. However, the mechanism of this side effect is not fully understood. Studies have shown that the propofol-induced hypotension may work by inhibiting myocardial contraction or vascular tension,[19,20] which may also be the mechanism of HSK3486-induced hypotension.

The interaction of HSK3486 and other concurrently administered drugs should be considered in clinical use. HSK3486 was suggested to be extensively metabolized similar to propofol to produce inactive metabolites by UDP-glucuronosyltransferases (UGTs) and CYP

enzymes. The major circulating metabolite M4 was a glucuronidation product of the phase II metabolic reaction, and was considered to be nonhypnotic and nontoxic. CYP2B6 was suggested to be the major subtype of CYP enzymes that mediated HSK3486 metabolism in previous in vitro studies. From a clinical perspective, low specificity among enzyme isoforms may contribute to reduced metabolic drug interactions of HSK3486 base on the knowledge of propofol and there is no evidence that moderate hepatic dysfunction or chronic renal failure impairs the metabolism and clearance of propofol.[21,22] However, pharmacological interaction should be considered, when taking drugs which may cause unconsciousness, hypotension, bradycardia and apnoea.

In summary, we report here for the first time the absorption, distribution, metabolism, excretion (ADME) and anaesthetic effects of HSK3486. HSK3486 showed good anaesthetic efficacy and safety at the study dose. The mean cumulative excreted radioactivity reached 87.3% in 240 hours, which showed a general mass balance of the drug. The main metabolic pathways of HSK3486 in humans were glucuronidation, oxidation and sulfation. The only major circulating metabolite in plasma was suggested to be nonhypnotic and nontoxic and finally excreted through urine. These results may translate to patients undergoing colonoscopy, gastroscopy or elective surgery due to the special pharmacological properties and clinical uses of HSK3486. However, due to the limitations of collection and detection methods, the study of HSK3486 excretion through the exhalation pathway needs to be further investigated.

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COMPETING INTERESTS

Pangke Yan, Xiao Liu and Shiping Ma are full-time employees of Sichuan Haisco Pharmaceutical Co., Ltd. All the other authors declare that they have no conflicts of interest that are directly relevant to the content of this article.

CONTRIBUTORS

Yicong Bian, Hua Zhang, Pangke Yan, Zheming Gu, Chenrong Huang and Liyan Miao were responsible for the study design; Yicong Bian, Hua Zhang, Sheng Ma, Yongyi Jiao, Xiao Liu, Shiping Ma, Yating Xiong, Chenrong Huang conducted the study; Yicong Bian, Yating Xiong, and Zhenwen Yu conducted data analyses; Yicong Bian and Liyan Miao were responsible for the manuscript writing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Qin L, Ren L, Wan S, et al. Design, synthesis, and evaluation of novel 2,6-disubstituted phenol derivatives as general anesthetics. *J Med Chem*. 2017;60(9):3606-3617.
2. Wei Y, Qiu G, Lei B, et al. Oral delivery of propofol with methoxymethylphosphonic acid as the delivery vehicle. *J Med Chem*. 2017;60(20):8580-8590.
3. von Richter O, Massimini G, Scheible H, Udvaros I, Johne A. Pimasertib, a selective oral MEK1/2 inhibitor: absolute bioavailability, mass balance, elimination route, and metabolite profile in cancer patients. *Br J Clin Pharmacol*. 2016;82(6):1498-1508.
4. Penner N, Xu L, Prakash C. Radiolabeled absorption, distribution, metabolism, and excretion studies in drug development: why, when, and how? *Chem Res Toxicol*. 2012;25(3):513-531.
5. Meng J, Liu XY, Ma S, et al. Metabolism and disposition of pyrotinib in healthy male volunteers: covalent binding with human plasma protein. *Acta Pharmacol Sin*. 2019;40(7):980-988.
6. Valentin J. The 2007 recommendations of the International Commission on Radiological Protection. ICRP publication 103. *Ann ICRP*. 2007;37(2-4):1-332.
7. ICRP. Radiation dose to patients from radiopharmaceuticals (addendum 1 to ICRP publication 53). 1992.
8. US FDA. 21 CFR 361.1. Radioactive Drugs for Certain Research Uses Available from:. https://www.ecfr.gov/cgi-bin/text-idx?SID=02ff833f9923cf785b195fc96d003c38&mc=true&node=se21.5.361_11&rgn=div8
9. Nijenhuis CM, Schellens JH, Beijnen JH. Regulatory aspects of human radiolabeled mass balance studies in oncology: concise review. *Drug Metab Rev*. 2016;48(2):266-280.
10. Hop CE, Wang Z, Chen Q, Kwei G. Plasma-pooling methods to increase throughput for in vivo pharmacokinetic screening. *J Pharm Sci*. 1998;87(7):901-903.
11. van Andel L, Zhang Z, Lu S, et al. Human mass balance study and metabolite profiling of (14)C-niraparib, a novel poly (ADP-ribose) polymerase (PARP)-1 and PARP-2 inhibitor, in patients with advanced cancer. *Invest New Drugs*. 2017;35(6):751-765.
12. Liao M, Watkins S, Nash E, et al. Evaluation of absorption, distribution, metabolism, and excretion of [(14)C]-rucaparib, a poly (ADP-ribose) polymerase inhibitor, in patients with advanced solid tumors. *Invest New Drugs*. 2020;38(3):765-775.
13. Valk BI, Absalom AR, Meyer P, et al. Safety and clinical effect of i.v. infusion of cyclopropyl-methoxycarbonyl etomidate (ABP-700), a soft analogue of etomidate, in healthy subjects. *Br J Anaesth*. 2018;120(6):1401-1411.
14. Harding SD, Sharman JL, Faccenda E, et al. The IUPHAR/BPS guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res*. 2018;46(1):D1091-D1106.
15. Alexander SPH, Mathie A, Peters JA, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Ion channels. *Br J Pharmacol*. 2019;176(Suppl 1):S142-228.
16. Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Hopkins K, Rowland M. Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol. *Xenobiotica*. 1988;18(4):429-440.
17. Sahinovic MM, Struys M, Absalom AR. Clinical pharmacokinetics and pharmacodynamics of propofol. *Clin Pharmacokinet*. 2018;57(12):1539-1558.
18. Grossherr M, Hengstenberg A, Meier T, Dibbelt L, Gerlach K, Gehring H. Discontinuous monitoring of propofol concentrations in

expired alveolar gas and in arterial and venous plasma during artificial ventilation. *Anesthesiology*. 2006;104(4):786-790.
19. Kanaya N, Gable B, Wickley PJ, Murray PA, Damron DS. Experimental conditions are important determinants of cardiac inotropic effects of propofol. *Anesthesiology*. 2005;103(5):1026-1034.
20. Nagakawa T, Yamazaki M, Hatakeyama N, Stekiel TA. The mechanisms of propofol-mediated hyperpolarization of in situ rat mesenteric vascular smooth muscle. *Anesth Analg*. 2003;97(6):1639-1645.
21. Moos DD. Propofol. *Gastroenterol Nurs*. 2006;29(2):176-178.
22. Dinis-Oliveira RJ. Metabolic profiles of propofol and fospropofol: clinical and forensic interpretative aspects. *Biomed Res Int*. 2018;2018:6852857.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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