Supplementary

Pharmacokinetic parameters of desloratadine and busulfan

The reported PK characters of desloratadine and busulfan are summarized in table S1.

Nonclinical experiments of desloratadine and busulfan

Drugs and reagents

The following drugs/reagents were used in the study: desloratadine citrate (Enrit Pharmaceutical Co., Anhui, China); busulfan for injection (Otsuka Pharmaceutical Co., Ltd, Zhejiang, China); busulfan standard (J&K Scientific Corporation, Beijing, China); verapamil (Thermo Scientific, Boston, MA, USA); propranolol (Sigma–Aldrich, St Louis, MO, USA); methanol/acetonitrile(chromatography grade; SK chemicals, Seoul, Korea); all other reagents and solvents were analytical grade.

Animals

Male Sprague-Dawley rats (weight 210±10 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were fed in an exhaust ventilation cage (EVC) system, where there were free food and water, and an acclimatization period of at least 3 days preceded the experiments. Animal experiments were performed under a project license (No. IACUC-DWZX-2020-694) granted by ethics board of Beijing Center for Drug Safety Evaluation and Research, Beijing Institute of Pharmacology and Toxicology, in compliance with National Research Council's Guide for the Care and Use of Laboratory Animals, which also in compliance with guidelines of Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

During acclimatization, jugular vein catheterization (JVC) was carried out with silica gel tubes (Skillsmodel Biotechnology Co., Peking, China) after pentobarbital anesthesia (50mg/kg), neck shaving and routine disinfection. Following JVC, the animals were maintained on regular infusion with heparin sodium dissolved into physiological saline prior to the start of treatment. All animals were housed in exhaust ventilated closed-system cage rack (EVC)with a 12-hour light/dark cycle. The room temperature and relative humidity were maintained in the range of 22±3 ℃ and 40–60% humidity. Experiments were performed the next day after JVC.

Dose

Twenty-four rats were randomly divided into 4 groups. Six per group were considered sufficient for evaluating model potency. Group A was administrated with desloratadine of 0.5 mg/kg for single bolus intravenous injection. Group B was administrated with desloratadine of 2 mg/kg for *p.o*. administration. Group C was administrated with busulfan of 2 mg/kg for single dose 1-h intravenous infusion and Group D was administrated with busulfan of 1 mg/kg with a 1-h intravenous infusion every 4 hours (six times daily) for a total of 7 doses. All the dosing solutions were diluted to the final concentration with physiological saline.

Collection of blood and urine samples

All the administrated rats were placed individually in metabolic cages to collect urine and feces at the prescribed time intervals. Meanwhile, about 150 μL of blood samples was taken from the JVC canal at the set time point after administration and placed into test tubes containing heparin. The same volume of heparin sodium solution was supplemented into the rats to prevent coagulation in the canal. The obtained whole blood samples were centrifuged for 10 min (2,500 g, 4 ℃) to separate the plasma before being stored at −20 ℃ until analysis.

To prevent the loss of urine, the rats were kept in metabolic cages throughout the experiment. Another concern was the possible residues of urinary drugs in the cages, which might cause loss on total excretion, also pollute the urine samples to be collected in the next round. Such pollution is quite likely at the beginning of experiments because of high drug excretion in urine. Thus, at least 2 sets of metabolic cages had been prepared for each animal. When urine was collected at the set time

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point, the animals were directly transferred into other "clean" metabolic cages without any contamination. The used one were flushed with 70 mL methanol: water (50:50 v/v). The flushing fluid was combined with the urine sample collected at the same time interval, and the total volume was recorded before all the samples were stored at −20 ℃. After collection, the used cages had to be cleaned thoroughly in time for the next collection period. It was worth noting that no liquid should be lost during the operation. The urinary concentration of the drug during the terminal phase was expected to be low, so the reduced volume of the flush liquid could be used to ensure the drug concentration was high enough for determination.

Plasma samples preparation

Busulfan was extracted from rat plasma using the protein precipitation method. 50 µL of rat plasma was spiked with 50 µL methanol and 200 µL internal standard (IS) working solution (propranolol in Methanol, 20 ng/mL). To prepare calibration point samples, a stock solution was diluted with methanol to serial working solutions that ranged from 1 to 5,000 ng/ mL, 50 µL of which was spiked into 50 µL of blank rat plasma and 200 µL IS working solution. The mixture was vortexed for 2 min and centrifuged at 20,000 g for 10 min before the supernatant was transferred to auto-sampler vials for LC-MS/MS analysis.

As for desloratadine, plasma samples were extracted by liquid-liquid extraction (LLE) and a 7-point standard curve (0.05, 0.25, 1.25, 6.25, 25, 125, 625 ng/mL) was prepared. 50 µL of plasma was mixed with 50 µL IS working solution (verapamil in methanol, 10 ng/mL), 50 µL of methanol or serial working solutions in methanol, vortexed, and extracted by 1 mL tert-butyl methyl ether (TBME). The samples were vortexed for 1 min, and the organic phase was collected after 10 min standing. The extraction procedures were repeated three times to improve extraction efficiency. The organic phase was merged and evaporated, and then re-dissolved with 100 µL 50% methanol (methanol: water 50:50 v/v). Samples were centrifuged at 20,000 g for 10 min at 4 ℃ and 90 µL of the supernatant was transferred into sample vials for LC-MS/MS analysis.

Urine samples preparation

All urine samples were unfrozen on ice and vortexed for 1 min before 1 mL of urine samples was mixed with 50 µL IS solution, 50 µL of methanol or serial working solutions in methanol, vortexed, and evaporated. After being re-dissolved in 1 mL distilled water, all samples were subjected to LLE as was mentioned above.

LC-MS/MS condition

Analysis was performed on an LC-MS/MS system composed of a binary LC-30AD delivery pump, a DUG-20A5R vacuum degasser, a CTO-20A column oven, a SIL-30AC auto-sampler, a CBM-20A system controller (Shimadzu, Japan) and an LCMS-8060 mass spectrometer (Shimadzu, Japan). The mass spectrometer was equipped with an electrospray ion (ESI) source working in the positive ion multiple reaction monitoring (MRM) mode. The main MS parameters of the analyst and the respective internal standard including the transition m/z, collision energy, Q1 and Q3 voltage were listed in Table S2.

For busulfan, the mobile phase was composed of solvent A (water containing 0.1 % formic acid and 2 mM ammonium formate) and solvent B (methanol). The chromatographic separation was performed on a Kinetex C18 column (2.6 µm, 50 mm ×3.0 mm, phenomenex, CA, USA) at a flow rate of 0.6 mL/min for 4.5 min kept at 40 ℃, using a gradient method of solvent B from 5% to 95% over 2.0 min, and kept for 0.5 min, and back to the initial condition over 2 min to equilibrate the column. The injection volume was set at 5 µL. The retention time was 1.63 min for busulfan and 2.06 min for IS.

For desloratadine, the mobile phase was composed of solvent A (water 0.1% formic acid with 5 mM ammonium formate) and solvent B (methanol containing 0.1% formic acid). The chromatographic separation was performed on a CAPCELLPAK C8 column (3.0 µm, 50 mm ×2.0 mm, Shiseido, Japan) at a flow rate of 0.6 mL/min for 4.0 min kept at 40 ℃, using a gradient method of solvent B from 10% to 95% over 2.0 min, and kept for 0.5 min, and back to the initial condition over 1.5 min to equilibrate the column. The injection volume was set at 2 µL. The retention time was 1.67 min for desloratadine and 1.75 min for IS.

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Clinical trials of Busulfan

Busulfan is a commonly used alkylating agent in the conditioning regimens of hematopoietic cell transplantation (HCT). Due to its wide pharmacokinetic (PK) variability and narrow therapeutic window, therapeutic drug monitoring (TDM) for busulfan is routinely used to individualize dosing and control the cumulative exposure.

This study was designed to assess the accuracy of PK profiles retro-constructed from urine data with comparison to results from TDM. Three patients who received intravenous busulfan conditioning regimens at Beijing Children's Hospital were enrolled from 1 March, 2022 to 31 June, 2022. The demographic data was summarized in Table S3. All patients received busulfan (Otsuka Pharmaceutical Co., Ltd., Zhejiang, China) by a 2-hour *i.v.* infusion every 6 hour and 12 or 16 doses in total. The initial dose was set as 0.8 mg/kg b.w., and subsequent doses were allowed to be adjusted by doctors according to TDM results.

The study was approved by the ethics committee of Beijing Children's Hospital (No. [2022]-E-047-Y), and written informed consent was obtained from the guardian of each patient. Based on the original TDM protocol, in which the blood sampling time point was set as 0.5, 1, 2, 2.5, 4, and 6 h after initiation of *i.v.* infusion of first dose, this study scavenged 3 daily blood samples that had been collected for clinical testing from day 2 to day 4. All urine samples within 48 h of the last administration were collected. During the sampling period, the subjects were allowed to urinate freely and all the collected urine had to be retained with accurate record of the collecting time and volume. Also, 2- and 6-h after the end of the last infusion were set as mandatory urine sampling points.

All the plasma or urine samples were stored at −20 ℃ until analysis. The quantitative method was the same as the one used in the pre-clinical study, except that the calibrate range was changed to 1–5,000 ng/mL, and the internal standard was busulfan-d8 with MS transition 272.1→159.1, as shown in Table S4.

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Table S1 Main pharmacokinetic parameters of desloratadine and busulfan

Parameters	Desloratadine		Busulfan	
	Rats	Human	Rats	Human
Structure				
Route of administration	Oral		*i.v.* infusion	
MW	310.8		246.3	
Compound type	Base			
pKa	9.7			
Absorption (t_{max}) (h)	~10 (21)	~3 (22)		
B/P (%)		2.2 (23)		1 (24)
$F_{u,p}$ (%)		0.16 (23)	0.9 (25)	0.4 (24)
V_{ss} (L/kg)		18.3 (23)	0.67 (26)	
Volume of distribution V/F (L/kg)	~52 (21)	~49 (27)		0.69–0.8 (24)
CL/F (L/h/kg)	~4.38 (21)	1.5~3 (28)	0.3 (26)	0.22 (29)
Metabolites (% of dose)		Extensive metabolism (30)		
Enzymes involved in metabolism		UGT1A1, 1A3, 2B15 (31), UGT2B10 (31), CYP2C8 (33)	GSTA1 (34)	GSTA1 (35)
Terminal elimination half-life (h)	~8.5 (21)	27 (22)		
Urinary excretion (% of radioactive dose)		41% (22,36)		30% (37)
Urinary excretion (% of dose)		<2% (38)		<2% (37)
Faecal excretion (% of radioactive dose)		47% (22,36)		
Faecal excretion (% of dose)		<7% (38)		Negligible (37)

MW, molecular weight, in Dalton; pKa, acid-base dissociation constant; B/P, blood plasma distribution ratio; Fu,p, fraction of unbound in plasma; Vss, volume of distribution at steady state; CL/F, bioavailability-corrected clearance.

Table S2 Mass spectrometric parameters for analytes in nonclinical research: precursor to fragment ion transition, voltage potential (Q1), collision energy (CE) and voltage potential (Q3)

Analytes	MRM Transition m/z (Q1→Q3)	CE (eV)	Q1 (V)	Q3 (V)	Retention time (min)
Desloratadine	311.30→259.05	–21	–16	–28	1.49
Verapamil	455.30→165.10	–29	–19	–15	1.74
Busulfan	264.10→151.10	–11	–14	–16	1.80
Propranolol	260.10→116.00	–19	–14	–13	2.20

MRM, multiple reaction monitoring.

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Table S3 Demographic characteristics and body measurements of subjects

Variable	Value
Age (years)	
N	3
Middium	11
Range	10–13
Gender, n	
Male	2
Female	1
Race, n	
Chinese	3
Weight (kg)	
Midieum	39.5
Range	36–47
Body mass index (kg/m^2)	
Midieum	1.26
Range	1.23–1.40

Table S4 Mass spectrometric parameters for analytes in clinical research: precursor to fragment ion transition, voltage potential (Q1), collision energy (CE) and voltage potential (Q3)

Analytes	MRM Transition m/z (Q1→Q3)	Q1 (V)	CE (eV)	Q3 (V)	Retention time (min)
Busulfan	264.1→151.1	–14	–11	–16	1.93
Busulfan-d8	272.1→159.1	–14	–19	–13	1.93

MRM, multiple reaction monitoring.

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