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EXPERT OPINION ON DRUG METABOLISM & TOXICOLOGY
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ORIGINAL RESEARCH

Mass balance and metabolite profiles in humans of tegoprazan, a novel potassium-competitive acid blocker, using ^{14}C-radiolabelled techniques

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ABSTRACT

Background: Tegoprazan (LXI-15028), a novel potassium-competitive acid blocker, has shown great efficacy in treating acid-related disorders. However, its metabolic and excretion characteristics are not fully understood.

Research design and methods: A single oral dose of 50 mg/150 µCi [^{14}C]tegoprazan was administered to six healthy subjects. Blood, urine and fecal samples were collected and measured for total radioactivity (TRA), tegoprazan and metabolites. Its safety was also assessed.

Results: The maximum concentrations (C_{max}) of tegoprazan and TRA in plasma were 634 ng/mL and 990 ng eq./mL, respectively, at 0.5 h post dose. Tegoprazan and its N-demethylation metabolite (M1) were the major drug-related compounds in plasma, accounting for 34.84% and 40.10% of TRA, respectively. The half-life ($t_{1/2}$) of TRA (8.72 h) was longer than that of tegoprazan (4.33 h) in plasma, indicating slower metabolite elimination. Tegoprazan was excreted through both the urine (50.51 ± 3.35%) and feces (47.26 ± 3.06%). The main metabolic pathways of tegoprazan are demethylation, oxidation, glucuronidation and sulfation. There were no serious adverse events observed in this study.

Conclusions: Tegoprazan is widely metabolized and excreted completely in humans. Tegoprazan and M1 were the primary compounds present in the circulation.

Clinical trial registration: www.clinicaltrials.gov identifier is NCT05883306.

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Mass balance; metabolites; pharmacokinetics; potassium-competitive acid blocker; tegoprazan

1. Introduction

Gastroesophageal reflux disease (GERD) is one of the most common gastrointestinal acid-related disorders [1,2]. The clinical symptoms of GERD include heartburn and regurgitation, and the consequent chest discomfort, chronic cough, hoarseness of voice and asthma [3]. Proton pump inhibitors (PPIs) are considered as effective acid-inhibitory drugs for relieving symptoms of GERD in clinical practice. However, up to 40% of daily PPI users have not adequately responded to PPI therapy in the last decade [3,4].

Compared with conventional PPIs, potassium-competitive acid blockers (P-CABs) exhibit strong advantages in inhibiting the secretion of gastric acid with favorable risk-benefits [5]. P-CABs are highly concentrated on the secretory canaliculus in parietal cells and are rapidly protonated, exhibiting a strong antisecretory effect by competitively blocking the availability of K^+ for H^+/K^+-adenosine triphosphate (ATP)ase [6,7]. The concentration of P-CABs in parietal cells is 100,000-fold greater than that in plasma, achieving its full effect after the first dose and maintaining its effect for both daytime and nocturnal hours [8,9]. Additionally, the pharmacokinetics and antisecretory activity of P-CABs are not affected by genetic polymorphisms or diet [3], which makes them convenient for clinical use. Moreover, clinical studies have shown that P-CABs have outstanding safety [10–12].

To date, several P-CABs have been marketed. For example, revaprazan was launched in Korea in 2007 for the treatment of gastroduodenal ulcers and gastritis [13]. In addition, vonoprazan fumarate was approved in Japan in 2014, and its curative effect for healing acid-related disorders and *H. pylori* eradication has been clinically confirmed [13–15].

Previous studies have demonstrated that once-daily administration of tegoprazan 50 or 100 mg has noninferior efficacy to that of esomeprazole 40 mg in healing erosive esophagitis (EE) [16], and to that of lansoprazole 30 mg in the treatment of gastric ulcers (GU) [17]. Moreover, tegoprazan 50 and 100 mg showed good therapeutic efficacy, and a favorable safety profile in patients with non-erosive reflux disease (NERD) [12]. To date, tegoprazan has been approved in Korea in 2018 for the treatment of EE, NERD and GU, and has been marketed in China in 2022 for healing EE and

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duodenal ulcer (DU). Tegoprazan may also be suitable for other indications such as *H. pylori* eradication, and a relevant clinical study (NCT05577468) is underway.

Several clinical trials of tegoprazan in humans reported that exposure to tegoprazan increased in a dose-proportional manner [9,18]. The median time to reach the maximum concentration (T_{max}) of tegoprazan was within 1 hour and the mean elimination half-life ($t_{1/2}$) ranged from 3.65 to 5.39 hours in a single-ascending-dose study [9]. However, the properties of its metabolism and excretion are still not fully understood. A human radiolabeled mass balance study is a feasible method to study the absorption, distribution, metabolism and excretion (ADME) properties of drugs [19–21], which can provide information on the overall pathways of metabolism and excretion of an investigational drug and assess which metabolites should be structurally characterized and which metabolites should undergo nonclinical safety assessment according to the Metabolites in Safety Testing (MIST) guidance [22,23]. In this mass balance study, a single oral dosage of 50 mg/150 μCi ^{14}C-labeled tegoprazan was administered to six healthy male subjects to identify circulating metabolites and determine the abundance of metabolites relative to the parent or total drug-related exposure, which would be helpful for evaluating whether renal or hepatic impairment studies or certain drug-drug interaction (DDI) studies should be recommended for investigational drugs.

2. Subjects and methods

2.1. Study design and subjects

This was a phase I, open-label, single-center study performed at the First Affiliated Hospital of Soochow University (Suzhou, China), approved by the Hospital Ethics Committee (approval number: 2022101) and conducted in compliance with the ethical principles of the Declaration of Helsinki.

Informed consent was signed by all subjects, aged 18 ~ 45 years with a body mass index (BMI) ranging from 19.0 to 26.0 kg/m^2. Six eligible male subjects were in good health based on vital signs, physical examinations, routine clinical laboratory tests, 12-lead electrocardiograms (ECGs), abdominal B-scan ultrasonography, chest CT, and urine drug tests that were performed within 7 days prior to drug administration. The exclusion criteria were as follows: a history of alcohol abuse; a history of habitual use of nicotine products; a history of allergic reactions related to PPIs or P-CABs; participation in any other clinical trials in the past 3 months; the use of hepatic microsomal enzyme inhibitors or inducers in the past 30 days; a need for any prescribed or nonprescribed concomitant medication within 14 days prior to study entry; and the administration of radiolabeled substances or exposure to significant radiation within the past 12 months.

Each subject received a single suspension orally at a dosage of 50 mg/150 μCi ^{14}C-labeled tegoprazan. The bottle containing the study drug was rinsed with pure water, and the subjects consumed the rinsing fluid (the total volume was approximately 240 mL) within 5 min. All of the subjects were required to fast for at least 10 h before dosing and 4 h after dosing, and were prohibited from drinking water for 1 h before and after drug administration. After administration, the empty bottles were recycled for quantification of residual radioactivity. The total amount of radioactivity in the preparation was subtracted from the residue in the bottle to obtain the actual amount of radioactivity taken by each subject. Combined with the specific activity, the actual dose administered would be calculated.

The statistical analysis sets were defined as follows:

The safety set (SS): all the enrolled subjects who received the study drug, and had at least one safety evaluation data; the pharmacokinetic concentration set (PKCS): all enrolled the subjects who received the study drug and had at least one valid quantitative concentration data during the trial; the mass balance set (MBS): all the enrolled subjects who received the study drug, collected at least one urine or fecal sample for at least one time period, and had at least one radioactive concentration data.

2.2. Sample collection

The blood, urine and fecal samples were collected within 0 ~ 192 h after administration, and the detailed time points and time intervals for sample collection were as follows:

Blood samples were taken at 0 h (before dosing), 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h, 48 h, 72 h, 120 h, 168 h and 192 h after dosing (8 mL per point) for radioactivity measurement and liquid chromatography tandem mass spectrometry (LC – MS/MS) analysis in plasma. Blood samples were collected at 1, 4, 8, 12, 24 and 48 h after dosing (10 mL per point) for biotransformation analysis in plasma. The collected blood samples were centrifuged at 2 ~ 8 ℃ and 2000 ± 20 g for 10 min. Then, the supernatant was collected to obtain plasma samples for analysis in an ice bath. Whole blood samples were collected at 0 h (before dosing), 1 h, 4 h, 8 h, 12 h, 24 h and 48 h postdose (2 mL per point) for radioactivity measurement in blood. EDTA-K_2 was used for anticoagulation in all the blood collection tubes. Urine and fecal samples were collected at predose and 0 ~ 4, 4 ~ 8, 8 ~ 12, 12 ~ 24, 24 ~ 48, 48 ~ 72, 72 ~ 96, 96 ~ 120, 120 ~ 144, 144 ~ 168 and 168 ~ 192 h postdose. All of the samples were stored at – 20 °C or below until analysis.

2.3. Radio-labelled tegoprazan and dosage form

The ^{14}C-labeled tegoprazan was synthesized by Wuxi Beita Pharmatech Co. and purified by Value Pharmaceutical Services Co. Figure 1 shows the chemical construction of tegoprazan and the labeled location of the ^{14}C atoms. The radiochemical purity of the ^{14}C-labeled tegoprazan was 100%, and the specific activity reached 55.96 mCi/mmol. Non-radiolabeled tegoprazan and blank excipients were supplied by Shandong Luoxin Pharmaceutical Group Stock Co., Ltd., and were used as a means of diluting ^{14}C-labeled tegoprazan prior to oral administration. In each preparation, the masses of tegoprazan and ^{14}C-tegoprazan were 48.96 mg and 1.04 mg, respectively.

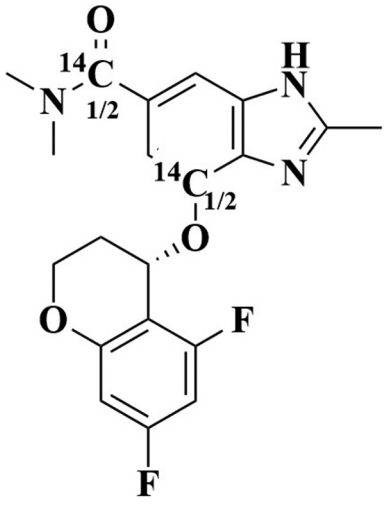

Figure 1. Chemical structure of ^{14}C-labeled tegoprazan.

2.4. Bioanalytical analysis

2.4.1. Radioactivity analysis

The blood, plasma, urine and fecal samples were analyzed for total radioactivity (TRA) using a liquid scintillation counter (LSC) (Tri-Carb 4910TR, PerkinElmer, U.S.A.). The fecal samples were first soaked with isopropanol/water (50/50, v/v) and then homogenized. The fecal homogenate samples were combusted using a biological oxidizer (HTC-501, Hualida, China) before LSC. Similarly, the blood samples were required to be burned before LSC. The plasma and urine samples were analyzed by LSC directly.

The amount of radioactivity remaining in empty bottles after administration was also quantified by LSC. The empty bottles were rinsed with absolute ethanol, and then samples were taken for analysis.

2.4.2. Quantification of tegoprazan and M1 in plasma

Determining of the concentrations of tegoprazan and N-demethylation metabolite of tegoprazan (M1) was performed by LC-MS/MS with a validated bioanalytical method. The plasma samples were processed by protein precipitation followed by entry into an XBridge C18 column (2.1 × 50 mm, 5 µm). A deuterium compound (tegoprazan-d6) was added as an internal standard (IS). The mobile phase was as follows: solution A, 5 mm ammonium formate aqueous solution containing 0.1% formic acid/acetonitrile; and solution B, methanol/acetonitrile/formic acid (*50/50/0.1, v/v/v*). Chromatographic separation was performed with gradient elution solvent, and the gradient elution program was as follows: 0–0.6 min: 0–15% B (*v:v*); 0.6–1.8 min: 15–40% B; 1.8–2.5 min: 40–95%; 2.5–3 min: 95% B; 3–3.1 min: 95–15% B; 3.1–4.5 min: 15% B. The total run time was 4.5 min, and the flow rate of the mobile phase was 0.8 mL/min.

An API-4000 triple quadrupole mass spectrometer, equipped with an electrospray ionization source in positive mode was used to carry out the tandem mass spectrometry experiments. Quantification was conducted by multiple reaction monitoring of the transitions of m/z 388.1→219.9 for tegoprazan, m/z 374.1→206.0 for M1, and m/z 394.2→225.8 for the IS, respectively.

The calibration ranges for tegoprazan and M1 in human plasma were 3.00 ~ 3000 ng/mL and 1.00 ~ 1000 ng/mL, respectively. The overall maximum coefficient of variation (CV%) for the calibration standards and quality control samples was 8.74%. The validation of the methodology for tegoprazan and M1 determination is provided in Table S1.

2.5. Metabolite profiling and identification

Four plasma samples at 0.25 h, 1 h, 4 h and 12 h were obtained by pooling equal volumes of plasma across individuals at the same time point. In addition, a plasma sample (0 ~ 48 h) was obtained by pooling plasma at each time point (0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h, 24 h and 48 h) of a volume proportional to the time interval used for calculating the area under the concentration-time curve (AUC) for each subject [24,25]. The radioactivity exposure in 0 ~ 48 h plasma samples accounted for more than 95% of the TRA in each subject.

The total radioactivity in the urine samples from 0 h to 48 h accounted for 98.22%~99.44% of the total radioactivity excreted from 0 h to 192 h, and the total radioactivity contained in 0 ~ 120 h fecal samples accounted for 98.53% ~100.00% of the total radioactivity excreted from 0 h to 192 h. Thus, urine samples from 0 h to 48 h and fecal samples from 0 ~ 120 h were selected from the six subjects for radioprofiling and identification. All of the fecal homogenate samples were extracted with acetonitrile. The selected urine samples (0 ~ 48 h) were mixed at an equal percentage of collection volume to obtain one pooled sample for each subject. Moreover, the urine samples were pooled across individuals at an equal volume to obtain one pooled sample per period of 0 ~ 8 h, 8 ~ 24 h, and 24 ~ 48 h. In the same way, fecal samples (0 ~ 120 h) were pooled at the same weight percentages across collection intervals (0 ~ 24 h, 24 ~ 48 h, 48 ~ 72 h, 72 ~ 96 h and 96 ~ 120 h) for each subject. In addition, fecal samples were pooled across individuals at an equal weight to obtain one pooled sample per period of 0 ~ 24 h, 24 ~ 48 h and 48 ~ 120 h.

The samples were injected into a reversed-phase (RP)-HPLC system (CBM-20A, Shimadzu, Japan) with a fraction collector (FOXY R2, TELEDYNE ISCO, U.S.A.) for fractionation and off-line detection. Separation was performed on a chromatographic column of Xbridge C18 (3.5 µm, 4.6 × 250 mm) with a guard column of Xbridge BEH C18 (3.5 µm, 3.9 × 5 mm) at 40 °C. The mobile phase A was water with 0.4% formic acid, the pH of which was adjusted to 3.20 with ammonia water. The mobile phase B was acetonitrile. The flow rate was 0.7 mL/min. The gradient elution is shown in Table S2. The fractions were collected at intervals of 15 seconds per well into 96-well microplates, and the plates were subsequently dried with a vacuum concentration system (SC250DDA–120, Thermo Savant, U.S.A.). The radioactivity in each fraction was measured by LSC using a microplate counter (2450–0120, PerkinElmer, U.S.A.). The data were reconstructed for offline radioactivity

detection by using ARC Convert software (version 3.0.2.379, AIM Research, USA).

Metabolite identification was performed by high-performance liquid chromatography combined with online radioactivity monitoring and high-resolution mass spectrometry. The LC conditions were the same as those for radioprofiling. The instrument parameters of mass spectrometry (Table S3) were optimized for maximal sensitivity of the compounds of interest.

2.6. Pharmacokinetic assessment and analysis

The pharmacokinetic parameters of tegoprazan, M1 and total radioactivity in plasma were derived from the pharmacokinetic concentration set. Noncompartmental analysis was performed by Phoenix WinNonlin® software (version 8.3, CERTARA, U.S.A.) and was applied to calculate pharmacokinetic parameters, including the maximum concentration (C_{max}), time to reach C_{max} (T_{max}), AUC from time zero to the time of the last quantifiable concentration (AUC_{0-t}), AUC from time zero to infinity ($AUC_{0-\infty}$), mean residence time (MRT), elimination half-life ($t_{1/2}$), apparent volume of distribution (V_z) and apparent clearance (CL). Summary pharmacokinetic parameters are presented as the mean and standard deviation of the mean. The AUC ratios of tegoprazan and M1 to the total radioactivity (as %AUC) in plasma were calculated by dividing the AUC_{0-t} values of tegoprazan, and M1 by the total radioactivity in plasma. The total radioactivity excreted in urine and feces was converted to the percentage of the actual administered radioactive dose (% dose). The total recovery of radioactivity was computed as the sum of the cumulative excretion (as %dose) in urine and feces.

The distribution of radioactivity in whole blood was estimated by the concentration of TRA in whole blood (CB)/concentration of TRA in plasma (CP) ratio, and the CB/CP ratio was calculated by dividing the concentration of total radioactivity in the blood at every time point by that in the plasma.

2.7. Safety assessment

Drug safety was evaluated via physical examination, laboratory tests, assessment of vital signs, and 12-lead ECGs, and the occurrence of any adverse events was recorded. The vital signs of the subjects were assessed at least once during each study day. Physical examinations, 12-lead ECGs and laboratory tests were performed during the screening period and before discharge. In addition, 12-lead ECGs were conducted at 0 h and 4 h after dosing. Adverse events were recorded throughout the study period. Safety outcomes are presented using descriptive statistics.

3. Results

3.1. Demographic characteristics and the actual dosage of drug administered

Six healthy males were enrolled in this study, and each subject received a single oral ^{14}C-labeled tegoprazan dose with 240 mL of water according to the protocol. The actual dosage of each subject ranged from 48.8 ~ 48.9 mg of tegoprazan containing 148 μCi of ^{14}C-labeled tegoprazan. The demographic characteristics of the volunteers are summarized in Table 1.

Table 1. Summary of the demographic characteristics.

Characteristics	Value
Sex, N (%)	
Male	6 (100)
Age (years)	
Mean (SD)	30.2 (5.49)
Median	30.5
Range	21 ~ 36
Height (cm)	
Mean (SD)	165.92 (2.782)
Median	166.75
Range	161.5 ~ 168.5
Weight (kg)	
Mean (SD)	56.12 (2.506)
Median	56.4
Range	52.8 ~ 58.6
BMI (kg/m^2)	
Mean (SD)	20.42 (1.25)
Median	20.2
Range	19.0 ~ 22.2

BMI body mass index, SD standard deviation.

3.2. Pharmacokinetics in plasma and blood

The concentrations of tegoprazan and the major metabolite M1 in plasma were analyzed by a validated LC-MS/MS method, and the concentrations of the total radioactivity in plasma and blood were detected by LSC. The concentration-time curves of tegoprazan, M1 and TRA in plasma and blood are shown in Figure 2(a). The pharmacokinetic parameters of total radioactivity, tegoprazan, and M1 in plasma are listed in Table 2. In plasma, the concentrations of total radioactivity and tegoprazan reached the peak rapidly, and the median T_{max} of both compounds was 0.500 h, which was earlier than that of M1 (4.00 h). The exposure (AUC_{0-t} and $AUC_{0-\infty}$) of TRA in plasma was higher than that of tegoprazan and the major metabolite M1. The exposure of tegoprazan and M1 accounted for 34.84% and 40.10% of the TRA, respectively. The mean $t_{1/2}$ values of TRA, parent drug and M1 in plasma were estimated to be 8.72 h, 4.33 h and 10.0 h, respectively.

The total radioactivity of whole blood samples was collected during 0 ~ 48 h after administration. As shown in Figure 2(a), the concentration of TRA in blood could be measured until 24 h postdose, and was lower than the limit of quantitation at 48 h. The mean blood-to-plasma ratios of total radioactivity at 1, 4, 8, 12 and 24 h were 0.841, 0.783, 0.714, 0.773 and 0.775, respectively, which were all lower than 1.

3.3. Mass balance

A total of 6 healthy subjects were included in the analysis set of mass balance. Since the percentage of total radioactivity determined in urine and feces of subject R001 after administration was more than 100%, normalization correction was carried out for subject R001 according to a total recovery rate of 100%. The cumulative radioactivity

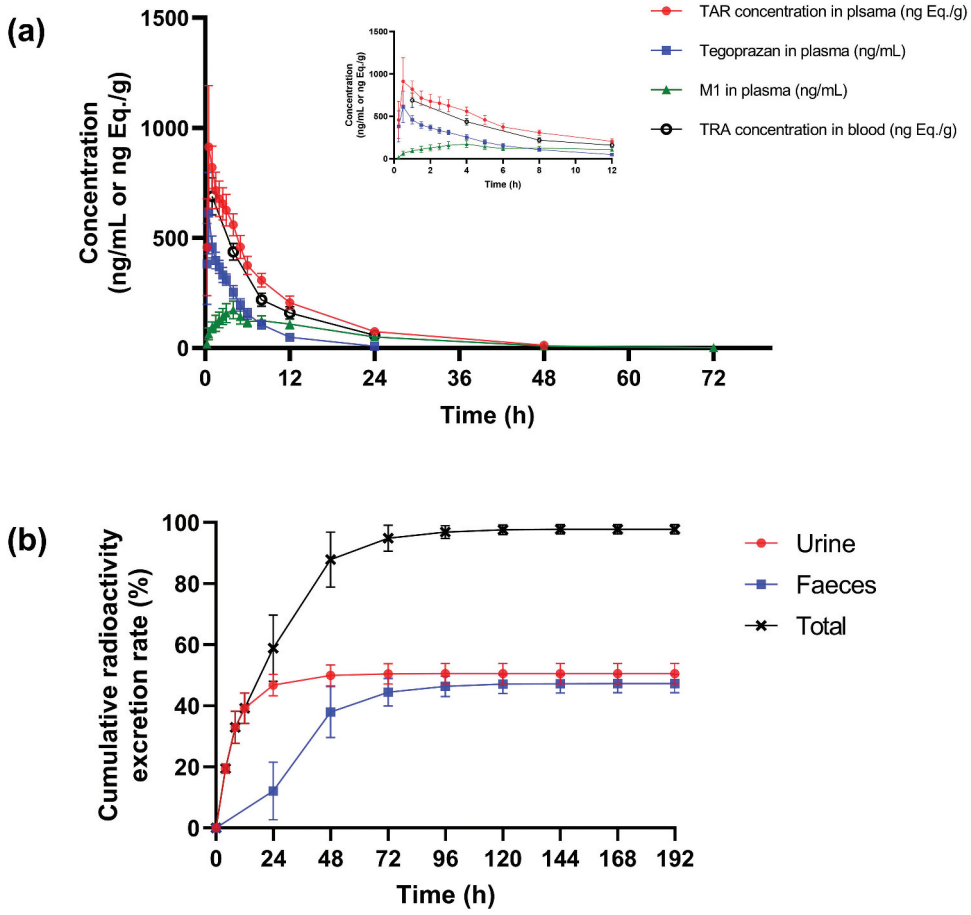

Figure 2. (a) Mean (±standard deviation) concentrations of total radioactivity, tegoprazan, and M1 in plasma and blood following a single oral administration of ^{14}C-labeled tegoprazan to healthy male volunteers. (b) Cumulative radioactivity excretion after a single oral administration of ^{14}C-labeled tegoprazan to healthy male volunteers.

Table 2. Pharmacokinetic parameters of total radioactivity, tegoprazan, and M1 in plasma following a single oral administration of ^{14}C-labeled tegoprazan to healthy male volunteers ($n = 6$).

Parameter	Unit	Total radioactivity	Tegoprazan	M1
T_{max} †	h	0.500 (0.500, 1.00)	0.500 (0.500, 1.00)	4.00 (3.00, 4.00)
C_{max}	ng Eq./mL or ng/mL	990 ± 218	634 ± 163	176 ± 41.0
AUC_{0-t}	h•ng Eq./mL or h•ng/mL	8020 ± 759	2790 ± 263	3210 ± 666
$AUC_{0-\infty}$	h•ng Eq./mL or h•ng/mL	8180 ± 836	2850 ± 288	3280 ± 683
MRT	h	9.78 ± 0.666	5.02 ± 0.549	14.7 ± 1.68
$t_{1/2}$	h	8.72 ± 0.841	4.33 ± 0.477	10.0 ± 0.843
V_z/F	L	77.1 ± 5.67	110 ± 8.30	NA
CL/F	L/h	6.16 ± 0.574	17.7 ± 1.84	NA

†T_{max} was described by median (minimum value and maximum value).
T_{max}: time to reach C_{max}; C_{max}: the maximum concentration; AUC_{0-t}: AUC from time zero to the time of the last quantifiable concentration; $AUC_{0-\infty}$: AUC from time zero to infinity; MRT: mean residence time, $t_{1/2}$: the elimination half-life; V_z: the apparent volume of distribution; CL: the apparent clearance.

excretion rates of all subjects after normalization correction are shown in Figure 2(b). The mean cumulative recovery rate of total radioactivity was 97.77% of administration dose at 192 h postdose, ranging from 96.04% to 97.94%. More than 90% of the administered radioactivity was recovered by 72 h after dosing. After 96 h postdose, less than 1% of the administered dose was excreted from each subject during each subsequent 24 h collection period over 2 consecutive days. The overall recovery rates in urine (50.51 ± 3.35%) and feces (47.26 ± 3.06%) were similar during the 0 ~ 192 h collection period.

3.4. Metabolite profiles and identification

The representative radio-chromatograms of pooled plasma (0 ~ 48 h), urine (0 ~ 48 h) and feces (0 ~ 120 h) are shown in Figure 3. The results (Table 3) revealed that in addition to the parent drug, a total of 20 radioactive metabolites were detected, including N-demethylation products (^{M1}), mono-oxidation at N-methyl products (M2), mono-oxidation products (M3), N-demethylation and mono-oxidation products (M4, M7 and M38), N-bis-demethylation products (M8), O-dealkylation products (M20), O-dealkylation and glucuronidation products (M13), di-oxidation and glucuronidation

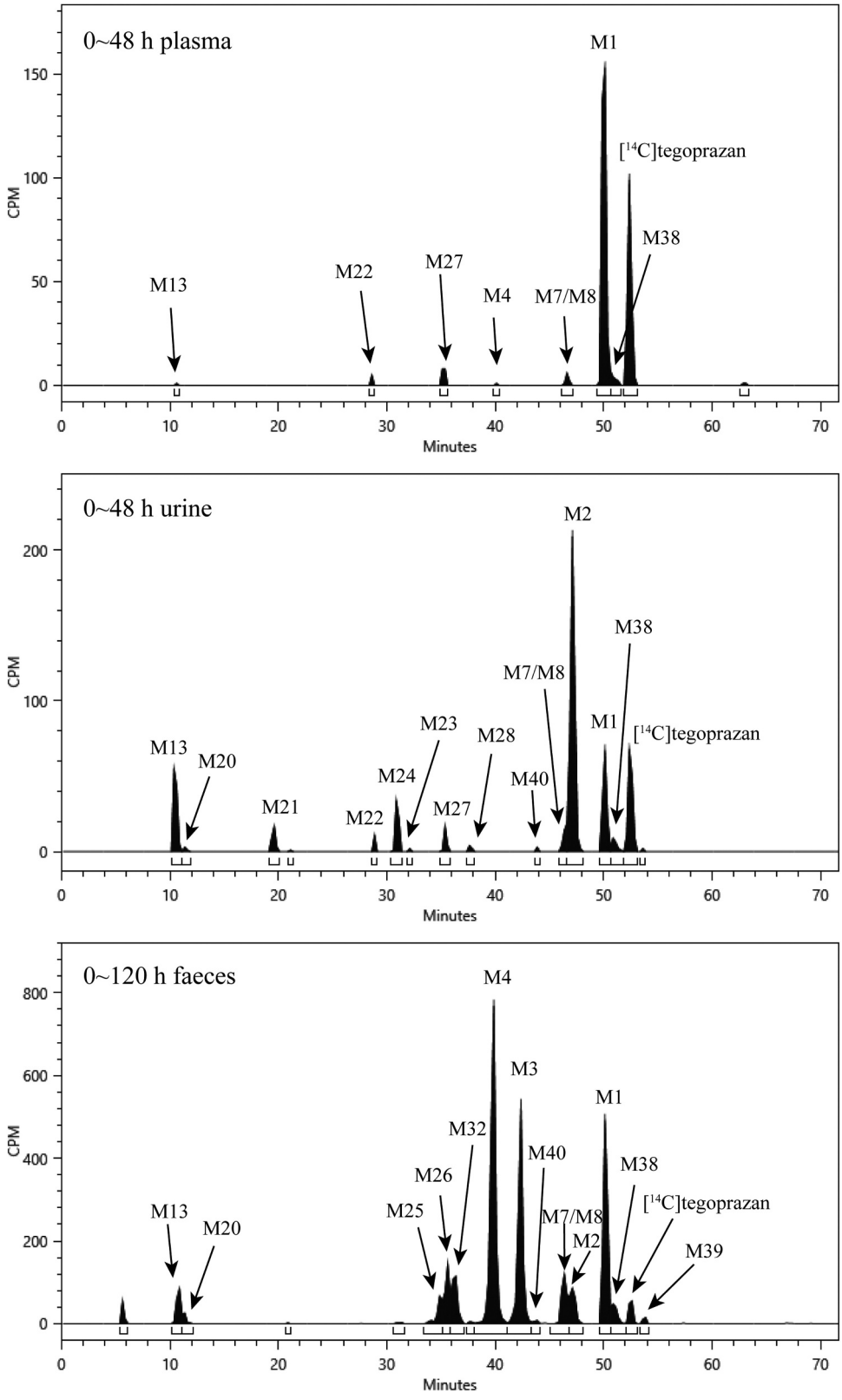

Figure 3. Representative radio-chromatograms of tegoprazan and its metabolites in pooled plasma (0 ~ 48 h), urine (0 ~ 48 h) and feces (0 ~ 120 h).

products (M21), further glucuronidation products (M22) of M4, further glucuronidation products (M23) of M3, di-oxidation and sulfation products (M24), N-bis-demethylation and mono-oxidation products (M25), N-demethylation and di-oxidation products (M26), and further sulfation products (M27) of M4, further sulfation products (M28) of M3, di-oxidation products (M32 and M40), and mono-oxidation and N-dealkylation products (M39). The proposed fragmentation pathways of tegoprazan and its metabolites upon MS/MS analysis are shown in Figure S1.

Table 3. Summary of ^{14}C-labeled tegoprazan and its metabolites in total plasma radioactive exposure (%AUC) and in urine and feces as a percentage of dose (%dose).

Metabolites	Structural modification	Plasma (0 ~ 48 h) %AUC	Urine (0 ~ 48 h) %Dose	Faeces (0 ~ 120 h) %Dose
Tegoprazan	Parent drug	40.41	5.92	1.44
M1	N-demethylation	52.56	6.9	10.54
M2	mono-oxidation at N-methyl	ND	19.76	1.55
M3	mono-oxidation	ND	ND	9.35
M4	N-demethylation and mono-oxidation	0.09	0.05	14.07
M7	N-demethylation and mono-oxidation	0.67	1.11	1.34
M8	N-bis-demethylation	0.67	1.11	1.34
M13	O-dealkylation and glucuronidation	0.7	6.86	1.46
M20	O-dealkylation	ND	0.35	0.39
M21	di-oxidation and glucuronidation	ND	0.95	ND
M22	N-demethylation, mono-oxidation and glucuronidation	0.5	0.63	ND
M23	mono-oxidation and glucuronidation	+	0.19	ND
M24	di-oxidation and sulfation	ND	3.33	ND
M25	N-bis-demethylation and mono-oxidation	ND	+	0.89
M26	N-demethylation and di-oxidation	ND	+	1.68
M27	N-demethylation, mono-oxidation and sulfation	2.43	1.56	+
M28	mono-oxidation and sulfation	+	0.38	+
M32	di-oxidation	ND	+	1.37
M38	N-demethylation and mono-oxidation	1.79	1.04	1.13
M39	mono-oxidation and N-dealkylation	+	0.12	0.18
M40	di-oxidation	ND	0.25	0.05
The sum of identified peaks		99.82	50.51	46.78

ND: Not detected; +: Only mass spectrum detected.

In the plasma sample (0 ~ 48 h pooling), the identified peaks of tegoprazan related radioactive substances accounted for 99.82% of the total radioactivity. The parent drug was one of the main radioactive components in circulating plasma, accounting for 40.41% AUC of the TRA. The major metabolite in plasma was M1 (accounting for 52.56% AUC of the TRA), the exposure level of which was 1.3-fold higher than that of tegoprazan. Several minor metabolites (M4, M7, M8, M13, M22, M27 and M38) accounted for 7.03% of the total AUC of the TRA, and each of them accounted for less than 5% AUC of the TRA. The metabolites M23, M28 and M39 were detected only by mass spectrometry, and could not be quantified by radio-chromatography. The AUC of the TRA did not exceed 0.06% for any unidentified peak.

The radioactive peaks identified in urine and feces accounted for 50.51% and 46.78% of the dose administered, respectively. Among them, the parent drug accounted for 5.92% of the dose in urine and 1.44% of the dose in feces. The metabolite M2 made up 19.76% of the dose and was the leading metabolite in urine. The secondary metabolites in urine were M1 and M13, accounting for 6.9% and 6.86% of the dose, respectively. The trace amounts of M4, M7, M8, M20, M21, M22, M23, M24, M27, M28, M38, M39 and M40 in urine accounted for 0.05 ~ 3.33% of the dose.The metabolites M25, M26 and M32 in urine were detected only by mass spectrometry without quantitative radioactive data. The major metabolites in feces were M1, M3 and M4, accounting for 10.54%, 9.35% and 14.07%, respectively. The radioactive percentages of M2, M7, M8, M13, M20, M25, M26, M32, M38, M39 and M40 at the dose ranged from 0.05% to 1.68%. Although the metabolites M27 and M28 in feces were detected by mass spectrometry, their radioactive percentages were not quantified.

Based on the metabolite identification mentioned above, the proposed metabolic pathways of tegoprazan in humans are shown in Figure 4. The main metabolic pathways were as follows: N-demethylation; mono-oxidation at N-methyl; other mono-oxidation or di-oxidation, and further glucuronidation or sulfation.

3.5. Safety outcomes

Six healthy subjects were tolerated the medicine well after a single oral administration of 50 mg/150 μCi ^{14}C-labeled tegoprazan suspension. During this study period, no severe adverse events occurred, and only one subject experienced one adverse event (elevated triglycerides), which was defined as a mild drug-related adverse event. This subject was cured without intervention treatment. In total, the incidence rate of adverse events was 16.7% in this study.

4. Discussion

This study is the first to report the pharmacokinetics, metabolism and mass balance of tegoprazan in humans. The dosage of 50 mg tegoprazan marketed in Korea and China was proven to be effective and safe for patients and healthy subjects [9,17,18,26]. Hence, in this study, 50 mg of tegoprazan was administered to volunteers. The dosimetry of subjects following the administration of a single dose of 50 mg/150 μCi ^{14}C-tegoprazan was calculated on the basis of data from a quantitative whole-body autoradiography (QWBA) study and a mass balance study in Long – Evans (LE) rats. The doses for active blood-forming organs, gonads, lens of the eye, the whole body and other organs were all far below Food and Drug Administration (FDA) constraints [27–29].

In the mass balance study, the overall recovery rate of dosed radioactivity was 97.77%±1.48% within 192 h, indicating that this study reached a mass balance perfectly. The

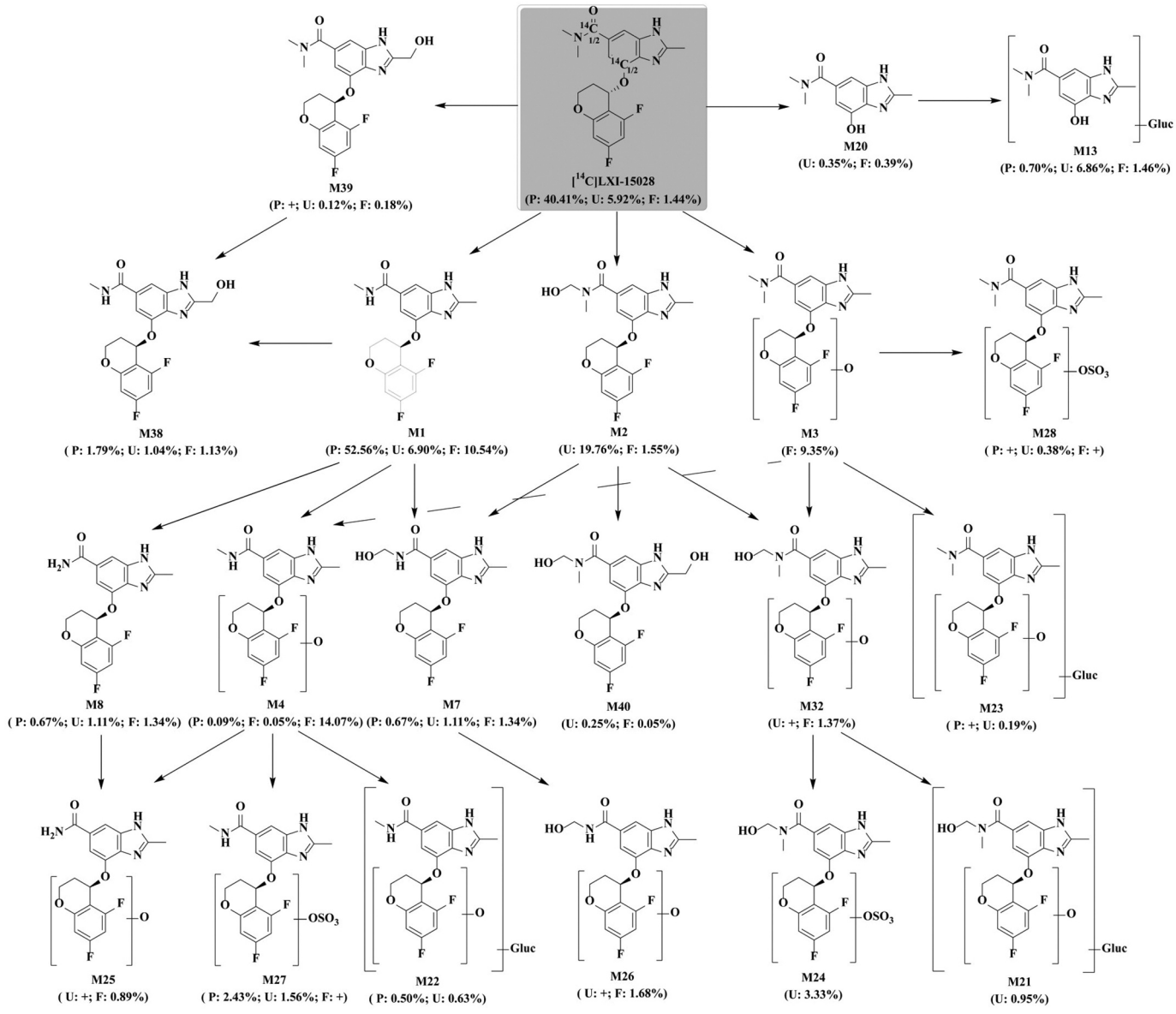

Figure 4. Proposed metabolic pathways of tegoprazan in humans (P: plasma, U: urine, F: feces).

cumulative recovery rates from urine and feces were 50.51% ±3.35% and 47.26%±3.06%, respectively, which suggested that the renal and fecal excretion jointly contributed to the elimination of tegoprazan. In bile duct-cannulated (BDC) male rats, through 120 hours postdose, the mean amount of the dosed radioactivity recovered in bile was 41.4%, and urine and fecal recoveries of radioactivity over this same 120 hour interval were 25.7% and 28.4% of the administered dose, respectively (unpublished data). The total amount of radioactivity recovered in bile and urine from BDC rats indicated that a minimum of 67% of the oral dose was absorbed in male rats. Considering the low proportion of the prototype drug in human excreta, it is speculated that the bioavailability in the human body is also high, and metabolism represents the major mechanism of clearance of tegoprazan in humans. Further studies of intravenous microdose isotope-labeled administration could be performed to determine its absolute bioavailability [30,31].

As shown in the concentration-time plots of tegoprazan and M1 in plasma (Figure 2), the PK characteristics of the parent drug and M1 were matched with the results from a previous single-ascending-dose study in healthy Chinese volunteers [18], which fully verified that the radioactivity preparation applied in this study could well mirror the behavior of the clinical preparation *in vivo*. The $AUC_{0\text{-}t}$ values of tegoprazan and M1 in plasma were estimated to be 2790 h•ng Eq./mL and 3210 h•ng Eq./mL, respectively, accordingly accounting for 34.84% and 40.10% of the AUC of total drug-related radioactive substances. The exposure ($AUC_{0\text{-}t}$) of total radioactivity was higher than the sum of the intact parent drug and M1, indicating that other metabolic substances might be present in plasma. Furthermore, the $t_{1/2}$ of the TRA (8.72 h) in plasma was longer than that of tegoprazan (4.33 h), suggesting that circulating metabolites were eliminated more slowly than the parent drug. The blood-to-plasma ratios ranged from 0.626 ~ 0.928, revealing that

tegoprazan-related substances were not inclined to be distributed in blood cells.

N-Demethylation was the main metabolic pathway of tegoprazan in plasma. On the basis of the FDA guidance for metabolites in safety, M1, the exposure of which exceeds 10% of the total drug-related exposure in humans, could raise safety concerns [32]. The data generated in animal studies (general toxicology, embryo-fetal development toxicology and carcinogenicity in mice, rats and dogs) suggested that the exposure of M1 produced by the no observed adverse effect level (NOAEL) was 12.5 to 286.7 times higher than that produced by the therapeutic dose in humans (data not shown). Owing to the differences in exposure between animals and humans, the safety of M1 has been well evaluated in animals and confirmed to be safe according to MIST guidance [22,33]. It has been reported that the CYP3A4 enzyme plays a principal role in the metabolism of tegoprazan [34,35]. Ngo et al. developed a physiologically based pharmacokinetic (PBPK) model for both tegoprazan and M1, but failed to predict the DDI profiles of M1 and CYP3A4 perpetrators due to the lack of robust clinical data [34]. The overall metabolic pathway of M1 identified in this study may be significant for PBPK model simulation. Considering the strong pharmacologic effects of parent drug and the various drug combinations for therapeutic purposes, investigating the effects of concomitant medications (CYP3A4 inhibitors or inducers) and gene polymorphisms of CYP3A4 on the pharmacokinetics of tegoprazan and M1 in the clinical phase is worthwhile. Further *in vivo* and *in vitro* studies are needed to explore whether tegoprazan and M1 inhibit or induce the CYP3A4 enzyme. Although the proportions of M2 and M3 in excreta were 21.31% and 9.35%, respectively, these two metabolites were not detected in plasma, which suggested that M2 and M3 May be produced by the metabolism of the tegoprazan in kidney and intestine.

The proposed metabolic pathways of tegoprazan are exhibited in Figure 4. Phase I metabolism, such as demethylation, dealkylation and oxidation, is the major metabolic pathway, and phase II metabolism, such as glucuronidation and sulfation, is the secondary metabolic pathway. Glucuronic acid conjugation usually transforms a compound into a hydrophilic material, facilitating the elimination of the drug from the body. In this study, glucuronic acid conjugates (M13, M22 and M23) were detected in plasma and urine, implying that these conjugates are transformed in liver and then entered systemic circulation. Notably, another glucuronide, M21, was present only in urine, which potentially results from the distribution of UGT-glucuronosyltransferases in kidney [36].

5. Conclusions

In conclusion, tegoprazan is well absorbed, widely metabolized and excreted completely in humans. The N-demethylation product (M1) of tegoprazan is the major circulating metabolite in plasma, which has been suggested to be fully evaluated in preclinical studies and considered safe in humans. The main metabolic pathways of tegoprazan in humans are demethylation, oxidation, glucuronidation and sulfation.

Abbreviations

ADMA	absorption, distribution, metabolism and excretion
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
AUC_{0-}	AUC from time zero to infinity
AUC_{0-t}	AUC from time zero to the time of the last quantifiable concentration
BDC	bile duct-cannulated
BMI	body mass index
CB	concentration of TRA in whole blood
CL	apparent clearance
C_{max}	the maximum concentration
CP	concentration of TRA in plasma
CV%	coefficient of variation
DDI	drug-drug interaction
DU	duodenal ulcer
ECGs	electrocardiograms
EE	erosive esophagitis
FDA	Food and Drug Administration
GERD	gastroesophageal reflux disease
GU	gastric ulcers
IS	internal standard
LC – MS/MS	liquid chromatography tandem mass spectrometry
LE	Long – Evans
LSC	liquid scintillation counter
MBS	Mass balance set
MIST	Metabolites in Safety Testing
MRT	mean residence time
NERD	non-erosive reflux disease
NOAEL	no observed adverse effect level
PBPK	physiologically based pharmacokinetic
P-CABs	potassium-competitive acid blockers
PK	pharmacokinetics
PKCS	Pharmacokinetic concentration set
PPIs	proton pump inhibitors
QWBA	quantitative whole-body autoradiography
RE	relative error
RP	reversed-phase
SD	standard deviation
SS	Safety set
$t_{1/2}$	elimination half-life
T_{max}	time to reach the maximum concentration
TRA	total radioactivity
V_z	apparent volume of distribution

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Author contributions statement

LY Miao, H Zhang, ZT Liu, F Xie, YH Wang, and CX Liu: substantial contributions to the conception or design of the work; ZM Gu, H Feng, and ZW Yu: the acquisition, analysis, or interpretation of data for the work; YC Bian, JJ Yuan, S Ma and J Nan: drafting the work or reviewing it critically for important intellectual content; H Zhang and LY Miao: final approval of the version to be published; LY Miao: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author, LY Miao. The data are not publicly available due to the containing information that could compromise the privacy of research participants.

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