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Preclinical pharmacokinetic study of a novel lipid-lowering agent, IMM-H007

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Abstract. – OBJECTIVE: To investigate the pharmacokinetic characteristics of absorption, distribution, metabolism, and excretion *in vivo* after oral administration and sublingual venous injection of the small molecule IMM-H007 in hamsters.

MATERIALS AND METHODS: Pharmacokinetic characteristics, including absorption, distribution, metabolism, and excretion, were studied *in vivo* by LC-MS/MS after oral administration and sublingual venous injection of IMM- hamsters. Furthermore, IMM-H007 stab in artificial gastric juices, artificial intestinal j , and Tris-HCl buffer was also analyzed.

RESULTS: There was no significant matrix impurity interference in golde ter whol blood as shown using MS/MS o detect the existence of these su nces. M-H007, MI, and MP exhibited goo earity i range of 1-500 ng/mL, 2-1000 and mL, respectively. The ma was 71.95-105.49%, and IMM-H , M1, a were stable during the proce sample d l. These results illustrate he HPLC MS/ nalytic method is sim re and sensitive and exhibits high sp icity a ich meets the clinical pharm kinetic requi ts of IMM-H007. IMM-H00 rapidly absorbe ough the oral route i amster The C_{max} and $AUC_{(0-t)}$ of the MI a P met tes in male and female hamste re i ased w increasing dosage and v rtiona he dose. In addition, T and we significantly prolonged ncrea ge, exhibiting linear dyna haract s and no significant gender diffe ces. Bioa ilability in male and female gol e after oral administration of I calculated using the sum of MI MP, resulting in 6.97% and 8.95%, respec- IMM-H007 and its metabolites were stable i HCl buffer, artificial gastric juices, and arti intestinal juices.

CONCLUSIONS: vide an experimental basi lucidating t terial pharmacodyna tions of IMM-H and predicting its ntial drug interactions.

Words
M-H007, Pre cal pharmacokinetic, Linear dyna haracter

Introduction

The social economy has conveyed great changes in people's life style. Particularly with the ageing population and the acceleration of urbanization, the epidemic trend of risk factors for vascular diseases in China is significant[1]. Currently, cardiovascular mortality accounts for the leading cause of overall death among both urban and rural residents. Furthermore, the burden of cardiovascular disease has become an increasingly major public health problem[2-4]. IMM-H007 is a novel AMP-activated protein kinase (AMPK) small molecule agonist that specifically binds to AMPKr subunits to activate AMPK. IMM-H007 is a novel type of lipid-lowering compound, and much research[5-8] has focused on the experimental study of IMM-H007. Pharmacological studies have shown that IMM-H007 inhibits fatty lesions induced by octadecenoic acid and accumulation of lipids in HepG2 cells[9]. Researchers[10-12] on the *in vivo* pharmacodynamics found that IMM-H007 reduces the concentration of serum triglycerides, total cholesterol, and low-density lipoproteins in golden hamsters with hyperlipidaemia. Mechanistic studies[13] suggest that IMM-H007 is a new type of AMP-activated protein kinase activator.

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In addition, preoperative pharmacokinetic studies have shown that IMM-H007 is a novel lipid-lowering compound with a distinct chemical structure, targets, and metabolic pathways from statins and is expected to be a new drug for the prevention and treatment of cardiovascular disease[14]. The pharmacokinetic characteristics of absorption, distribution, metabolism, and excretion *in vivo* after oral administration and sublingual venous injection of IMM-H007 in hamsters were investigated herein using LC-MS/MS. Our findings provide an experimental basis for elucidating the pharmacodynamics activity and predicting potential drug interactions.

Materials and Methods

Drugs and Reagents

IMM-H007 (Batch No. 20120401, 99.7% purity), Ml, MP, and the internal standard WS070119 were supplied by the Institute of Medicine of the Chinese Academy of Medical Sciences (Beijing, China). Formic acid and heparin sodium were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium carboxy methyl cellulose and sodium fluoride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methanol, acetonitrile (chromatography pure, Merck, Darmstadt, Germany), and other reagents were of analytical grade. Ultra-pure water was sourced from a Milli-Q water instrument (Millipore, Billerica, MA, USA).

Experimental Apparatus

Sartorius-precision weighing scales were from Sartorius (Goettingen, Germany). Sartorius ultrasound equipment was from Sanyo (Osaka, Japan). The GL-88B combination mixer was from Nanjing Jiancheng Tech (Nanjing, China). The TGL-16B centrifuge was from Shanghai Anting Scientific Instrument Factory (Shanghai, China). The Thermo-Biofuge-PrimoR refrigerated centrifuge and LC-MS/MS liquid chromatography-mass spectrometry analyzer were from Thermo-Fisher (Waltham, MA, USA) and included the following: surveyor autosampler, surveyor liquid pump, TSQ Quantum Access™ Triple Quadrupole Mass Spectrometer, Electrospray Ionization (ESI), and X calibur 1.4 software (used for collection and analysis).

Experimental Animals

Experimental hamsters were 6-8 weeks old and weighed 100-120 g. Animals were randomly grouped for experiments. Hamsters were fed in a plastic box and with standard specific pathogen-free (SPF) feed and were given free access to water. Room temperature was maintained at 20-25°C and 50-60% humidity. Animals were kept on a 12 h light/dark cycle, and ventilation frequency was 10-20 times/h. The velocity was 0.1-0.2 m/s, and the cleanliness reached 10,000. The ammonia concentration was ≤ 14 mg/m^3, and the noise level was ≤ 60 decibels. Illumination was 150-300 lx, and normal illumination was 15-20 LX. The fasting was carried twice per week, and cages were sterilized with high pressure after cleaning. This study was approved by the Animal Ethics Committee of China Pharmaceutical University Animal Center.

Experimental Methods

(1) Chromatographic conditions included use of the following: ReproSil 120 C18-AQ; mobile phase: A (0.1% formic acid water), B (0.1% formic acid methanol); flow rate: 0.2 mL/min; column temperature: 30°C; injection volume: 5 μL; and injection temperature: 10°C.

(2) Mass spectrometry conditions included the following: electrospray ion source, 3700 V spray voltage, the sheath gas and auxiliary gas were nitrogen, the pressure and air flow rate were 30 psi and 10 L/min; the capillary temperature was 350°C; and the collision gas pressure was 1.5 mTorr.

(3) Standard product preparation included accurate weighing of standard products. Acetonitrile was added (10 mL) to a volumetric flask that was the corresponding concentration of the reserving solution. Next, the reserving solution was precisely drawn. Acetonitrile was then diluted into a standard solution of suitable concentration by means of gradient dilution.

(4) The matric effect value was calculated by comparing the peak area ratios of whole blood samples and working fluids of IMM-H007, M1, and MP QC samples with low, medium, and high concentrations.

(5) To determine stability, five samples were taken from each IMM-H007, M1, and MP QC sample with low, medium, and high concentrations. The stability of IMM-H007, M1, and MP was determined by placing the sample concentrations in an auto sampler for 24 h. Before investigation, samples were placed in an ice bath for 1 h, subjected to repeated freezing and thawing (three times), as well as short-term placed and long-term placed.

Table I. Regression equations of IMM-H007, M1 and MP in hamster blood and liver.

Sample type	Substance	C(ng/mL)	Equation	R^2	LLOQ
Blood	IMM-H007	1-500	Y=0.0123654X+0.0154658	0.9991	1
	M1	2-1000	Y=0.0098745X+0.0162354	0.9980	2
	MP	10-5000	Y=0.0046542X+0.00123549	0.9991	10
Liver	IMM-H007	1-500	Y=0.0135648X+0.0178946	0.9996	
	M1	2-1000	Y=0.0124685X+0.0156987	0.99	
	MP	10-5000	Y=0.00329307X-0.00055849	0	10

Results

The Linear Relationship between Prototype Drug IMM-H007 and Metabolites M1 and MP in Whole Blood and Tissue Samples

The prototype drug IMM-H007 and its metabolites M1 and MP exhibited a good linear relationship in whole blood and tissue samples from hamsters at concentration ranges of 1-500, 2-1000, and 10-5000 ng/mL, respectively. The correlation coefficient of standard curves (r^2) was greater than 0.99. The minimum limit of quantification (LLQQ) for these three groups was 1, 2, and 10 ng/mL, respectively, which met the requir[illegible]t of sensitivity administration of whole blo[illegible] ples after hamsters were administered the [illegible]a- tion. Regression equations for the prototype [illegible] IMM-H007 and its metabolites M1 and MP [illegible] biological sample are shown in Table I.

The Linear Relationship[illegible]twe[illegible]he Prototype Drug IMM-[illegible]7 and Metabolites M1 and [illegible]n Fa[illegible] Urine Samples

M228, M244, M2[illegible]M404, M[illegible]nd M536 in faeces and urine [illegible]es from han[illegible]xhibited good linear rela[illegible]t concentratio[illegible]nges of 0.5-50, 1-200, 0.01-0.[illegible]20, 0[illegible]0, and 0[illegible]9 μg/mL, respectively[illegible]e c[illegible] coeffi[illegible] of the standard curve[illegible] was grea[illegible]n 0[illegible]he minimum limit o[illegible]tification (LL[illegible]0.5, 1, 0.01, 0.2, 0.5, a[illegible]/mL, respectiv[illegible], meeting the requiren[illegible]of sen[illegible] administration of metabolites in faeces and uri[illegible]les after administration of t[illegible]cation in ham[illegible]Regression equations f[illegible]228, M244, M344, M404, M404, and M536 in [illegible]ological sampl[illegible]re shown in Table II.

[illegible]rix Effects[illegible]d Stability

[illegible]lute ma[illegible] effect values of whole blood samp[illegible] medium, and high quality control concentrations (2, 50, and 200 ng/mL) of IMM-H007, [illegible]M1, and MP are shown in Table III. The results show [illegible]thod has no effect on determination of the [illegible]trix [illegible]ect, suggesting that the method is accurate and reliable. Samples with low, medium, and high quality control concentrations (IMM-H007: 2, 50, and 200 ng/mL; M1: 5, 100, and 500 ng/mL; MP: 20, [illegible]00, and 2000 ng/mL) were placed for 1 h in an ice bath, subjected to repeated freeze-thaw cycles (three times), placed at -80°C for 30 days, and were tested for stability by placement in an auto sampler for 24 h. The results are shown in Table IV and indicate that IMM-H007, M1, and MP remained stable during the above-mentioned treatments.

Table II. R[illegible]sion equations o[illegible]M244, M344, M404, M440 and M536 in urine and feces samples.

Meta[illegible]es	C (μg/mL)	Equation	R2	LLOQ (μg/mL)
M[illegible]	0.5-5[illegible]	Urine: Y=0.01834592X+0.00385974 Feces: Y=0.0160358X+0.00446871	0.9991 0.9980	0.5
M244	[illegible]	Urine: Y=0.00465823X+0.0492689 Feces: Y=0.0579846X+0.0196438	0.9980 0.9983	1
	[illegible]1-0.5	Urine: Y=0.0062938X-0.00062851 Feces: Y=0.00171527X-0.000302987	0.9999 0.9996	0.01
M[illegible]	0.2-20	Urine: Y=0.0729184X+0.0283431 Feces: Y=0.475842X+0.0117105	0.9996 0.9989	0.2
[illegible]40	0.5-50	Urine: Y=0.029874X+0.00390267 Feces: Y=0.29687X+0.0024684	0.9998 0.9998	0.5
	0.2-20	Urine: Y=0.009534581+0.0204985X Feces: Y=0.01264587X+0.0150378	0.9990 0.9978	0.2

Table III. Matrix effects of IMM-H007, M1 and MP in hamster blood (n=5).

Sample	C (ng/mL)	Peak area of matrix sample	Peak area of standard sampl	Matr ffect	Precision (RSD%)
IMM-H007	2	60896±5623	84654±126		8
	50	1365894±89561	1645612±7	8	7
	200	5365489±198542	6356412±	84.41	4
MI	2	81235±4652	94584 456	85.89	4
	50	1592548±400254	1874562 598742	84.95	2
	200	7845652±295641	87 84568	89.21	4
MP	2	61203±2365	365±8546	84.58	4
	50	1985641±395461	9754±56894	110.94	21
	200	7056945±298543	9745±79845	105.49	5

Table IV. Stability of IMM-H007, M1 and MP in hamster blood under different co (n=5).

Sample	C (ng/mL)	Ice bath for 1 h		ee freez raw cycles		Store at -80°C (30 days)		Autosampler at 10°C (24 h)	
		Measured (Mean±SD)	Accu (R.	asured	Accuracy (R.E.%)	Measured (Mean±SD)	Accuracy (R.E.%)	Measured (Mean±SD)	Accuracy (R.E.%)
IMM-H007	2	1.898±0.301	.6	2.156±0.015	7.1	2.179±0.165	9.2	2.23±0.21	12.5
	50	52.98±1.26	6.54	12±1.04	2.1	47.8±3.94	-4.2	44.2±1.3	-11.7
	200	221.6±3.7	.2	.6±3.6	9.4	182.5±2.6	-8.5	184.0±6.8	-8.2
M1	5	4.962±0.5		5.03±0.384	0.4	4.65±0.4	-6.2	5.0±0.41	-0.2
	100	108.89± .45	9.	108.56±3.89	9.1	118.26±3.59	11.56	106.14±1.49	6.0
	500	522. 2	4.5	528.6±7.7	5.6	514.1±8.1	2.8	558.2±10.9	12.4
MP	20	21 .23	7.1	21.87±1.09	9.2	18.45±1.02	-8.4	18.5±1.02	-9.4
	500	5 9±3.20	13.26	554.26±16.48	10.84	456.59±35.26	-8.2	567.9±8.26	13.7
	2000	5±25	8	2165.1±26.4	8.2	2094.2±19.6	4.2	2189.2±20.8	9.8

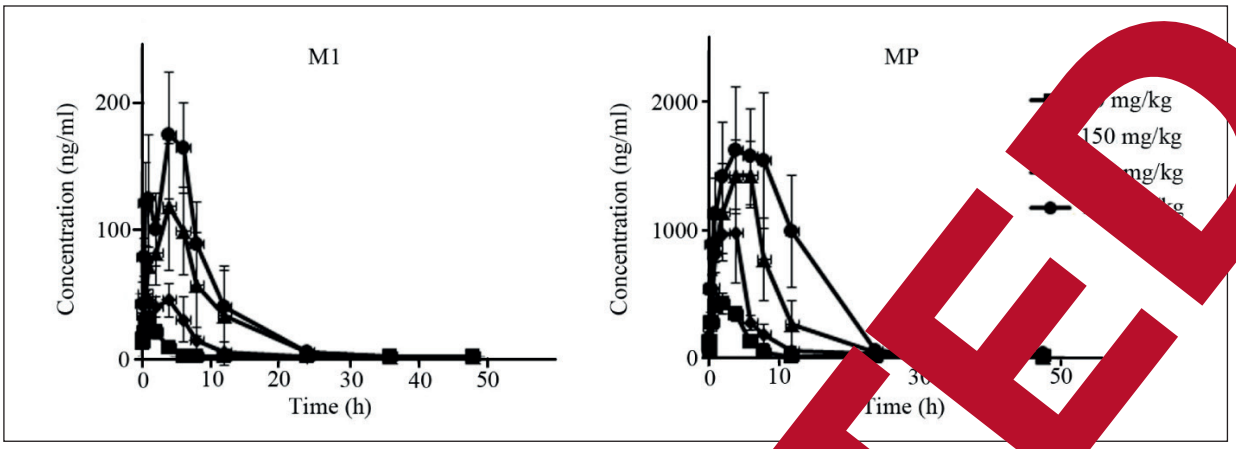


Figure 1. Mean blood concentration-time profiles of M1 and MP in male h… oral IMM-H007 administration at doses of 50, 150, 450, and 900 mg/kg (n=5).

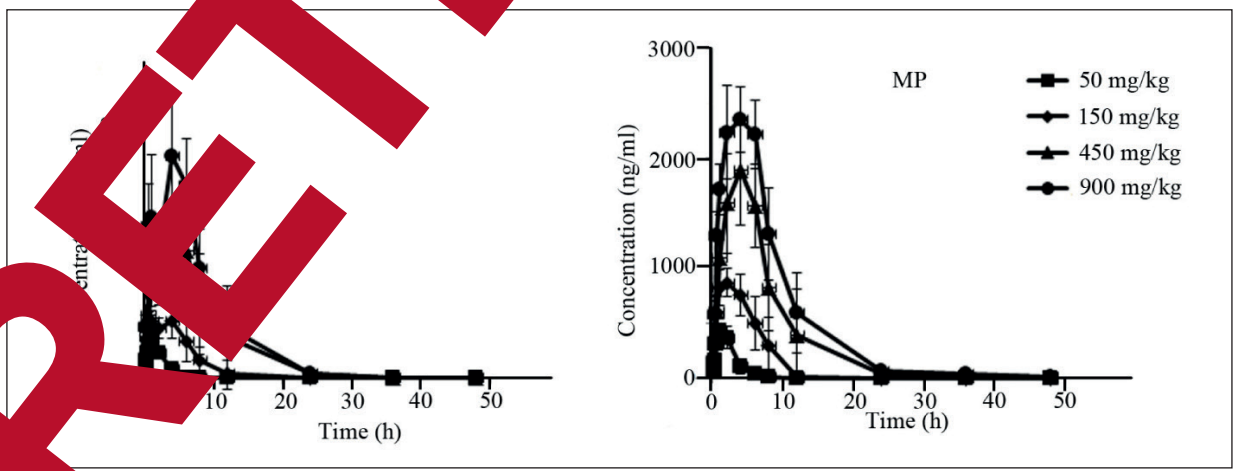
Pharmacokinetics Study of Oral and Intravenous Administration of IMM-H007 in Hamsters

Pharmacokinetics of Oral Administration of IMM-H007 in Hamsters

After hamsters were treated with different … of IMM-H007 (50, 150, 450, and 900 m… curve of whole blood concentrations was …ed (Figure 1 and Figure 2). According to the … time curve for Ml and MP after oral administra… of different doses of IMM-H007 (50, 150, 45… and 900 mg/kg) in male and …hamsters, the Ml and MP pharmacoki… para…rs were calculated using the non-c…artment …odel of Phoenix WinNonLin so… Rele… cokinetic parameters … she… …les V-VIII.

T… …lationship between the average peak concen-…on C_{max}, AUC… of Ml and MP, and the dosage …hown in Fig… 3. Hamsters rapidly absorbed …-H007 after … administration, and the me-ta… M1 an… could be detected in blood 3 min … …ration in each dose group. Twelve to thirty-six hours after administration, the plasma …ncentration of the metabolic products M1 and MP …o the detection limit. The concentration … …g prototype at each time point was close to or below the detection limit.

After oral administration of different IMM-H007 doses (50, 150, 450, and 900 mg/kg) to male hamsters, C_{max} of the M1 metabolite was 35.78 ± 10.82, 82.65 ± 28.15, 123.46 ± 30.87, and 209.46 ± 59.87 ng/mL, while $T_{1/2}$ was 1.38 ± 0.06, 4.36 ± 1.18, 6.19 ± 0.67, and 3.66 ± 0.87 h,



Fi… Mean blood concentration-time profiles of M1 and MP in female hamsters after oral IMM-H007 administration at doses …0, 150, 450, and 900 mg/kg (n=5).

Table V. Pharmacokinetic parameters of M1 in male hamsters after oral administration of IMM-H007 at 50, 150, 450, 900 mg/kg (n=5).

Parameters	50 mg/kg	150 mg/kg	450 mg/kg	900 mg/kg
$T_{1/2}$ (h)	1.38±0.06	4.36±1.18	6.19±0.67	3.66±0.8
T_{max} (h)	1.02±0.84	2.39±1.68	4.79±0.99	5.91±3.2
C_{max} (ng/mL)	35.78±10.82	82.65±28.15	123.46±30.87	209.4 ±59.87
$AUC_{(0-t)}$ (h*ng/mL)	143.58±12.09	402.69±120.39	982.81±236.42	234 856.14
$AUC_{(0-\infty)}$ (h*ng/mL)	149.74±13.05	420.36±120.69	996.57±230.14	±798.38
Vz (L/kg)	0.87±0.06	2.04±0.82	4.21±10.6	.15±0.4
CLz (L/h/kg)	0.36±0.04	0.38±0.10	0.45±0.10	0.43±
$MRT_{(0-t)}$ (h)	2.86±0.22	4.19±0.68	6.76±0.97	
$MRT_{(0-\infty)}$ (h)	3.07±0.19	4.47±0.79	7.04±0.69	

Table VI. Pharmacokinetic parameters of MP in male hamsters after oral adm of IMM-H007 150, 450, 900 mg/kg (n=5).

Parameters	50 mg/kg	150 mg/kg	450 mg/kg	mg/kg
$T_{1/2}$ (h)	1.42±0.64	5.16±1.08	.65	0.84
T_{max} (h)	1.87±0.56	2.71±1.28	4.79±1.32	4.78±2.98
C_{max} (ng/mL)	479.23±58.24	1123.48±279.36	1579.64±209.67	1865.37±459.87
$AUC_{(0-t)}$ (h*ng/mL)	2130.46±436.52	5897.61±1024.16	3249.87±2360.9	23048.97±6983.27
$AUC_{(0-\infty)}$ (h*ng/mL)	2197.64±504.93	5798.46±1023.97	97.64±2309.2	23129.83±6974.38
Vz (L/kg)	0.06±0.01	0.20±0.08	±0.07	0.30±0.08
CLz (L/h/kg)	0.03±0.01	0.03±0.00		0.05±0.01
$MRT_{(0-t)}$ (h)	2.86±0.46		7.19±0.91	8.16±0.89
$MRT_{(0-\infty)}$ (h)	3.27±1.19		7.56±0.78	8.32±0.94

Table VII . Pharmacokinetic parameters of M1 in fema after oral administration of IMM-H007 at 50, 150, 450, 900 mg/kg (n=5).

Parameters	50 mg/	150 mg/	450 mg/kg	900 mg/kg
$T_{1/2}$ (h)	1.36	1.94±0.37	2.56±0.69	3.76±0.43
T_{max} (h)	0 .19		4.86±1.99	4.65±0.92
C_{max} (ng/mL)	30		130.46±39.12	187.34±51.68
$AUC_{(0-t)}$ (h*ng/mL)	46±	300.04±109.37	1033.32±498.67	1587.64±423.15
$AUC_{(0-\infty)}$ (h*ng/mL)	83.67±17.8	320.97±100.34	1067.85±485.37	1597.64±428.37
Vz (L/kg)	1.17±0.36	39±0.46	1.89±0.97	3.27±0.79
CLz (L/h/kg)	3±0.13	0.53±0.10	0.51±0.22	0.58±0.21
$MRT_{(0-t)}$ (h)	.28	3.68±0.78	5.78±1.83	6.57±0.99
$MRT_{(0-\infty)}$ (h	2.3	4.47±0.72	6.07±1.67	6.67±1.03

Table V harmac inetic parameters of MP in female hamsters after oral administration of IMM-H007 at 50, 150, 450, 900 mg/k).

Pa rs	g/kg	150 mg/kg	450 mg/kg	900 mg/kg
(h)	.20±0.09	3.56±0.67	6.73±2.34	9.67±2.43
	0.99±0.09	2.34±1.11	4.45±1.57	4.32±1.94
C /mL)	426.39±73.98	912.31±150.12	1978.46±379.52	2546.31±364.35
AU (h*ng/mL)	1304.65±286.36	5963.46±1876.45	16435.23±3765.48	24653.46±4987.56
)	1334.89±267.48	6045.31±1879.46	16423.87±3970.46	24361.21±4974.2
L/kg)	0.06±0.0.2	0.16±0.04	0.26±0.07	0.56±0.29
(L/h/kg)	0.04±0.03	0.03±0.01	0.03±0.01	0.04±0.02
(h)	2.16±0.22	4.67±0.56	6.75±1.34	7.27±0.68
MR (h)	2.30±0.25	4.98±0.32	7.21±1.27	7.68±0.64

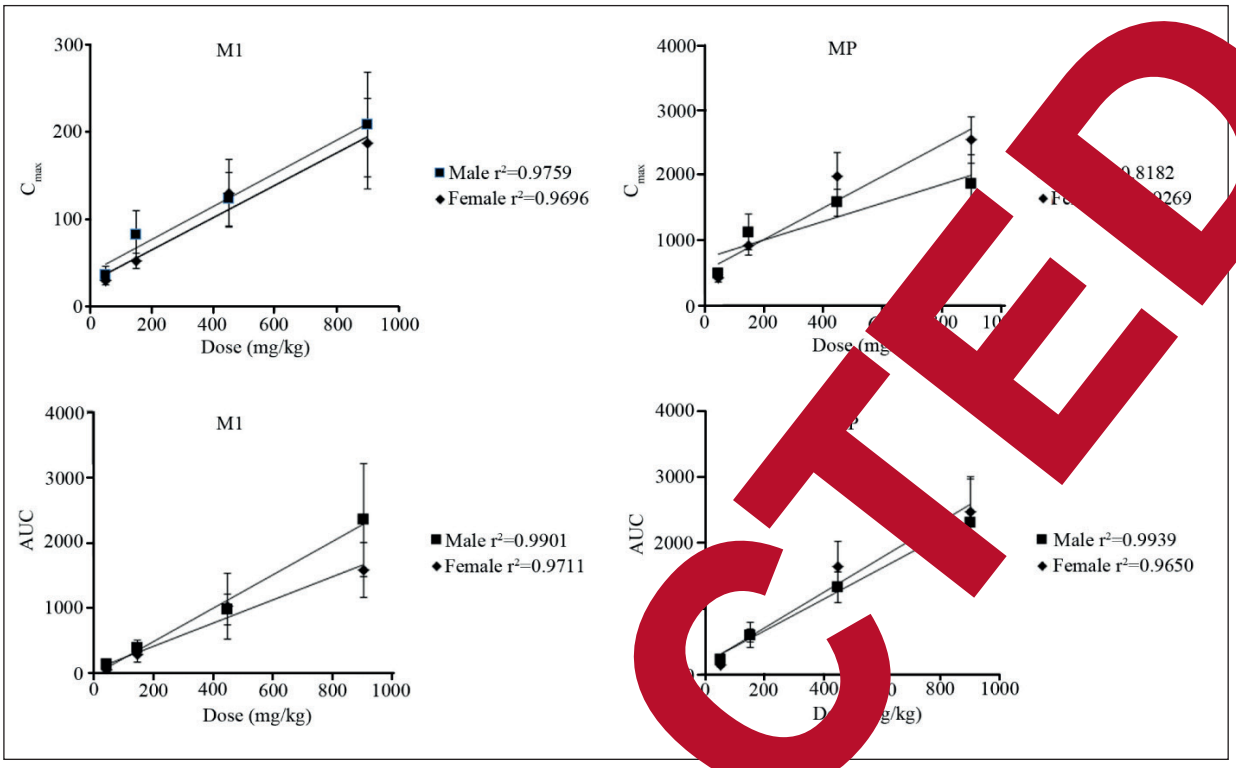


Figure 3. Relationship betwee[illegible]mg/kg) and Cmax and AUC(0-t) of M1 and MP.

respectively. $AUC_{(0-t)}$ was 143.58 ± 12.09, 4[illegible] ± 120.39, 982.81 ± 236.42, and 2345.64 ± 85[illegible] h * ng/mL, while $MRT_{(0-t)}$ was 2.86 ± 0.22, 4.19 ± 0.68, 6.76 ± 0.97, and 7.64 ± 0[illegible]ectively. C_{max} of the MP metabolite [illegible]479.[illegible] 58.24, 1123.48 ± 279.36, 1579.64 ± [illegible]09.67 a[illegible]1865.37 ± 459.87 ng/mL; $T_{1/2}$ wa[illegible] ± 0.64, 7.28 ± 0.65, and 4.96 ± [illegible]24 h, [illegible]was 2130.46 ± 436.52, 5897.61 ± [illegible]4.16, 15[illegible]7 ± 2360.98, and 23048.97 ± 6[illegible]7 h * Ng/mL. $MRT_{(0-t)}$ was 2.86 ± 0.46, [illegible]0.67, 7.19 ± 0.[illegible]nd 8.16 ± 0.89 h, resp[illegible]ely. [illegible] and $AUC_{(0-t)}$ of the M1 and MP metabolites [illegible]le hamsters after IMM-H0[illegible]ral administrati[illegible]MM-H007 increased [illegible]n increasing dose, $MRT_{(0-t)}$ increased slight[illegible]ith in[illegible]sing dose, and in the 50-900 mg[illegible]dose [illegible]ge, the [illegible]1 and MP metabolites [illegible]xhibite[illegible]inear kinetic process *in vivo*. [illegible]oral [illegible]inistration of different [illegible]H007 [illegible]150, 450, and 900 mg/kg) to f[illegible]e hamst[illegible]he C_{max} of the M1 metabolite was [illegible]43±5.82, 52.46±8.37, 130.46±39.12, and 18[illegible]/mL, while $T_{1/2}$ was 1.36±0.24, [illegible]±0.37, 2.56±0.69, and 3.76±0.43 h, respectively. $AUC_{(0-t)}$ was 73.46±16.37, 300.04±109.37, [illegible]±498.67, and 1587.64±423.15 h*ng/mL, whi[illegible]$RT_{(0-t)}$ was 1.67±0.28, 3.68±0.78, 5.78±1.83, and [illegible]±0.99 h. C_{max} of the MP metabolite was 426.39±73.98, 912.31±150.12, 1978.46±379.52, and 2546.31±364.35 ng/mL, while $T_{1/2}$ was 1.20±0.09, 3.56±0.67, 6.73±2.34, and 9.67±2.43 h. $AUC_{(0-t)}$ was 1304.65±286.36, 5963.46±1876.45, 16435.23±3765.48, and 24653.46±4987.56 h*ng/mL, while $MRT_{(0-t)}$ was 2.16±0.22, 4.67±0.56, 6.75±1.34, and 7.27±0.68 h. The C_{max} and $AUC_{(0-t)}$ of the M1 and MP metabolites in female hamsters after oral IMM-H007 administration increased with increasing dose, and $MRT_{(0-t)}$ increased slightly with increasing dose. In the 50-900 mg/kg dose range, the M1 and MP metabolites exhibited a linear kinetic process *in vivo*. There was no significant gender difference.

Changes in Blood Concentration and the Drug-Time Curve after Intravenous IMM-H007 Injection at Different Time Points in Hamsters

The drug-time curve of whole blood at each time point in golden hamsters after intravenous IMM-H007 injection (5 mg/kg) is shown in Figure 4. According to the drug-time curve of Ml and MP metabolites after IMM-H007 sublingual venous injection (5 mg/kg) in male and female golden hamsters, Ml and MP pharmacokinetic parame-

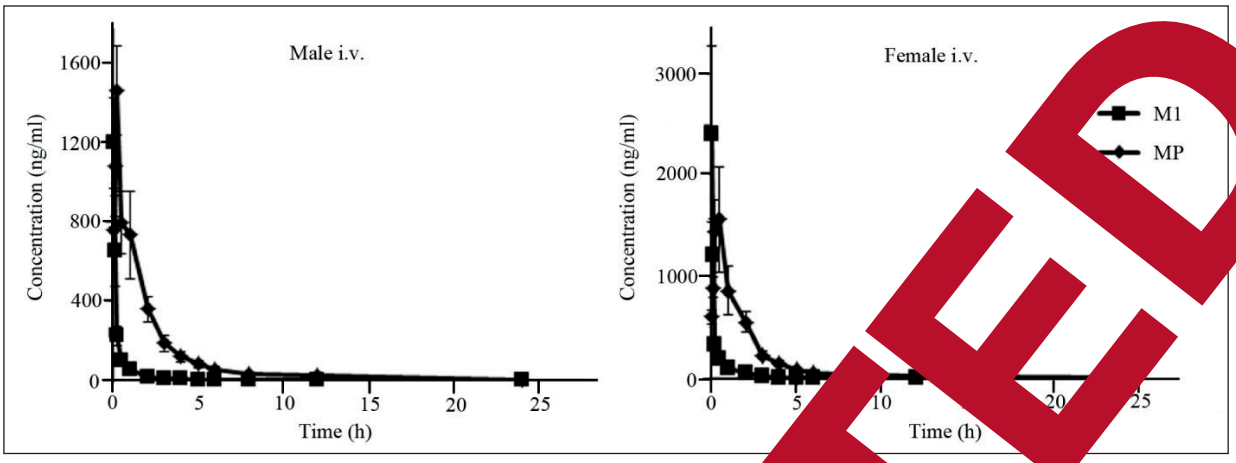


Figure 4. Mean blood concentration-time profiles of M1 and MP in male a… ma… …ters after a single i.v. administration of IMM-H007 at 5 mg/kg (n=5).

ters were calculated using the non-compartmental model of Phoenix WinNonLin software. Relevant pharmacokinetic parameters are shown in Table IX. After IMM-H007 injection into the sublingual vein of male and female golden hamsters, the drug prototype and Ml and MP metabolites were detected in the blood at two minutes. …ty minutes after administration, the drug p… was close to the detection limit. Twelve to …n-ty-five hours after administration, Ml and … metabolites were close to the detection limit. … minutes after administration in mal… hamsters, t… average blood concentration of … type was 21.05 ng/mL, the C_{max} of Ml… MP … abolites was 1198.69 and 1465.23 n… L, $T_{1/2}$ w… 11 and 2.33 h, $AUC_{(0-t)}$ was 332.2… 2274.… and $MRT_{(0-t)}$ was 1.09 … 12.0… tively. Two minutes after admi… …tion in… e hamsters, the average blood … centration o… rototype was 7.58 ng/mL … of Ml and M… metabo-li… as 2406.58 and 1569.37 ng/mL, $T_{1/2}$ was 2.11 … 2.34 h, AUC … was 569.29 and 2874.66 ng/mL … and $MRT_{(0-t)}$ … 0.79 and 1.97 h, respectively. … bioavailabil… after oral administration of I… H007 (50… /kg) in male golden hamsters for I… as 6.97%, and the bioavailability in female golden hamsters was 8.95%. Calculation … the area under the drug curve of the Ml and MP … abolites in whole blood to determine the … vailability of IMM-H007 is shown below.

$$F_{male,M1}(\%) = \frac{5mg/kg \times 73.06ng/ml*h}{50mg/kg \times 332.47ng/ml*h} = 2.19\%$$

$$F_{female,M1}(\%) = \frac{5mg/kg \times 132.08ng/ml*h}{50mg/kg \times 600.02ng/ml*h} = 2.20\%$$

$$F_{male,MP}(\%) = \frac{5mg/kg \times 1079.58ng/ml*h}{50mg/kg \times 2258.71ng/ml*h} = 4.78\%$$

$$F_{female,MP}(\%) = \frac{5mg/kg \times 1901.83ng/ml*h}{50mg/kg \times 2819.45ng/ml*h} = 6.75\%$$

Table IX. Ph… acokinetic para… of M1 and MP in male and female hamster after a single i.v. administration of IMM-H007 at 5 mg/kg…

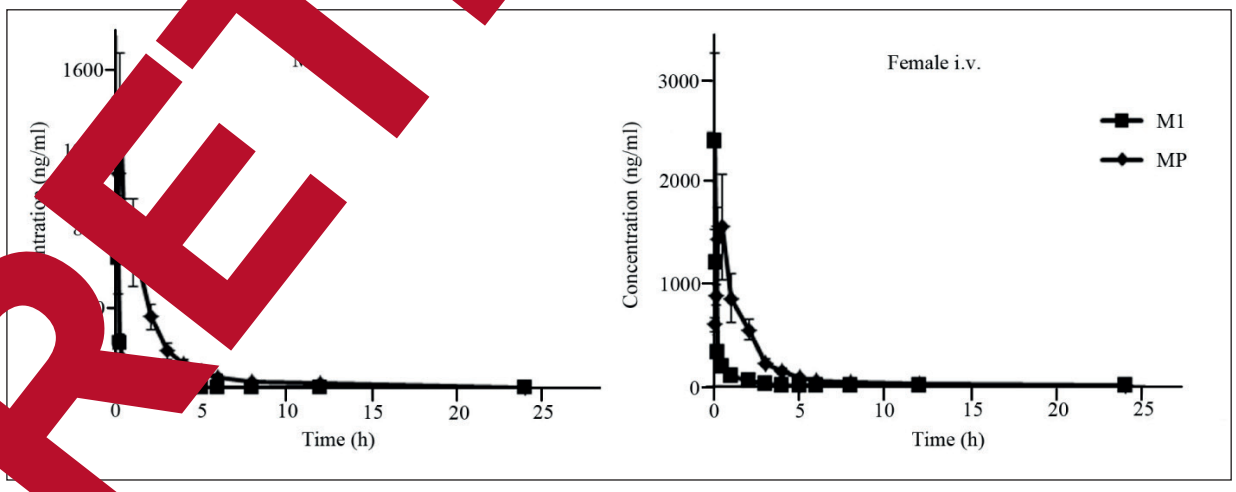
Para… …rs	Male		Female	
	…M1	MP	M1	MP
$T_{1/2}$ (h)	…11±0.56	2.33±0.26	2.11±0.12	2.34±0.18
…(h)	0.03±0.00	0.25±0.00	0.03±0.00	0.35±0.03
C_{max} …/mL)	1198.69±223.65	1465.23±256.58	2406.58±896.25	1569.37±489.56
AU… (h*ng/mL)	332.26±55.25	2274.35±336.21	569.29±198.55	2874.66±546.21
A… …)	339.65±55.26	2345.61±336.78	609.58±199.25	2985±523.86
…L/kg)	44.05±6.89	0.01±0.00	22.89±8.12	5.79±1.52
…z (L/h/kg)	15.03±2.21	0.00±0.00	8.49±2.56	1.79±0.36
… (h)	1.09±0.22	2.08±0.06	0.79±0.12	1.97±0.15
M… …(h)	1.48±0.36	2.65±0.15	0.97±0.23	2.35±0.2

Stability of IMM-H007 in Artificial Gastric Juices, Artificial Intestinal Juices, and Tris-HCl Buffer

The stability of IMM-H007, Ml, and MP in artificial gastric juices, artificial intestinal juices, and Tris-HCI buffer is shown in Table X and Figure 5. IMM-H007, M1, and MP were stable in artificial gastric juices, artificial intestinal juices, and Tris-HCl buffer. After incubating for 4 h in artificial gastric juices, artificial intestinal juices, and buffer, they remained 118.20%/102.38%/101.60%,92.67% /94.10%/107.42%, and 89.82%/98.40%/103.64%, respectively. After incubating with the artificial gastric juices, artificial intestinal juices, and buffer, neither the primary drug nor the metabolites were significantly reduced, and no other metabolite was produced, indicating that the prototype drugs and metabolites were less affected by gastrointestinal pH changes.

Discussion

Early pharmacological researches have shown that the M1 and MP metabolites are pr[illegible]ly the *in vivo* active metabolites of the p[illegible] drug IMM-H007. In this study, the rapid, [illegible]si-tive, and reliable LC-MS/MS method was [illegible]l to detect the prototype drug IMM-H007 and [illegible] metabolites, M1 and MP, at the same time. As t[illegible] plasma content of MP in mice [illegible]w and it primarily exists in whole bl[illegible] this [illegible]eriment utilized whole blood for pl[illegible]macokin[illegible]studies. The Guidelines of Non-[illegible]l Pha[illegible] Study in Chemical D[illegible]gs, [illegible]ted by the State Food and Dru[illegible]ministr[illegible]ndicate that a variety of factors could affect the determination of biological samples due to low conce[illegible] drugs, endogenous substances (suc[illegible] inorg-ic salts, lipids, proteins, metabol[illegible], individual differences, and so on. Therefo[illegible]is necessary to establish a suitable biological [illegible] analysis method and to confirm th[illegible]ethod a[illegible]g to the structure, biological m[illegible], and expe[illegible] centration range of the [illegible]pound[illegible]der exa[illegible] tion. Among them, [illegible]eatmen[illegible] the sample is a prerequisite for es[illegible]ing[illegible]ologic[illegible]mple analysis method[illegible] Com[illegible]used p[illegible]atment methods inclu[illegible]rotein p[illegible]tio[illegible], organic solvent, liqu[illegible]uid extracti[illegible]solid-phase extraction[illegible]rge differen[illegible] between the chemica[illegible]operti[illegible] IMM-H007 and its metabolites Ml and M[illegible]-H007 and M1 being non[illegible]MP having hi[illegible]larity), the recovery r[illegible] the liquid-liquid extraction method is low. [illegible]ddition, due t[illegible] IMM-H007 being unstable at [illegible]m temperatu[illegible] whole blood, this step needs [illegible] performed[illegible] an ice bath. Therefore, for th[illegible]erimen[illegible] chose the method of protein precip[illegible]ganic solvent. The experimental results showed that the absolute matrix effect value of IMM-H007, M1, and MP in low, medium, and [illegible]ntrations of whole blood samples was [illegible].49%, and the test value was basically unchanged, indicating that the LC-MS/MS method established in this research is accurate and reliable for detection of the target compounds and can be applied to the study of pharmacokinetics *in vitro* and *in vivo*. Pharmacokinetics are examined to understand the pharmacological characteristics of a drug and to explain the pharmacological effects of a drug in the body by understanding the absorp-



Figu[illegible]. Stability of IMM-H007, M1, and MP in artificial gastric juices, artificial intestinal juices, and Tris-HCl buffer solution.

Table X. Stability of IMM-H007, M1 and MP in artificial gastric juice, artificial intestinal juice and Tris-HCl buffer solution.

Matrix	Time	IMM-H007 (Mean±SD)			M (Mean±SD)			MP (Mean±SD)		
		IMM-H007	M1	MP	IMM-H		MP	IMM-H007	M1	MP
Artificial gastric juice	0 h	4.23±0.02	ND	ND	ND	5.27±0.24	ND	ND	ND	4.91±0.02
	2 h	4.24±0.20	ND	ND		5.17±0.17	ND	ND	ND	4.66±0.16
	4 h	5.00±0.12	ND	ND		5.51±0.11	ND	ND	ND	4.41±0.13
Artificial intestinal juice	0 h	4.42±0.16	ND	ND	ND	5.95±0.08	ND	ND	ND	5.64±0.06
	2 h	4.46±0.16	ND	ND	ND	5.62±0.07	ND	ND	ND	5.83±0.08
	4 h	4.30±0.11	ND		ND	5.67±0.19	ND	ND	ND	5.55±0.26
Tris-HCl buffer solution	0 h	4.37±0.13	ND		ND	5.08±0.05	ND	ND	ND	4.94±0.23
	2 h	4.38±0.08	ND	N	ND	5.65±0.11	ND	ND	ND	5.15±0.26
	4 h	4.44±0.06	N		ND	5.81±0.01	ND	ND	ND	5.12±0.26

tion, distribution, metabolism, and excretion of the drug *in vivo*[15]. Early drug evaluation, drug design, dosage form modification, optimization of dosing regimen, and guiding clinical rational drug use are of great significance. It is generally believed that the linear kinetic process *in vivo* should have the following characteristics: concentration of drug in the blood decreases exponentially with time; amount of drug transferred in unit time decreases with time; transfer percentage remains unchanged in unit time; half-life is independent of the dose; and $AUC_{(0-t)}$ is proportional to the dose[16]. In the efficacy test using golden hamsters, 50 mg/kg were used as an oral dose and administered twice daily. Therefore, this study selected four doses of 50, 150, 450, and 900 mg/kg for pharmacokinetic studies. After IMM-H007 administration in male and female golden hamsters, the $AUC_{(0-t)}$ of the major metabolites MP and M1 in the whole blood increased with increasing dose with good correlation, indicating that the metabolism of M1 and MP is essentially a linear kinetic process *in vivo* in the selected dose range. Since the blood prototype drug concentration was low, close to, or below, the detection limit after oral or intra... IMM-H007 administration, we used M1 ... to calculate the bioavailability, which was ...% and 4.78%, respectively, in the whole blood of ... golden hamsters and 2.20% and 6.75%, respectively, in female golden hamsters. The bioavailabili... of male and female golden ha... s 6.97% and 8.95% using the sum o... and ..., which was relatively low. There ... three m... possible factors that dictate bio... ility: and chemical propert... o... pound: stereo-chemical struct... solubi... rmeability, etc. (2) gastroint... l environm... here are many transport... mall intestina... pithelial cells, such a... g p, ... affect the transport of drugs in the gastrointe... tract. In addition, abundant ...stinal bacteria ... o involved in the met... ism of drugs *in vivo*. Other factors may also i... de path... gical conditions, drug interactio... l indi... al diff... nces. The specific reasons f... bioava... ity of IMM-H007 need t... be inv... ed fo... study of bile excretion ... testina... etabolism. The drug-time cur... f the pri... metabolites in whole blood afte... l administration and sublingual venous in... M-H007 in golden hamsters was ...gular, likely due to the heterogeneity of gas... testinal absorption and reabsorption of the d... tissue. After drugs are discharged from the g... ic juices into the intestine, a large number of drugs are re-transformed into non-ionic forms due to the neutral pH of the intestinal f... point, transmembrane transport an... bsorpt... are facilitated, so that the plasm... ncentration... has multiple peaks. In addition... fferent parts of the gastrointestinal tract, the org... s exhibit differential permeability fo... rtain di... t can also cause irregular curv... ecause high... trations of drugs in the ...ue ma... be re-rel... into the blood and t... redistri... d throughout the body. This phe... n ... causes ...gular changes for the d... tim... The ri... ring of IMM-H007 co... is H ace... r l... s, which, like other e... tructures, ar... ydrolyzed in animal ... plasma both ... *vitro* and i*n vivo*. Th... re, th... of IMM-H007 biological transformation of *in v*... ological samples helps to ... nd its metab... athway *in vivo*. In th... ork, the relationship between stability and ...ual transforma...on of IMM-H007 and its major ...abolites M1 ... MP was studied in *in vitro* ...gical sample... ing *in vitro* thermolysis. The re... howed t... MM-H007, M1, and MP were stabl... gastric juices, artificial intestinal juices, and Tris-HCl buffer, suggesting that the ...totype drug and metabolites were less affected ... in gastrointestinal pH and digestive ...zym...

Conclusions

In this research, an efficient, sensitive, and reliable LC-MS/MS method was established and applied to pharmacokinetics and related studies after oral or sublingual intravenous IMM-H007 administration in hamsters. The experimental results demonstrated that IMM-H007 is rapidly absorbed through the oral route in hamsters. The C_{max} and $AUC_{(0-t)}$ of the metabolites Ml and MP in male and female hamsters were increased with increasing dose and were proportional to the dose, and $T_{1/2}$ and $MRT_{(0-t)}$ were significantly prolonged with increasing dose, which basically show linear dynamic characteristics and no significant gender differences. The bioavailability in male and female golden hamsters after oral IMM-H007 administration was calculated using the sum of Ml and MP, resulting in 6.97% and 8.95%, respectively. The IMM-H007 and its metabolites were stable in Tris-HCl buffer, artificial gastric juices, and artificial intestinal juices. These findings provide reference for clinical application research.

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Conflict of Interests

The authors declare that they have no conflicts of interest.

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