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Metabolites characterization of a novel DPP-4 inhibitor, imigliptin in humans and rats using ultra-high performance liquid chromatography coupled with synapt high-resolution mass spectrometry

Yang Liu[a,b], Xueting Yao[a,b], Ling Song[a,b], Hongzhong Liu[a,b], Qian Zhao[a,b], Ji Jiang[a,b], Gang Ni[d], Chongtie Shi[c], Xifeng Ma[c], Huimin Zhou[c], Dongyang Liu[a,b,*], Pei Hu[a,b,**]

[a] Clinical Pharmacology Research Center Phase I Unit, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100032, China
[b] Beijing Key Laboratory of Clinical PK and PD Investigation for Innovative Drugs, Beijing 100032, China
[c] XuanZhu Pharma Co., Ltd., Jinan, Shandong, 250101, China
[d] Institete of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, China

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ABSTRACT

Imigliptin has been reported as a novel dipeptidyl-peptidase-IV (DPP-4) inhibitor to treat type 2 Diabetes Mellitus (T2DM), and is currently being tested in clinical trials. In the first human clinical study, imigliptin was well tolerated and proved to be a potent DPP-4 inhibitor. Considering its potential therapeutic benefits and promising future, it is of great importance to study the metabolite profiles in the early stage of drug development. In the present study, a robust and reliable analytical method based on the ultra-high performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF MS) method combined with MassLynx software was established to investigate the characterization of metabolites of imigliptin in human and rat plasma, urine and feces after oral administration. As a result, a total of 9 metabolites were identified in humans, including 6, 9 and 8 metabolites in human plasma, urine, and feces, respectively. A total of 11 metabolites were identified in rats, including 7, 10 and 8 metabolites in rat plasma, urine, and feces, respectively. In addition, 6 of the metabolites detected in humans and rats were phase I metabolites, including demethylation, carboxylation, hydroxylation and dehydrogenation metabolites, and 5 of the metabolites were phase II metabolites, including acetylation and glucuronidation. There was no human metabolite detected compared to those in rats. The major metabolites detected in human plasma (M1 and M2) were products resulting from acetylation, and hydroxylation followed by dehydrogenation. M1 was the major metabolite in rat plasma. M2 and the parent drug were the major drug-related substances in human urine. The parent drug was the major drug-related substances in rat urine. M2, M5 (hydroxylation product) and M6 (2 × hydroxylation and acetylation product) were the predominant metabolites in human feces. M2 and M5 were the major metabolites in rat feces. In addition, renal clearance was the major route of excretion for imigliptin.

1. Introduction

Diabetes has emerged as a major public health problem worldwide; type 2 Diabetes Mellitus (T2DM) is the most common form of diabetes mellitus, which accounts for 80–90% of all diabetes cases and its incidence is increasing [1]. The high frequency of complications associated with the disease leads to significant decrease of life expectancy [2]. As a relatively efficacious therapeutic option, DPP-4 inhibitor suppresses the degradation of GLP-1 to exhibit its bioactivity, including glucose-induced stimulation of insulin biosynthesis and secretion, regulation of gene expression, trophic effects on β cells, inhibition of appetite, and slowing of gastric

* Corresponding author at: Clinical Pharmacology Research Center Phase I Unit, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing 100032, China. Tel: +86 10 6915 8359; fax: +86 10 6915 8365.
** Corresponding author at: Clinical Pharmacology Research Center Phase I Unit, Peking Union Medical College Hospital and Chinese Academy of Medical Sciences, Beijing 100032, China. Tel: +86 10 6915 8366; fax: +86 10 6915 8365.
E-mail addresses: liudongyang@pumch.cn (D. Liu), hubei01_pumch@163.com (P. Hu).

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emptying. Therefore, dipeptidyl-peptidase-IV (DPP-4) inhibitor can decrease blood glucose effectively [3–5].

Imigliptin hydrochloride is a novel DPP-4 inhibitor, which was chemically synthesized by Shandong Xuanzhu Pharma Inc. (Shandong, China) and is currently undergoing phase I clinical trial as an investigational new drug for the potential use in T2DM [6]. In the first human (FIH), single dose escalation clinical study, the LC–MS/MS determination method of imigliptin and its three metabolites was developed and applied to investigate the pharmacokinetic characteristics of imigliptin [7], which suggested that the three metabolites (KBP-3926, KBP-3902, KBP-5493) were not the major metabolites in human. Moreover, the exposure of the three metabolites was less than 5% of parent drug. In addition, mean percentage of drug unchanged in urine (%) from 0 to 72 h was around 40%, which suggested that the metabolism also played an important role in the elimination of imigliptin. The guidance on metabolites in safety testing (MIST) of U.S. Food and Drug Administration [8], and International Conference on harmonization (ICH) [9] encouraged the identification of differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process. Thus, the identification of its metabolites in humans and rats should be investigated at early phase of drug development for safety and efficacy evaluation.

The accurate mass measurements and valuable fragmentation information provided by high-resolution mass spectrometry (HR-MS) significantly contribute to the metabolite characterization [10–15]. In the present study, ultra-high performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF MS) was employed to screen and characterize the metabolites of imigliptin in human and rat plasma, urine and feces. In addition, the data were acquired in the MS^E method, which has been applied to record non-targeted metabolites and facilitated the rapid metabolites identification. A total of 11 metabolites were observed, and five reference substances were synthesized to identify the metabolites, of which two metabolites (M1 and M2) were found to be the major systemic metabolites in human. Metabolic pathways of imigliptin and fragmentation patterns of metabolites were summarized.

2. Experimental

2.1. Reagents and chemicals

Imigliptin (M0, KBP-3853) hydrochloride (purity 99.4%), its metabolites M1 (XZP-3605, purity 100.3%), M2 (XZP-3882, purity 97.7%), M3 (KBP-5493, purity 95.8%), M4 (KBP-3902, purity 96.8%), and M5 (KBP-3926, purity 98.9%) were provided by Shandong Xuanzhu Pharma Inc. (Shandong, China). Formic acid, ammonium formate, and ammonia water were all analytical grade and purchased from Sigma-Aldrich Co. LLC. and Sinopharm Chemical Reagent Co., Ltd (Beijing, China), respectively. Methanol and acetonitrile were of chromatographic grades and were obtained from Burdick&Jackson Lab (New Jersey, USA). Water was prepared by a Milli-Q water purifying system (Millipore, Bedford, USA).

2.2. Clinical study and subjects

The clinical study was conducted at the Clinical Pharmacology Research Center of the Peking Union Medical College Hospital, which was in full compliance with the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines, and all subjects signed the Informed Consent Form before the study. This was a randomized, double-blind, placebo-controlled, single-ascending-dose study in which 60 healthy Chinese subjects were enrolled and received either placebo or oral doses of imigliptin hydrochloride (5–600 mg). Plasma samples were collected at pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h post-dose (doses ranging from 5 to 50 mg), and pre-dose, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, 36, 48, 72, 96 and 120 h post-dose (doses ranging from 100 to 400 mg). The plasma samples used in this study were collected from one subject in 100 mg dose group, at pre-dose and 0.5 h post-dose. Urine samples were collected at pre-dose and over the following post-dose intervals: 0–4, 4–8, 8–12, 12–24, 24–48, 48–72 h (5–50 mg), and pre-dose, 0–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120 h (100–600 mg). The urine samples used in this study were collected from one subject in 100 mg dose group, at pre-dose and 0–24 h post-dose. Feces samples were collected at pre-dose and within 72 h post-dose (25, 50 mg), and within 120 h post-dose (100 mg). The feces samples used in this study were collected from two subjects in 100 mg dose group, at pre-dose, 0–24 and 24–48 h post-dose. All samples were stored at −80 °C.

2.3. Preclinical rat samples collection

The biospecimen of imigliptin in rats was provided by Shandong Xuanzhu Pharma Inc. (Shandong, China). The Sprague-Dawley rats (160–180 g) were randomly divided into four groups with 6 animals per group (n = 3/gender) and administered a single oral dose of imigliptin (4, 10, 25, and 50 mg/kg). Plasma samples were collected at pre-dose and 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dose. The plasma samples used in this study were collected from one rat in 50 mg/kg dose group, at pre-dose, 0.5 and 1 h post-dose. Another 6 rats (200–220 g, n = 3/gender) were administered a single 20 mg/kg oral dose of imigliptin. Urine samples were collected at pre-dose and over the following post-dose intervals: 0–4, 4–8, 8–24 h. Feces samples were collected at pre-dose and within 96 h post-dose. The urine and feces samples in this study were from two rats.

2.4. Sample preparation

A total of 0.2 mL plasma sample was mixed with a 3-fold volume of methanol. After centrifugation at 13.000 rpm for 10 min, the supernatant was evaporated to dryness under a nitrogen stream at 35 °C. These residues were reconstituted with 100 μL of acetonitrile/water containing 5 mM ammonium acetate (5:95, v/v). 50 μL of urine sample was mixed with 450 μL reconsitituted solution. After centrifugation at 13.000 rpm for 10 min, the supernatant was directly injected. Feces samples were weighed at first, and then 4 mL water was added to each 1 g feces, followed by homogenization for 15 min. Then 10 mL of the homogenate was centrifuged for 10 min (3000 rpm, 4 °C). 0.5 mL of the supernatant was mixed with a 3-fold volume of methanol. After centrifugation at 13.000 rpm for 10 min, the supernatant was evaporated to dryness under a nitrogen stream at 35 °C. These residues were reconstituted with 100 μL of acetonitrile/water containing 5 mM ammonium acetate (5:95, v/v).

2.5. UHPLC/Q-TOF HRMS analysis

Samples extracted from biological matrix were analyzed by UHPLC/Q-TOF HRMS method. The UHPLC–MS analysis was performed on a Waters Acquity UHPLC system coupled with a Waters SYNAPT G2-Si High Resolution Mass Spectrometry equipped with electrospray ionization (ESI) source (Waters Corporation, MA, USA).

Chromatographic separation was carried out on an ACQUITY UHPLC BEH C_{18} (50 mm × 2.1 mm, 1.7 μm) column at 35 °C with a flow rate of 0.5 mL/min. The mobile phase consisted of water containing 5 mM ammonium acetate (pH = 7) (A) and acetonitrile (B). The linear gradient profile started from 5% B, followed by a linear