European Journal of Pharmacology 788 (2016) 54–64

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Endocrine pharmacology

Pharmacological profiles of gemigliptin (LC15-0444), a novel dipeptidyl peptidase-4 inhibitor, *in vitro* and *in vivo*

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ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form
8 June 2016
Accepted 10 June 2016
Available online 11 June 2016

Chemical compound studied in this article:
Gemigliptin (PubChem CID: 11953153)
Vildagliptin (PubChem CID: 11077541)
Sitagliptin (PubChem CID: 11591741)

Keywords:
Dipeptidyl peptidase-4 inhibitor
Gemigliptin
Incretin
Type 2 diabetes mellitus

ABSTRACT

Gemigliptin, a novel dipeptidyl peptidase (DPP)-4 inhibitor, is approved for use as a monotherapy or in combination therapy to treat hyperglycemia in patients with type 2 diabetes mellitus. In this study, we investigated the pharmacological profiles of gemigliptin *in vitro* and *in vivo* and compared them to those of the other DPP-4 inhibitors. Gemigliptin was a reversible and competitive inhibitor with a K_i value of 7.25 ± 0.67 nM. Similar potency was shown in plasma from humans, rats, dogs, and monkeys. The kinetics of DPP-4 inhibition by gemigliptin was characterized by a fast association and a slow dissociation rate compared to sitagliptin (fast *on* and fast *off* rate) or vildagliptin (slow *on* and slow *off* rate). In addition, gemigliptin showed at least $>23{,}000$-fold selectivity for DPP-4 over various proteases and peptidases, including DPP-8, DPP-9, and fibroblast activation protein (FAP)-α. In the rat, dog, and monkey, gemigliptin showed more potent DPP-4 inhibitory activity *in vivo* compared with sitagliptin.

In mice and dogs, gemigliptin prevented the degradation of active glucagon-like peptide-1 by DPP-4 inhibition, which improved glucose tolerance by increasing insulin secretion and reducing glucagon secretion during an oral glucose tolerance test. The long-term anti-hyperglycemic effect of gemigliptin was evaluated in diet-induced obese mice and high-fat diet/streptozotocin-induced diabetic mice. Gemigliptin dose-dependently decreased hemoglobin A1c (HbA1c) levels and ameliorated β-cell damage. In conclusion, gemigliptin is a potent, long-acting, and highly selective DPP-4 inhibitor and can be a safe and effective drug for the long-term treatment of type 2 diabetes.

1. Introduction

Type 2 diabetes mellitus is a complex and chronic disease caused by insulin resistance in the peripheral tissues, the inability of the pancreatic β-cells to produce enough insulin to overcome this resistant state, or inappropriate glucagon secretion (DeFronzo, 1988). In recent studies, incretin deficiency/resistance, neurotransmitter dysfunction, and abnormal kidney reabsorption of glucose were also shown to play a key role in type 2 diabetes pathophysiology (Defronzo, 2009). Therefore, pharmacological intervention based on the known pathogenic abnormalities is an important part of the treatment of type 2 diabetes, including lifestyle modifications, such as changes in diet and physical activity levels. Unfortunately, traditional anti-diabetic agents, such as metformin, sulfonylureas, glinides, thiazolidinediones, α-glucosidase inhibitors, and insulin, have several limitations, including weight gain, hypoglycemia, gastrointestinal intolerance, and fluid retention, which may potentially reduce adherence to therapy (Cefalu, 2007).

The dipeptidyl peptidase (DPP)-4 inhibitors are a relatively new class of oral anti-diabetic agents and are widely used in various combination regimens because of their robust efficacy, good tolerability, and overall favorable safety profiles. They prevent the inactivation of endogenously released incretin hormones and prolong their physiological action. Glucagon-like peptide-1 (GLP-1) is the primary incretin hormone released from the L cells and regulates blood glucose via stimulation of glucose-dependent insulin secretion and inhibition of glucagon secretion (Drucker and Nauck, 2006; Nauck et al., 1997; Willms et al., 1996). It was also shown to restore β-cell mass in multiple preclinical studies (De Leon et al., 2003; Wang and Brubaker, 2002; Xu et al., 1999). Consequently, DPP-4 inhibitors improve glycemic control with a

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http://dx.doi.org/10.1016/j.ejphar.2016.06.016
0014-2999/

low risk of hypoglycemia and a neutral effect on body weight based on their incretin-based mechanism of action.

DPP-4 inhibitors with different chemical structures have been developed by several pharmaceutical companies for the treatment of type 2 diabetes. There are eleven DPP-4 inhibitors currently available (sitagliptin, vildagliptin, saxagliptin, linagliptin, alogliptin, anagliptin, teneligliptin, trelagliptin, omarigliptin, evogliptin, and gemigliptin) for clinical use. Although DPP-4 inhibitors share a common mode of action, they differ in terms of their chemical structure, pharmacokinetic, pharmacodynamic and safety profiles, and clinical characteristics (Deacon, 2011). All DPP-4 inhibitors provide sustained DPP-4 inhibition over 24 h with once-daily or once-weekly dosing, except vildagliptin and anagliptin (twice daily) (Addy et al., 2016; Filippatos et al., 2014; Kaku, 2015; McCormack, 2015). The different DPP-4 inhibition profiles of these members have also been associated with different effects on glycemic variability (Rizzo et al., 2012). In addition, saxagliptin and vildagliptin are less selective with regard to inhibition of DPP-8/9, which has been associated with multi-organ toxicities in a non-clinical study (Lankas et al., 2005). These differences should be considered when deciding which DPP-4 inhibitor is appropriate for the individual patient.

Gemigliptin (Zemiglo®, LG Life Sciences, Daejeon, Korea) is a novel DPP-4 inhibitor that was approved for use in patients with type 2 diabetes mellitus in June 2012 in Korea. In our previous clinical studies, gemigliptin improved glycemic control with a low risk of hypoglycemia and a neutral effect on body weight in patients with type 2 diabetes (Kim et al., 2013). It can also be used without the inconvenience of dose adjustment in patient with renal impairment (Shon et al., 2014). Gemigliptin has a half-life of approximately 17 h in humans and can be administered once daily as 50 mg tablet at any time regardless of food intake. The aim of this study is to evaluate the pharmacological profiles of gemigliptin (LC15-0444), a novel DPP-4 inhibitor, *in vitro* and *in vivo* compared with those of the other DPP-4 inhibitors. In addition, we also evaluated the chronic effects of gemigliptin on glycemic control and β-cell function in diabetic mice.

2. Materials and methods

2.1. Chemicals

Gemigliptin (LC15-0444), was synthesized by LG Life Sciences Co., Ltd. (Daejeon, Korea). Sitagliptin (Kim et al., 2005) and vildagliptin (Villhauer et al., 2003) were also synthesized in our laboratories with $>98\%$ purity as confirmed by high-performance liquid chromatography (HPLC) analysis. Gly-Pro-7-amino-4-methylcoumarin (AMC) and Gly-Pro-p-nitroaniline (pNA) were purchased from Bachem (Torrance, CA, USA). Gly-Pro-7-amino-4-trifluoromethyl coumarin (AFC) was purchased from Enzyme Systems Products (Dublin, CA, USA). Streptozotocin (STZ) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. In vitro studies

2.2.1. In vitro enzyme inhibition assay

The DPP-4 enzyme was isolated from Sf21 insect cells transfected with cloned human DPP-4 using an insect virus vector. Another human recombinant DPP-4 was purchased from Enzo Life Sciences (Farmingdale, NY, USA). Human DPP-2, DPP-8, and DPP-9 were also isolated using a similar method to that used for DPP-4. All other enzymes were purchased from Calbiochem (Darmstadt, Germany), except for trypsin, which was from Sigma (St. Louis, MO, USA), and fibroblast activation protein (FAP)-α from Enzo Life Sciences (Farmingdale, NY, USA). A synthetic peptide, Gly-Pro-AFC, was used as a substrate to assess the activity of DPP-2, DPP-4, DPP-8, and DPP-9. The substrate for both elastase and cathepsin G was methoxysuccinyl-Ala-Ala-Pro-Val-AFC, while those for urokinase and trypsin were benzoyl-lle-Glu-Gly-Arg-pNA · HCl and benzyloxycarbonyl-Val-Gly-Arg-AFC, respectively. The inhibitory effect on each enzyme was measured using a fluorescence microplate reader (SpectraMax Gemini, Molecular Devices, Sunnyvale, CA, USA) and represented as IC_{50} values (the concentration of compounds showing 50% inhibition of enzyme activity). IC_{50} values were calculated using a curve-fitting program (GraphPad, La Jolla, CA, USA).

2.2.2. Ex vivo DPP-4 inhibition assays

DPP-4 activity in plasma was measured using a continuous fluorometric assay with the substrate Gly-Pro-AMC as previously described (Villhauer et al., 2003). The amount of AMC produced was analyzed using a FlexStation II384 instrument from Molecular Devices (Sunnyvale, CA, USA) with excitation at 360 nm and emission at 460 nm.

2.2.3. Enzyme kinetics assays

The enzyme kinetic analysis of gemigliptin on DPP-4 was performed using a continuous spectrophotometric assay with the substrate Gly-Pro-pNA as previously described (Wang et al., 2012). Briefly, inhibition of DPP-4 activity was determined by measuring the absorbance (at 390 nm) resulting from the cleavage of the substrate Gly-Pro-pNA by the DPP-4 enzyme under steady-state conditions. Enzyme activity was defined as the slope (in mOD/min) from 5 to 15 min. The inhibition pattern was evaluated using a Lineweaver-Burk plot, and the K_i was determined using a curve-fitting program (GraphPad, La Jolla, CA, USA).

2.2.4. Binding assays

The kinetic study of interactions between the DPP-4 enzyme and DPP-4 inhibitors was performed using a surface plasmon resonance system provided by Biacore 2000 (GE Healthcare Bio-Sciences, Pittsburgh, USA), an instrument that measures the interaction between biological substances in real time and analyzes the coupling and segregation process between substances present on the surface of the sensor chip using a special optical phenomena called SPR (surface plasmon resonance). After immobilizing the human DPP-4 on the chip using 10 mM sodium acetate (pH 5.0) as the coupling buffer, gemigliptin, sitagliptin, or vildagliptin (0, 31.25, 62.5, and 125 nM) was loaded onto the sensor chip. The kinetic values of DPP-4 inhibitors were determined using Biacore 2000 (GE Healthcare Bio-Sciences, Pittsburgh, USA) and were calculated by its program.

2.3. In vivo studies

2.3.1. Ethics statement

All animal experimental procedures were approved by the LG Life Sciences Institutional Animal Care and Use Committee and were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" and institutional standard operating procedures (SOPs).

2.3.2. Effect on plasma DPP-4 activity in rat, dog, and monkey

Gemigliptin, sitagliptin or vehicle (DW, distilled water) was orally administered to male Sprague-Dawley (SD) Crl: CD® rats (Orient Bio Inc., Seongnam, Korea), male beagle dogs (Beijing Marshall Biotechnology Co., Ltd., Beijing, China), and male cynomolgus monkeys (CSIM Co., Ltd., Beijing, China). To determine the pharmacokinetic/pharmacodynamic relationship, the plasma concentration of DPP-4 inhibitors and their inhibition of plasma DPP-4 activity were monitored for 24 h following the oral