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DA-1229, a novel and potent DPP4 inhibitor, improves insulin resistance and delays the onset of diabetes

Mi-Kyung Kim, Yu Na Chae, Ha Dong Kim, Eun Kyoung Yang, Eun Jung Cho, Song-hyen Choi, Ye-Hwang Cheong, Hae-Sun Kim, Heung Jae Kim, Yeong Woo Jo, Moon-Ho Son, Soon-Hoe Kim, Chang Yell Shin *

Dong-A Pharm. Research Center, 47-5 Sanggal-dong, Giheung-gu, Yongin-si, Gyeonggi-do, 446-905, Republic of Korea

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ABSTRACT

Aim: To characterize the pharmacodynamic profile of DA-1229, a novel dipeptidyl peptidase (DPP) 4 inhibitor.
Main methods: Enzyme inhibition assays against DPP4, DPP8 and DPP9. Antidiabetic effects of DA-1229 in HF-DIO mice and young db/db mice.
Key findings: DA-1229 was shown to potently inhibit the DPP4 enzyme in human and murine soluble forms and the human membrane-bound form with IC_{50} values of 0.98, 3.59 and 1.26 nM, respectively. As a reversible and competitive inhibitor, DA-1229 was more selective to human DPP4 (6000-fold) than to human DPP8 and DPP9. DA-1229 (0.1–3 mg/kg) dose-dependently inhibited plasma DPP4 activity, leading to increased levels of plasma GLP-1 and insulin, and thereby lowering blood glucose levels in mice. In high fat diet-fed (HF) mice, a single oral dose of 100 mg/kg of DA-1229 reduced plasma DPP4 activity by over 80% during a 24 h period. Long-term treatment with DA-1229 for 8 weeks revealed significant improvements in glucose intolerance and insulin resistance, accompanied by significant body weight reduction. However, it remains unclear whether there is a direct causal relationship between DPP4 inhibition and body weight reduction. In young db/db mice, the DA-1229 treatment significantly reduced blood glucose excursions for the first 2 weeks, resulting in significantly lower levels of HbA1c at the end of the study. Furthermore, the pancreatic insulin content of the treatment group was significantly higher than that of the db/db control.
Significance: DA-1229 as a novel and selective DPP4 inhibitor improves the insulin sensitivity in HF mice and delays the onset of diabetes in young db/db mice.

Introduction

Incretins are a group of gastrointestinal hormones that increase the amount of insulin released from pancreatic beta cells. Enhancing incretin action is considered to be a novel, yet promising therapeutic approach to the treatment of type 2 diabetes. Therefore, intense research efforts have been made to evaluate incretin-based therapies for clinical development (Drucker and Nauck, 2006).

Glucagon-like peptide 1 (GLP-1) is an incretin hormone released from gut endocrine L cells in response to food ingestion, and serves as a modulator of glucose-stimulated insulin secretion from pancreatic beta cells (Drucker, 2007). GLP-1 has a clearly established role in glucose homeostasis by stimulating insulin biosynthesis and secretion, and inhibiting glucagon release (Deacon et al., 1999; Drucker, 1998). In addition, GLP-1 has been suggested to reduce insulin resistance (Parlevliet et al., 2010).

Active GLP-1 is degraded in the blood stream by dipeptidyl peptidase 4 (DPP4) (Pauly et al., 1996), a serine protease present in the circulation in both soluble and membrane-bound forms (Lambeir et al., 2003). DPP4 renders incretin hormones inactive and truncated by cleaving dipeptides from the N-terminal ends. The degradation of GLP-1 by DPP4 is rapid, and thus explains the short half-life of GLP-1 (Mentlein et al., 1993). Hence an inhibitor of DPP4 can increase the half-life of active GLP-1 and potentially prolong its anti-diabetic effects (Drucker and Nauck, 2006; Mest and Mentlein, 2005). For the past decade, DPP4 inhibitors have emerged as an orally available incretin-based therapy, and in 2006, Sitagliptin was approved as the first-in-class drug in the treatment of type 2 diabetes. Exenatide, a degradation-resistant GLP-1 receptor agonist with a circulating half-life of 60–90 min, shares approximately 50% of its amino acid sequence with mammalian GLP-1, and was approved for the treatment of type 2 diabetes in 2005 (Chen and Drucker, 1997; Kolterman et al., 2005). Exenatide was reported to delay the onset of diabetes in 8 week-old db/db mice through a mechanism involving Akt1 and an expansion of the functional beta-cell mass (Wang and Brubaker, 2002).

* Corresponding author. Tel.: +82 31 280 1362; fax: +82 31 282 8564.
E-mail address: pharm91@donga.co.kr (C.Y. Shin).

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In recent years, several DPP4 inhibitors have been approved or are in the late stages of development for the treatment of type 2 diabetes. DA-1229, a novel and orally available DPP4 inhibitor under development to improve glycemic control in type 2 diabetic patients, was synthesized in an effort to search for more potent and selective DPP4 inhibitors.

In the present study, to ascertain its therapeutic potential, we studied the effects of DA 1229 on insulin resistance and development of diabetes in HF and db/db mice.

Materials and methods

Materials

DA-1229 [(R)-4-[(R)-3-amino-4-(2, 4, 5-trifluorophenyl)-butanoyl]-3-(t-butoxymethyl)-piperazin-2-one, purity≥97.0%; Fig. 1A] tartrate salt was synthesized in Dong-A Pharm. Research Center. Recombinant human and murine soluble DPP4 were purchased from R&D systems (Minneapolis, MN). The human membrane bound form of DPP4 was prepared from Caco-2 cell extracts as previously described (Alfalah et al., 2002). Human DPP8 and DPP9 were prepared from CHO-K1 cells transiently transfected with the human DPP8 or DPP9 expression vectors using lipofectamine and PLUS reagents (Invitrogen, Carlsbad, CA). The full length expression vectors for human DPP8 and DPP9 were purchased from RZPD (Berlin, Germany). To increase the expression efficiency, each target gene was transferred to the pcDNA3.1/V5 vector (Invitrogen, Carlsbad, CA). For DPP8, the target sequence was restored to its wild type state by single point mutagenesis. Crude cell lysates were used as the enzyme sources. Gly-Pro-7-amido-4-methylcoumarin (Gly-Pro-AMC), as a substrate for DPP4, was obtained from Merck (Frankfurt, German). Unless otherwise specified, the used substrates were products of Bachem (Bubendorf, Switzerland). The other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

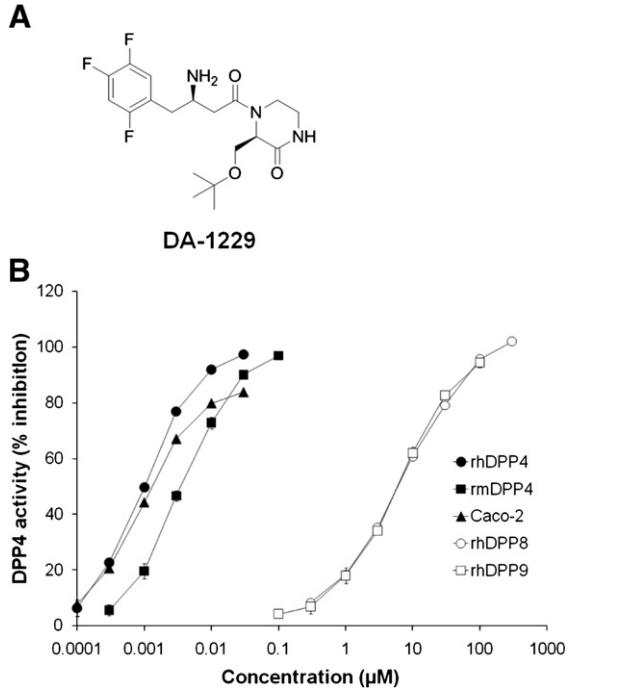

Fig. 1. Chemical structure (A) and the potency and selectivity of DA-1229 in vitro (B). Recombinant enzymes were pre-incubated with DA-1229. The reaction was initiated by adding fluorogenic substrates, such as Gly-Pro-AMC for DPP4 and DPP9 or Ala-Pro-AFC for DPP8. The liberation of 4-methylcoumarine (AMC) or 4-trifluoromethylcoumarin (AFC) was monitored by the increase in fluorescence intensity. The results were represented as the percentage inhibition vs. the control. The data is reported as the mean ± S.E.M. (N = 2–4). The symbols are as follows; ● recombinant human DPP4, ■ recombinant mouse DPP4, ▲membrane bound DPP4, ○ recombinant human DPP8, □ recombinant human DPP9.

Enzyme inhibition assay in vitro

Each enzyme assay was carried out as described elsewhere (Edosada et al., 2006; Lankas et al., 2005; Leiting et al., 2003; Pham et al., 1998).

DPP4

To measure the activity of DPP4, fluorogenic assay was employed using Gly-Pro-AMC, which is cleaved by the enzyme to release fluorescent aminoethylcoumarin (AMC). Compounds were incubated with recombinant DPP4 or Caco-2 lysate and 50 μM Gly-Pro-AMC in a buffer containing 25 mM Tris/HCl, pH 8.0 (1 mg/ml bovine serum albumin was added only for recombinant human DPP4). The assay was performed at 25 °C for 1 h in a total reaction volume of 200 μl. Liberated AMC was detected using an excitation wavelength of 360 nm and an emission wavelength of 465 nm.

DPP8

Crude lysate from human DPP8-overexpressed CHO-K1 cells exhibited approximately 5-fold higher fluorescence than mock lysate 1 h after reaction at 37 °C, where enzyme activity increased in a time-dependent manner. Compounds were tested against lysate containing human DPP8 in 50 mM HEPES buffer, pH 8.0 using 100 μM Ala-Pro-7-amino-4-trifluoromethylcoumarin at 37 °C for 1 h (excitation/emission: 400/505 nm).

DPP9

Crude lysate from human DPP9-overexpressed CHO-K1 cells showed over 10-fold higher fluorescence than mock lysate 1 h after reaction at 25 °C, where enzyme activity increased in a time-dependent manner. Compounds were tested against lysate containing human DPP9 in 50 mM HEPES buffer, pH 8.0 using 100 μM Gly-Pro-AMC at 25 °C for 1 h (excitation/emission: 360/465 nm).

At the end of incubation, fluorescence intensity was measured using a SpectraFluor (Tecan, Mannedorf, Switzerland). The results were represented as the IC_{50} values or the inhibition percentage of enzyme activity. The IC_{50} values were calculated using a nonlinear regression method (SigmaPlot 10.0, SPSS).

Enzyme kinetic assay

The enzyme kinetic study for DPP4 was conducted using a slight modification of the method reported by Kakkar et al. (1999). Different concentrations of DA-1229 were incubated with 20 ng/ml of human DPP4 enzyme and the indicated concentration of Gly-Pro-AMC for 1 h at 25 °C in a 200 μl reaction volume. The fluorescence intensity (excitation wavelength, 360 nm; emission wavelength, 465 nm) was measured every 4 min. The initial reaction rates were calculated and the results were expressed in a Lineweaver–Burk plot (v_0^{-1} vs. $[S]^{-1}$). The Ki value was calculated from a Dixon plot (v_0^{-1} vs. [I]).

The reversibility test was conducted using a slight modification of the method reported by Holt et al. (1992). Eighty ng/ml of the human DPP4 enzyme was preincubated either in the presence or absence of the inhibitor. The final concentrations of DA-1229 and diisopropyl fluorophosphates (DIFP), an irreversible inhibitor, were 100 nM and 270 μM, respectively, which were 100 times higher than their IC_{50} values. After 2 h incubation at 25 °C, the enzyme-inhibitor complex in solution was injected into a Slide-A-Lyzer® Dialysis Cassette (a molecular weight cut-off of 10,000; Pierce, Rockford, IL, USA), and dialyzed against 1000 volumes of the assay buffer (25 mM Tris, pH 8.0) at RT for 30 h. The samples were taken at the indicated time points, and the remaining DPP4 activity was measured using the aforementioned method.