ABSTRACT

BI 1356 [proposed trade name ONDERO; (R)-8-(3-amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione] is a novel dipeptidyl peptidase (DPP)-4 inhibitor under clinical development for the treatment of type 2 diabetes. In this study, we investigated the potency, selectivity, mechanism, and duration of action of BI 1356 in vitro and in vivo and compared it with other DPP-4 inhibitors. BI 1356 inhibited DPP-4 activity in vitro with an IC₅₀ of approximately 1 nM, compared with sitagliptin (19 nM), alogliptin (24 nM), saxagliptin (50 nM), and vildagliptin (100 nM). BI 1356 was a competitive inhibitor, with a Kᵢ of 1 nM. The calculated kₗₒᵣₜ rate for BI 1356 was 3.0 × 10⁻⁵/s (versus 2.1 × 10⁻⁵/s for vildagliptin). BI 1356 was ≥10,000-fold more selective for DPP-4 than DPP-8, DPP-9, amino-peptidases N and P, prolyl oligopeptidase, trypsin, plasmin, and thrombin and was 90-fold more selective than for fibroblast activation protein in vitro. In HanWistar rats, the DPP-4 inhibition 24 h after administration of BI 1356 was more profound than with any of the other DPP-4 inhibitors. In C57BL/6J mice and Zucker fatty (fa/fa) rats, the duration of action on glucose tolerance decreased in the order BI 1356 > (sitagliptin/saxagliptin) > vildagliptin. These effects were mediated through control of glucagon-like peptide-1 and insulin. In conclusion, BI 1356 inhibited DPP-4 more effectively than vildagliptin, sitagliptin, saxagliptin, and alogliptin and has the potential to become the first truly once-a-day DPP-4 inhibitor for the treatment of type 2 diabetes.

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The online version of this article (available at http://jpet.aspetjournals.org) contains supplemental material.

ABBREVIATIONS: GLP, glucagon-like peptide; GIP, glucose-dependent insulinotropic peptide; DPP, dipeptidyl peptidase; BI 1356, (R)-8-(3-amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione; FAP, fibroblast activation protein; OGTT, oral glucose tolerance test; NVP-DPP728, 1-[2-[(S-cyanopyridin-2-yl)amino]ethylamino]acetyl-2-(S)-pyrrolidinedecarbonitrile; AUC, area under the curve.
Dipeptidyl peptidase (DPP) 4 (EC3.4.14.5) is an N-terminal dipeptidyl exopeptidase that exists as both a membrane-bound protein and as a soluble protein in plasma. Under physiological conditions, GLP-1 is rapidly truncated by DPP-4, which is located on the capillary endothelium proximal to the L-cells from which GLP-1 is secreted in the ileum (Ogata et al., 1989; Pauly et al., 1996). Inactivation of GLP-1 and GIP can be successfully prevented by DPP-4 inhibitors, leading to potentiation of their biological activity in various species. Low-molecular weight reversible inhibitors of DPP-4 have been studied for several years (Weber, 2004). DPP-4 inhibitors have been shown clinically to lower blood glucose, increase glucose tolerance, and improve insulin responses to oral glucose challenges in patients with type 2 diabetes (Pratley and Salsali, 2007). BI 1356 (proposed trade name ON-DERO) is a novel xanthine-derived DPP-4 inhibitor that differs structurally from other DPP-4 inhibitors known to be in later stages of clinical development or launched (Fig. 1A) (Eckhardt et al., 2007). In the series of investigations reported here, we describe the in vitro and in vivo profile of BI 1356, in turn comparing it with the DPP-4 inhibitors vildagliptin, sitagliptin, saxagliptin, and alogliptin.

Materials and Methods

This series of investigations looked at the in vitro activity profile of BI 1356 against DPP-4 enzyme extracted from human colon carcinoma (Caco-2), DPP-2 from baby hamster kidney, DPP-8 and DPP-9 from baculovirus-infected Sf9 insect cell cultures, and fibroblast activation protein (FAP) from CDSHuFAP cells as well as against porcine prolyl oligopeptidase, bovine trypsin and thrombin, and human aminopeptidase N, aminopeptidase P, and plasmin. The in vivo profile was investigated in male C57BL/6J mice (9–11 weeks; Janvier Laboratories, Le Genest-St-Ise, France), HanWistar rats (180–190 g; Charles River, Germany), and Zucker fatty rats (9–12 weeks; Charles River, Sulzfeld, France). Animal procedures were approved by the local animal ethics committee and complied with National Institutes of Health guidance (Institute of Laboratory Animal Resources, 1996).

In several of these experiments, BI 1356 was compared with other DPP-4 inhibitors. BI 1356, vildagliptin (Villhauer et al., 2003), sitagliptin (Kim et al., 2005), saxagliptin (Augeri et al., 2005), and alogliptin (Feng et al., 2007) were provided by the Department of Chemical Research, Boehringer Ingelheim Pharma GmbH and Co. KG (Biberach an der Riss, Germany).

In vivo studies, compound administration was by oral gavage using 0.5% aqueous hydroxyethylcellulose as vehicle. Blood glucose was measured with a glucometer or using an automated analyzer after dilution with hemolysis reagent (Cobas Integra 400 plus; Roche Diagnostics, Indianapolis, IN). Commercially available enzyme-linked immunosorbent assay kits were used to measure rat GLP-1 (7–36)amide (Linco Research Inc., St. Charles, MO), rat insulin (Crystal Chem Inc., Downers Grove, IL), and rat pancreatic glucagon (Yanaihara Institute Inc., Awaka, Japan).

In Vitro DPP-4 Inhibition Assay. BI 1356 was extracted from confluent Caco-2 cells. After 5 min of incubation at room temperature with lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 0.04 U/ml aprotinin, 0.5% Nonidet P40, pH 8.0), cells were centrifuged at 35,000 × g at 4°C for 30 min, and the supernatant was stored at −80°C. Assays were performed by mixing 20 μl of appropriate compound dilutions with 50 μl of the substrate for the DPP-4 enzyme, H-Ala-Pro-7-amido-4-trifluoromethylcoumarin (Bachem, Bubendorf, Switzerland; final concentration in the assay, 100 μM) and 30 μl of the Caco-2 cell extract (diluted 1000-fold with 100 mM Tris-HCl, 100 mM NaCl, pH 7.8). Plates were incubated at room temperature for 1 h, and fluorescence was measured at excitation/emission wavelengths of 405/535 nm using a SpectraMax GeminiXS (Molecular Devices, Sunnyvale, CA).

Dissociation kinetics of inhibitors from the DPP-4 enzyme was determined after a 1-h preincubation of Caco-2 cell extracts with high inhibitor concentrations (30 nM for BI 1356, 3 μM for vildagliptin). The enzymatic reaction was then started by adding the substrate H-Ala-Pro-7-amido-4-trifluoromethylcoumarin after a 3000-fold dilution of the preincubation mixture with assay buffer. Under these conditions, the difference in DPP-4 activity at a certain time point in the presence or absence of an inhibitor reflects the amount of this inhibitor still bound to the DPP-4 enzyme. Maximal reaction rates (fluorescence units/sec-onds × 1000) in 10-min intervals were calculated using the SoftMax software of the SpectraMax and corrected for the rate of an uninhibited reaction (∆Fcontrol = ∆Fcontrol − ∆Vinhibitor)/(vcontrol).

Effect on Plasma DPP-4 Activity in Rats. Freely fed Han-Wistar rats were administered either vehicle or single oral doses of a
DPP-4 inhibitor. Blood samples were drawn from the retrobulbar venous plexus under isoflurane anesthesia at serial time points up to 24 h postdose. EDTA plasma was frozen for ex vivo measurement of DPP-4 activity, which was assayed in 5-fold diluted plasma after 10-min incubation as described above.

**Oral Glucose Tolerance Test in Mice.** Overnight fasted C57BL/6J mice were challenged 45 min after compound administration with an oral glucose load (2 g/kg). Blood samples for glucose measurement were obtained by tail bleed predose and at serial time points after the glucose load. To evaluate the duration of the effect on glucose tolerance, vehicle or DPP-4 inhibitors were administered 16 h before the glucose challenge.

**Oral Glucose Tolerance Test in Zucker Fatty Rats.** The animals were cannulated for blood sampling before the oral glucose tolerance test (OGTT). An indwelling catheter was inserted under anesthesia in the right carotid artery, exteriorized through the skin at the back of the neck, and connected to a swivel system. The OGTT was performed 3 days after surgery in overnight fasted, awake, freely moving rats. Vehicle or BI 1356 was orally administered either 24 h or 30 min before an oral glucose challenge. Blood samples were immediately supplemented with 100 μM DPP-4 inhibitor NVP-DPP728 (Villhauer et al., 2002) as well as with a protease inhibitor cocktail (obtained from Sigma-Aldrich, St. Louis, MO) to prevent degradation of active GLP-1.

**Effect on Basal GLP-1 Levels in Zucker Fatty Rats.** Animals were cannulated for blood sampling as described above. BI 1356 was orally administered at a dose of 3 mg/kg in the afternoon, and food was subsequently withdrawn. Serial blood samples were taken the next morning (15–23 h after administration). The animals received regular chow diet 17 h postdose and were allowed to eat ad libitum for the rest of the study.

**Data Analysis.** The two-sided unpaired Student’s t test was used for statistical comparison of the control group and the active group with the 5% level as the limit of statistical significance. The half-maximal inhibitory concentration (IC_{50}), median effective dose (ED_{50}), and k_{off} rates were calculated with the Prism program (GraphPad Software Inc., San Diego, CA).

**Supplemental Data.** Supplemental data include two figures and a description of in vitro inhibition assays for DPP-2, -8, -9, FAP, aminopeptidases N and P, prolyloligopeptidase, plasmin, trypsin, and thrombin and can be found with this article online.

**Results**

**BI 1356 Is a Potent and Competitive DPP-4 Inhibitor in Vitro.** BI 1356 inhibited DPP-4 activity in vitro in several independent experiments with IC_{50} values of 0.4, 0.5 (Fig. 1B), 0.9, and 1.1 nM (mean IC_{50}, approximately 1 nM). When tested under identical experimental conditions, vildagliptin, saxagliptin, sitagliptin, and alogliptin exhibited a comparable maximal efficacy for in vitro inhibition of the DPP-4 protease. However, the potency of BI 1356 was higher than for each of the other DPP-4 inhibitors, which yielded IC_{50} values of 19 (sitagliptin), 24 (alogliptin), 50 (saxagliptin), and 62 (vildagliptin) nM, respectively (Fig. 1B). When the DPP-4 enzyme initial reaction rates in the presence of various concentrations of substrate and BI 1356 or vildagliptin were analyzed according to Lineweaver-Burk and Eadie-Hofstee, the data indicated that both compounds inhibit the DPP-4 enzyme by competition for the substrate binding site. Assuming competitive inhibition, the calculated K_i values confirmed the higher potency of BI 1356 versus vildagliptin (K_i = 1 and 10 nM, respectively; Supplemental Figs. S1 and S2).

**BI 1356 Has a Long Off-Rate from the DPP-4 Enzyme.** Figure 2 shows that the enzyme inhibition rate declines faster for vildagliptin than for BI 1356. The calculated k_{off} rate for BI 1356 is 3.0 × 10^{-7}s^{-1}, which is approximately 10-fold slower than the off-rate for vildagliptin (2.1 × 10^{-4}s^{-1}).

**Selectivity of BI 1356.** BI 1356 possesses at least 10,000-fold selectivity for DPP-4 relative to other dipeptidyl peptidases (Table 1), including DPP-8 and DPP-9. Likewise, the selectivity of BI 1356 versus aminopeptidases N and P, prolyloligopeptidase, and the proteases trypsin, plasmin, and thrombin is more than 10,000-fold. BI 1356 inhibited FAP with an IC_{50} of 89 nM (approximately 90-fold selectivity versus DPP-4). Vildagliptin, sitagliptin, and alogliptin had IC_{50} values for FAP inhibition > 10 μM and saxagliptin > 1 μM (data not shown).

**BI 1356 Is a Potent and Long-Acting DPP-4 Inhibitor in Vivo.** In HanWistar rats, BI 1356 dose-dependently inhibited the DPP-4 enzyme in plasma within 30 min of administration (Fig. 3). Doses of 3 and 10 mg/kg achieved approximately 90% inhibition of plasma DPP-4 activity was calculated to be 0.3 mg/kg 7 h postdose and 0.9 mg/kg 24 h postdose. A full dose range for inhibition of plasma DPP-4 activity in the Han-Wistar rat was also established for saxagliptin, alogliptin, vildagliptin, and sitagliptin up to 24 h after administration (data not shown). Figure 4 shows as examples the remaining plasma DPP-4 activity 7 and 24 h after dosing of 1 or 10 mg/kg.

**Table 1**

<table>
<thead>
<tr>
<th>Peptidase</th>
<th>IC_{50} Values of Triplicate Determinations</th>
<th>nM</th>
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</thead>
<tbody>
<tr>
<td>DPP-2</td>
<td>&gt;100,000</td>
<td></td>
</tr>
<tr>
<td>DPP-8</td>
<td>&gt;100,000</td>
<td></td>
</tr>
<tr>
<td>DPP-9</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>FAP</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>Aminopeptidase N</td>
<td>&gt;100,000</td>
<td></td>
</tr>
<tr>
<td>Aminopeptidase P</td>
<td>&gt;10,000</td>
<td></td>
</tr>
<tr>
<td>Prolyloligopeptidase</td>
<td>&gt;100,000</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>&gt;100,000</td>
<td></td>
</tr>
<tr>
<td>Plasmin</td>
<td>&gt;100,000</td>
<td></td>
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<tr>
<td>Thrombin</td>
<td>&gt;100,000</td>
<td></td>
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</tbody>
</table>
mg/kg for the various inhibitors. Plasma DPP-4 activity 7 h postdose is comparably inhibited by 70 to 80% with the lower dose and by approximately 90% with the higher dose for BI 1356, saxagliptin, alogliptin, and vildagliptin. Sitagliptin produced less inhibition at the respective doses. However, when plasma DPP-4 activity was analyzed 24 h after dosing, the inhibition achieved by BI 1356 was more profound than with any of the other DPP-4 inhibitors. The ratio between the ED50 values obtained 24 and 7 h after dosing confirms that BI 1356 exhibits the lowest decline in potency with time (Table 2).

A Long-Lasting Effect on Glucose Tolerance Distinguishes BI 1356 from Other DPP-4 Inhibitors. When BI 1356 was given orally at a dose of 1 mg/kg 45 min before the glucose challenge, glucose excursion was significantly reduced by approximately 50% (Fig. 5, A and B). The same improvement of glucose tolerance was also observed for vildagliptin, sitagliptin, and saxagliptin at the identical dose. After prolonging the time between compound administration and glucose challenge to 16 h, BI 1356 at 1 mg/kg was still able to significantly reduce glucose excursion by approximately 20 to 30% (Fig. 6). However, vildagliptin (Fig. 6, A and D), sitagliptin (Fig. 6, B and D), or saxagliptin (Fig. 6, C and D) did not yield any improvement of glucose tolerance 16 h after administration of the same dose. Sitagliptin and saxagliptin showed comparable long-lasting effects as BI 1356 only when the dose administered was 10-fold increased. Vildagliptin remained without long-lasting effect on glucose tolerance even at a dose of 10 mg/kg. The duration of action decreases in the order BI 1356 > (sitagliptin, saxagliptin) > vildagliptin.

**Effects of BI 1356 on Glucose-Stimulated GLP-1 and Insulin Levels in Vivo.** Total glucose excursion was reduced by 29% during an OGTT in Zucker rats 30 min after dosing of BI 1356 (Fig. 7, A and C). This glucose suppression was preceded by an increase in peak levels of active GLP-1, whereby its AUC was increased by 136% (Fig. 7, D and F).

### Table 2

<table>
<thead>
<tr>
<th>BI 1356</th>
<th>Saxagliptin</th>
<th>Alogliptin</th>
<th>Vildagliptin</th>
<th>Sitagliptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 h Postdose</td>
<td>0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>24 h Postdose</td>
<td>0.9</td>
<td>2.7</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Ratio 24/7 h</td>
<td>3</td>
<td>27</td>
<td>100</td>
<td>28</td>
</tr>
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</table>

Fig. 3. Inhibition of DPP-4 activity ex vivo in plasma obtained from HanWistar rats after single oral administration of BI 1356 at different doses. Data are means ± S.E.M. (n = 5/group).

Fig. 4. Inhibition of plasma DPP-4 activity in HanWistar rats after single oral dosing of various inhibitors. The compounds were administered at 1 and 10 mg/kg, and DPP-4 activity in plasma was measured 7 and 24 h after dosing. Data are means ± S.E.M. (n = 5–10/group).

Fig. 5. OGTT in C57BL/6J mice after oral administration of various DPP-4 inhibitors at a dose of 1 mg/kg (A). Compound administration was 45 min before the glucose challenge. The suppression of reactive glucose AUC between 0 and 120 min is shown in B. Data are means ± S.E.M.; n = 6/group; ***, p < 0.001 versus control.
Subsequent to the GLP-1 rise, peak insulin levels were elevated. The insulin AUC was 47% above control animals (Fig. 7, G and I). These data support the association between inhibition of the DPP-4 enzyme by BI 1356, an increase in plasma GLP-1 levels followed by an increase in plasma insulin, and the resulting improvement of glucose tolerance.

During another OGTT 24 h after administration of BI 1356, total glucose excursion was significantly suppressed by 12% (Fig. 7, B and C). As in the acute setting, peak levels for active GLP-1 (Fig. 7E) and insulin (Fig. 7H) were elevated after the glucose challenge, and the corresponding AUC values were increased by 77% for GLP-1 (Fig. 7F) and by 26% for insulin (Fig. 7I). These data suggest that the underlying mechanism for improvement of glucose tolerance by BI 1356 is identical immediately (30 min) and 24 h after dosing.

**Effect of BI 1356 on Basal GLP-1 Levels.** A careful investigation of the GLP-1 time course in the OGTT in Zucker fatty rats (Fig. 7, D and E) revealed a tendency for elevated active GLP-1 levels after administration of BI 1356 but before the glucose challenge. Furthermore, active GLP-1 levels remained at their elevated basal level after the glucose-induced peak. In contrast, basal insulin levels were not increased, and peak levels returned to control values (Fig. 7, G and H).

After an overnight fast, basal active GLP-1 levels were elevated (Fig. 8A). The rise in active GLP-1 after feeding a normal chow diet was much less pronounced than the sharp peak after a glucose challenge (Fig. 7E). However, the concentration of active GLP-1 in the plasma remained elevated in the animals having been pretreated with BI 1356 compared with control animals in all prandial and postprandial phases throughout the day. Fasting and postprandial blood glucose levels were not different between the groups (data not shown).

In contrast to active GLP-1, insulin levels were not increased in the fasting state after treatment with BI 1356 (Fig. 8B). However, when feeding was resumed, insulin concentrations increased sharply above control levels and returned to baseline control values in the following postprandial phase. A second peak in insulin levels was observed in animals that had received BI 1356, 5 h after feeding was resumed. Plasma glucagon levels tended to be lower in the postprandial phase (0.20 versus 0.27 ng/ml; data not shown), in accordance with an effect of increased active GLP-1 on inhibition of glucagon release.

**Discussion**

Inhibition of DPP-4 augments the action of GLP-1 and returns glucose homeostasis toward physiological control levels (Pratley and Salsali, 2007). Several DPP-4 inhibitors are in development or approved for use in the treatment of type 2 diabetes. Comparisons between DPP-4 inhibitors (or other similar compounds) are complicated by variations in experimental conditions described, so we tested BI 1356, vildagliptin, sitagliptin, saxagliptin, and alogliptin under identical conditions. BI 1356 inhibited DPP-4 more potently than all other inhibitors tested, with IC_{50} and K_i values of approximately 1 nM. BI 1356 also demonstrated an approximately 10-fold slower rate of decline in DPP-4 enzyme inhibition than vildagliptin. Because vildagliptin dissociates faster from the DPP-4 enzyme than BI 1356, it can also be more readily displaced by a substrate, and this is reflected in the large difference of the K_i and IC_{50} values for vildagliptin (10
versus 62 nM) because $K_i$ (in contrast to $IC_{50}$) is independent from the substrate concentration present in the assay for DPP-4 activity.

Both vildagliptin and BI 1356 competitively inhibit DPP-4, but unlike vildagliptin, BI 1356 remained tightly but not irreversibly associated with the DPP-4 enzyme, even in the presence of only low free inhibitor concentrations. Assuming that the association with the DPP-4 enzyme is similar for BI 1356 and vildagliptin, the 10-fold lower $k_{off}$ rate of BI 1356 could explain its lower $K_i$ value. Tight binding inhibitors are important from a pharmacological perspective because once bound to their target, they inhibit the enzyme function even after the free drug has been cleared from the circulation or the specific site of action. Vildagliptin has been hailed as a once-daily DPP-4 inhibitor (Demuth et al., 2005; Ristic et al., 2005). We have shown that although BI 1356 does not undergo irreversible binding, it is more potent and longer acting than vildagliptin. These characteristics may support a longer, truly once-a-day, 24-h inhibition clinical profile.

The potency and duration of BI 1356 action was confirmed in rats. Plasma DPP-4 inhibition was comparable for BI 1356, saxagliptin, alogliptin, and vildagliptin 7 h postdose (sitagliptin produced less inhibition, in agreement with its reported high clearance in rats; Kim et al., 2005). However, BI 1356 had the longest duration of action and achieved more profound plasma DPP-4 inhibition 24 h after dosing than any of the other DPP-4 inhibitors tested. Furthermore, this translated into longer lasting improvement of glucose tolerance in C57BL/6J mice (compared with sitagliptin, saxagliptin, and vildagliptin) and Zucker fatty rats, a model of obesity and insulin resistance; Pénaud et al., 1991). BI 1356 inhibited plasma DPP-4 activity and glucose excursion even after 24 h.
ment of glucose tolerance and augmentation of the insulin secretion, and that the elevated insulin levels contributed to the improvement of glucose tolerance. Indeed, the improvement of glucose tolerance by BI 1356 was shown in the animals that had received BI 1356 is assumed to represent a second major prandial phase. These data are in line with the glucose-dependent insulinoform action of active GLP-1, which does not result in permanently elevated insulin levels.

Small amounts of active GLP-1 are permanently secreted by intestinal cells but are rapidly degraded by DPP-4. We postulate that longer lasting inhibition of DPP-4 by BI 1356 causes minor amounts to accumulate, resulting in elevated levels of active GLP-1 even in the absence of nutrient stimuli. These increased basal GLP-1 levels are expected to exert positive effects on pancreatic α- and β-cells. In α-cells, they diminish glucagon secretion, which is elevated in diabetics, leading to an increase in hepatic glucose production. In β-cells, they stimulate neogenesis and regeneration, partly by inhibiting apoptosis. It is possible therefore to postulate that increasing basal levels of GLP-1 may provide the conditions for β-cell regeneration (Farilla et al., 2003; Li et al., 2003). It may also be possible that the elevated basal GLP-1 levels enhance the readiness and responsiveness of the β-cell and contribute to the rise in insulin after the glucose stimulus. To our knowledge, our data show for the first time an increase in basal GLP-1 levels even 24 h after single dosing of a DPP-4 inhibitor.

An important feature of any new agent is its selectivity, and this may be particularly true of the DPP-4 inhibitors class. DPP-4 is a member of a family of serine peptidases including FAP and DPP-8 and -9 (Park et al., 1999; Abbott et al., 2000; Ajami et al., 2004). DPP-8 and -9 inhibition is associated with multiorgan toxicities and profound immuno-toxicity in rats and dogs (Lankas et al., 2005). Given the high degree of homology of DPP-8 and DPP-9 across species and the finding that a selective DPP-8 and -9 inhibitor attenuates T-cell activation in a human in vitro system, it is reasonable to speculate that some liabilities of DPP-8 and -9 inhibition may be observed in humans (Lankas et al., 2005). In the present study, we demonstrated that BI 1356 possesses at least 10,000-fold selectivity for DPP-4 relative to DPP-8 and -9.

BI 1356 was less selective for FAP than for other serine proteases (IC₅₀ = 89 nM, which is approximately 90-fold selectivity versus DPP-4), in contrast to vildagliptin, sitagliptin, alogliptin (IC₅₀ values for FAP inhibition > 10 μM), and saxagliptin (IC₅₀ > 1 μM; data not shown). FAP is expressed by the activated stromal fibroblasts in many epithelial cancers but not by most normal adult human tissues examined (Rettig et al., 1988; Garin-Chesa et al., 1990; Dolznig et al., 2005). FAP expression is regulated during embryonic development and wound healing; however, the biological function...
of FAP is still unclear. An inconspicuous phenotype was observed in the fap−/− mice (Niedermeyer et al., 2001). It is noteworthy that a postulated tumor suppressor activity of FAP seems to be independent of its enzymatic activity (Ramirez-Montagut et al., 2004). It is suggested, therefore, that the tumor suppressor activity of FAP would not be affected by BI 1356 even if it would be present at FAP-inhibiting concentrations. However, in clinical studies, the steady-state plasma concentration of BI 1356 at therapeutically relevant doses was approximately 10-fold lower than the IC50 value for FAP, and BI 1356 was well tolerated up to very high doses (Forst et al., 2007).

In summary, BI 1356 is a competitive, selective, potent, and long-acting DPP-4 inhibitor with long-lasting effects on glucose tolerance through control of GLP-1 and insulin. BI 1356 has a low dissociation rate from the DPP-4 enzyme, is more potent than other DPP-4 inhibitors, and is therefore expected to be effective at low therapeutic doses. Its long duration of action means it has the potential to be the first truly once-a-day DPP-4 inhibitor for the treatment of type 2 diabetes, providing full 24-h DPP-4 inhibition coverage and a rise in basal GLP-1 levels. The effects of BI 1356 after multiple dosing in different animal models of diabetes are the subject of further studies, which are currently ongoing.

Acknowledgments

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References


Address correspondence to: Dr. Leo Thomas, Boehringer Ingelheim Pharma GmbH and Co. KG, Department of Metabolic Diseases Research, Birkendorfer Straße 65, D-88397 Biberach, Germany. E-mail: leo.thomas@boehringer-ingelheim.com