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Discovery of a novel protein tyrosine phosphatase-1B inhibitor, KR61639: potential development as an antihyperglycemic agent

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Abstract

Protein tyrosine phosphatase-1B (PTP-1B), a negative regulator of insulin signaling, may be an attractive therapeutic target for type 2 diabetes mellitus. High throughput screening (HTS) for PTP-1B inhibitors using compounds from the Korea Chemical Bank identified several hits (active compounds). Among them, a hit with 1,2-naphthoquinone scaffold was chosen for lead development. KR61639, {4-[1-(1*H*-indol-3-yl)-3,4-dioxo-3,4-dihydro-naphthalen-2-ylmethyl]-phenoxy}-acetic acid *tert*-butyl ester, inhibited human recombinant PTP-1B with an IC_{50} value of 0.65 μM in a noncompetitive manner. KR61639 showed modest selectivity over several phosphatases and increased insulin-stimulated glycogen synthesis in HepG2 cells and stimulated 2-deoxyglucose uptake in 3T3/L1 adipocytes. In addition, in vivo study using ob/ob mouse demonstrated that KR61639 exerted a hypoglycemic action when given orally. Thus, KR61639 may be a good starting point for lead optimization in developing a novel antidiabetic agent.

Keywords: PTP-1B (protein tyrosine phosphatase-1B); 1,2-Naphthoquinone; Deoxyglucose uptake; Glycogen incorporation; ob/ob mouse

1. Introduction

Protein tyrosine phosphatases (PTPs) are involved in the downregulation of cellular signal transduction mediated by receptor tyrosine kinases such as insulin receptor and epidermal growth factor receptor (Burke and Zhang, 1998). PTP-1B, a member of the PTP family, is thought to function as a negative regulator of insulin signal transduction. PTP-1B directly interacts with activated insulin receptor or insulin receptor substrate-1 (IRS-1) to dephosphorylate phosphotyrosine residues, resulting in downregulation of insulin action (Goldstein et al., 2000).

PTP-1B $_{-/-}$ mice show enhanced insulin sensitivity in glucose and insulin tolerance tests, and the insulin sensitivity remains without weight gain if the mice are fed a high fat diet, indicating that PTP-1B is a major player in the modulation of insulin sensitivity (Elchebly et al., 1999; Klaman et al., 2000). Increased expression of PTP-1B in adipose tissue and muscle of obese humans and rodents is thought to be related to insulin resistance (Wu et al., 2001), whereas the increased insulin sensitivity from weight loss is accompanied by reduced PTP-1B activity (Ahmad et al., 1997). Further evidence for the involvement of PTP-1B in insulin resistance was provided by cell line studies. PTP-1B overexpression in rat primary adipose tissues and 3T3/L1 adipocytes has been shown to decrease insulin-sensitive Glut4 translocation (Chen et al., 1999) and insulin receptor and IRS-1 phosphorylation (Venable et al., 2000), respectively.

Recent reports indicate that treatment of diabetic animals with PTP-1B antisense oligonucleotides ameliorates hyperglycemia and hyperinsulinemia, and clinical trials for these oligonucleotides are in progress (Zinker et al., 2002). Vanadium-containing compounds, non-selective inhibitors of PTPs, have also been shown to normalize blood glucose level in diabetes (Evans and Jallal, 1999). Several PTP-1B inhibitors have been described, including difluoromethylene phosphonates, 2-carbomethoxybenzoic acids, 2-oxalylaminobenzoic acids and several lipophilic compounds (Burke et al., 1994; Liljebris et al., 2002; Andersen et al., 2000; Wrobel et al., 1999).

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Previously, we reported the structure–activity relationship of 1,2-naphthoquinones derived from HTS as PTP-1B inhibitors with a novel scaffold (Ahn et al., 2002). In the present study, we further characterized various biological effects of KR61639, the representative of the 1,2-naphthoquinone scaffold compounds derived from the HTS hit. In vitro enzyme inhibition assay and the cell-based assay indicate that the compound is functionally active as a PTP-1B inhibitor, and in vivo animal study shows that the compound acts as a hypoglycemic agent.

2. Materials and methods

2.1. Materials

Phosphotyrosyl dodecapeptide, TRDIpYETDpYpYPRK, corresponding to amino acids 1142–1153 of the insulin receptor regulatory domain, was custom-synthesized by AnaSpec (San Jose, CA). All chemicals were purchased from commercial suppliers: Tris, HEPES, dimethylsulfoxide (DMSO), dithiothreitol, EDTA, NaCl, $MnCl_2$, KCl, $CaCl_2$, $MgSO_4$, Na_2HPO_4, bovine serum albumin, $NaHCO_3$, malachite green, glycogen, methylisobutylxanthine (IBMX), dexamethasone, ammonium molybdate, phloretin, and protein phosphatase 1 (PP1) (Sigma-Aldrich); isopropyl β-D-thiogalactoside (IPTG) and protein phosphatase 2A (PP2A) (Promega); fluorescein diphosphate (FDP) (Molecular Probe); phosphate-buffered saline (PBS), Dulbecco's modified Eagle's media (DMEM), MEM, FBS and insulin (Gibco BRL); leukocyte antigen-related PTP (LAR), vaccinia H1-related phosphatase (VHR) and *Yersinia enterocolitica* PTP (Yop) (Biomol); 2-deoxy-D-[^{3}H]glucose and D-[U-^{14}C]glucose (Perkin Elmer); carboxymethylcellulose (CMC) (Showa). The catalytic domains of CD45 phosphatase (residues 641–1268), Cdc25A (residues 336–523) and Cdc25B (residues 378–566) phosphatases were expressed in *E. coli* and purified on a glutathione Sepharose column. Experimental conditions for animal studies conformed with the European Community guidelines for the use of experimental animals.

2.2. PTP-1B HTS and enzyme assay

In order to carry out PTP-1B HTS, the cDNA encoding the catalytic domain of PTP-1B (residues 1–322) was cloned into the *Nde*I site of pET14b, and expressed in *E. coli* BL21. After overnight culture with 0.1 mM IPTG for induction, the histidine-tagged PTP-1B fusion protein was purified from bacterial lysates by using a nickel-chelated affinity column (Lee et al., 1998). PTP-1B HTS was done by using compounds from Korea Chemical Bank with HTS automation instruments. The enzyme assay for PTP-1B was carried out on 96-well plates. To each well (final volume: 200 μl) was added 20 μM FDP and 0.1 μg of PTP-1B in a buffer containing 30 mM Tris (pH 8.0), 75 mM NaCl, 0.67 mM EDTA and 1 mM dithiothreitol with or without test compounds. Following incubation at room temperature for 1 h, the fluorescence released by enzyme catalysis was measured at 485 nm (excitation) and 538 nm (emission) by using a fluorometer (Synergy HT, BioTek).

To confirm the inhibitory activity of the compounds selected from the initial HTS, an assay based on the dodecapeptide substrate was performed, where the release of inorganic phosphate was monitored by the malachite green-ammonium molybdate method (Wrobel et al., 1999). After preincubation (10 min at 37 °C) of the enzyme (0.5 μg) with or without inhibitors in a buffer containing 30 mM Tris–HCl (pH 7.4), 2 mM EDTA and 1 mM dithiothreitol (total reaction volume: 395 μl), aliquots (39.5 μl) were taken and incubated with the peptide substrate (final concentration: 50 μM) for 30 min at 37 °C. The reaction was terminated by the addition of the malachite green (0.45%)-ammonium molybdate (4.2%) reagent. The color was allowed to develop at room temperature for 30 min, and the absorbance at 620 nm was measured using a plate reader (Benchmark, BioRad). In all enzyme assays, the DMSO concentration was 5% and did not affect enzyme activity.

2.3. PTP isozyme assay of KR61639

For CD45 and LAR enzyme assays, each enzyme (CD45, 0.05 μg/well; LAR, 0.4 μg/well) was incubated in a buffer containing 30 mM Tris–HCl (pH 7.0), 75 mM NaCl, 0.67 mM EDTA, 1 mM dithiothreitol and 20 μM FDP with or without the inhibitor. For PP1 and PP2A, PP1 (0.2 μg/well) or PP2A (0.02 μg/well) was incubated in a buffer containing 25 mM Tris–HCl (pH 7.5), 5 mM dithiothreitol, 0.5 mM $MnCl_2$ and 35 μM FDP. For Cdc25A and Cdc25B, Cdc25A (1.0 μg/well) or Cdc25B (0.2 μg/well) was incubated in a buffer containing 30 mM Tris–HCl (pH 8.5), 75 mM NaCl, 0.67 mM EDTA, 1 mM dithiothreitol and 20 μM FDP. For Yop, the enzyme (5 ng/well) was incubated in a buffer containing 50 mM sodium citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, 1 mM dithiothreitol and 20 μM FDP. For VHR, the enzyme (0.1 μg/well) was incubated in a buffer containing 50 mM HEPES (pH 7.2), 1 mM EDTA, 1 mM dithiothreitol, 0.05% NP-40 and 20 μM FDP. Incubation was continued for 30 min (CD45, LAR, PP1 and PP2A) or 60 min (Cdc25A, Cdc25B, Yop and VHR) at room temperature, and the fluorescence released from FDP was measured at 485 nm (excitation) and 538 nm (emission).

2.4. Inhibition kinetics

Initial rates at five different inhibitor concentrations (0, 1, 2.5, 5 and 7.5 μM) were measured. For each inhibitor concentration, measurements were performed at five different concentrations of FDP (2, 5, 10, 20 and 40 μM) in a buffer containing 50 mM Tris (pH 7.5), 125 mM NaCl and 1 mM

dithiothreitol. The inhibition pattern was evaluated, and K_i was determined using a direct curve-fitting program (GraFit Software).

2.5. Glucose incorporation into glycogen

HepG2 cells (human hepatoma cells) were seeded into six-well plates to a seeding density of 400,000 cells/well. Subconfluent cells were washed three times with serum-free MEM medium containing 0.05% FBS and incubated with 2 ml of the medium for 4 h. Cells were then incubated with or without the inhibitor for 1 h, and further incubated with or without 10 nM insulin for 30 min at 37 °C. D-[U-^{14}C]glucose (0.4 μCi) was added to each well and incubated for a further 3 h. At the end of the incubation, cells were washed with ice-cold PBS and then solubilized with 1 ml of 0.03% sodium dodecyl sulfate (SDS). The solubilized cell lysates were transferred to 15-ml Falcon tubes and boiled for 60 min in the presence of 100 μl glycogen (20 mg/ml). After adding 3 ml of 95% alcohol and shaking at 4 °C overnight, glycogen was pelleted, and radioactivity from the recovered glycogen was counted.

2.6. Deoxyglucose uptake in 3T3/L1 adipocytes

3T3/L1 preadipocytes were seeded into 24-well plates to a seeding density of 20,000 cells/well. After being grown to confluency, preadipocytes were differentiated by the addition of 5 μg/ml insulin, 0.5 mM IBMX and 0.25 μM dexamethasone for 4–7 days. The differentiated 3T3/L1 adipocytes were incubated with serum-free DMEM for 4.5 h, and washed with the Krebs ringer bicarbonate buffer (KRB; 118 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM Na_2HPO_4, 2% bovine serum albumin, 0.5 mM glucose, 25 mM $NaHCO_3$, pH 7.4). The washed cells were incubated in the same buffer with 10 nM insulin with or without the inhibitor. After a 30-min incubation at 37 °C, 0.1 mM 2-deoxy-D-[^{3}H]glucose (2 μCi) in KRB was added to the incubation medium. After incubation for 10 min at 37 °C, cells were treated with ice-cold KRB containing 0.2 mM phloretin, washed with KRB three times, and solubilized by 0.1% SDS. Radioactivity was counted for the measurement of glucose uptake.

2.7. In vivo glucose lowering effect

Ob/ob mice were bred at in-house facilities. Mice of 9–10 weeks were used to assess the effect of the inhibitor on blood glucose level after their body weights and blood glucose levels were matched. The inhibitor suspended in 0.5% CMC was administered orally to the mice once a day. At the 1st, 4th and 7th days, blood glucose concentration was measured. Control mice received the vehicle (0.5% CMC) only.

2.8. Statistical analysis

Data are expressed as means ± S.E.M., and statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test.

3. Results

3.1. PTP-1B HTS

To discover novel scaffolds for PTP-1B inhibitors, a chemical library of approximately 40,000 compounds (Korea Chemical Bank) was screened by HTS techniques using FDP as the substrate. Of the compounds tested, 72 inhibited the enzyme with IC_{50} values less than 10 μM: the most active one had an IC_{50} value of 1.7 μM. The hit rate of the assay was 0.18%. Among five novel scaffolds discovered, a 1,2-naphthoquinone derivative was chosen for further evaluation. Several hit derivatives of the 1,2-naphthoquinone were tested for PTP-1B inhibitory activity, and KR61639 was selected as a lead compound. The structure of KR61639 is shown in Fig. 1A.

As shown in Fig. 2A, KR61639 inhibited PTP-1B in a concentration-dependent manner, and its IC_{50} value was determined to be 0.65 μM. As reference compounds, vanadate and KR61170 were used. KR61170, a 11-arylbenzo[*b*]naphtho[2,3-*d*]furan derivative (the structure shown in Fig. 1B), was previously reported to be a potent PTP-1B inhibitor with an IC_{50} value of 83 nM (Wrobel et al., 1999). We synthesized KR61170 in our laboratory, and used it as one of the reference compounds. Under our experimental conditions, KR61170 and vanadate showed IC_{50} values of 0.26 and 4.6 μM, respectively.

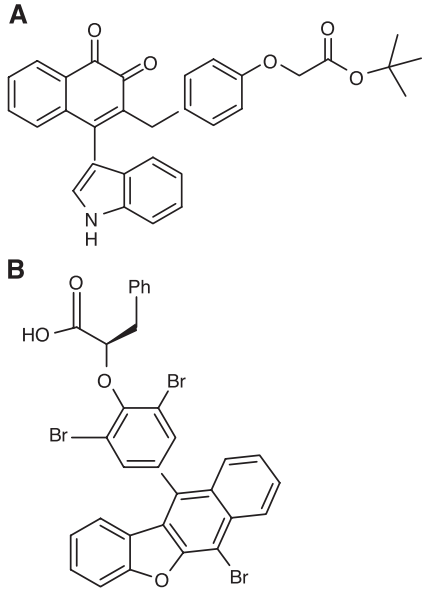

Fig. 1. Chemical structures of KR61639 (A) and KR61170 (B).

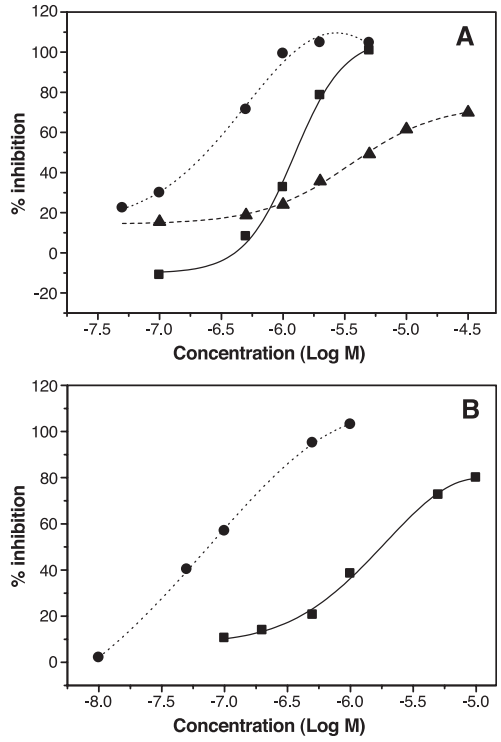

Fig. 2. Concentration-dependent inhibition of PTP-1B by KR61639. (A) Effect of KR61639 on PTP-1B using FDP as the substrate. PTP-1B was incubated with FDP in the presence or absence of various concentrations of KR61639, KR61170, and vanadate. After a 1-h incubation at room temperature, fluorescence released from FDP by enzyme reaction was measured. Symbols: KR61170 (●), KR61639 (■), Vanadate (▲). (B) Effect of KR61639 on PTP-1B using insulin receptor dodecapeptide as the substrate. PTP-1B was preincubated in the presence or absence of KR61639 or KR61170, and an aliquot of the preincubation mixture was incubated with phosphotyrosyl insulin receptor dodecapeptide for 30 min. Inorganic phosphate released from phosphotyrosine of the substrate peptide was measured by malachite green-ammonium molybdate method. Symbols: KR61170 (●), KR61639 (■).

Since the insulin receptor is a physiological substrate of PTP-1B, we carried out the PTP-1B activity assay with the phosphorylated insulin receptor dodecapeptide (TRDI-pYETDpYpYPRK) in addition to the FDP assay. The dodecapeptide corresponds to amino acids 1142–1153 of the insulin receptor regulatory domain. Similar to the results with FDP as the substrate, KR61639 showed potent inhibitory activity with an IC_{50} value of 2.65 μM (Fig. 2B). As a reference compound, KR61170 was included in the assay and had an IC_{50} of 70 nM, which is similar to the value reported previously (Wrobel et al., 1999). These results suggest that KR61639 acts as a true PTP-1B inhibitor.

3.2. Selectivity of KR61639 against other phosphatases

One of the obstacles in developing PTP-1B inhibitors for clinical use is the selectivity of the compounds against other phosphatases. To examine the selectivity profile of KR61639 in vitro, we evaluated the effect of KR61639 on various phosphatases. As shown in Table 1, KR61639 exhibited clear selectivity against LAR and Cdc25A, where the selectivity was about 10- to 30-fold. The compound had modest selectivity against CD45, Yop, VHR, PP2A and PP1, whereas KR61639 was equally potent against Cdc25B. By comparison, we examined the effect of KR61170 on the phosphatases. In addition to PTP-1B, KR61170 inhibited CD45, Cdc25B and Yop as well as the structurally unrelated serine/threonine phosphatases PP2A and PP1 (Table 1). The inhibition of PP2A and PP1 by PTP inhibitors may be due to unavoidable similarity in the active site pocket that should fit the phosphate group in the phosphorylated serine/threonine or tyrosine substrates.

Table 1
Selectivity of KR61639 and KR61170 for various phosphatases

Phosphatase	IC_{50} (μM)	
	KR61639	KR61170
hPTP-1B	0.65 ± 0.2	0.29 ± 0.1
CD45	2.10 ± 0.9	0.41 ± 0.2
LAR	10.5 ± 1.7	10.8 ± 2.3
Cdc25A	20.1 ± 2.4	1.5 ± 0.3
Cdc25B	0.67 ± 0.1	0.17 ± 0.1
PP1	3.90 ± 2.5	1.71 ± 0.4
PP2A	6.38 ± 0.8	10.0 ± 0.3
VHR	3.10 ± 1.6	3.08 ± 0.6
Yop	2.25 ± 1.4	0.20 ± 0.2

Enzyme assay was done with FDP as a substrate as described under Materials and methods. Results are expressed as means ± S.E.M. for three experiments.

3.3. Inhibition kinetics of KR61639

To examine whether KR61639 inhibits PTP-1B by interacting with the enzyme's active site, we tested the inhibition kinetics of KR61639 with FDP as the substrate. KR61639 inhibited PTP-1B in a noncompetitive manner under our experimental conditions (Fig. 3). The K_i value was estimated to be 0.39 μM.

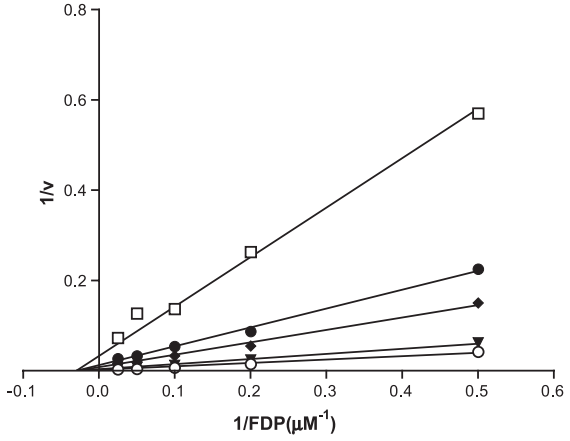

Fig. 3. Inhibition kinetics of KR61639. At various fixed concentrations of KR61639, initial velocity was determined with various concentrations of FDP. Symbols: 7.5 μM KR61639 (□), 5 μM (●), 2.5 μM (◆), 1 μM (▼), 0 μM (○).

3.4. Glycogen incorporation assay

Since PTP-1B is an intracellular enzyme, it is important for PTP-1B inhibitors to penetrate cell membranes. As a functional assay, we examined the effect of KR61639 on insulin-stimulated glycogen synthesis in HepG2 cells, one of the representative metabolic actions of insulin. As shown in Fig. 4, KR61639 enhanced insulin-stimulated glycogen synthesis in a concentration-dependent manner. A significant enhancing effect was observed with 10 μM KR61639, where the effect was about 130% of the effect of insulin as a control. The effect of KR61639 was further increased at 50 μM. This result suggests that KR61639 efficiently penetrates cell membranes and increases insulin signaling via inhibition of PTP-1B-mediated dephosphorylation of the enzyme's substrates including insulin receptor. KR61639 alone did not affect glycogen synthesis in HepG2 cells, indicating that stimulation of insulin signaling by PTP1B inhibitors may require a certain level of insulin receptor activation that cannot be achieved by a basal level activity of the receptor.

3.5. Deoxyglucose uptake in 3T3/L1 adipocytes

To further examine the cellular effects of KR61639, we tested whether KR61639 was capable of stimulating the deoxyglucose uptake induced by insulin in 3T3/L1 adipocytes. Previously, it was shown that insulin enhances deoxyglucose uptake in 3T3/L1 adipocytes, possibly via Glut4 translocation to the cell surface (Pessin et al., 1999). As shown in Fig. 5, KR61639 increased insulin-stimulated deoxyglucose uptake in 3T3/L1 adipocytes (Fig. 5). Interestingly, the effect of KR61639 on deoxyglucose uptake was more potent at 10 μM than at 50 μM.

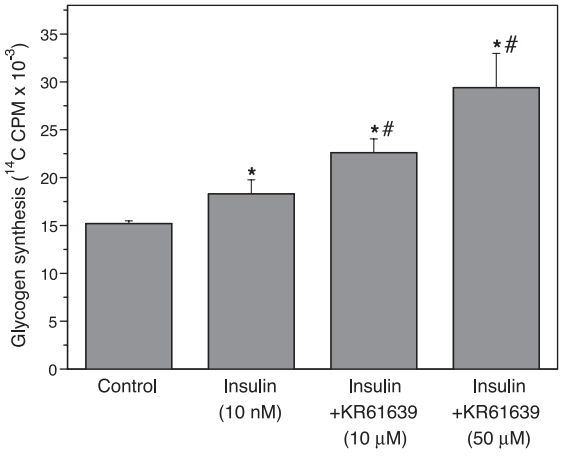

Fig. 4. Effect of KR61639 on insulin-stimulated glycogen incorporation. HepG2 cells were treated with or without KR61639 in the presence of 10 nM insulin. To observe a stimulating effect in insulin-mediated signaling, we used insulin concentrations lower than the saturating concentrations (100 nM–1 μM). When we treated with saturating concentrations, we were able to observe the expected 1.5- to 2-fold greater response (data not shown). After further incubation with D-[U-^{14}C]glucose for 3 h, radioactivity recovered in glycogen was measured. Values represent the means ± S.E.M. for three experiments done in duplicates (*P<0.05 vs. control, $^{\#}P$<0.05 vs. insulin alone).

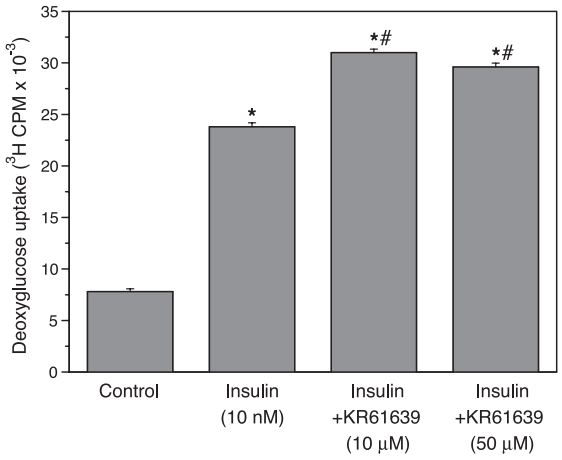

Fig. 5. Effect of KR61639 on insulin-stimulated deoxyglucose uptake. After differentiation to adipocytes, 3T3/L1 adipocytes were incubated with 10 nM insulin in the presence or absence of KR61639 for 30 min. Cell lysates were counted for radioactivity after incubation with 2-deoxy-D-[^{3}H]glucose for 10 min. Values represent the means ± S.E.M. for three experiments done in duplicate (*P<0.05 vs. control, $^{\#}P$<0.05 vs. insulin alone).

3.6. In vivo glucose lowering effect of KR61639

Since KR61639 was active in cell-based functional assays, we next investigated the in vivo efficacy of KR61639. KR61639 was given orally to ob/ob mice, a strain widely used as an animal model for type 2 diabetes, for 7 days with a once a day dosing regimen. As shown in Fig. 6, KR61639 exhibited blood glucose lowering activity at an oral dose of 100 mg/kg. At the 7th day, KR61639 reduced the blood glucose level by about 43%. Under the same condition, the positive control, KR61170, exhibited potent antihyperglyce-

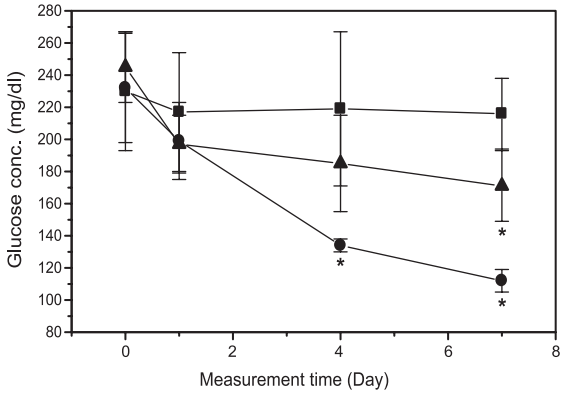

Fig. 6. In vivo glucose-lowering effect of KR61639. KR61639 (100 mg/kg) or KR61170 (10 mg/kg) was administered to ob/ob mouse orally once a day. At 1st, 4th and 7th days, blood glucose concentration was measured. Data represent the means ± S.E.M. for three experiments (N=6). Symbols: vehicle (■), KR61639 (▲), KR61170 (●) (*P<0.05 vs. control).

mic activity, reducing the blood glucose level to normal value at the 7th day with 10 mg/kg dose. In both KR61639 and 61170, the antihyperglycemic effect was statistically negligible upon acute treatment (1 day). The necessity of chronic treatment for the in vivo effect may be due to the complex downstream processes for the normalization of glucose homeostasis after stimulation of insulin signaling. The less-optimal pharmacokinetic profile of the compounds also could contribute to the delayed in vivo response.

4. Discussion

Owing to the active role of PTPases in various diseases, efforts for the development of PTPases inhibitors have been intensified (Burke and Zhang, 1998). Of the PTPases, PTP-1B appears to be an attractive therapeutic target because the PTP-1B levels in muscles and adipose tissues correlate with the degree of insulin resistance in subjects with obesity or diabetes (Ahmad et al., 1997). Furthermore, a study with PTP-1B knock-out mice presented evidence that selective PTP-1B inhibitors can serve as antidiabetic agents without overt toxicity (Elchebly et al., 1999; Klaman et al., 2000). However, the development of orally active and selective PTP-1B inhibitors has been a great challenge.

KR61639 in the present study exhibited selectivity against several well-known tyrosine or dual specific phosphatases (CD45, LAR, VHR, Yop, Cdc25A and Cdc25C) and serine/threonine phosphatases (PP1 and PP2A). In comparison, the reference compound KR61170 did not show selectivity against CD45, Cdc25B and Yop. Although the selectivity of KR61639 for Cdc25B was not as good as for other tested phosphatases, it should be possible to confer the necessary selectivity after further development exploiting differences in active site structures between PTP1B and Cdc25B (Reynolds et al., 1999). Furthermore, the discovery of the second aryl phosphate binding site near the PTP-1B active site in a recent crystallographic study of the PTP-1B/bis-(*p*-phosphophenyl)methane (BPPM) complex provides new possibilities for the design of selective PTP-1B inhibitors (Puius et al., 1997).

Because PTP-1B is an intracellular enzyme, potential PTP-1B inhibitors need to have good cellular penetration properties. Most of the reported PTP-1B inhibitors contain either phosphate groups or phosphate-mimicking groups that have negative charges. While the charged groups may confer on the inhibitors a high enzyme-binding affinity, molecules with charged groups have difficulty penetrating cellular membranes, resulting in poor efficacy and poor absorption in vivo. KR61639, which lacks known phosphate-mimicking groups, shows a potent inhibitory activity against PTP-1B. To confirm the cellular permeability of the compound, we evaluated the effect of KR61639 in two different cell-based assays including the glucose incorporation assay and the deoxyglucose uptake assay. As expected from the structure, KR61639 was found to penetrate cellular membranes effectively. Although KR61639 was active in both the glucose incorporation and the deoxyglucose uptake assays, the potency of the compound was somewhat weaker in the deoxyglucose uptake assay. The phenomenon may be due to the limited effect of PTP-1B in the regulation of glucose transport including the deoxyglucose uptake assay, as reported in previous studies where PTP-1B overexpression did not impair glucose transport (Venable et al., 2000).

The in vivo efficacy of KR61639 was determined by using the ob/ob mouse model. Ob/ob mice manifest insulin resistance, and PTP activity is elevated, which should reduce phosphorylation of the insulin receptor, thereby contributing to the insulin resistance in this model (Sredy et al., 1995). Oral administration of KR61639 (100 mg/kg) reduced the blood glucose level to 57% of untreated ob/ob mice, proving the in vivo efficacy of the compound. Since we used a high dose of KR61639, we cannot exclude the possibility that the blood glucose-lowering effect of KR61639 may be due to inhibition of other protein phosphatases and/or activation of peroxisome proliferator-activated receptor (PPAR)-γ. Recently, the in vivo glucose level lowering effect of KR61170 has been partially attributed to its activation of PPAR-γ (Johnson et al., 2002).

When evaluating HTS hits, it is essential to examine their kinetic behavior before further development since compounds found from HTS hits can interact with the target enzyme by various mechanisms (Doman et al., 2002). From a kinetics study, we found that KR61639 inhibited PTP-1B in a noncompetitive manner, indicating that KR61639 may bind to the enzyme–substrate complex or interact with the secondary aryl phosphate-binding site near the active site pocket of the enzyme. KR61639 did not appear to disrupt enzyme folding, as judged by the absence of an increase in the turbidity of the solution or protein precipitation.

In summary, using HTS techniques, we discovered a non-peptide, small-molecule inhibitor of PTP-1B that functions as an oral glucose-lowering agent. Further optimization for better potency and selectivity is under progress. Ultimately, these inhibitors may provide a new therapeutic option for type 2 diabetes and possibly for other metabolic syndromes.

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