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Full paper

Therapeutic effects of the allosteric protein tyrosine phosphatase 1B inhibitor KY-226 on experimental diabetes and obesity via enhancements in insulin and leptin signaling in mice

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ABSTRACT

The anti-diabetic and anti-obesity effects of the allosteric protein tyrosine phosphatase 1B (PTP1B) inhibitor 4-(biphenyl-4-ylmethylsulfanylmethyl)-*N*-(hexane-1-sulfonyl)benzoylamide (KY-226) were pharmacologically evaluated. KY-226 inhibited human PTP1B activity (IC_{50} = 0.28 μM), but did not exhibit peroxisome proliferator-activated receptor γ (PPARγ) agonist activity. In rodent preadipocytes (3T3-L1), KY-226 up to 10 μM had no effects on adipocyte differentiation, whereas pioglitazone, a PPARγ agonist, markedly promoted it. In human hepatoma-derived cells (HepG2), KY-226 (0.3–10 μM) increased the phosphorylated insulin receptor (pIR) produced by insulin. In *db/db* mice, the oral administration of KY-226 (10 and 30 mg/kg/day, 4 weeks) significantly reduced plasma glucose and triglyceride levels as well as hemoglobin A1c values without increasing body weight gain, while pioglitazone exerted similar effects with increases in body weight gain. KY-226 attenuated plasma glucose elevations in the oral glucose tolerance test. KY-226 also increased pIR and phosphorylated Akt in the liver and femoral muscle. In high-fat diet-induced obese mice, the oral administration of KY-226 (30 and 60 mg/kg/day, 4 weeks) decreased body weight gain, food consumption, and fat volume gain with increases in phosphorylated STAT3 in the hypothalamus. In conclusion, KY-226 exerted anti-diabetic and anti-obesity effects by enhancing insulin and leptin signaling, respectively.

1. Introduction

Diabetes is now regarded as one of the most serious global health issues and affects approximately several million adults. Type 2 diabetes causes hyperglycemia due to impaired insulin secretion and/or resistance, which leads to severe complications including cardiovascular diseases, nephropathy, retinopathy, and peripheral neuropathy. Hyperglycemia is treated with insulin, insulin secretagogues, such as sulfonylureas, glinides, and dipeptidyl-peptidase IV inhibitors, and glucagon-like peptide-1 analogues, as well as insulin sensitizers, including peroxisome proliferator-activated receptor (PPAR) γ agonists, and insulin-independent drugs, such as sodium glucose co-transporter 2 inhibitors. Insulin sensitizers are beneficial for the treatment of diabetic patients with insulin resistance and hyperinsulinemia because they lower plasma glucose levels without increasing insulin levels; hyperinsulinemia is associated with a risk of developing obesity and cardiovascular diseases.[1] Although pioglitazone, a PPARγ agonist, has been used as an effective insulin sensitizer, and was shown to prevent macrovasculopathy in diabetic patients,[2] it may cause various adverse effects including edema, obesity, and bone loss.[3–5] PPARγ agonists are considered to increase fat mass by promoting adipocyte differentiation.[6,7]

Abbreviations: EDTA, ethylenediaminetetraacetic acid; FBS, fetal bovine serum; GLUT4, glucose transporter type 4; GPDH, glycerol-3-phosphate dehydrogenase; HbA1c, hemoglobin A1c; HFD, high-fat diet; IBMX, 3-isobutyl-1-methylxanthine; IR, insulin receptor; IRS, insulin receptor substrate; JAK2, Janus kinase 2; KY-226, 4-(biphenyl-4-ylmethylsulfanylmethyl)-N-(hexane-1-sulfonyl)benzoylamide; OGTT, oral glucose tolerance test; pAkt, phosphorylated Akt; pIR, phosphorylated IR; *p*NPP, *p*-nitrophenol phosphate; PPAR, peroxisome proliferator-activated receptor; pSTAT3, phosphorylated STAT3; PTP1B, protein tyrosine phosphatase 1B; STAT3, signal transducer and activator of transcription 3; TG, triglyceride.

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Based on this background, safer insulin sensitizers independent of the activation of PPARγ need to be developed. Protein tyrosine phosphatase 1B (PTP1B), a non-receptor type tyrosine phosphatase, has been attracting attention as a novel target of insulin sensitizers because it mediates the dephosphorylation of key proteins in insulin and leptin signaling.[8,9] In the insulin signal cascade, the insulin-bound insulin receptor (IR), insulin receptor substrate (IRS), and Akt are sequentially phosphorylated, resulting in glucose uptake via the translocation of glucose transporter type 4 (GLUT4). In the leptin signal pathway, leptin binding to leptin receptors activates Janus kinase 2 (JAK2), which phosphorylates the receptor, thereby recruiting signal transducer and activator of transcription 3 (STAT3). STAT3 is phosphorylated by JAK2, which regulates appetite and energy expenditure. The genetic deletion of PTP1B increases insulin and leptin sensitivity,[10,11] driving the race to develop PTP1B inhibitors.[12,13] An antisense oligonucleotide specific for PTP1B was previously shown to increase insulin signaling, and, thus, insulin sensitivity, resulting in the amelioration of hyperglycemia in diabetic mice.[14] Although a large number of small molecule inhibitors have been reported,[12,13] only three compounds were entered into the clinical trial stage: trodusquemine, a naturally occurring allosteric inhibitor,[15,16] ertiprotafib, a non-competitive inhibitor with multiple actions,[17] and JTT-551, a mixed type inhibitor.[18,19] However, the clinical development of these compounds was suspended, possibly due to weak efficacy or unexpected adverse effects in patients. Various small-molecule allosteric PTP1B inhibitors have been synthesized; however, their anti-diabetic and anti-obesity effects have not yet been elucidated in detail.[13] KY-226, a novel benzoylsulfonamide derivative, was recently reported to be a non-competitive allosteric PTP1B inhibitor.[20]

The present study was undertaken in order to examine the effects of KY-226 on enzymes, cells, experimental diabetes, and obesity, and to elucidate the mechanisms underlying its ability to enhance insulin and leptin signaling.

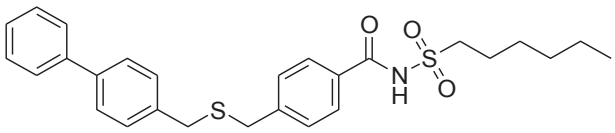

Fig. 1. Chemical structure of KY-226.

2. Materials and methods

2.1. Materials and animals

KY-226, pioglitazone, and farglitazar were synthesized in our laboratories. HepG2 cells (the European Collection of

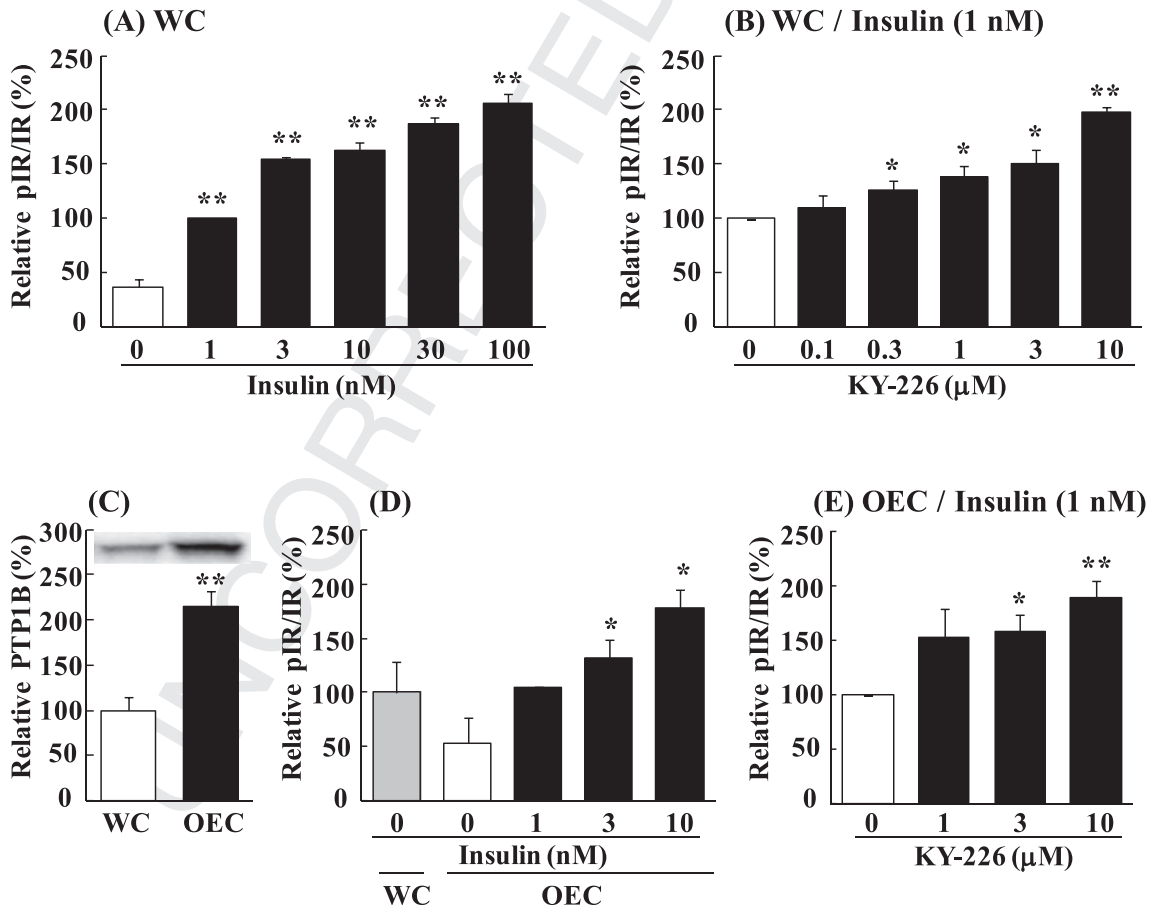

Fig. 2. Effects of KY-226 on insulin signaling in HepG2 cells with or without the overexpression of PTP1B. (A) Phosphorylation of insulin receptors induced by insulin (1–100 nM) in HepG2 cells. (B) Enhancement of insulin-induced phosphorylation by KY-226 (0.1–10 μM) in HepG2 cells. (C) PTP1B protein levels in HepG2 cells with or without the overexpression of PTP1B. (D) Phosphorylation of insulin receptors induced by insulin (1–10 nM) in HepG2 cells with the overexpression of PTP1B. (E) Enhancement of insulin-induced phosphorylation by KY-226 (1–10 μM) in HepG2 cells with the overexpression of PTP1B. A, B, D, E; Phosphorylation levels (pIR/IR) in insulin (1 nM)-treated cells were taken as 100%. C; The PTP1B expression level in HepG2 cells was taken as 100%. WC: wild cells, OEC: PTP1B-overexpressing cells. Values are the mean ± S.E.M. A: n = 3–6, B: n = 8, C: n = 3, D: n = 4–7, E: n = 6–8. A and D: *P < 0.05, **P < 0.01 vs. Insulin (0 nM), Student's *t*-test for paired data. B and E: vs. KY-226 (0 μM), Student's *t*-test for paired data. C: vs. WC. Student's *t*-test for unpaired data.