HIS-388, a Novel Orally Active and Long-Acting 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitor, Ameliorates Insulin Sensitivity and Glucose Intolerance in Diet-Induced Obesity and Nongenetic Type 2 Diabetic Murine Models

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ABSTRACT

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is considered a potential therapeutic target in the treatment of type 2 diabetes mellitus. In this study, we investigated the pharmacological properties of HIS-388 (N-[(1R,2S,3S,5aS,7aS)-5-hydroxyadamantan-2-yl]-3-(pyridin-2-yl) isoxazole-4-carboxamide), a newly synthesized 11β-HSD1 inhibitor, using several mouse models. In cortisone pellet-implanted mice in which hypercortisolism and hyperinsulinemia occur, single administration of HIS-388 exhibited potent and prolonged suppression of plasma cortisol and lowered plasma insulin levels. These effects were more potent than those achieved using the same dose of other 11β-HSD1 inhibitors (carbenoxolone and compound 544 [3-[(1s,3s)-adamantan-1-yl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine], indicating that HIS-388 potently and continuously suppresses 11β-HSD1 enzyme activity in vivo. In diet-induced obese mice, HIS-388 significantly decreased fasting blood glucose, plasma insulin concentration, and homeostasis model assessment–insulin resistance score, and ameliorated insulin sensitivity. In addition, HIS-388 significantly reduced body weight and suppressed the elevation of blood glucose during the pyruvate tolerance test. In nongenetic type 2 diabetic mice with disease induced by a high-fat diet and low-dose streptozotocin, HIS-388 also significantly decreased postprandial blood glucose and plasma insulin levels and improved glucose intolerance. The effects of HIS-388 on glucose metabolism were indistinguishable from those of an insulin sensitizer, pioglitazone. Our results suggest that HIS-388 is a potent agent against type 2 diabetes. Moreover, amelioration of diabetic symptoms by HIS-388 was at least in part attributable to an antiobesity effect or improvement of hepatic insulin resistance. Therefore, potent and long-lasting inhibition of 11β-HSD1 enzyme activity may be an effective approach for the treatment of type 2 diabetes and obesity-associated disease.

Introduction

The prevalence of obesity and type 2 diabetes mellitus has dramatically increased in recent years, causing a global burden (Whiting et al., 2011; Goto et al., 2013). Excessive lipid accumulation in adipose and peripheral tissues resulting in obesity is caused by excess nutrition and/or a sedentary lifestyle.

Obesity, especially visceral obesity, is closely associated with metabolic diseases, such as type 2 diabetes mellitus. Type 2 diabetes mellitus is characterized by impaired insulin secretion from pancreatic β cells and insulin resistance in the liver, muscle, and adipose tissue (Kahn et al., 2006). Insulin resistance is a key feature of the disease and is defined as a state in which more than normal levels of insulin are required to obtain biologic effects (Ruderman et al., 2013). Insulin resistance also results in compensatory hyperinsulinemia, leading to pancreatic β-cell dysfunction and glucose intolerance (Abdul-Ghani et al., 2006). Therefore, improvements of insulin resistance and obesity are considered to be effective strategies for the treatment of type 2 diabetes.

Thiazolidinediones (TZDs), such as pioglitazone or rosiglitazone, are used as potent insulin sensitizers for the treatment of type 2 diabetes. The mechanism of action of TZDs is thought to be a combination of insulin sensitization and antiobesity effects. However, TZDs have adverse effects such as fluid retention, edema, and weight gain, which limit their utility. Therefore, the development of a novel class of drugs with insulin-sensitizing effects and antiobesity effects has gained attention.

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ABBREVIATIONS: AUC, area under the curve; BVT116429, (S)-2-((S)-1-(2-fluorophenyl)ethylamino)-5-methyl-5-(trifluoromethyl)thiazol-4(5H)-one; BVT2733, 3-chloro-2-methyl-N-[(4-[(4-methylpiperazin-1-yl)-2-oxoethyl]-1,3-thiazol-2-yl)benzenesulfonamide; CBX, carbenoxolone; Cpd544, compound 544, 3-[(1S,3S)-adamantan-1-yl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepine; DIO, diet-induced obesity; ELISA, enzyme-linked immunosorbent assay; HFD, high-fat diet; HIS-388, N-[(1R,2S,3S,5aS,7aS)-5-hydroxyadamantan-2-yl]-3-(pyridin-2-yl) isoxazole-4-carboxamide; HOMA-IR, homeostasis model assessment–insulin resistance; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; HTRF, homogenous time-resolved fluorescence; ITT, insulin tolerance test; KR-66344, 2-(3-benzoyl)-4-hydroxy-1,1-dioxo-2H-1,2-benzothiazine-2-yl-1-phenylethanone; MS, micromomes; OGTT, oral glucose tolerance test; PFT, pyruvate tolerance test; STZ, streptozotocin; TZD, thiazolidinedione.
diabetes (Derosa and Maffioli, 2012). TZDs are known to have a high affinity and agonistic effect on peroxisome proliferator–activated receptor γ (Lehmann et al., 1995), thereby promoting adipose differentiation and increasing the number of small adipocytes that are more sensitive to insulin (Moller, 2001; Arner, 2003). The small insulin-sensitive adipocytes generated by TZDs seem to correlate with amelioration of insulin resistance (Okuno et al., 1998; Evans et al., 2004). Moreover, TZD treatment leads to a decrease of circulating serum triglyceride and free fatty acids levels and downregulates the production of adipokines, such as tumor necrosis factor-α or resistin (Arner, 2003). Meanwhile, a substantial number of TZD-treated patients encounter adverse side effects, such as fluid retention, weight gain, and congestive heart failure (Yang and Soodvilai, 2008). Therefore, clinical use of TZDs in patients with heart failure, a past history of heart failure, or renal dysfunction has been limited.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a key enzyme that catalyzes the conversion of the active glucocorticoid cortisol from its inactive metabolite cortisone (Harno and White, 2010). 11β-HSD1 is abundant in the liver and adipose tissue, and higher adipose 11β-HSD1 activity is associated with features of the metabolic syndrome (Lindsay et al., 2003). 11β-HSD1–deficient mice have normal or minimally increased plasma glucocorticoid levels but cannot regenerate glucocorticoid within cells in the liver and adipose tissue. As a result, they are protected against insulin resistance, hyperglycemia, and weight gain induced by a high-fat diet (Kotelevtsev et al., 1997). Conversely, mice overexpressing 11β-HSD1 in adipose tissue have increased intra-adipose glucocorticoid concentrations despite unchanged plasma levels. These mice show remarkable features of the metabolic syndrome, such as obesity, insulin resistance, glucose intolerance, and hyperglycemia (Masuzaki et al., 2001, 2003; Paterson et al., 2004). These findings suggest that 11β-HSD1 is a potential therapeutic target for the treatment of type 2 diabetes.

Many selective 11β-HSD1 inhibitors have been explored as a new approach for the treatment of type 2 diabetes. In fact, several 11β-HSD1 inhibitors, such as compound 544 (Cpd544; 3-[(1S,3S)-adamantan-1-yl]-6,7,8,9-tetrahydro-5H-[1,2,4](triazol-4,3-a)-azepine), BVT2733 (3-chloro-2-methyl-N-[4-(2-(4-methylpiperezin-1-yl)-2-oxoetoxy)-1,3-thiazol-2-yl]benzenesulfonamide), and KR-66344 [2-(3-benzoyl)-4-hydroxy-1,1-dioxo-2-yl]-1-phenylethanone], improve insulin sensitivity or glucose intolerance in several animal models (Alberts et al., 2003; Hermanowski-Vosatka et al., 2005; Park et al., 2011). Although BVT116429 [(S)-2-[(S)-1-(2-fluorophenyl)ethylamino]-5-methyl-5(trifluoromethyl)thiazol-4(5H)-one] shows a high potency for 11β-HSD1 inhibition in vitro, it has no effect on glucose intolerance in type 2 diabetic mice (Sundbom et al., 2008). Thus, although compounds show a high potency for 11β-HSD1 inhibition in vitro, it is unclear whether these compounds have an equivalent effect on insulin resistance or glucose intolerance in vivo. Recently, we synthesized a novel 11β-HSD1 inhibitor, HIS-388 (N-[(1R,2s,3S,5s,7s)-5-hydroxyadamantan-2-yl]-3-(pyridin-2-yl) isoxazole-4-carboxamide). In this study, we investigated the pharmacological properties of HIS-388 in several animal models. First, we assessed the effect of HIS-388 on in vivo 11β-HSD1 enzyme activity in mice with cortisone-induced hypercortisolism and hyperinsulinemia. Furthermore, we explored whether HIS-388 can improve insulin sensitivity and glucose intolerance in two different animal models, namely mice with diet-induced obesity (DIO) or the nongenetic type 2 diabetes murine model, with the latter produced by the combination of a high-fat diet (HFD) and low doses of streptozotocin (HFD/STZ mice).

### Materials and Methods

**Chemicals.** HIS-388 (Fig. 1) and Cpd544 were synthesized in the Pharmaceutical Laboratories of Toray Industries, Inc. (Kanagawa, Japan). Carbenoxolone (CBX), pioglitazone, and 21-day slow-release cortisone pellet were purchased from Sigma-Aldrich Japan (Tokyo, Japan), Kemprotex Limited (Middlesbrough, UK), and Innovative Research of America (Sarasota, FL), respectively. Other chemicals were purchased from Sigma-Aldrich Japan. In the enzyme assay, HIS-388, CBX, and Cpd544 were dissolved in dimethylsulfoxide. For in vivo experiments, HIS-388, CBX, Cpd544, and pioglitazone were suspended in 0.5% methylcellulose. To prepare the nongenetic type 2 diabetes animal model involving HFD/STZ mice, STZ was dissolved in saline.

**In Vitro Enzyme Assay.** The 11β-HSD1 enzyme assay was carried out using human and mouse liver microsomes (MS). Human MS (mixed sex) was purchased from Xenotech (Lexena, KA). The MS protein (50 μg/ml), substrate cortisone (160 nM), and NADPH (200 nM), obtained from Sigma-Aldrich Japan, were added to an assay buffer (20 mM HEPES with 5 mM EDTA, pH 6.0) with or without 11β-HSD1 inhibitors and incubated for 2 hours at 37°C. The amount of cortisol in the reaction mixture was measured by homogenous time-resolved fluorescence (HTRF) assay system (Cisbio, Bedford, MA). Mouse MS was prepared according to a method described previously (Kusihoro et al., 2004). The MS protein (10 μg/ml) from ICR mouse liver, substrate 11-dehydrocorticosterone (20 nM) obtained from Innovative Research of America, and NADPH (200 μM) were added to the assay buffer and incubated for 2 hours at 37°C. The amount of corticosterone was measured by enzyme-linked immunosorbent assay (ELISA; Cayman Chemical, Ann Arbor, MI). The percentage inhibition was calculated and plotted against test compound concentration to generate the IC₅₀.

**Animals.** This study was reviewed by the Animal Care and Use Committee and approved by the head of the test facility and performed in accordance with the Guideline for Animal Experiments, Research and Development Division, Toray Industries, Inc. All mice were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan) and group-housed in cages at a temperature of 22–24°C with a 12-hour light/dark cycle (lights on at 7:00 AM) for at least 1 week before experiments. Mice were given access to food and water ad libitum.

**Cortisone-Implanted Mice.** Ten-week-old male C57BL/6J mice were anesthetized with pentobarbital sodium (70 mg/kg i.p.). The back skin of the neck was cut open, and a subcutaneous longitudinal pocket was created about 3 cm beyond the incision site toward the lower back. A cortisone pellet (1.5 or 35 mg) was implanted, and the incision site was closed using wound glue. Sham-operated mice did not have cortisone pellet implants. After more than 3 days of recovery from surgery, single doses of HIS-388, CBX, and Cpd544 were orally administered at a dose of 30 mg/kg in cortisone pellet (35 mg)–implanted mice to investigate the ability of compounds to inhibit in vivo 11β-HSD1 enzyme activity. Whole blood was sampled from the tail vein before and after administration of compounds at different time points (0, 1, 4, 8, and 24 hours). Plasma cortisol and insulin levels

![Fig. 1. Chemical structure of HIS-388.](image-url)
TABLE 1
In vitro 11β-HSD1 inhibitory activity of HIS-388

<table>
<thead>
<tr>
<th>Compound</th>
<th>Liver MS IC50 nmol/l</th>
<th>hHSD1</th>
<th>mHSD1</th>
</tr>
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<tr>
<td>HIS-388</td>
<td>14.7</td>
<td>82.0</td>
<td></td>
</tr>
<tr>
<td>CBX</td>
<td>550</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Cpd544</td>
<td>20.9</td>
<td>269</td>
<td></td>
</tr>
</tbody>
</table>

Statistical Analysis. Data were expressed as mean ± S.E.M. Homeostasis model assessment–insulin resistance (HOMA-IR) score as an insulin resistance index was calculated according to the formula (Lee et al., 2002; Pickavance et al., 2005): fasting blood glucose (mg/dl) × fasting plasma insulin (ng/ml)/22.5. Areas under the curve (AUCs) were calculated using trapezoidal rule from the blood glucose change in ITT, PTT, and OGTT in DIO or HFD/STZ mice and the temporal changes in plasma cortisol level in cortisone-implanted mice. Statistical analysis was performed using one-way analysis of variance followed by Dunnett’s or Tukey’s multiple comparison for three or more groups or using the t test for two groups. P values <0.05 were considered statistically significant.

Results

In Vitro 11β-HSD1 Inhibitory Effect of HIS-388. We assessed the in vitro enzyme activity by HTRF or ELISA methods using human or mice liver MS. CBX and Cpd544 were quantified at all time points and 24 hours after administration of compounds, respectively.

Mice with Diet-Induced Obesity. Four-week-old male C57BL/6J mice were given regular chow (CRF-1; Oriental Yeast Co., Tokyo, Japan) or a HFD (D12492; Research Diets, Inc., New Brunswick, NJ) for 12–13 weeks. To evaluate the efficacy of HIS-388 (30 mg/kg, 100 mg/kg) against insulin resistance and obesity, mice were weighed regularly to allow accurate HIS-388 dosing, which was orally administrated once daily for 14 days (days 0–13). After the final dosing of HIS-388, mice were deprived of food for 18 hours. Blood glucose concentration was then measured, and whole blood was sampled via the tail tip. To compare the efficacy of HIS-388 (30 mg/kg) and pioglitazone (30 mg/kg) in DIO mice, these compounds were orally administrated once daily for 14 days (days 0–13). After the final administration, blood glucose level was determined, and whole blood was collected under a fasting condition. The insulin tolerance test (ITT) was performed under fasting conditions by intraperitoneal injection of regular human insulin (0.3 U/kg Humulin; Eli Lilly Japan, Kobe, Japan). The concentration of blood glucose was measured before and after insulin injection at different time points (30, 60, 90, and 120 minutes). To clarify the effect of HIS-388 on hepatic insulin resistance, the pyruvate tolerance test (PTT) was performed. Basal blood glucose level was determined, and 1.5 g/kg sodium pyruvate (dissolved in saline) was injected into the peritoneum of overnight-fasted mice. The concentration of blood glucose was measured at 30, 60, 90, 120, and 180 minutes after sodium pyruvate injection.

HFD/STZ, Nongenetic Type 2 Diabetes Mice. Nongenetic type 2 diabetes mice were prepared as described previously (Luo et al., 1998; Mu et al., 2009) with slight modifications. In brief, 5-week-old male C57BL/6J mice received regular chow (CRF-1; Oriental Yeast Co.) or a HFD (D12492; Research Diets, Inc.) for 4 weeks to generate peripheral insulin resistance. The mice were given an intraperitoneal injection of STZ at 100 mg/kg and maintained for 14 days under the above condition. Control mice did not receive STZ administration or a HFD. HIS-388 (30 mg/kg) and pioglitazone (30 mg/kg) were orally administered once daily for 14 days (days 0–13). After the final administration, blood glucose concentration was determined, and whole blood was sampled under fasting conditions. To evaluate the efficacy of HIS-388 (30 mg/kg, 100 mg/kg) against insulin resistance and obesity, mice were weighed regularly to allow accurate HIS-388 dosing, which was orally administrated once daily for 14 days (days 0–13). After the final dosing of HIS-388, mice were deprived of food for 18 hours. Blood glucose concentration was then measured, and whole blood was sampled via the tail tip. To compare the efficacy of HIS-388 (30 mg/kg) and pioglitazone (30 mg/kg) in DIO mice, these compounds were orally administrated once daily for 14 days (days 0–13). After the final administration, blood glucose level was determined, and whole blood was collected under a fasting condition. The insulin tolerance test (ITT) was performed under fasting conditions by intraperitoneal injection of regular human insulin (0.3 U/kg Humulin; Eli Lilly Japan, Kobe, Japan). The concentration of blood glucose was measured before and after insulin injection at different time points (30, 60, 90, and 120 minutes). To clarify the effect of HIS-388 on hepatic insulin resistance, the pyruvate tolerance test (PTT) was performed. Basal blood glucose level was determined, and 1.5 g/kg sodium pyruvate (dissolved in saline) was injected into the peritoneum of overnight-fasted mice. The concentration of blood glucose was measured at 30, 60, 90, 120, and 180 minutes after sodium pyruvate injection.

Measurement of Food Intake. Four-week-old male C57BL/6J or 6N mice were treated with regular chow for 11 weeks (normal) or HFD for 40 weeks (DIO). These mice were used for the measurement of food intake. Vehicle or HIS-388 was orally administrated once daily for 7 days in normal mice (30 or 100 mg/kg) and for 12 days in DIO mice (30 mg/kg). Food intake was calculated as the difference between the food provided and the food remaining on an arbitrary day.

Blood Sample Assay. Blood glucose level was measured with an automatic glucometer (Precision Xceed; Abbott Diabetes Care Ltd., Alameda, CA). The plasma was obtained by centrifuge of collected whole blood samples. Plasma cortisol and insulin concentrations were quantified by the HTRF assay system (Cisbio) and ELISA (Shibayagi, Gunma, Japan), respectively.
were used as reference compounds. HIS-388 displayed inhibitory effects on 11β-HSD1 enzyme activity, and the IC₅₀ values were 14.7 and 82.0 nmol/l in human and mice, respectively. The inhibitory effects of HIS-388 and CBX on murine 11β-HSD1 enzyme activity were equivalent; however, Cpd544 had lower potency than HIS-388 (Table 1).

**Effect of HIS-388 on In Vivo 11β-HSD1 Activity in Cortisone-Implanted Mice.** To evaluate the efficacy of HIS-388 against in vivo 11β-HSD1 enzyme activity, we examined its effect on plasma cortisol and insulin concentration in slow-release cortisone pellet-implanted mice. In cortisone-implanted mice, the plasma cortisol and insulin, but not glucose levels, were increased in a cortisone dose-dependent manner, indicating that in vivo 11β-HSD1 enzyme activity contributes to this phenomenon (Fig. 2). Since the high-dose (35 mg) cortisone pellet strongly elevated plasma concentrations of cortisol and insulin, we adopted this dose for the following evaluation. We found a remarkable decrease of plasma cortisol concentration after HIS-388 administration. The efficacy of CBX was weaker than HIS-388 (Fig. 3, A and B). Although Cpd544 reduced plasma cortisol concentration at 1 hour after administration, this effect rapidly disappeared 4 hours after administration (Fig. 3, C and D). Moreover, HIS-388, but not CBX and Cpd544, decreased plasma insulin levels at 24 hours after administration (Fig. 3, E and F), indicating that HIS-388 has a potent and continuous suppressive effect on 11β-HSD1 enzyme activity in vivo.

**Effect of HIS-388 on Obesity and Insulin Resistance in DIO Mice.** To test the effect of HIS-388 on obesity and insulin resistance, HIS-388 was given to DIO mice for 14 days. A significant decrease in body weight was observed in HIS-388-treated DIO mice in a dose-dependent fashion (Fig. 4A). HIS-388 also significantly reduced fasting blood glucose levels at a high dose (100 mg/kg) (Fig. 4B). Moreover, fasting plasma insulin levels and HOMA-IR score in HIS-388-treated DIO mice were significantly lower in a dose-dependent manner compared with vehicle-treated mice (Fig. 4, C and D).

**Effect of HIS-388 on Food Intake.** In normal mice, there were no differences in food intake and body weight after HIS-388 (30 and 100 mg/kg) treatment (Fig. 5, A and B). In DIO mice, food intake remarkably decreased on day 2 after HIS-388 (30 mg/kg)
administration. From day 2 onward, slight reduction was observed on days 7 and 12 compared with vehicle-treated mice (Fig. 5C). Weight reduction was observed in HIS-388–treated DIO mice, followed by a decrease in food intake (Fig. 5D).

**Comparative Study of the Therapeutic Efficacy of HIS-388 and Pioglitazone against Insulin Resistance in DIO Mice.** Because the inhibition of 11\(\beta\)-HSD1 by HIS-388 ameliorated obesity and insulin resistance in DIO mice (Fig. 4), we conducted a comparative study of the therapeutic efficacy of HIS-388 (30 mg/kg per day) and pioglitazone (30 mg/kg per day) against insulin resistance in DIO mice. A significant decrease in body weight was observed in DIO mice treated with HIS-388, as shown above. Meanwhile, pioglitazone treatment slightly increased body weight (Fig. 6A). Fasting blood glucose, insulin levels, and HOMA-IR score were significantly decreased in HIS-388–treated DIO mice, the results of which were indistinguishable from those of pioglitazone treatment (Fig. 6, B–D). HIS-388 ameliorated insulin sensitivity to the same degree as pioglitazone in ITT (Fig. 6, E and F). Furthermore, we carried out the PTT in DIO mice to estimate the site of action of HIS-388 in the liver. During the PTT, blood glucose levels of HIS-388–treated DIO mice were lowered, and the AUC was significantly decreased compared with those of vehicle-treated DIO mice (Fig. 6, G and H).

**Comparative Study of the Therapeutic Efficacy of HIS-388 and Pioglitazone against Glucose Intolerance in HFD/STZ, Nongenetic Type 2 Diabetes Mice.** We next examined the therapeutic efficacy of HIS-388 compared with pioglitazone in the nongenetic type 2 diabetes model involving HFD/STZ mice, which is produced by combined HFD feeding and STZ injection. Injection of 100 mg/kg STZ did not affect postprandial blood glucose and plasma insulin levels. In mice treated with HFD, postprandial plasma insulin, but not blood glucose level, was significantly increased compared with those in control mice (Fig. 7, A and B). Furthermore, an increase in the postprandial blood glucose level and a decrease of the postprandial plasma insulin level were observed in HFD/STZ (vehicle treatment) mice compared with those in mice that were fed a HFD (Fig. 7, A and B). During OGTT, blood glucose levels and glucose AUC were significantly increased in HFD/STZ (vehicle treatment) mice, although no significant changes were seen in mice treated with STZ or HFD compared with control mice (Fig. 7, C and D). These results indicated that combined HFD and STZ injection produced mild hyperglycemia, mild hyperinsulinemia, and glucose intolerance, and HIS-388 decreased postprandial blood glucose and insulin levels in HFD/STZ mice (Fig. 7, A and B). In addition to these effects, HIS-388 significantly decreased blood glucose AUC in the OGTT compared with that in vehicle-treated mice. The effects on glucose intolerance in HIS-388–treated HFD/STZ mice were indistinguishable from those in pioglitazone-treated mice, indicating that HIS-388 has a similar efficacy to pioglitazone in this murine model (Fig. 7, C and D).

**Discussion**

It has been reported that the small molecule 11\(\beta\)-HSD1 inhibitor ameliorated insulin sensitivity or glucose intolerance in rodent models related to diabetes or obesity (Anagnostis et al., 2013). For instance, Cpd544 ameliorated insulin sensitivity and hyperglycemia and reduced body weight with improvement of the lipid profile in DIO mice (Hermanowski-Vosatka et al., 2005).
BVT2733 increased insulin sensitivity and decreased endogenous glucose production under a euglycemic, hyperinsulinemic state in KKAY mice (Alberts et al., 2003). KR-66344 also improved glucose intolerance and suppressed adipocyte differentiation in ob/ob mice (Park et al., 2011). Although BVT116429 has selective and potent 11β-HSD1 inhibitory effect in vitro, BVT116429 had no effect on glucose intolerance in type 2 diabetic mice (Sundbom et al., 2008). Therefore, in addition to in vitro 11β-HSD1 inhibitory activity, another pharmacological property may be involved in the improvement of glucose intolerance or insulin resistance. In the present study, we demonstrated that HIS-388, a novel 11β-HSD1 inhibitor that exhibits potent and long-lasting suppression of in vivo 11β-HSD1 enzyme activity, has therapeutic efficacy against insulin resistance and glucose intolerance indistinguishable from those of a potent insulin sensitizer, pioglitazone, in DIO mice and nongenetic type 2 diabetes mice.

We examined the effect of HIS-388, which inhibits 11β-HSD1 enzyme activity with a potency equivalent to that of CBX but greater than that of Cpd544 in vitro, on in vivo 11β-HSD1 enzyme activity using the cortisol pellet implant murine model. This model shows an increase of plasma cortisol and insulin levels. These alterations are attributed to the conversion of inactive cortisone to active cortisol via an in vivo 11β-HSD1 enzyme reaction (Bhat et al., 2008). In the present study, plasma cortisol and insulin levels were increased in a dose-dependent manner in mice with cortisol pellet implants. These results were consistent with those of a previous report; therefore, we applied this animal model to the evaluation of in vivo 11β-HSD1 enzyme activity, including the evaluation of potency and long-acting effects. Single dosing of HIS-388 suppressed the increase of plasma cortisol level in this model. The suppressive effect of HIS-388 on plasma cortisol level was more potent or longer than those of CBX or Cpd544. Moreover, HIS-388, but not CBX or Cpd544, decreased plasma insulin levels. These data suggest that HIS-388 has a potent and long-acting inhibitory effect on 11β-HSD1 enzyme activity in vivo.

To assess the effect of HIS-388 on obesity and insulin resistance, we next examined the efficacy of HIS-388 (30 and 100 mg/kg) in DIO mice. HIS-388 reduced body weight in a dose-dependent manner in DIO mice, which suggests a potent antiobesity effect. This weight reduction was not observed in normal C57BL/6J mice treated with HIS-388 (30 and 100 mg/kg) (Fig. 5A). These data indicated that the weight reduction effect of HIS-388 is a characteristic phenomenon in DIO and possibly other disease model mice. In general, food intake is closely related to body weight change. Some 11β-HSD1 inhibitors exhibit reduction of food intake in DIO mice (Hermanowski-Vosatka et al., 2005; Wang et al., 2006). In contrast, overexpression of 11β-HSD1 in adipose tissue is associated with increased food intake (Masuzaki et al., 2001). These reports indicate that 11β-HSD1 activity is involved in food intake regulation. HIS-388 decreased food intake in DIO mice but not in normal mice (Fig. 5, A and C), indicating that our data are consistent with the results of the previous report. Therefore, this effect, at least in part, may be involved in the decrease of weight reduction in DIO mice. HIS-388 dose-dependently decreased blood glucose and plasma insulin levels in the fasting state and HOMA-IR score in DIO mice. In preliminary study, we found that single administration of HIS-388 dose-dependently inhibited 11β-HSD1 enzyme activity; however, it is not completely inhibited at the dose of 30 mg/kg 6 hours after administration in

![Fig. 5. Effects of HIS-388 on food intake and body weight in normal or DIO mice. In normal mice, HIS-388 (30 and 100 mg/kg) was orally administrated for 7 days. In DIO mice, HIS-388 (30 mg/kg) was orally administrated for 12 days. Food intake and body weight were measured on days 0, 1, 3, 5, 7 (normal mice) or days 0, 2, 7, 12 (DIO mice). Food intake (A and C) and body weight (B and D) in normal and DIO mice are shown. Values are means ± S.E.M. N = 5–9 animals per group.](image-url)
normal mice ex vivo experiment. Therefore, we speculate that the dose of 100 mg/kg of HIS-388 has a longer duration of suppressed 11\(\beta\)-HSD1 enzyme activity compared with the dose of 30 mg/kg. 11\(\beta\)-HSD1 knockout mice are resistant to HFD-induced obesity and insulin resistance (Wamil et al., 2011). The results of 100 mg/kg of HIS-388 in DIO mice were like the phenotype of 11\(\beta\)-HSD1 knockout mice treated with HFD. Taken together, we consider that HIS-388 improved obesity and insulin resistance via inhibition of 11\(\beta\)-HSD1 enzyme activity in DIO mice.

To evaluate the therapeutic efficacy of HIS-388, we next compared the effect of HIS-388 and the potent insulin sensitizer, pioglitazone, on insulin resistance in DIO mice. HIS-388 reduced weight gain in DIO mice that corresponded to the dosing period. In contrast, a slight increase in body weight was observed in

![Fig. 6. Effects of HIS-388 and pioglitazone on body weight, diabetic parameters, insulin sensitivity, and hepatic glucose production in mice with DIO. HIS-388 (30 mg/kg) and pioglitazone (Pio; 30 mg/kg) were administered orally once daily for 14 days. After the final administration, mice were deprived of food for 18 hours, and blood samples were collected for measurement of blood glucose and plasma insulin. To assess insulin sensitivity and hepatic glucose production in DIO mice, the ITT or PTT was carried out. Body weight change (A), fasting blood glucose (B), fasting plasma insulin (C), HOMA-IR index (D), blood glucose levels during ITT (E), blood glucose AUC (F) in ITT, blood glucose levels during PTT (G) and blood glucose AUC (H) in PTT are shown. Values are means ± S.E.M. *P < 0.05; **P < 0.01 compared with the vehicle-treated group by t test or Dunnett’s test. N = 8 animals per group.](image-url)
pioglitazone-treated DIO mice. This weight gain was considered to be an adverse side effect of pioglitazone that is seen in clinical practice (Gillies and Dunn, 2000). As for insulin resistance, HIS-388 ameliorated parameters of insulin resistance such as blood glucose, plasma insulin levels in the fasting state, and the HOMA-IR score and enhanced insulin sensitivity to the same degree as that of pioglitazone. Glucocorticoids contribute to increased hepatic glucose production in diabetes and counteract the actions of insulin (Friedman et al., 1993). Accordingly, 11β-HSD1–deficient mice or treatment with an 11β-HSD1 inhibitor in the type 2 diabetes model displayed amelioration of hepatic insulin resistance (Morton et al., 2001; Alberts et al., 2003). These reports imply that hepatic 11β-HSD1 activation leads to insulin resistance in the liver. Thus, to reveal the pharmacological characteristics of HIS-388, we performed the PTT in DIO mice. After pyruvate injection, blood glucose levels in HIS-388–treated DIO mice were lower than those in vehicle-treated mice. A significant decrease in blood glucose AUC was also observed in HIS-388–treated DIO mice. Taken together, these results suggest that the ameliorative effect of HIS-388 on insulin resistance is comparable to that of pioglitazone in DIO mice. Moreover, it is considered that the insulin-sensitizing effects of HIS-388 may be at least in part due to its antiobesity effect and improvement of hepatic insulin resistance via inhibition of 11β-HSD1 enzyme activity in DIO mice.

Combined HFD and low-dose STZ elicited mild hyperglycemia, insulin deficiency, and reduction of glucose uptake in rodents, which partly mimic human type 2 diabetes. Thus, HFD/STZ mice are regarded as a nongenetic type 2 diabetes model (Mu et al., 2009). To further evaluate the therapeutic effect of HIS-388 from the point of glucose control, we compared the effect of HIS-388 with pioglitazone on glucose intolerance in HFD/STZ mice. HIS-388 reduced postprandial blood glucose and plasma insulin levels and improved glucose intolerance. These effects of HIS-388 were indistinguishable from those of pioglitazone treatment, indicating that HIS-388 may have a therapeutic efficacy against glucose intolerance that is almost equal to that of pioglitazone in the nongenetic type 2 diabetes model. The effects of HIS-388 on biochemical parameters, postprandial blood glucose, and insulin levels was observed to
the same degree as those of the potent insulin sensitizer pioglitazone, thus suggesting that its mechanism of action might be through improved insulin sensitivity. Cpd544 decreased blood glucose levels (postprandial and fasting), increased insulin sensitivity, and improved glucose intolerance in HFD/STZ mice by twice daily administration (Hermanowski-Vosatk et al., 2005). By focusing on the administration frequency in this animal model, pharmacological efficacy of HIS-388 could be achieved by once daily administration. These results suggest that potent and long-acting inhibition of 11β-HSD1 may contribute to the efficacy on insulin sensitivity and glucose intolerance in HFD/STZ mice.

In conclusion, we found that HIS-388, a novel 11β-HSD1 inhibitor, exhibited potent and long-lasting 11β-HSD1 enzymatic inhibition in vivo. HIS-388 also ameliorated insulin sensitivity and glucose intolerance that was indistinguishable from the effects of the potent insulin sensitizer pioglitazone in DIO mice and nongenetic type 2 diabetic mice. In addition, we also found that HIS-388 has additional pharmacological effects including an antiobesity effect and an ameliorative effect on hepatic insulin resistance. These findings suggest that HIS-388, which provides potent and long-acting enzyme inhibition of 11β-HSD1, could represent a new therapeutic approach for the treatment of type 2 diabetes and/or obesity-associated diseases.

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