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)-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 lipids 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 mellitus, obesity, and dysglycemia. However, the long-term use of TZDs has been associated with adverse effects such as edema, weight gain, bone fractures, and fluid retention. Therefore, there is a need for the development of new agents with improved safety profiles. Thiazolidinedione–hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitors are a novel class of agents that target this enzyme, which plays a key role in the regulation of insulin sensitivity and glucose metabolism. In this study, we investigated the pharmacological properties of HIS-388 (N-[(1R,2S,3S)-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.
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 (Kotel'nov 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-5-(trifluoromethyl)thiazol-4(5H)-one), shows a high potency for 11β-HSD1 inhibition in vitro, as does 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-5-(trifluoromethyl)thiazol-4(5H)-one) shows a high potency for 11β-HSD1 inhibition in vitro, as does 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-5-(trifluoromethyl)thiazol-4(5H)-one). A binary salt of Cortisone-Implanted Mice.

Fig. 1. Chemical structure of HIS-388.
TABLE 1
In vitro 11β-HSD1 inhibitory activity of HIS-388

<table>
<thead>
<tr>
<th>Compound</th>
<th>Liver MS IC50 (nmol/l)</th>
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<tbody>
<tr>
<td>HIS-388</td>
<td>hHSD1 14.7 mHSD1 82.0</td>
</tr>
<tr>
<td>CBX</td>
<td>5.50 115</td>
</tr>
<tr>
<td>Cpd544</td>
<td>20.9 269</td>
</tr>
</tbody>
</table>

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 also 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 15 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 collected under a fasting condition. The oral glucose tolerance test (OGTT) was performed under fasting conditions 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 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 11β-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 11β-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 cortisone 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 cortisone 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, over-expression 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 DIO mice.
normal mice ex vivo experiment. Therefore, we speculate that the dose of 100 mg/kg of HIS-388 has a longer duration of suppressed 11β-HSD1 enzyme activity compared with the dose of 30 mg/kg. 11β-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 11β-HSD1 knockout mice treated with HFD. Taken together, we consider that HIS-388 improved obesity and insulin resistance via inhibition of 11β-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

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**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.
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, $11\beta$-HSD1–deficient mice or treatment with an $11\beta$-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 $11\beta$-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 antidiabetic effect and improvement of hepatic insulin resistance via inhibition of $11\beta$-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

Fig. 7. Effects of HIS-388 and pioglitazone on glucose intolerance in HFD/STZ mice. Either HIS-388 (30 mg/kg) or pioglitazone (Pio; 30 mg/kg) was administered orally once daily for 15 days. After the final administration, blood samples were collected to assess the postprandial blood glucose and insulin levels. Afterward, the OGTT was carried out after 18 hours of food deprivation to determine glucose intolerance. Postprandial blood glucose (A), postprandial plasma insulin (B), blood glucose levels during OGTT (C), and blood glucose AUC (D) are shown. Values are means ± S.E.M. **$P < 0.01$ compared with the vehicle-treated group by Dunnett’s test; ††$P < 0.01$ compared with control, STZ, HFD, and vehicle-treated groups by Turkey’s test. $N = 6–8$ animals per group.
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/DZT mice by twice daily administration (Hermanowski-Vosatka et al., 2005). By focusing on the administration frequency in this animal model, pharmacological efficacy of HIS-388 may contribute to the efficacy on insulin sensitivity and glucose intolerance in HFD/DZT 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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Authorship Contributions

Participated in research design: Okazaki, Takahashi, Iwamura, Kumagai, Kainoh.
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Performed data analysis: Okazaki, Takahashi.
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References

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