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FR258900, a Novel Glycogen Phosphorylase Inhibitor Isolated from Fungus No. 138354

II. Anti-hyperglycemic Effects in Diabetic Animal Models

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Abstract A novel glycogen phosphorylase inhibitor FR258900 was isolated from the cultured broth of a fungal strain No. 138354. We examined the hypoglycemic effects of FR258900 in diabetic animal models. FR258900 treatment significantly reduced the plasma glucose concentrations during oral glucose tolerance tests in diabetic mice models, including *db*/*db* mice and STZ-induced diabetic mice. Furthermore, FR258900 treatment resulted in rapid decrease in the plasma glucose levels in *db*/*db* mice. These improvements in glucose disposal were accompanied by increased liver glycogen contents, suggesting that the glucose lowering effects of FR258900 were attributed to suppressed hepatic glycogen breakdown and increased hepatic glycogen synthesis. Taken together, our results suggest that glycogen phosphorylase is a potentially useful target in new therapies against diabetes.

Keywords fungal metabolite, glycogen phosphorylase inhibitor, hypoglycemic effect

Introduction

In the prior paper [1], we showed that a novel glycogen phosphorylase inhibitor FR258900 could stimulate glycogen synthesis in primary rat hepatocytes. So far, several compounds have been reported as a potent glycogen phosphorylase inhibitor, and recently some inhibitors were shown to have hypoglycemic activity in a rodent model of type 2 diabetes [2]. In the present study, we evaluated the effect of FR258900 on hepatic glycogen synthesis *in vivo* and the biological activities in diabetic mice models.

Materials and Methods

Animals

Male C57BL/6J mice were purchased from Charles River Japan Inc., Atsugi, Japan. Male C57BL/KsJ-*db*/*db* mice were purchased from Jackson Laboratories, Bar Harbor, ME, USA. Diabetes was induced in C57BL/6 mice by streptozotocin (STZ) treatment. Male, 8 week-old, C57BL/6 mice were treated intraperitoneally with 175 mg/kg streptozotocin (Sigma, St. Louis, MO) suspended in 0.05 M citrate buffer (pH 4.5). Blood glucose levels were monitored, and mice exhibiting hyperglycemia (500～600 mg/dl plasma glucose) were used for the experiment, a week after streptozotocin administration.

In Vivo Hypoglycemic Activity

(i) Glucagon-induced hyperglycemia model[3]: Male C57BL/6 mice (8 weeks of age) were treated subcutaneously with FR258900 dissolved in saline containing 10% HCO-60 (Nikko Chemicals Co., Ltd.,

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Tokyo, Japan). After 15 minutes, glucagon (100 μg/kg) dissolved in 0.01 M HCl/saline was administered subcutaneously. Blood samples were collected at 0, 15, 30, and 60 minutes after glucagon administration, and plasma glucose levels were measured using Glucose-test Wako (Wako Pure Chemical Industries).

(ii) Oral glucose tolerance test: Male C57BL/6 mice (8 weeks of age), male diabetic C57BL/KsJ-*db*/*db* mice (12 weeks of age), and STZ-induced diabetic mice (9 weeks of age) had fasted for 6 hours and were given FR258900 dissolved in 10% HCO-60/saline by subcutaneous injection. After 15 minutes, mice were treated orally with 2 g/kg glucose, and blood samples were collected from the orbital vein at 0, 15, 30, 60, and 120 minutes after glucose administration. Plasma glucose levels were determined as described above.

(iii) Hypoglycemic activity with C57BL/KsJ-*db*/*db* mice: Diabetic male C57BL/KsJ-*db*/*db* mice (10 weeks of age) had fasted for 2 hours and were treated subcutaneously with FR258900 dissolved in 10% HCO-60/saline. At 0, 1, 2, 4, and 6 hours after administration, blood samples were collected and plasma glucose levels were determined.

Measurement of Liver Glycogen Content

Male C57BL/6 mice (8 weeks of age) and male diabetic C57BL/KsJ-*db*/*db* mice (8 weeks of age) had fasted for 6 hours and were treated subcutaneously with FR258900 in 10% HCO-60/saline. After 20 minutes, mice were treated orally with 2 g/kg glucose. After 2 hours, mice were anesthetized, and the liver was dissected out and stored at −80°C until use. The liver was homogenized with a polytron homogenizer in 3% perchloric acid, and the glycogen content in the homogenate was determined enzymatically as described previously [4]. Results are expressed as mg glucose equivalent of glycogen/g weight.

Results

The Effect of FR258900 on Glucagon-induced Hyperglycemia

To investigate whether FR258900 can inhibit glycogen phosphorylase activity *in vivo*, we examined the effect of FR258900 on glucagons-induced hyperglycemia. Glucagon stimulates glycogen degradation in the liver *via* activating glycogen phosphorylase, resulting in hyperglycemia. As shown in Figure 1, the glucagon-induced hyperglycemia in C57BL/6 mice was significantly suppressed by FR258900, suggesting that FR258900 could suppress glycogenolysis and hepatic glucose output *in vivo*.

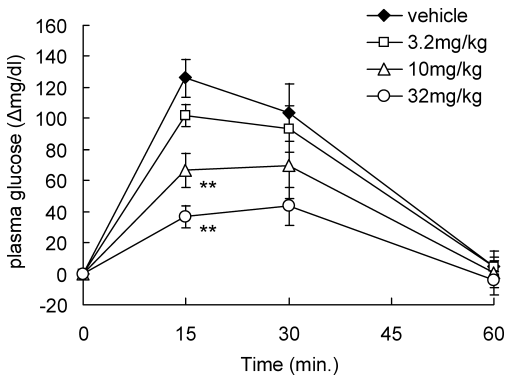

Fig. 1 The effect of FR258900 on glucagon-induced hyperglycemia in C57BL/6 mice. FR258900 was injected subcutaneously, followed by glucagon challenge as described in the Materials and Methods. The data are expressed as mean±S.E.M. of individual delta plasma glucose concentrations from basal (pre-glucagon) levels (n=5). $^{**}p<0.01$ compared with vehicle-treated group.

The Effect of FR258900 on Oral Glucose Tolerance Test

To investigate the possible effects of FR258900 on hepatic glucose uptake, we performed oral glucose tolerance test (OGTT) in normal or diabetic mice. As shown in Figure 2a, the plasma glucose concentrations after the OGTT were significantly reduced in FR258900-treated C57BL/6 mice. Furthermore, FR258900 treatment also reduced the plasma glucose concentrations in both *db*/*db* mice, an animal model of type 2 diabetes, and STZ-induced diabetic mice, a model of type 1 diabetes (Figure 2, b and c). These results suggested that FR258900 treatment can improve postprandial hyperglycemia in diabetics.

Next we examined the effect of FR258900 on hepatic glycogen synthesis after the OGTT. Liver glycogen content was increased after oral glucose administration in vehicle-treated C57BL/6 mice (Figure 3a). In contrast, liver glycogen content was not increased in *db*/*db* mice (Figure 3b), suggesting that hepatic glycogen synthesis in the postprandial state is impaired in a diabetic condition. FR258900 treatment significantly increased liver glycogen concentration after the OGTT in both C57BL/6 mice and *db*/*db* mice (Figure 3, a and b). These results suggested that FR258900 improved oral glucose disposal by increasing hepatic glycogen synthesis.

Hypoglycemic Effect of FR258900 in *db*/*db* Mice

We next examined the glucose lowering effect of FR258900 in diabetic *db*/*db* mice. As shown in Figure 4, the plasma glucose concentrations in *db*/*db* mice were acutely and significantly reduced after a single administration of FR258900. In parallel, liver glycogen content was elevated two hours after FR258900 administration from

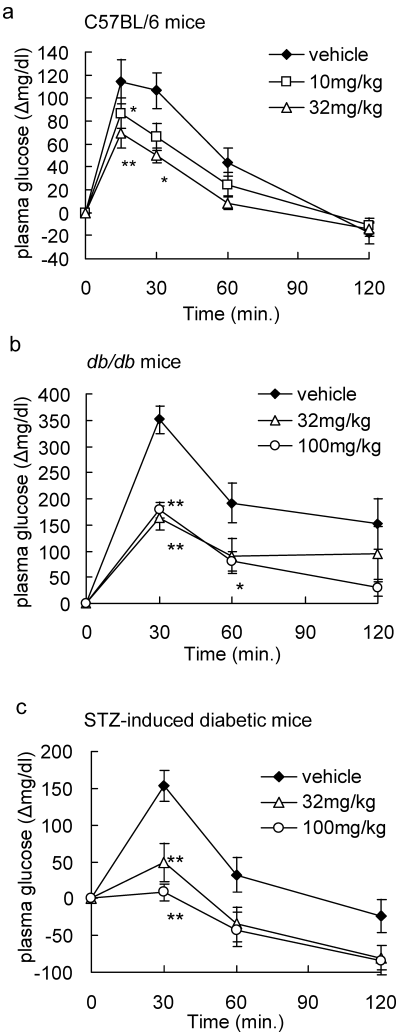

Fig. 2 FR258900 ameliorates postprandial hyperglycemia in normal and diabetic mice. Plasma glucose levels during the oral glucose tolerance test in C57BL/6 mice (a), *db*/*db* mice (b), and STZ-induced diabetic mice (c). The data are expressed as mean±S.E.M. of individual delta plasma glucose concentrations from basal (at 0 minute) levels (n=5). *$p<0.05$; **$p<0.01$ compared with vehicle-treated group.

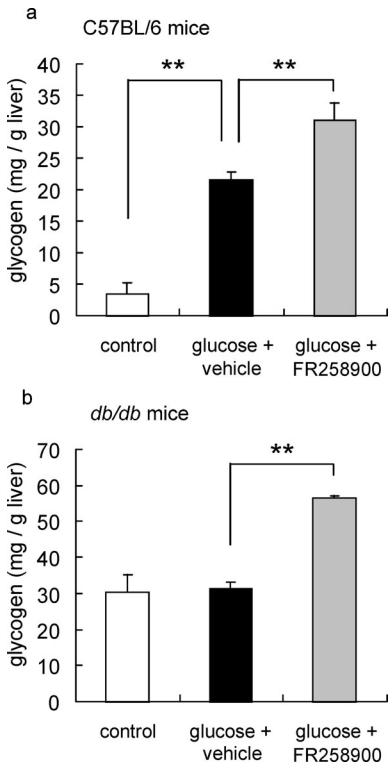

Fig. 3 The effect of FR258900 on hepatic glycogen synthesis after the OGTT. Liver glycogen contents after oral glucose administration in C57BL/6 mice (a) and *db*/*db* mice (b) treated with vehicle or FR258900 (100 mg/kg). Liver glycogen contents before glucose administration are displayed as control (white bar). The data are expressed as mean±S.E.M. (n=3). **$p<0.01$ compared with glucose-administrated vehicle-treated group.

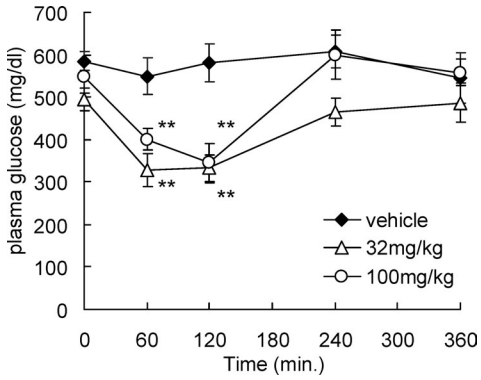

Fig. 4 Hypoglycemic effect of FR258900 in *db*/*db* mice. Plasma glucose levels in *db*/*db* mice treated with vehicle or FR258900. The data are expressed as mean±S.E.M. (n=5). **$p<0.01$ compared with vehicle-treated group.

25.9±2.6 mg/g liver in the vehicle-treated *db*/*db* mice to 41.5±4.8 mg/g liver in the 32 mg/kg FR258900-treated *db*/*db* mice. These results suggested that glucose lowering by FR258900 might be associated with inhibition of glycogenolysis.

Discussion

In diabetic patients, hepatic glucose production is significantly elevated relative to non-diabetic subjects, which contributes to diabetic hyperglycemia [5]. Hepatic glucose production is the net sum of two metabolic processes performed by the liver, gluconeogenesis and glycogenolysis, and the relative contributions of each to hepatic glucose production have been difficult to estimate

[6]. In the present study, we demonstrated that FR258900 treatment significantly lowered plasma glucose levels in diabetic mice by inhibition of hepatic glycogenolysis, suggesting that glycogenolysis is quantitatively important to hepatic glucose production. A recent study has estimated that glycogenolysis contributes about 60% of the hepatic glucose production in overnight fasted type 2 diabetes patients [7]. Furthermore, it has been reported that a substantial portion of gluconeogenesis appears to be cycled through the glycogen pool prior to efflux from the liver [8], and that glycogen phosphorylase inhibitors indirectly inhibit gluconeogenesis by disrupting the glucose/glycogen cycling involved in hepatic glucose production [9]. Thus, inhibition of glycogenolysis by glycogen phosphorylase inhibitor may prove beneficial in the treatment of type 2 diabetes.

Additionally, we demonstrated that FR258900 treatment activated hepatic glycogen synthesis in diabetic mice model, and resulted in significant improvement in oral glucose disposal. As we described in the prior paper [1], inhibition of glycogen phosphorylase by FR258900 might result in the indirect activation of glycogen synthase, accompanied by increased hepatic glucose uptake. Both liver glycogen synthesis and the regulation of hepatic glucose production are defective in patients with type 2 diabetes. Hence, our results suggest that glycogen phosphorylase is a potentially useful target in new therapies against type 2 diabetes.

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