Brain-derived neurotrophic factor (BDNF) prevents the development of diabetes in prediabetic mice

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ABSTRACT

We previously reported that peripheral injection of brain-derived neurotrophic factor (BDNF) exhibits hypophagic and hypoglycemic effects in obese hyperglycemic animals, indicating its antiobesity and antidiabetic effects. Since previous studies were focused on the effect of BDNF on overt diabetic animals with severe hyperglycemia, there was no evidence whether BDNF is effective or not for the development of diabetes in prediabetic animal models. Therefore, we evaluated the effect of BDNF on preventing the development of diabetes in db/db mice. First, we characterized age-related changes in the pathophysiology of diabetes in db/db mice. We chose 8 week-old db/db mice as the early diabetic stage (early intervention study) and 4 week-old db/db mice as the prediabetic stage (prevention study). Next, we examined the effects of BDNF on the progression of diabetes in early diabetic db/db mice. In the early intervention study using 8 week-old db/db mice, intermittent treatment with BDNF prevented the deterioration in hyperglycemia. Lastly, we examined the preventive effects of BDNF on the development of diabetes in prediabetic db/db mice. In the prevention study using 4 week-old db/db mice, treatment with BDNF prevented the age-related increase in blood glucose concentration. These results showed for the first time that BDNF prevents the development of diabetes in prediabetic db/db mice.

Type 2 diabetes is progressive disorder initially characterized by impaired glucose tolerance (IGT) and compensatory hyperinsulinemia and, in the later stages, by severe insulin resistance and impaired β-cell function (7). The syndrome is associated with hyperglycemia, dyslipidemia, hypertension, and obesity and, in the long term, micro- and macrovascular complications, resulting in impaired life quality and increased mortality (5–7, 10). IGT was first introduced 1979 by the National Diabetes Data Group (16) and the World Health Organization (WHO) Expert Committee on Diabetes (23) as an intermediate category covering the gray area between unequivocal diabetes mellitus and normal glucose tolerance. Early stage of diabetes also carries an increased risk of development of cardiovascular disease and forms part of the “metabolic syndrome” (9). Therefore, it is very important to treat early stage of diabetes as well as hyperglycemia in overt diabetes.

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, which includes nerve growth factor, neurotrophin-3 and neurotrophin-4/5 (1, 13, 14, 19). BDNF promotes neurite outgrowth, provides trophic support to certain neurons in the central and peripheral nervous systems, and is efficacious in the treatment of neurological disorders (18, 26).

In addition to the efficacy of BDNF in neurological disorders, we and others reported that peripheral injection of BDNF exhibits hypophagic and hypoglycemic effects in obese hyperglycemic animals but
not in normal animals, indicating its antiobesity and antidiabetic effects (15, 17, 21). The efficacy of BDNF in ameliorating glucose metabolism was observed not only in the case of the repetitive administration but also in the case of the intermittent administration (25). Moreover, we have previously shown that concomitant administration of BDNF with insulin enhances the hypoglycemic action of insulin in streptozotocin (STZ)-induced type 1 diabetic mice (22) and BDNF administration protected against pancreatic exhaustion in obese diabetic mice (24). Since previous studies were focused on the effect of BDNF on diabetic animals with severe obesity and hyperglycemia, there was no evidence whether BDNF is effective or not for the development of diabetes in prediabetic animal models.

In the current study, we characterized age-related changes in the pathophysiology of diabetes in \( \text{db/db} \) mice. Next, to evaluate the preventive effect of BDNF on the development of diabetes, we examined the effect of BDNF on glycemic control in early diabetic or prediabetic \( \text{db/db} \) mice. We presented here for the first time that BDNF prevented the development of diabetes of \( \text{db/db} \) mice.

**MATERIAL AND METHODS**

*Animals.* Male C57BL/KsJ-\( \text{db/db} \) mice were obtained from Clea Japan Inc. (Tokyo, Japan). Animals were housed under a temperature-, humidity-, and light-controlled room with a daily cycle of 12 h light and 12 h darkness. They were given free access to water and diet (CE-2; Clea Japan). All animal experiments were conducted in accordance with the guidelines of the Dainippon Sumitomo Pharma Committee on Animal Research.

*BDNF administration.* Human recombinant BDNF (N-terminal methionine-free; Regeneron Pharmaceuticals, Tarrytown, NY, USA) was administered twice a week subcutaneously to 4 or 8 week-old \( \text{db/db} \) mice at 20 mg/kg. A solution containing 0.01% Tween 80 and 1% Mannitol in PBS (phosphate-buffered saline; 10 mM phosphate and 150 mM NaCl, pH 7.0) was used as the vehicle.

*Measurement of blood glucose concentration, pancreatic and plasma insulin concentrations.* Blood was sampled from the tail vein, and blood glucose concentrations were measured by the Antsense II blood glucose analyzer (Bayer Medical, Tokyo, Japan). Pancreas tissues were weighed, homogenized and extracted with acid ethanol solution (conc. HCl: ethanol:distilled H\(_2\)O = 1.5 : 75 : 23.5) and the insulin concentration in the supernatant was measured. The pancreatic and plasma insulin concentrations were determined by Enzyme-Linked ImmunoSorbent Assay (ELISA) (Levis-insulin-mouse; Shibayagi, Gunma, Japan).

*Oral glucose tolerance test (OGTT).* After 8 weeks of treatment, oral glucose tolerance test was conducted as follows. After overnight fasting, 3 g/kg D-glucose was administered orally to overnight-fasted \( \text{db/db} \) mice. Blood glucose concentrations were measured at 0 (before), 45, 90, and 135 min after glucose administration.

*Statistical analysis.* All the data are presented as mean ± SD. Differences between individual groups were analyzed by Student’s \( t \)-test. The statistical calculations were performed using SAS software (SAS Institute, Cary, NC, USA), and \( P < 0.05 \) was considered statistically significant.

**RESULTS**

*The development of diabetes in \( \text{db/db} \) mice.* To investigate the protective effect of BDNF against the development of diabetes, first we characterized age-related changes in the pathophysiology of diabetes in \( \text{db/db} \) mice. We measured the body weight, blood glucose concentration, plasma and pancreatic insulin concentrations of \( \text{db/db} \) mice and non-diabetic control \( \text{db/m} \) mice in the period of 11 weeks from 5 to 16 weeks old. As shown Fig. 1A, the initial (5 weeks old) body weight in \( \text{db/db} \) mice was already significantly higher than that in \( \text{db/m} \) mice (28.5 ± 1.0 vs. 22.1 ± 1.0 g). The increment in body weight of \( \text{db/db} \) mice during the experimental period was greater than that of \( \text{db/m} \) mice. As a consequence, 16 week-old \( \text{db/db} \) mice exhibited severe obesity compared with age-matched \( \text{db/m} \) mice (48.7 ± 2.8 vs. 29.4 ± 1.9 g) (Fig. 1A). The initial (5 weeks old) blood glucose concentration in \( \text{db/db} \) mice was slightly high compared with that in \( \text{db/m} \) mice (193 ± 31 vs. 147 ± 12 mg/dL). \( \text{db/db} \) mice showed a rapid increase in blood glucose concentration until 10 weeks old, whereas \( \text{db/m} \) mice maintained normoglycemia (428 ± 33 vs. 157 ± 13 mg/dL) (Fig. 1B). After 10 weeks old, \( \text{db/db} \) mice exhibited severe hyperglycemia and kept it for the experimental period (Fig. 1B).

Plasma insulin concentration of 5 week-old \( \text{db/db} \) mice was already extremely high compared with that in \( \text{db/m} \) mice (53.59 ± 17.58 vs. 1.29 ± 0.74 ng/
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Dose-dependently decreased, with a significant effect at 10 mg/kg or more administered compared with that in the vehicle-treated db/db mice (25). Based on this result, we examined the efficacy of BDNF administered twice a week to early diabetic 8 week-old db/db mice at 20 mg/kg for 8 weeks. As shown Fig. 2A, an age-related increase in blood glucose concentration was observed in the vehicle-treated db/db mice. In contrast, BDNF-treated db/db mice maintained the initial blood glucose concentration during the treatment period, showing the prevention against the progression of diabetes. Body weight and food intake of the BDNF-treated db/db mice also tended to be lower than those of the vehicle-treated db/db mice (Figs. 2B and 2C). We also performed OGTT after 8 weeks administration of BDNF. After overnight fasting, blood glucose concentrations in the BDNF-treated db/db mice were significantly lower than those in the vehicle-treated db/db mice (206 ± 96 vs. 320 ± 49 mg/dL) (Fig. 3A). The area under the curve (AUC) of blood glucose concentration of the BDNF-treated db/db mice was

Effect of twice-a-week administration of BDNF on glycemic control in early diabetic db/db mice (early intervention study)

We have previously showed that the intermittent administration of BDNF ameliorates glucose metabolism in overt diabetic mice (25). In the previous study, BDNF was administered twice a week to overt diabetic 10 week-old db/db mice. In the BDNF-treated mice, the blood glucose concentration dose-dependently decreased, with a significant effect at 10 mg/kg or more administered compared with that in the vehicle-treated db/db mice (25). Based on this result, we examined the efficacy of BDNF administered twice a week to early diabetic 8 week-old db/db mice at 20 mg/kg for 8 weeks. As shown Fig. 2A, an age-related increase in blood glucose concentration was observed in the vehicle-treated db/db mice. In contrast, BDNF-treated db/db mice maintained the initial blood glucose concentration during the treatment period, showing the prevention against the progression of diabetes. Body weight and food intake of the BDNF-treated db/db mice also tended to be lower than those of the vehicle-treated db/db mice (Figs. 2B and 2C). We also performed OGTT after 8 weeks administration of BDNF. After overnight fasting, blood glucose concentrations in the BDNF-treated db/db mice were significantly lower than those in the vehicle-treated db/db mice (206 ± 96 vs. 320 ± 49 mg/dL) (Fig. 3A). The area under the curve (AUC) of blood glucose concentration of the BDNF-treated db/db mice was

Fig. 1  Body weight (A), blood glucose concentration (B), and plasma (C) and pancreatic (D) insulin concentrations of db/db mice and db/m mice. Data are expressed as mean ± SD (n = 8). **P < 0.01 vs. db/m mice by Student's t-test.
concentrations in the vehicle-treated db/db mice in increased from 5 to 11 weeks old, and were kept at high level (Fig. 4A). Interestingly, BDNF treatment mostly prevented the age-related increase in blood glucose concentration during the experimental period (Fig. 4A). Moreover, body weight of the BDNF-treated db/db mice was lower than that of the vehicle-treated db/db mice during the experimental period (Fig. 4B). These results demonstrated that intermittent administration of BDNF prevents the development of diabetes in prediabetic db/db mice.

DISCUSSION

In this study, we characterized age-related changes in the pathophysiology of diabetes in db/db mice.
The age-related progression in diabetes of db/db mice were summarized by showing plasma insulin and blood glucose concentrations (Fig. 5). In the relatively young db/db mice from 5 to 9 weeks old, hyperinsulinemia and the gradual increase in blood glucose concentration were observed. Therefore, we supposed that db/db mice in this period are early diabetes and that db/db mice younger than 5 weeks old are prediabetes. Our present results agree with other reports that the development of diabetes in db/db mice was initially characterized by impaired glucose tolerance and hyperinsulinemia associated with decreasing insulin sensitivity (4, 8). After 9 weeks old, the age-related decrease in plasma insulin concentration was observed in db/db mice, and hyperglycemia continued. Pancreatic insulin content in
BDNF prevents the development of diabetes in early diabetic and prediabetic mice. These data suggest that BDNF may be of therapeutical merit in the treatment of prediabetes and early diabetes such as metabolic syndrome.

REFERENCES


