Effect of Oophorectomy and Estrogen Administration on Diabetic Pathogenesis in Female Spontaneously Diabetic Torii Rats

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Abstract: In order to examine the effect of sex hormones on diabetic pathogenesis in female Spontaneously Diabetic Torii (SDT) rats, we performed oophorectomy on female SDT rats, administered female hormones after the oophorectomy, and measured body weight changes, plasma glucose concentration, and pancreatic histopathology. At 26 weeks of age, the body weight was significantly heavier in the oophorectomized group and significantly lighter in the estradiol benzoate (EB) administration group than in the sham-operated control group. Although glucose concentration did not significantly change in the oophorectomized group, it was significantly lower in the EB administration group than in both control and the oophorectomized group. Severe histopathological change was observed in the oophoretomized group but not in the EB administration group. Thus, EB blocked weight gain and pancreatic islet change that was induced by oophoretomy and it also lowered glucose concentration. These results suggest that estrogen plays a preventive role for diabetic pathogenesis in female SDT rats.

Keywords: Female SDT rats, oophorectomy, estrogen, body weight, plasma glucose, pancreatic histopathology.

INTRODUCTION

The Spontaneously Diabetic Torii (SDT) rat serves as a new model for non-obese type 2 diabetes mellitus. It is an inbred rat strain established from the Sprague-Dawley rat by Shinohara, who is affiliated with the Research Laboratories of Torii Pharmaceutical Co., Ltd., Japan [1, 2]. As a result of chronic severe hyperglycemia, SDT rats develop diabetic retinopathy [1-10], diabetic peripheral neuropathy [8, 9] and diabetic nephropathy [11, 12]. It has been reported that male SDT rats begin showing pancreatic islet histopathology including hemorrhage in the pancreatic islets at 8-10 weeks of age, and inflammatory cell infiltration with fibroblasts infiltrating the pancreatic islets by 16 weeks of age [1, 2, 13]. Prior to the onset of diabetes, glucose intolerance with impaired insulin secretion [1, 2, 13] and impaired lipid catabolism [14] are observed. Genetic analysis for diabetes in SDT rats identified significant quantitative trait loci (QTL) (*Gisdt1*, *Gisdt2* and *Gisdt3*) [15], and (*Dmsdt1*, *Dmsdt2*, *Dmsdt3* and *Dmsdt4*) [16] for glucose intolerance. A major locus on chromosome 3 (*Dmsdt1*) was identified as a dominantly acting SDT allele that causes islet inflammation and fibrosis [16]. At the same time, female SDT rats manifest pancreatic islet histopathology and glucose intolerance similar to that seen in male rats only at 25 weeks of age [17]. Additionally, the incidence of diabetes in female SDT rats is 33.3% at 65 weeks of age, lower compared to the 100% seen in male SDT rats; thus, a gender difference clearly exists in terms of the incidence of diabetes in these rats [1].

There has been no detailed report on sex differences conducted to evaluate the role of sex hormone in the development of diabetes in SDT rats, although a sex difference in the incidence of diabetes has been noted. To examine this phenomenon, in this study oophorectomies were performed on female SDT rats, followed by administration of female sex hormones, and subsequent effects were observed on diabetic pathogenesis factors such as body weight, plasma glucose concentration, and pancreatic histopathology.

MATERIALS AND METHODOLOGY

Animal Care

The experiments were conducted in compliance with the Guidelines for Animal Experimentation of CLEA Japan, Inc. A total 18 number of female SDT rats were reared at the Nogawa Branch of the Technical Service Dept., CLEA Japan, Inc. (Kanagawa, Japan). All the rats were maintained in animal facilities under specific pathogen-free conditions with a temperature range from 20 to 26°C, humidity 50% to 70%, and lighting 07:00-19:00h; they were provided with a commercial pellet diet (CE-2, CLEA Japan, Tokyo, Japan),

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and tap water *ad libitum* from an automated sterilized water supply system.

Experimental Design

The experimental population was divided into three groups: a sham-operated control group, an oophorectomized group, and a group given estradiol benzoate (EB) after oophorectomy (EB administration group). Each group consisted of 6 animals. At 4 weeks of age, the skin of the back midline of the sham-operated group was incised and the right and left back muscles were sutured under anesthesia using ketalar (Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan). Ooporectomies were performed on the oophorectomized group using a similar operative procedure under ketalar anesthesia at 4 weeks of age. EB (β-estradiol 3-benzoate; EB, Sigma Chemical Co., USA) was dissolved in corn oil and 50.0 μg was injected subcutaneously into the dorsal region of each animal in the EB administration group once a week from 5 weeks to 25 weeks of age.

Measurement of Body Weight and Plasma Glucose Concentration

The body weights of test animals were measured every two weeks from 4 weeks to 26 weeks of age. After measuring the body weight of all animals for each group after 16 hours of fasting at 26 weeks, rats were sacrificed and blood samples were taken from the abdominal aorta under ether anesthesia, followed by centrifugation, and the plasma glucose concentration was measured with an autoanalyzer (COBAS®MIRA Plus, Roche, Tokyo) using the hexokinase method.

Histopathological Examinations

The pancreases of all 26-week-old animals were fixed in 10% neutral buffered formalin. The fixed specimens were embedded in paraffin, sliced into 5 μm sections and stained with hematoxylin and eosin for histopathological examination.

Statistical Analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analysis of the difference between mean values was performed using the Tukey-Kramer test. Probability values of $P<0.05$ were considered significant.

RESULTS

Body Weight and Plasma Glucose Concentration

Body weight changes are shown in (Fig. **1**) and (Fig. **2A**). At 26 weeks of age, the mean body weight was significantly heavier in the oophorectomized group 406 ± 22.5 g and significantly lighter in the EB administration group 209 ± 13.8 g than in the sham-operated control 355 ± 15.4 g. The measured values for plasma glucose concentration in each experimental animal at 26 weeks of age are shown in (Fig. **2B**). Although the plasma glucose concentration did not significantly change in the oophorectomized group 130.9 ± 16.6 mg/dl, it was significantly lower in the EB administration group 100 ± 3.4 mg/dl than in both sham-operated control 139.6 ± 8.4 mg/dl and the oophorectomized group.

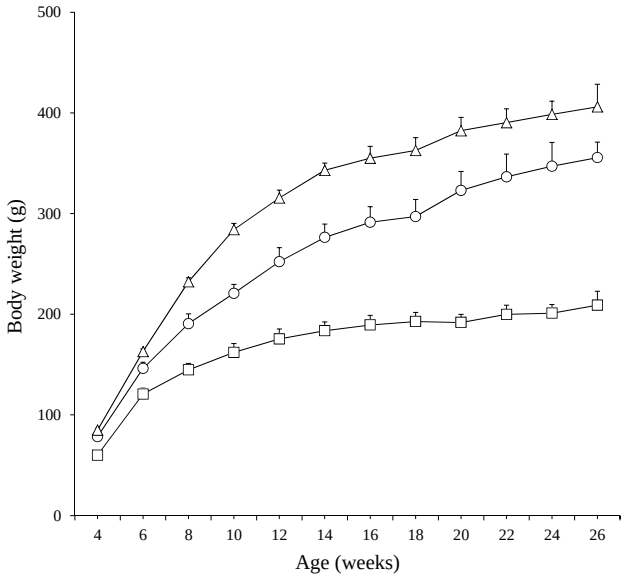

Fig. (1). Chronological changes in body weights of the sham-operated female rat group (○), oophorectomized female rat group (△), and the female rats oophorectomized with estradiol benzoate group (□) from 4 weeks to 26 weeks of age (n = 6 per group). Control female SDT rats were sham-operated. Points and bars represent the mean ± SD.

Histopathological Findings

In histopathological observations of the pancreatic islets at 26 weeks of age, inflammatory cell infiltration, proliferation of fibroblasts, and hemosiderin deposition were observed in and around the pancreatic islets in the sham-operated control (Fig. **3**). In addition, in the oophorectomized group, highly aggravated histopathological findings of inflammatory cell infiltration and proliferation of fibroblasts were observed compared to the sham-operated control (Fig. **4**). On the other hand, the structure of the pancreatic islets in the EB administration group did not show any histopathological changes (Fig. **5**).

DISCUSSION

Thus far, the effects of female hormones on diabetic pathogenesis have been examined in experimental diabetic rats by streptozotocin (STZ) administration [18] and in spontaneously diabetic rats such as Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats [19], Wistar diabetic fatty rats [20] and eSS rats [21]. From the research findings in these diabetic models, increases in body weight and plasma glucose concentration and worsening of glucose tolerance following oophorectomy were reported. It was also shown that administration of estrogen to oophorectomized rats can inhibit the increase in body weight and curtail the worsening of glucose tolerance.

Although the findings in the female SDT rats in the present study were mostly the same as the findings in these prior experiments, no notable hyperglycemia leading to development of diabetes was found in the oophorectomized

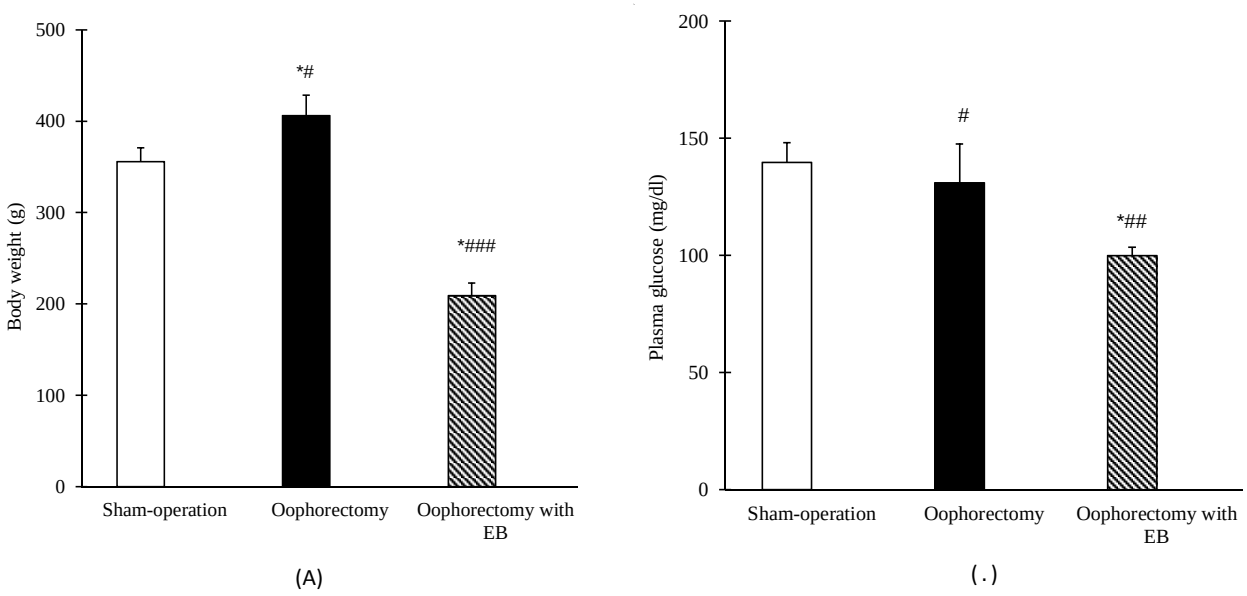

Fig. (2). (**A**) Body weight and (**B**) fasting plasma glucose concentrations of female SDT rats sham-operated, oophorectomized and oophorectomized with estradiol benzoate administration at 26 weeks of age (n = 6 per group). Data are expressed as mean ± SD. EB: Estradiol benzoate. *P<0.01 *versus* sham-operation group, $^{\#}$P<0.01 *versus* oophorectomy with EB group, oophorectomy group, $^{\#\#}$P<0.01 *versus* oophorectomy group.

rats, and no clear histopathology in the pancreatic islets was observed in the EB administration group. With respect to the oophorectomized rats, a picture of aggravated fibrosis was observed in and around the pancreatic islets. Body weights in the oophorectomized group increased significantly compared to those in the sham-operated contrors, so it is possible that the increase in body weight caused by oophorectomy affected the β-cells of the pancreatic islets by way of changes in the insulin sensitivity and *via* the impact of other factors. Detailed analysis of the reasons for this will be a subject for future investigations.

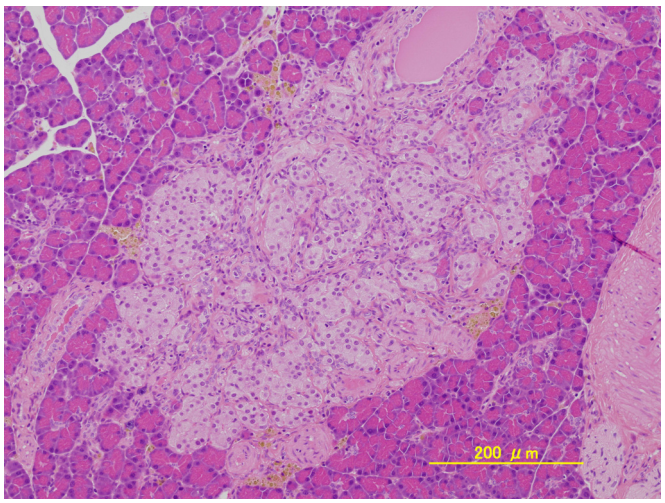

Fig. (3). Histopathological findings of the pancreas in sham-operated females at 26 weeks of age. Inflammatory cell infiltration, proliferation of fibroblasts and hemosiderin deposition were observed in and around the pancreatic islets. Hematoxylin and eosin (original magnification ×140); bar, 200μm.

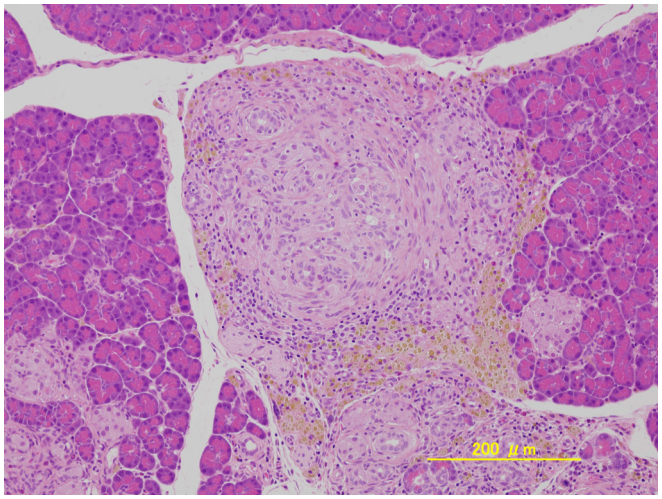

Fig. (4). Histopathological findings of the pancreas in oophorectomized females at 26 weeks of age. Histopathological findings observed in oophorectomized females were exacerbated in terms of inflammatory cell infiltration and proliferation of fibroblasts. Hematoxylin and eosin (original magnification × 140); bar, 200 μm.

Estrogen is commonly known to raise insulin sensitivity. For instance, although glucose uptake in the skeletal muscles of pregnant rats normally decreases, it has been indicated that glucose uptake in the skeletal muscles increased and glucose utilization in peripheral areas improved when the skeletal muscles were cultured in the presence of estrogen [22]. When estrogen was administered to rats after oophorectomy, insulin sensitivity improved, the insulin/glucagon ratio in the portal vein increased, and fasting plasma glucose concentrations decreased due to

inhibition of gluconeogenesis in the liver [23]. In addition, when estrone (E1) was administered to *db*/*db* mice, elevated levels of insulin in the plasma and hyperglycemia were prevented, atrophy of pancreatic islets lessened in the E1 administration *db*/*db* mouse group, and morphology was maintained compared to the *db*/*db* mice. The E1 administration *db*/*db* mouse group recovered to an extent similar to normal (+/+) mice when comparing insulin secretion from isolated pancreatic islets against glucose, and E1 administration protected pancreatic islets improved insulin secretion [24]. Furthermore, it was recently reported that transgenic strains of aromatase-deficient ($ArKO^{-/-}$) mice are vulnerable to β-cell apoptosis and prone to insulin-deficient diabetes after exposure to acute oxidative stress with STZ. In these mice, estradiol treatment reverses STZ-induced β-cell apoptosis, helps sustain insulin production, and prevents diabetes. *In vitro*, in mouse pancreatic islets and β-cells exposed to oxidative stress, estradiol prevents apoptosis and protects insulin secretion. Estradiol protection is partially lost in β-cells and islets treated with an estrogen receptor-α antagonist and in estradiol-deficient (αERKO) islets. Therefore, αERKO mice are no longer protected by estradiol and display a gender-nonspecific susceptibility to oxidative injury, which precipitates β-cell apoptosis and insulin-deficient diabetes [25]. Additionally, in a wide range of classical rodent models of β-cell failure with different pathogenic mechanisms, such as mice with STZ-induced diabetes [25, 26], transgenic mice [25, 27], Zucker Diabetic Fatty rats [28] and OLETF rats [19, 29] 17β-estradiol (E2) at physiological concentrations protects pancreatic β-cells against glucolipotoxicity, oxidative stress, and apoptosis [30].

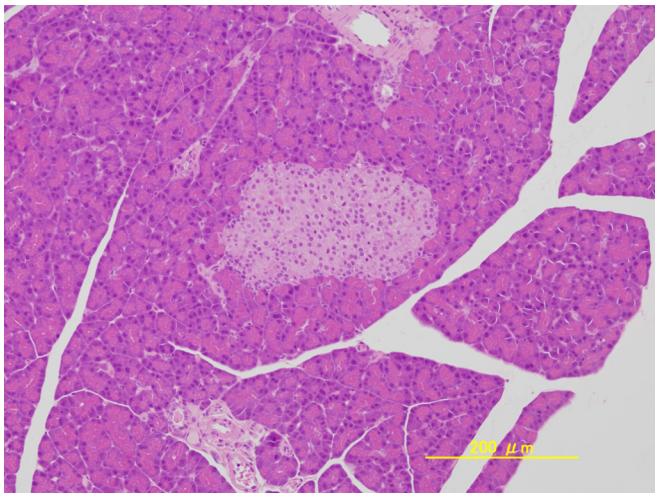

Fig. (5). Histopathological findings of the pancreas in females oophorectomized with estradiol benzoate administration at 26-weeks of age. Histopathological abnormalities were seldom observed. Hematoxylin and eosin (original magnification ×140); bar, 200 μm.

These findings suggest that steroid hormones, especially female sex hormones, act prophylactically against diabetes. In the female SDT rat, it is also possible that estrogen prevents the progression of diabetic pathogenesis *via* action on insulin or by directly affecting the pancreatic islets. Thus, further detailed examination is necessary regarding the action of estrogen on pancreatic islet cells.

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