Diabetes-Associated Complications in Spontaneously Diabetic Torii Fatty Rats

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Abstract: The Spontaneously Diabetic Torii (SDT) fatty rat, established by introducing the fa allele of the Zucker fatty rat into the SDT rat genome, is a new model of obese type 2 diabetes. The SDT-fa/fa (SDT fatty) rat shows overt obesity, and hyperglycemia and hyperlipidemia are observed at a young age as compared with the SDT-+/+ (SDT normal) rat. However, the features of the diabetic complications in the SDT fatty rat have not been reported. In the present study, the incidence and the progression of diabetic complications in the SDT fatty rat were examined, and compared with those of the SDT normal rat. Renal function parameters, such as blood urea nitrogen, urine volume and urinary protein, increased from 4 weeks of age in the SDT fatty rat, and pathological findings in the renal tubule were observed from 8 weeks. Furthermore, cataract was observed in the SDT fatty rat from 8 weeks of age, and prolongation of peak latencies on electroretinograms was observed at 16 and 24 weeks of age. On the other hand, in the SDT normal rat, renal or ocular changes were observed from 24 weeks of age. With early incidence of diabetes mellitus, diabetes-associated complications in the SDT fatty rat were seen at younger ages than those in the SDT normal rat. In conclusion, the SDT fatty rat is expected to be a useful model for the analysis of diabetic complications and the evaluation of drugs related to metabolic diseases.

Key words: diabetes, diabetic nephropathy, ERG, SDT fatty rat, SDT normal rat

Introduction

Sustained hyperglycemia causes severe diabetic microvascular complications, such as retinopathy, peripheral neuropathy, and nephropathy. In the diabetic states, multiple mechanisms have been implicated in glucose-mediated vascular damage and contribute to diabetic microvascular complications. Moreover, because of difficulties in achieving complete euglycemia, a new approach to preventing diabetic complications is required [15]. Diabetic animal models have a critical role in the elucidation of the mechanisms of diabetic complications.

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and the development of novel drugs as treatments.

The SDT normal rat is a useful model of non-obese type 2 diabetes that spontaneously develops hyperglycemia and glucose intolerance resulting from decreased insulin secretion accompanying β-cell degeneration. The SDT normal rat develops diabetes from about 20 weeks of age [8, 20]. It is worthy of note that the SDT normal rat shows ocular complications such as venous dilation and meandering vascular networks, which are caused by hyperglycemia [16]. The Spontaneously Diabetic Torii (SDT) fatty rat was established by introducing the fa allele of the Zucker fatty rat into the SDT normal rat genome [9]. The SDT fatty rat develops diabetes from 5 weeks of age, and the development time is much earlier than that of the SDT normal rat [9].

In the present study, we investigated diabetic complications in the SDT fatty rat by comparing them with those in age-matched SDT normal rats, and examined the utility of the SDT fatty rat as a model of diabetic complications.

Materials and Methods

Animals

Male SDT-fa/fa (SDT fatty) rats and age-matched SDT-+/+ (SDT normal) rats from our colonies were used. The SDT fatty rats and SDT normal rats were selected by genetic analysis. Genotyping of the fa locus was performed according to a PCR-restricted fragment length polymorphism (RFLP) method [9]. The rats were housed in a climate-controlled room with a temperature of 23 ± 3°C, humidity of 55 ± 15%, and a 12-h lighting cycle. A basal diet (CRF-1, Charles River Japan, Yokohama, Japan) and water were provided ad libitum. All the experiments received prior approval from the committee for the human care and use of animals of our laboratory, in accordance with the Standards Relating to the Care and Management of Experimental Animals (Notification No.6, March 27, 1980, of the Prime Minister’s Office of Japan).

Biological parameters

Body weight, food intake, biochemical parameters, and renal parameters were evaluated at 4, 6, 8, 16, 24, and 32 weeks of age. Blood samples were collected from the tail vein of rats. Serum glucose, insulin, triglyceride (TG), total cholesterol (TC), and blood urea nitrogen (BUN) levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi 7170S; Hitachi, Tokyo, Japan) as biochemical parameters.

Urine volume, urinary protein, and creatinine clearance were evaluated as renal parameters. Urine samples were collected by placing the animals in metabolic cages with feed and water for 8 h. Urinary protein was measured with a commercial kit (Tonein-TPII; Otsuka, Tokushima, Japan). Urinary creatinine and serum creatinine were measured using commercial kits (Roche Diagnostics, Basel, Switzerland) and an automatic analyzer (Hitachi 7170S; Hitachi, Tokyo, Japan). Creatinine clearance (mL/h·g) was calculated by dividing the 8-h urinary excretion of creatinine by the serum creatinine concentration and body weight.

Electroretinogram (ERG)

ERGs were obtained at 6, 8, 16, 24, and 32 weeks of age using the method of Segawa et al. [18], with minor modification. Rats adapted to darkness for at least 60 min, then anaesthetized with an intraperitoneal injection of 50 mg/kg ketamine (Sankyo, Tokyo, Japan). Monocular recordings were obtained with the pupil maximally dilated by instillation of 0.5% tropicamide. A 40 J xenon discharge lamp (MES-3102; Nihon-Koden, Osaka, Japan) was flashed once, 30 cm above the eye, after dark adaptation. The potential between the corneal contact lens electrode and the reference electrode (a gold ring embedded in a hard contact lens with the reference electrode; Kyoto Contact Lens Laboratories, Kyoto, Japan) was amplified (AVB-10; Nihon-Koden) and displayed on an oscilloscope (VC-11; Nihon-Koden) with low (15 Hz) and high (1 kHz) band-pass filters. Peak latency was measured as the interval between the stimulus onset and the peak of the corresponding b-waves, and latencies were designated as OP1, OP2, OP3, and OP4, in the order of superimposition. Peak latency in this experiment was represented as the summed potential ∑(OP1–OP4).

Tissue sampling and histopathology

Necropsy was performed on SDT fatty rats and SDT
normal rats at 8, 16, 24, 32, and 40 weeks of age (n=3–4 for each age). All animals were sacrificed by exsanguination under light ether anesthesia.

The kidneys were immediately sampled and fixed in 4% paraformaldehyde. After resection, the tissue was paraffin-embedded by standard techniques and thin-sectioned (3 to 5 µm). The sections were stained with hematoxylin and eosin (HE) and periodic acid Schiff (PAS). Those samples were all examined histopathologically in a blind manner, and the findings were graded from normal (−) to severe (+++). A grade of + was used to represent focal or weakly positive sections, ++ was used to represent half of the section area positive, and +++ was used to represent more than half of the whole area positive in a tissue section. For the glomerular findings in kidneys, more than one hundred glomeruli per animal were observed in this study.

The eyes were sampled and fixed in 4% glutaraldehyde/10% neutral-buffered formalin, then embedded in paraffin, sectioned and stained with HE. The samples were all examined histopathologically in a blind manner, and the findings were graded from normal (−) to severe (+++). The criteria for grading cataract in the cortex of the lens were as follows: + for a few lesions of small size seen in the focal area, ++ for some of the lesions of medium size seen multifocally, +++ for numerous lesions of large size seen widespread and diffusely.

Statistical analysis

The results of biological parameters are expressed as the mean ± standard deviation (SD). Statistical analysis of differences between mean values was performed using an F-test, followed by Student’s t-test or Aspin-Welch’s t-test. Differences were considered significant at P<0.05.

Results

Body weight and food intake

SDT fatty rats showed polyphagia immediately after weaning, and food intake from 4 to 24 weeks of age increased significantly as compared with that in SDT normal rats. Total food intake increased by about 2-fold between 4 and 16 weeks of age (Fig. 1B). Body weight showed a significant increase as compared with that in SDT normal rats during the observation period except at 16 weeks of age (Fig. 1A). The SDT fatty rats showed overt polyphagia and obesity.

Biochemical parameters

Serum glucose levels in SDT fatty rats were elevated from 6 weeks, whereas in SDT normal rats they were elevated from 24 weeks of age (Fig. 1C). The SDT fatty rats showed hyperinsulinemia from 4 to 8 weeks of age, but after 16 weeks their insulin levels decreased to levels similar to those in SDT normal rats (Fig. 1D). Lipid parameters, serum TG and TC levels, in SDT fatty rats from 4 weeks of age were significantly higher than those in SDT normal rats (Figs. 1E and 1F). In other monitoring the biochemical parameters at 32 weeks of age for the Sprague-Dawley (SD) rat, the control animal of the SDT normal rat, are as follows: glucose, 133 ± 1 mg/dL; insulin, 4.0 ± 0.3 ng/mL; triglyceride, 240 ± 31 mg/dL; total cholesterol, 93 ± 8 mg/dL. (Data represent means ± SD, n=6). Glycolipid disorder in SDT fatty rats was obviously promoted as compared with SDT normal rats.

Renal parameters

Serum BUN levels in SDT fatty rats were significantly higher than those in SDT normal rats during the observation period, except at 24 weeks of age (Fig. 2A). Urinary volume and protein levels increased from 4 weeks, and those after 16 weeks of age showed remarkable elevation as compared with those in SDT normal rats (Figs. 2B and 2C). Urinary protein in SDT fatty rats increased from 1.2 mg/creatinine to 29.7 mg/creatinine. Creatinine clearance in SDT fatty rats was significantly higher at 16 weeks of age, but was significantly lower than that in SDT normal rats at 32 weeks of age (Fig. 2D). Increases in renal parameters such as BUN, urine volume and urine protein were shown by SDT fatty rats.

Electroretinogram

SDT fatty rats at 16 and 24 weeks of age showed a prolongation of the peak latencies (142.6 ms and 149.3 ms, respectively) of oscillatory potentials as compared with those of age-matched SDT normal rats (131.3 ms and 133.6 ms, respectively) (Fig. 3). Since the peak
Fig. 1. Changes of biological parameters (A, Body weight; B, Food intake; C, Glucose; D, Insulin; E, Triglyceride; F, Total cholesterol) in SDT fatty rats and SDT normal rats. SDT fatty rats showed overt polyphagia and obesity. Glycolipid disorder in SDT fatty rats was obviously promoted as compared with SDT normal rats. Data shown as mean ± SD (n=5–9). *P<0.05, **P<0.01; significantly different from the SDT normal rat.
Fig. 2. Changes of renal parameters (A, Blood urea nitrogen; B, Urine volume; C, Urinary protein; D, Creatinine clearance) in SDT fatty rats and SDT normal rats. Blood urea nitrogen, urine volume and urinary protein in SDT fatty rats tended to increase during the experimental period. Data shown as mean ± SD (n=5–9). *P<0.05, **P<0.01; significantly different from the SDT normal rat.

Fig. 3. Changes of peak latencies of oscillatory potentials on ERG in SDT fatty rats and SDT normal rats. A prolongation of the peak latencies in SDT fatty rats was shown at 16 and 24 weeks of age. Data shown as mean ± SD (n=4–8). *P<0.05; significantly different from the SDT normal rat.

Histopathological analyses

In SDT fatty rats, histopathological examination of the kidneys revealed changes in the glomeruli from 16 weeks, and in the renal tubules from 8 weeks of age (Table 1). In kidneys of age-matched SDT normal rats, glomerular changes were observed from 32 weeks of age, and renal tubular lesions from 24 weeks of age. In the glomeruli of SDT fatty rats, glomerulosclerosis was observed from 16 weeks of age, and the sclerosis progressed with aging. In SDT normal rats, glomeruloscler-
Table 1. Histopathological findings of kidney in SDT fatty rats and SDT normal rats

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<td></td>
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<td>SDT fatty rat</td>
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<td>Glomeruli</td>
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<td>Glomerulosclerosis, diffuse</td>
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<td>Glomerulosclerosis, nodular</td>
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<tr>
<td>Renal Tubule</td>
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<tr>
<td>Armanni-Ebstein change</td>
<td>3</td>
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<td>Tubular dilation, diffuse</td>
<td>2</td>
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<tr>
<td>Hyaline cast, diffuse</td>
<td>4</td>
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<td>SDT normal rat</td>
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<td>Hyaline cast, diffuse</td>
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Each of the findings were qualitatively divided into four grades: normal (–), slight (+), moderate (++), or severe (+++).

Fig. 4. Histological analysis of glomeruli. PAS stain. A, SDT fatty rat (24 weeks of age); B, SDT fatty rat (40 weeks of age); C, SDT normal rat (24 weeks of age); D, SDT normal rat (40 weeks of age). Glomerular lesions in SDT fatty rats were observed from 16 weeks of age, whereas such lesions in SDT normal rats were observed from 32 weeks of age. Bar=20 µm. Diffuse glomerulosclerosis was observed in the SDT fatty rat and the SDT normal rat (arrow heads). Nodular lesions were observed only in a few glomeruli of SDT fatty rats (arrows).
Atherosclerosis was slightly observed at 32 and 40 weeks of age (Table 1, Fig. 4). Nodular lesions were observed at 40 weeks of age in SDT fatty rats, but were not seen in SDT normal rats (Figs. 4B and 4D). In the renal tubules, glycogen deposition in the tubular epithelium (Armanni-Ebstein lesions) and tubular dilation were noted from 8 weeks of age in SDT fatty rats, and the changes progressed from 8 to 16 weeks of age (Table 1). There was an increased number of hyaline casts from 16 weeks of age. In age-matched SDT normal rats, renal tubular lesions were observed from 24 weeks of age (Fig. 5).

In SDT fatty rats, histopathological findings in the lens, including hyperplasia of epithelium, vacuolation of fiber and morgagnian globules, were observed from 8 weeks of age, and these changes progressed with aging (Table 2, Fig. 6). In SDT normal rats, ocular lesions in the lens were observed from 24 weeks of age.

SDT fatty rats showed renal and ocular lesions from a younger age than in SDT normal rats.

**Discussion**

The SDT normal rat, a model of non-obese type 2 diabetes, was established in 1997 by Shinohara *et al.* [20]. More recently, Masuyama *et al.* produced the SDT fatty rat by introducing the fa allele of the Zucker fatty rat into the SDT normal rat genome [9]. SDT fatty rats develop diabetes from 5 weeks of age, with cumulative incidence of diabetes reaching 100% by 16 weeks of age. SDT fatty rats show manifest obesity, hyperglycemia, and hyperlipidemia. In this study, we investigated the characteristics of SDT fatty rats, chiefly for diabetes-asso-

![Fig. 5. Histological analysis of renal tubules. HE stain. A, SDT fatty rat (16 weeks of age); B, SDT fatty rat (24 weeks of age); C, SDT normal rat (16 weeks of age); D, SDT normal rat (24 weeks of age). Renal tubular changes such as Armanni-Ebstein change (single arrows) and tubular dilation (double arrows) were observed from 8 weeks of age in SDT fatty rats. On the other hand, such changes in SDT normal rats were observed from 24 weeks of age. Bar=50 µm.](image-url)
Table 2. Histopathological findings of eye in SDT fatty rats and SDT normal rats

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<th>Groups</th>
<th>Pathological findings</th>
<th>Weeks of age</th>
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<td>SDT fatty rat</td>
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<td>Retina</td>
<td>Rosette formation/ irregularity</td>
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<td></td>
<td>Protrude, papilla</td>
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<tr>
<td>Lens</td>
<td>Cataract</td>
<td>3 1 0 0 3 1 0</td>
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<td></td>
<td>Degeneration of lens fiber (vacuolation/Morgans body)</td>
<td>2 2 0 0 1 3 0</td>
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<td>Hypertrophy/proliferation, epithelium</td>
<td>4 0 0 0 4 0 0</td>
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SDT normal rat

| Retina          | Rosette formation/ irregularity                 | 4 0 0 0 4 0 0 | 0 4 0 0 3 0 0 | 0 3 0 0 3 1 0 |
|                 | Protrude, papilla                              | 4 0 0 0 4 0 0 | 0 4 0 0 3 0 0 | 0 4 0 0 4 0 0 |
| Lens            | Cataract                                       | 4 0 0 0 4 0 0 | 0 1 3 0 0 2 1 | 0 0 0 3 1 0 |
|                 | Degeneration of lens fiber (vacuolation/Morgans body) | 4 0 0 0 4 0 0 | 0 0 4 0 0 3 0 | 0 0 4 0 0 |
|                 | Hypertrophy/proliferation, epithelium           | 4 0 0 0 4 0 0 | 0 0 4 0 0 3 0 | 0 0 4 0 0 |

Each of the findings were qualitatively divided into four grades: normal (–), slight (+), moderate (++), or severe (+++).

In the present study, SDT fatty rats showed hyperglycemia and hyperlipidemia with polyphagia and obesity immediately after weaning (4 weeks of age). These changes in SDT fatty rats were almost consistent with the results for SDT fatty rats reported by Masuyama et al. [9]. In the Zucker diabetic fatty (ZDF) rat, plasma triglyceride and cholesterol levels increased at 6 weeks, whereas glucose levels increased after 12 weeks of age [1, 23]. We have also experienced that the blood glucose levels of ZDF rats elevate after 9 or 10 weeks of age in other experiments. Wistar fatty (WF) rats were developed by Ikeda et al., by crossbreeding Wistar Kyoto rats and Zucker fatty rats [4]. In WF rats, the plasma glucose level increases after 8 or 10 weeks of age [4, 7, 22]. Serum insulin levels in the SDT fatty rats increased between 4 and 8 weeks of age, but the levels had dramatically decreased after 16 weeks of age. On the other hand, the plasma insulin levels of ZDF rats was maintained at high levels as compared with those in lean control rats [1]. In WF rats, the plasma insulin level was elevated until 16 weeks of age, but thereafter decreased [4]. Hyperglycemia from a young age is a distinctive feature of SDT fatty rats, and the early expression of hyperglycemia may be caused by hyperphagia, from 4 weeks of age, and genetic weakness of the pancreatic function, which was indicated by the rapid decline of the insulin level between 8 and 16 weeks of age.

In SDT fatty rats, renal function as assessed by BUN, urine volume and urine protein deteriorated from 4 weeks of age. In ZDF rats, an increase of urinary albumin excretion was shown at 8 weeks of age [23], and an increase of urinary protein excretion was observed at 40 weeks of age [1]. Moreover, WF rats showed polyuria or proteinuria after 12 weeks of age [13, 24]. The deterioration in these renal parameters from an early stage in SDT fatty rats is considered to be associated with the occurrence of hyperglycemia and hyperlipidemia from a young age.

In the renal tubule of SDT fatty rats, renal tubular lesions such as Armanni-Ebstein change and tubular dilation were observed from 8 weeks of age, and similar lesions were observed in SDT normal rats at 24 weeks of age (Table 1, Fig. 5). Furthermore, in the glomeruli of SDT fatty rats, glomerulosclerosis was slightly observed from 16 weeks of age, and nodular lesions were
observed at 40 weeks of age. In the SDT normal rats, glomerulosclerosis was slightly observed at 32 and 40 weeks of age, but nodular lesions were not observed (Table 1, Fig. 4). Nodular lesions in SDT normal rats have been observed at 68 weeks of age [14]. SD rats have not shown renal lesions from at ages younger than 68 weeks of age [14]. Renal histological lesions of the glomerulus and tubule in ZDF rats were observed after 20 weeks of age [2, 17]. Moreover, in WF rats, the renal lesions of the glomerulus and tubule were shown at 15 and 18 weeks of age [11, 24]. It is considered that the nephropathy of the SDT fatty rat was expressed early, as compared with the other diabetic models, because of hyperglycemia from a younger age. The histopathological features of the kidney were not different between the SDT fatty rat and the SDT normal rat; however, the pathological degree was more progressed in the SDT fatty rat. Further pathological examination after 40 weeks of age is considered to be necessary for confirmation of the final changes in kidneys of the SDT fatty rat.

In humans, the histopathologic features of glomerular lesions are generally divided into 3 types: diffuse lesions, nodular lesions, and exudative lesions [5]. Of these, the predominant change in long-standing diabetes is diffuse lesions. Diffuse glomerulosclerosis, including thickening of the glomerular basement membrane or increased mesangial matrix, and glomerular hypertrophy were observed in the SDT fatty rats (Figs. 4A and 4B). In the SDT fatty rats, the onset and progression of diffuse le-
sions was similar to that in human diabetes. The histopathological findings correlated with increase in urinary protein and albumin excretion, and are probably related to the increases in glomerular pressure seen early after diabetes onset [10, 12]. Aramani-Ebstein lesion has also been observed in human diabetic nephropathy [6], and the change in the SDT fatty rat was similar to that in humans. Since renal lesions in both the glomerulus and tubule of SDT fatty rats are similar to those in humans and occur from a young age, it is expected that the SDT fatty rat will be very useful as a model of diabetic nephropathy. Some early diabetic patients show elevation of the glomerular filtration rate [3], and the creatinine clearance in SDT fatty rats also increased at 16 weeks of age.

Cataract in SDT fatty rats was observed from 8 weeks of age, and the change progressed with aging. In SDT normal rats, a similar change was observed from 24 to 40 weeks of age (Table 2, Fig. 6). Moreover, SD rats have not shown ocular changes before 68 weeks of age [14]. The histopathological features of cataract were not different between the SDT fatty rat and the SDT normal rat, whereas the pathological degree was more progressed in the SDT fatty rat. Lens opacity and histopathological findings such as vacuolation and formation of Morgan’s body, have also been found in the eyes of ZDF rats [19, 21]. Histological changes, such as tractional retinal detachment and thickening, and distortion of the retina, were observed in SDT normal rats at 70 weeks of age [20], whereas those retinal changes were not observed in the SDT fatty rats up to 40 weeks of age. Further investigation after 40 weeks of age for the SDT fatty rat is essential for evaluation of retinal changes. Since the peak latency of SDT fatty rats on ERG was retarded at 16 and 24 week of age, functional abnormality of the retina may have occurred. The peak latency of the SDT normal rat showed a prolongation at 44 weeks of age [16]. There was no difference in the peak latency at 32 weeks of age between the SDT normal rat group and the SDT fatty rat group because of the prolongation of the peak latency in the SDT normal rat group.

Diabetes-associated complications in SDT fatty rats are found at a younger age than in SDT normal rats. The early onset of diabetes-associated complications has advantages for the use SDT fatty rats in diabetes research. In conclusion, the SDT fatty rat is a useful model for the evaluation of the effects of drugs and the investigation of diabetic complications. This rat model has promise in the further elucidation of the pathogenesis of human diabetic nephropathy and in new drug discovery.

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References


