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The spontaneously diabetic Torii rat with gastroenteropathy

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Abstract

The spontaneously diabetic Torii (SDT) rat was recently recognized as a new animal model of non-obese type 2 diabetes. As the severe diabetic ocular complications seen in SDT rats already have been investigated, we examined another common diabetic complication, gastroenteropathy. Male SDT rats developed diabetes at 20 weeks and diarrhea at 28 weeks of age. Gastrointestinal motility was evaluated at 28 weeks by measuring the distance of small intestinal transit by oral administration of the non-absorbed marker, arabic gum. SDT rats exhibited greater intestinal transit distance than control SD rats (54.1 ± 2.6% versus 43.0 ± 1.2%). Insulin treatment of SDT rats begun at 20 weeks of age produced improved stool and reduced intestinal transit distance (41.4 ± 0.3%). Morphologically, the SDT rats exhibited longer villi and heavier weight of intestine compared to control SD rats. These results suggest that the SDT rat may be a useful animal model for studies of diabetic gastroenteropathy.

Keywords: Diabetes mellitus; Gastroenteropathy; Diarrhea; Intestinal motility

1. Introduction

Diabetic animal models can be made by chemical treatment, pancreas resection, or careful crossbreeding to exhibit spontaneous development, and are especially helpful for analysis of the pathogenesis of the complications of human type 2 diabetes. There are several established lines of spontaneously diabetic animal models of the disease, including the GK rat, the Wistar fatty rat, and the OLETF rat [1–3], but recently the spontaneously diabetic Torii (SDT) rat has been recognized as an inbred animal model [4–6]. It was reported that SDT male rats develop hyperglycemia and hypoinsulinemia without obesity at about 20 weeks of age. SDT male rats develop hyperglycemia and hypoinsulinemia at about 20 weeks of age. Interestingly, they also exhibit severe diabetic ocular complications such as cataracts, proliferative retinopathy,

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and retinal detachment that resemble human diabetic ocular complications [4]. In the present study, we studied whether they develop gastroenteropathy with diarrhea by morphological and functional analysis.

2. Materials and methods

2.1. Animals

Male SDT rats were obtained from Research Laboratories of Torii Pharmaceutical Co. Ltd. Age-matched male Sprague Dawley (SD) rats (Crj: CD(SD)IGS, Charles River Japan, Kanagawa, Japan) were used as control animals. All rats were housed in a pathogen free, air-controlled room (25 ± 2 °C and 50% humidity) with 12-h light/12-h dark cycle. The studies were performed in accordance with the Declaration of Helsinki. The diagnostic criteria for diabetes were the presence of glucosuria and hyperglycemia characterized by non-fasting blood glucose concentration exceeding 200 mg/dl. The diabetic rats were divided into two groups, untreated SDT rats ($n = 8$) and insulin-treated SDT (SDT-INS) rats ($n = 8$). The plasma glucose concentration was measured and doses of ultralente insulin (2–12 U/day: Shimizu Pharmaceutical, Shizuoka, Japan) was determined using a sliding scale from 20 weeks of age.

2.2. Intestinal motility of small intestine

Transit distance through the stomach and small intestine was measured by a non-absorbed-marker (10% charcoal suspension in 5% gum Arabic), as previously described [7–9]. Briefly, food was withheld for 16 h before the experiment, with free access to drinking water, and the rats received the suspension orally by gavage using a straight blunt-ended feeding needle. After 20 min, the animals were killed by cervical dislocation, and the entire gastrointestinal tract was removed. The distance from the pylorus to the front of the charcoal bolus and the ileocecal junction was measured. The rate of transit was determined as [(distance to charcoal front)/(length of small intestine)] × 100 (%) [9].

2.3. Weight of bowel segment

The animals were anesthetized with pentobarbital administrated intramuscularly. Ten-centimeter segments of jejunum, beginning 5 cm distal to the ligament of Treitz and 10-cm segments of ileum, ending 5 cm proximal to the ileocecal valve (measured as accurately as possible by ruler; the intestine in these conditions is in a relaxed, non-contracted state), were removed and weighed. The full length of the villi from the tip to the bottom was removed by scraping firmly with a glass slide and weighed.

2.4. Morphometric measurements

The circumference of the small intestine was determined by measuring the width at three points of the intestine, which was flattened and stapled onto cardboard. The sheets of intestine were then fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin. Mucosal thickness (villus tip to crypt bottom) was measured on at least three representative villus-crypt units per section; only well-oriented sections were used. All measurements were made by the same researcher on coded sections [10].

2.5. Estimation of blood glucose levels and fecal water content

Blood glucose levels were measured using Novo-assist (LIFE SCAN Inc., Milpitas, CA) to sample from the tail vein. Feces were sampled immediately after defecation.

Fecal water content was calculated as [(fecal net weight − fecal dry weight after being freeze-dried)/fecal net weight] × 100 (%). Body weight and blood glucose levels were measured every week from 14 to 28 weeks of age. The percentage of fecal water content was measured at 14 and 28 weeks of age.

2.6. Fecal fat content

Feces were lyophilized and ground. Fat content in feces was determined by extraction with diethyl ether after acid hydrolysis (4 M HCl) for 30 min [11].

2.7. Statistical analyses

Significant difference was determined using one-factor ANOVA followed by *t*-test. P values of <0.05 were considered statistically significant. All values are expressed as mean ± standard error of the mean (S.E.M., n = number).

2.8. Ethical considerations

All studies were performed in the laboratories of the Department of Diabetes and Clinical Nutrition, Kyoto University, in accordance with the Declaration of Helsinki.

3. Results

3.1. Body weight and blood glucose

Body weights of the control SD rats and the SDT and SDT-INS rats are compared in Fig. 1A. The average body weight of SD, SDT, and SDT-INS rats was similar at 18 weeks of age (525 ± 3, 534 ± 5, and 535 ± 10 g, respectively). The body weights of both the SDT and SDT-INS rats then became significantly less than controls. At 28 weeks of age, the body weight of the SDT rats was significantly lower than both SD (447 ± 33 g versus 657 ± 8 g, $P < 0.01$) and SDT-INS rats (447 ± 3 g versus 607 ± 9 g, $P < 0.01$). Although the body weight of the SDT-INS rats increased after 20 weeks of age, it was