Difference in glucose intolerance between C57BL/6J and ICR strain mice with streptozotocin/nicotinamide-induced diabetes

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(Received 26 September 2011; and accepted 6 December 2011)

ABSTRACT

Blood glucose and plasma insulin levels between C57BL/6J and ICR strain mice with nicotinamide (NA) and streptozotocin (STZ)-induced diabetes were compared to establish a suitable strain of the experimental diabetic mouse model. The mice were intraperitoneally treated twice with STZ (100 mg/kg) 15 min after injection of NA (120 mg/kg) at a 1-day interval, and non-fasting blood glucose level was then weekly monitored for 5 weeks. The blood glucose level in ICR mice gradually increased and was about 2-times higher than that in C57BL/6J mice at the end of the observation. The plasma insulin level in ICR mice was comparatively low, compared with that in C57BL/6J mice. ICR mice were also markedly glucose-intolerant when oral glucose tolerance test was performed. These results indicate that ICR strain is more sensitive than C57BL/6J strain as a mouse model with NA/STZ-induced mild diabetes.

Diabetes mellitus is a group of metabolic diseases developed by impaired insulin secretion of pancreatic β -cells and disrupted insulin action in the target tissues such as the liver, muscle and adipose tissue (2, 5, 15). This disease represents diabetes-specific microvascular pathology in the retina, renal glomerulus and peripheral nervous system, and which is a leading cause of blindness, end-stage renal disease and neuropathy (3). Diabetes mellitus also has a direct relevance to accelerated atherosclerosis (17). As the result, patients with diabetes mellitus have a much higher risk of myocardial infarction and stroke (17), increasing cases of the disease all over the world with the global figure of the patients set to rise from the current estimate of 150 million to 220 million in 2010, and 300 million in 2025 (1, 9). Therefore, it is necessary to establish the animal

model suitable for prevention of diabetes mellitus.

Streptozotocin (STZ) is an agent frequently used to induce experimental diabetes in mammals (14). This chemical specifically damages pancreatic β -cells and initiates hyperglycemia by insulin deficiency (13). A single intraperitoneal injection of STZ at the dose of 100 mg/kg is enough for mice to cause hyperglycemia (10). However, there are differences among various inbred strains of mice in the severity of hyperglycemia and insulinitis by STZ (12). Although STZ dose- and time course-dependently causes hyperglycemia at doses of 100–200 mg/kg in ICR mice, there is little dependence between the effective doses or time course and the severity in BALB/c and C57BL/6 inbred mice (7). Recently, the simultaneous administration of nicotinamide (NA) with STZ to C57BL/6J mice at the dose of 120 mg/kg showed protecting the pancreatic β -cells partially from the oxidative damage and inducing mild diabetes in comparison with the treatment of STZ alone (11). In view of the dose-effect relation of STZ, ICR that is a common non-inbred closed colony may be more preferable than C57BL/6 as a strain of the ex-

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perimental diabetes mouse model by the simultaneous treatment of NA and STZ (NA/STZ-treatment). In the present study, the blood glucose and plasma insulin levels between C57BL/6J and ICR strain mice after NA/STZ-treatment were compared.

Permission for animal experiments in this study was obtained from the Animal Experiment Room Administration Committee of Setsunan University, and the experiments were conducted according to the Animal Experiment Guidelines of the Faculty of Pharmaceutical Sciences, Setsunan University. Specific pathogen-free male C57BL/6J (5 weeks old, about 20 g) and ICR mice (5 weeks old, about 25 g) were purchased from Japan SLC Inc. (Shizuoka, Japan). The animals were maintained at $23 \pm 1^\circ\text{C}$, approximately 40 % relative humidity and a light/dark cycle of 12 h each, and had *ad libitum* access to γ -ray-irradiated pelleted rodent chow (Type NMF; Oriental Yeast Co., Tokyo, Japan) and tap water. The mice were acclimated for 1 week prior to use. C57BL/6J and ICR mice were fasted for 16 h and then intraperitoneally injected with NA (Sigma, St. Louis, MO, USA) dissolved in saline at the dose of 120 mg/kg body weight. The mice were intraperitoneally injected with STZ (Sigma) freshly dissolved in 50 mmol/L citrate buffer (pH 4.5) at the dose of 100 mg/kg body weight 15 min after the treatment of NA. After an interval of 1 day, NA and STZ were administered again in the same manner. After the treatment of NA/STZ, non-fasting blood glucose level was weekly monitored for 5 weeks. Blood was drawn from tail-vein at 10:00–12:00, and blood glucose level was measured directly by a glucose oxidase method using Glucose Pilot (Aventir Biotech, LLC, West Carlsbad, CA, USA). Plasma insulin level was measured 5 weeks after the treatment of NA/STZ. Blood was drawn from the abdominal aorta of the mice under pentobarbital euthanasia and centrifuged at $700 \times g$ for 10 min at 4°C , and plasma was stored at -80°C . The plasma insulin level was measured with the ELISA kit (Shibayagi Co., Ltd., Gunma, Japan). Oral glucose tolerance test (OGTT) was performed by oral administration of glucose at a dosage of 2000 mg/kg body weight to overnight-fasted mice 5 weeks after the treatment of NA/STZ. Blood was collected from the tail vein 0, 30, 60, 90 and 120 min after glucose loading, and blood glucose level was determined using Glucose Pilot (Aventir Biotech, LLC). Values in the figures are expressed as the means $\pm$ SD. Statistical analysis was carried out by one-way analysis of variance (ANOVA) with Bonferroni correction. The *P* level was set at 0.05 or 0.01.

To compare glucose intolerance between C57BL/6J and ICR strain mice with NA/STZ-induced mild diabetes, the mice received twice intraperitoneal injections of 100 mg/kg STZ 15 min after the injection of 120 mg/kg NA at an interval of 1 day, and levels of blood glucose and plasma insulin were measured. As shown in Fig. 1, the blood glucose levels in C57BL/6J and ICR mice gradually increased throughout the 5-week observation period. The blood glucose level in ICR mice was about 2 times higher than that in C57BL/6J mice at the end of observation period. As shown in Fig. 2, the plasma insulin level in ICR mice was low, compared with that in C57BL/6J mice. In OGTT by oral glucose loading of 2000 mg/kg, as shown in Fig. 3, the blood glucose level in ICR mice was also significantly higher than that in C57BL/6J mice.

STZ specifically produces free radicals in pancreatic β -cells and causes fragmentation of DNA chains, resulting in destruction of the cells by necrosis (13). These processes evoke activation of poly(ADP-ribose)-polymerase to repair the damaged DNA, and a large amount of intracellular nicotinamide adenine dinucleotide (NAD) is consumed for this restoration (14). The intake of NA effectively supplements consumption of NAD (16), which may lead to protection of pancreatic β -cells against the disorder by STZ treatment. Therefore, the induction of experimental diabetes by NA/STZ-treatment is considered to be restricted and may simulate type 2 diabetes-like symptoms. In our preliminary study, NA/STZ-treatment to ICR strain mice indeed caused mild diabetic condition in comparison with the treatment

Fig. 1 Effect of NA/STZ-treatment on blood glucose level of C57BL/6 and ICR mice. C57BL/6 (○) and ICR (●) mice. The values are mean $\pm$ SD ($n = 6-7$). Significantly different from C57BL/6 mice at ** $P < 0.01$. Significantly different from the mice before treatment with NA/STZ at †† $P < 0.01$; †† $P < 0.01$.

of STZ alone (data not shown). In the present study, the non-fasting blood glucose level in ICR mice received NA/STZ-treatment was about 520 mg/dL at the end of the 2-wk observation, whereas that in C57BL/6J mice showed about 280 mg/dL (Fig. 1). This indicates that both strain mice may fall into diabetic condition 2-wk after the treatment, when 220–240 mg/dL in the non-fasting blood glucose level is defined as the threshold because the 200 mg/dL is regarded as diabetic condition in rats (6) and the normal levels in rodents is a little higher than that in humans.

Fig. 2 Effect of NA/STZ-treatment on plasma insulin level of C57BL/6 and ICR mice. Plasma insulin level was determined 5 weeks after NA/STZ-treatment. The values are mean $\pm$ SD ($n=5-6$). Significantly different from C57BL/6 mice at $**P < 0.01$.

Fig. 3 Effect of NA/STZ-treatment on OGTT in C57BL/6 and ICR mice. OGTT was performed 5 weeks after NA/STZ-treatment. Blood samples were collected at 0, 30, 60, 90 and 120 min after oral glucose loading of 2000 mg/kg. C57BL/6 (○) and ICR (●) mice. The values are mean $\pm$ SD ($n=6-7$). Significantly different from C57BL/6 mice at $*P < 0.05$; $**P < 0.01$. Significantly different from the mice before NA/STZ-treatment at $††P < 0.01$; $†P < 0.01$.

In the diabetic mouse model induced by STZ, pancreatic insulin-immunoreactive cells tend to be disrupted and plasma insulin is depleted until undetectable level (8). However, the insulin level in plasma of ICR mice after NA/STZ-treatment was detectable even though the level was lower than that of C57BL/6J mice (Fig. 2). This result indicates that the simultaneous treatment of NA indeed partially protects the islets from oxidative damage. Furthermore, the blood glucose level after the load in ICR mice treated with NA/STZ was higher than that in C57BL/6J mice (Fig. 3). In ICR mice, the STZ-induced diabetes is shown to be a non-insulin-dependent type that is characterized by impaired insulin response to glucose stimulation with a decrease in the number of insulin-immunoreactive cells and a relative increase in the number of glucagon-immunoreactive cells (8). Even by the simultaneous treatment of NA with STZ, the diabetes type seems to be a non-insulin-dependent type because the plasma insulin levels 120 min after glucose load in NA/STZ-treated mice decreased only slightly, like the case of STZ-treatment alone (data not shown).

Compared with C57BL/6J mice, ICR mice seems to be more sensitive to STZ because there are differences among mouse strains in poly(ADP-ribose)-polymerase activities and NAD consumption in pancreatic β -cells that are related to sensitivity of their mice to STZ (4). NA/STZ-treated ICR mice are likely to induce severe diabetes mellitus within a shorter term than C57BL/6J mice. ICR mice as an animal model with NA/STZ-induced mild diabetes also will give a possible experimental advantage of much quantity of blood and tissues, as their body sizes are larger than C57BL/6J mice.

Acknowledgements

The authors are grateful to Miss K. Furukawa, Mr. R. Maitani, Miss K. Takemura and Mr. T. Tanaka for their expert technical assistance. This work was supported in part by grants in aid for Scientific Research (KAKENHI 20590128) from Japan Society for the Promotion of Science.

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