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Sensitization to alloxan-induced diabetes and pancreatic cell apoptosis in acatalasemic mice

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ABSTRACT

Human acatalasemia may be a risk factor for the development of diabetes mellitus. However, the mechanism by which diabetes is induced is still poorly understood. The impact of catalase deficiency on the onset of diabetes has been studied in homozygous acatalasemic mutant mice or control wild-type mice by intraperitoneal injection of diabetogenic alloxan. The incidence of diabetes was higher in acatalasemic mice treated with a high dose (180 mg/kg body weight) of alloxan. A higher dose of alloxan accelerated severe atrophy of pancreatic islets and induced pancreatic β cell apoptosis in acatalasemic mice in comparison to wild-type mice. Catalase activity remained low in the acatalasemic pancreas without the significant compensatory up-regulation of glutathione peroxidase or superoxide dismutase. Furthermore, daily intraperitoneal injection of angiotensin II type 1 (AT1) receptor antagonist telmisartan (0.1 mg/kg body weight) prevented the development of alloxan-induced hyperglycemia in acatalasemic mice. This study suggests that catalase plays a crucial role in the defense against oxidative-stress-mediated pancreatic β cell death in an alloxan-induced diabetes mouse model. Treatment with telmisartan may prevent the onset of alloxan-induced diabetes even under acatalasemic conditions.

1. Introduction

Diabetes mellitus is a worldwide disease and one of the major causes of death, thus it is essential to clarify the pathogenesis and effective preventive or therapeutic measures of the disease. Diabetes is characterized by progressive β cell loss and, it is widely accepted that reactive oxygen species (ROS) contribute to pancreatic cell or tissue damage and dysfunction both in type 1 and 2 diabetes, even though the underlying mechanisms differ [1].

The degree of oxidative stress and the severity of subsequent tissue injury may depend on an imbalance between the excessive production of ROS and antioxidant defense within the pancreatic islet. These antioxidants include the enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX), which detoxify ROS. Catalase (E.C.1.11.1.6) is a major enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) and plays a role in cellular antioxidant defense mechanisms [2]. The main reaction of catalase is the catalytic reaction ($2H_2O_2 \rightarrow O_2 + 2H_2O$) and it is essential for the removal of excessive H_2O_2 and for regulation of the H_2O_2 concentration in signaling pathways [3]. Catalase limits the accumulation of H_2O_2 generated by various oxidases in tissue and serves as a substrate for the Fenton reaction to produce the highly injurious hydroxyl radicals.

Genetic defects of catalase were first documented by Takahara [4] in Japanese patients who exhibited a deficiency of catalase enzyme activity in their blood (acatalasemia). The short-time clinical manifestations of human acatalasemia after exposure to H_2O_2 or infection with peroxide-generating bacteria such as streptococci appear predominantly in the mouse. Oral ulcerations, alveolar gangrene and atrophy resulting in a loss of teeth have been reported. A high frequency (12.7%) of diabetes mellitus and deleterious changes in lipid and carbohydrate metabolism is observed in Hungarian acatalasemia, thus suggesting that this inherited disorder may be a risk factor for the development of diabetes or atherosclerosis, and catalase deficiency may not be the benign disorder [5].

ROS are involved in many of the angiotensin II (Ang II) signaling pathways. Ang II stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity via the AT1 receptor to produce the superoxide anion, H_2O_2 and hydroxyl radicals [6]. The existence of

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a local renin–angiotensin system (RAS) is now recognized in pancreas, the activities of which are subjected to regulation by physiological and pathophysiological stimuli such as hypoxia, pancreatitis, islet transplantation, hyperglycemia and diabetes mellitus [7]. Thus, the increased RAS in pancreas may aggravate pancreatic cell damage induced by ROS, and the blockade of RAS may be useful for preventing diabetes.

The present study hypothesized that a functional catalase deficiency would render the pancreas more susceptible to oxidant tissue injury, and the effect of dysregulation of the antioxidant system on the onset of diabetes was investigated using an acatalasemic mouse strain induced by the diabetogenic compound alloxan. The acatalasemic mice differentially express their catalase activity in a tissue specific manner. The catalase activities in these mice range from 1%–2% of the normal mice in red blood cells, to approximately 20% in kidney, or to nearly normal levels in liver [8,9]. Alloxan is a mild oxidant which shows the selective toxicity to pancreatic β cells. The diabetologic action of alloxan is initiated by the generation of ROS [10]. In addition, since ROS are associated with many Ang II signaling pathways, we investigated whether the AT1 receptor antagonist, telmisartan, could inhibit the alloxan-induced hyperglycemia in acatalasemic mice.

2. Materials and methods

2.1. Animals and experimental protocol

Male wild-type mice (C3H/AnLCs^{a}Cs^{a}) and male homozygous acatalasemic mutant mice (C3H/AnLCs^{b}Cs^{b}) were used at the age of 8 to 10 weeks old. All animals were housed in a group of five and fed standard laboratory chow and water. Diabetes mellitus was induced by the intraperitoneal injection with 120 or 180 mg/kg body weight (BW) of alloxan (2,4,5,6-Tetraoxypyrimidine) (Sigma-Aldrich Co., St. Louis, MO) dissolved in phosphate buffered saline (PBS) at the first two consecutive days of experimental protocol. In the control group of mice, the same volume of PBS was injected intraperitoneally. Each group consisted of 15 to 20 mice. The BW was measured at day 0 and 7 of protocol. The blood glucose concentration was determined by a portable glucose meter using Glutest Sensor (Sanwa Kagaku Kenkyusho Co., Nagoya, Japan) at day 0, 2 and 7, using tail tip blood. Plasma insulin at day 7 was measured using a rat insulin radioimmunoassay kit (Linco Research Inc., St. Charles, MO). The pancreases were dissected out at day 7 under pentobarbital anesthesia. The development of diabetes was defined as over 200 mg/dl of blood glucose concentration [11]. Telmisartan (BIBR 277) was dissolved in PBS, adjusted to pH 8.0, and injected intraperitoneally daily from 1 day before administration of 180 mg/kg BW of alloxan to day 7, at a dose of 0.1 mg/kg BW [12]. A vehicle-treated group received the intraperitoneal injection of PBS alone. In this treatment experiment, mice were divided into 8 subgroups ($N=10$ to 15/group). The BW and blood glucose concentration was checked as described above. Streptozotocin (STZ) is another prominent diabetogenic compounds. In a pilot study, the intraperitoneal injection of different concentrations of STZ (120 to 160 mg/kg of BW) did not sensitize acatalasemic mice to diabetes (Supplementary Fig. 1). Therefore, we utilized alloxan-induced diabetes model in this study. The experimental protocol was approved by the Ethics Review Committees for Animal Experimentation of Okayama University Graduate School.

2.2. Light microscopic studies

Formalin-fixed, paraffin-embedded 3-μm sections were assessed using periodic acid-Schiff (PAS) stain. Each tissue section was observed using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) with a high-resolution digital camera system (Penguin 600CL; Pixera Co., CA). The measurement of the pancreatic islets size was performed using a Microanalyzer program (version 1.1; Nippon Poladigital Co., Tokyo, Japan).

2.3. Apoptosis detection

DNA fragmentation associated with apoptosis was detected in situ by the addition of nucleotides to free 3′ hydroxyl groups in DNA as described previously [13,14]. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was performed using a MEBSTAIN Apoptosis Kit Direct (Medical and Biological Laboratories, Nagoya, Japan).

2.4. Catalase, GPX and SOD activity

After harvesting pancreatic tissue, the samples were immediately snap frozen in liquid nitrogen and stored at -80 °C until assayed. The catalase activity was determined by measuring the removal rate of 70 μM H_2O_2 based on a method described previously [15,16]. The activity of GPX or SOD was measured as described previously [15,17].

2.5. RNA extraction and quantitative real-time PCR analysis of catalase in isolated pancreatic islets

Before harvesting pancreatic tissue, mice were perfused with saline and the samples were fixed in PAX gene™ Tissue Containers (Qiagen, Valencia, CA). Total RNA was extracted from pancreatic islets dissected by the laser-capture microdissection (LCM) technique as described previously [18,19], using LCM Staining Kit (Applied Biosystems, Foster City, CA), PAX gene™ Tissue miRNA Kit (Qiagen) and PALM MicroBeam System (Carl Zeiss Inc., Bernried, Germany). Real-time PCR was carried out as described previously [12,15,19,36]. TaqMan PCR primers and labeled probes were purchased from Applied Biosystems and the oligonucleotide primers used for PCR were custom-ordered from Nihon Gene Research Lab's Inc. (Sendai, Miyagi, Japan) [20]. The amount of PCR product was normalized with β-actin to determine the relative expression ratios for each mRNA in relation to β-actin mRNA.

2.6. Statistical analyses

The data, presented as the mean ± SEM, were analyzed by the Mann–Whitney *U* test using the Stat View statistical software package (Hulkins, Tokyo, Japan). *P* values <0.05 were considered to be statistically significant.

3. Results

3.1. Acatalasemia promotes alloxan-induced diabetes in mice

Alloxan is an oxidative stress agent that is relatively specific to, and destroys the insulin-producing pancreatic β cells, so alloxan-induced diabetes is considered to be a model of type 1 diabetes mellitus [21]. Hyperglycemia was not observed in the control mice in both groups ($N=15$ to 20 animals in each group). The incidence of diabetes at 7 days after intraperitoneal injection with 120 mg/kg BW of alloxan (ALX 120) was 0% in the wild-type mice group, 7.7% in the acatalasemic mice group, 31.6% in the wild-type mice treated with ALX 180, and 72.2% in the acatalasemic mice treated with ALX 180. There were no significant changes in body weight throughout the experiment in the wild-type mice, while a significant decrease in body weight was observed in the acatalasemic mice at 7 days after injection with ALX 180 (Table 1). Blood glucose significantly increased in both groups at 7 days after the administration of ALX 180. Moreover, the elevation of blood glucose in the acatalasemic mice was remarkable at 2 and 7 days after injection with ALX 180 in comparison to that in the wild-type mice (Fig. 1). The concentrations of plasma insulin at day 7