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Antioxidant α-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes

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Abstract We have shown recently that oxidative stress by chronic hyperglycemia damages the pancreatic β-cells of GK rats, a model of non-obese type 2 diabetes, which may worsen diabetic condition and suggested the administration of antioxidants as a supportive therapy. To determine if natural antioxidant α-tocopherol (vitamin E) has beneficial effects on the glycemic control of type 2 diabetes, GK rats were fed a diet containing 0, 20 or 500 mg/kg diet α-tocopherol. Intraperitoneal glucose tolerance test revealed a significant increment of insulin secretion at 30 min and a significant decrement of blood glucose levels at 30 and 120 min after glucose loading in the GK rats fed with high α-tocopherol diet. The levels of glycated hemoglobin A1c, an indicator of glycemic control, were also reduced. Vitamin E supplementation clearly ameliorated diabetic control of GK rats, suggesting the importance of not only dietary supplementation of natural antioxidants but also other antioxidative intervention as a supportive therapy of type 2 diabetic patients.

Key words: Oxidative stress; Type 2 diabetes; α-Tocopherol; Glucose metabolism; Insulin

1. Introduction

Overloads of reactive oxygen species (ROS) that exceed the capacity of the antioxidant system induce oxidative stress in cells. Oxidative stress is associated with a number of pathological conditions such as inflammation, atherosclerosis, carcinogenesis, aging and reperfusion injury [1]. Oxidative stress may also play a role in the pathophysiology of diabetes mellitus, because prolonged exposure to hyperglycemia causes non-enzymatic glycation of proteins through Maillard's reaction and the resulting products such as Schiff base and Amadori products can lead to the production of ROS [2–4]. We and other investigators have shown that the level of 8-hydroxy-2′-deoxyguanosine [5], a marker for oxidative stress, is increased either in the urine or blood mononuclear cells of type 2 diabetic patients [6,7]. Furthermore, Rehman et al. reported that multiple DNA base oxidation products are elevated in white blood cell DNA from patients with type 2 diabetes [8].

The GK rat is an inbred rat strain developed by selective breeding of an outbred colony of Wistar strain rats with high glucose levels on oral glucose tolerance test. This is one of the most reliable models for type 2 diabetes because of the many primary features manifested, including fasting hyperglycemia, impaired insulin response to glucose, hepatic and peripheral insulin resistance, and the typical complications [9–14]. We have reported recently that the pancreatic β-cells of GK rats, a model of non-obese type 2 diabetes, are oxidatively stressed, and that chronic hyperglycemia is responsible for the oxidative stress, causing cytotoxicity in the pancreatic β-cells [15]. In addition, transfection of antioxidant enzymes in insulin producing cell lines is reportedly effective against the toxic action of ROS [16,17]. These observations suggest that administration of antioxidants to type 2 diabetics may have beneficial effects on glycemic control.

α-Tocopherol (vitamin E) is a major peroxyl radical scavenger in cell membranes [18,19]. It inhibits or interrupts chain reactions of lipid peroxidation, which is the basis for its function as an antioxidant. Various studies have evaluated the antioxidant effects of α-tocopherol in prevention of coronary heart disease and diabetic complications [20–25]. In the present study, we have examined the effect of dietary α-tocopherol supplementation on the diabetic control of GK rats.

2. Materials and methods

2.1. Animals

A total of 24 male GK rats were used in the experiments. Five-week-old GK rats were divided into three groups and fed the following semisynthetic basic diet AIN-76 (Funahashi, Chiba, Japan) [26,27] with or without α-tocopherol supplementation for 4 weeks: (1) α-tocopherol-deficient diet ($n=8$);(2) α-tocopherol-sufficient diet containing 20 mg/kg diet of DL-α-tocopheryl acetate ($n=8$) as a control diet; (3) α-tocopherol-supplemented diet containing 500 mg/kg diet of DL-α-tocopheryl acetate ($n=8$). The animals were given tap water ad libitum, and maintained at a 12 h light and dark cycle in an air-conditioned room (22–24°C) during the experiments.

2.2. Intraperitoneal glucose tolerance test (IPGTT)

After α-tocopherol dietary supplementation, 20% D-glucose solution (2 g/kg body weight) was injected intraperitoneally to the animals ($n=5$ for each experimental group) in the fasting state. Blood samples were obtained from the tail vein at 0, 15, 30, 60 and 120 min after the injection. The blood glucose concentration was determined by an enzyme-electrode method and the plasma insulin concentration by an EIA method with insulin assay kit (Morinaga, Kanagawa, Japan).

2.3. Measurement of glycated hemoglobin (HbA1c)

On the day after IPGTT, blood samples were obtained from heart

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under pentobarbital anesthesia (40 mg/kg body weight). HbA1c was determined by an affinity chromatography method with Glyc-Affin.GHb kit (Seikagaku-kogyo Co. Ltd., Tokyo, Japan) [28].

2.4. Measurement of pancreatic content of α-tocopherol

The pancreases of the animals ($n=3$ for each experimental group) were removed under pentobarbital anesthesia and frozen. The content of α-tocopherol in the pancreas was determined by the high performance liquid chromatography method [29]. The protein determinations were performed by the Lowry method.

2.5. Statistical analysis

Data are presented as means ± S.E.M. Statistical analyses were performed by an unpaired t test.

3. Results

3.1. Glucose tolerance

To determine the effect of α-tocopherol on the function of the pancreatic β-cells in GK rats, we carried out IPGTT by administration of 2 g D-glucose per kg body weight ($n=5$ for each experimental group). While the fasting blood glucose levels in α-tocopherol-supplemented GK rats were not significantly different from those in α-tocopherol-deficient and -sufficient GK rats, the peak levels of blood glucose in α-tocopherol-supplemented GK rats were significantly lower than in α-tocopherol-deficient and -sufficient GK rats (24.2 ± 0.43 mM vs. 26.2 ± 0.57 mM and 26.8 ± 0.61 mM at 30 min, respectively, $P<0.05$) (Fig. 1). The blood glucose levels at 60 min in α-tocopherol-supplemented GK rats were significantly lower than in α-tocopherol-deficient GK rats (23.9 ± 0.66 mM vs. 26.4 ± 0.57 mM, $P<0.05$). The blood glucose levels at 120 min in α-tocopherol-supplemented GK rats were significantly lower than in α-tocopherol-deficient and -sufficient GK rats (16.7 ± 0.54 mM vs. 19.2 ± 0.79 mM and 18.6 ± 0.47 mM, respectively, $P<0.05$). In addition, the peak levels of plasma insulin in α-tocopherol-supplemented GK rats were significantly higher than in α-tocopherol-deficient and -sufficient GK rats (2125 ± 69.5 pg/ml vs. 1696.2 ± 127.9 pg/ml and 1696.4 ± 138 pg/ml, respectively, $P<0.05$).

3.2. Evaluation of diabetic control

No significant differences in body weight were observed among α-tocopherol-deficient, -sufficient and -supplemented GK rats (Table 1). To evaluate the effect of α-tocopherol on diabetic control, we measured HbA1c of GK rats after supplementary diet ($n=7$ for each experimental group). The levels of HbA1c in α-tocopherol-supplemented GK rats were significantly lower than in α-tocopherol-deficient and -sufficient GK rats ($P<0.0005$ and $P<0.05$, respectively). The levels of HbA1c in α-tocopherol-sufficient GK rats were significantly lower than in α-tocopherol-deficient GK rats ($P<0.05$).

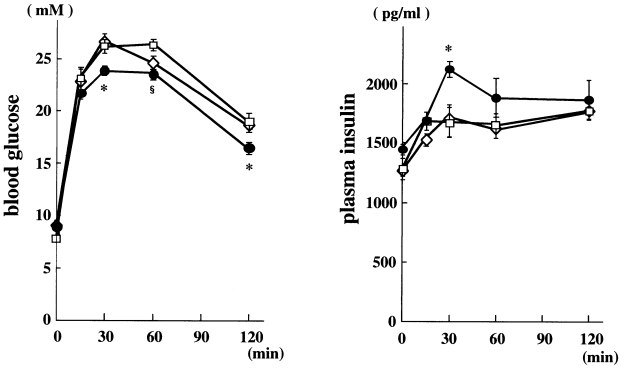

Fig. 1. Glucose tolerance test. Blood glucose (left) and plasma insulin (right) levels after intraperitoneal glucose loading in α-tocopherol-deficient (open square), -sufficient (open lozenge) and -supplemented (closed circle) GK rats. *, $P<0.05$ versus α-tocopherol-deficient and -sufficient GK rats. §, $P<0.05$ versus α-tocopherol-deficient GK rats (mean ± S.E.M.).

3.3. Pancreatic contents of α-tocopherol

We measured the α-tocopherol contents of pancreas (Table 1); it was not possible to determine the α-tocopherol content of the pancreatic islets directly because of the limited volume of samples. The pancreatic α-tocopherol contents in α-tocopherol-supplemented GK rats were significantly higher than in α-tocopherol-sufficient and -deficient GK rats ($P<0.001$ and $P<0.0005$, respectively).

4. Discussion

Because expression of antioxidant enzymes in the pancreatic islets is reportedly very low [30,31], the pancreatic β-cells are thought to be especially vulnerable to the attacks of ROS. We have previously reported that nitric oxide suppresses glucose metabolism and results in inhibition of insulin secretion from the pancreatic β-cells [32]. We have more recently reported that the chronic hyperglycemic state of type 2 diabetes induces the oxidative stress on the pancreatic β-cells, causing cytotoxicity that might worsen clinical diabetic states. These findings suggest that antioxidant supplementation to type 2 diabetes may be effective to improve diabetic states.

In the present study, GK rats were fed a diet with or without antioxidant α-tocopherol supplementation. α-Tocopherol was indeed accumulated in the pancreas of α-tocopherol-supplemented GK rats. IPGTT revealed that glucose tolerance in α-tocopherol-supplemented GK rats was ameliorated compared to that in α-tocopherol-deficient or -sufficient GK rats. Blood glucose levels were decreased significantly at 30 min and 120 min in α-tocopherol-supplemented GK rats. Plasma insulin levels in α-tocopherol-supplemented GK rats

Table 1
Biochemical measurements in α-tocopherol-deficient, -sufficient and -supplemented GK rats

	α-Tocopherol-deficient GK rat	α-Tocopherol-sufficient GK rat	α-Tocopherol-supplemented GK rat
Body weight (g)	201.9 ± 7.6 ($n=8$)	211.3 ± 3.9 ($n=8$)	217.5 ± 3.4 ($n=8$)
HbA1c (%)	10.5 ± 0.88 ($n=7$)	9.3 ± 0.3[a] ($n=7$)	8.5 ± 0.15[b,c] ($n=7$)
Pancreatic content of α-tocopherol (μg/g protein)	5.2 ± 0.38 ($n=3$)	39.9 ± 3.1[b] ($n=3$)	254 ± 24[b,d] ($n=3$)

[a] $P<0.05$.
[b] $P<0.0005$ vs. α-tocopherol-deficient GK rats.
[c] $P<0.05$.
[d] $P<0.001$ vs. α-tocopherol-sufficient GK rats.

at 30 min were significantly higher than the others. As a result of amelioration of defects in glucose metabolism, the levels of HbA1c were decreased in α-tocopherol-supplemented GK rats.

There has been a controversy on whether supplementation of α-tocopherol is effective on decreasing protein glycation in type 2 diabetes [33]. Fuller et al. reported that α-tocopherol could not decrease protein glycation but reduced oxidation of low density lipoprotein [34]. Our study revealed that reduction of oxidative stress by α-tocopherol decreased blood glucose levels through improvement of insulin secretion, resulting in decrement of glycation of hemoglobin in type 2 diabetes.

In conclusion, these findings suggest that accumulated α-tocopherol in the pancreatic islets had scavenging effects of ROS, and resulted in improvements of glucose tolerance and of diabetic control. It is not known so far how hyperglycemia causes oxidative stress in the pancreatic β-cells. This signaling pathway should be elucidated. Our observations indicate that antioxidant α-tocopherol supplementation to type 2 diabetes has beneficial effects on diabetic control as a supportive therapy. Tanaka et al. [35] reported recently that *N*-acetyl-L-cysteine (NAC) or aminoguanidine prevent glucose-induced oxidative stress in HIT-T15 cells and Zucker diabetic fatty rats. The combined treatment of α-tocopherol with other natural antioxidants or antioxidant agents, such as vitamin C, carotenoids, polyphenols, probucol, NAC, aminoguanidine, etc., may lead to easier and more stable control of type 2 diabetic patients.

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