Protection Against the Diabetogenic Effect of Feeding tert-Butylhydroquinone to Rats Prior to the Administration of Streptozotocin

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We determined whether an oral administration of the synthetic antioxidant, tert-butylhydroquinone (TBHQ), or the naturally occurring lipoxygenase inhibitor, curcumin, to rats would provide protection against the diabetogenic effect of streptozotocin (STZ). Male Sprague-Dawley rats were fed on an AIN-76M-based purified diet containing 0.0028% TBHQ or on the purified diet with a daily intragastric administration of curcumin (200 mg/kg of body weight) for one week while receiving intravenously administered STZ. The rats fed on the TBHQ-containing diet were resistant to diabetes development when compared with the rats fed on the TBHQ-free diet and had a higher body weight gain and lower serum glucose concentration. Glucose-stimulated insulin secretion from the pancreatic islet in the rats that had received TBHQ was higher than that in the control rats. The rats receiving curcumin showed no beneficial effect on these diabetic symptoms. These findings provide direct evidence for the suggestion that dietary supplementation of an antioxidant may exert a preventive effect on the diabetogenic action of free-radical producers.

Key words: diabetes; tert-butylhydroquinone; curcumin; pancreatic islet; insulin release

Oxygen free radicals (OFRs) have been found to be involved in a wide variety of cellular functions, but they can be both essential and highly toxic to cellular homeostasis. They are implicated in the pathophysiology of various disease states, including diabetes mellitus. Pancreatic beta-cell death underlies the pathogenesis of type I (insulin-dependent) diabetes mellitus, and OFRs have been implicated in beta-cell destruction. Pancreatic beta-cell death that occurs with type I diabetes may occur via an immune or non-immune mechanism. Pancreatic beta-cell lysis by cytotoxic T-lymphocytes and cytokine-mediated lysis by macrophages and helper T-cells through an OFR-mediated mechanism have been well document-

ed. Theoretically, therefore, antioxidant supplementation may be of benefit to the vascular function in diabetes. In the Cambridge Heart Antioxidant Study (CHAOS), vitamin E supplementation has been shown to be of some benefit to non-diabetic patients with coronary artery disease as it decreased the rate of non-fatal myocardial infarction. In a streptozotocin-diabetic animal (an animal model of uncontrolled diabetes mellitus), while antioxidant therapy did not improve hyperglycemia itself or the vascular endothelial dysfunction in the small mesenteric arteries of streptozotocin (STZ)-diabetic rats, it did ameliorate the oxidative stress, as reflected in the decreased level of plasma 8-epi-prostaglandin F$_2$-\(
\alpha\)
 and the in vitro cytotoxicity of diabetic lipoproteins. These results from humans and experimental animals suggest that, after the diabetes had been established, an antioxidant had no effect on the subsequent course of the disease as monitored by the serum glucose level or vascular function, despite the concomitant decrease in oxidative stress.

From the nutritional point of view, it is absolutely essential for an effective protective system against the destruction of beta-cells to function. The studies by Slonim et al., Sandler and Andersson and Robbins et al. have shown that pretreating animals with vitamin E, dimethyl urea (a hydroxyl-radical scavenger) or superoxide dismutase prevented or inhibited the diabetogenic action of STZ. In these studies, vitamin E and dimethyl urea were administered intraperitoneally, and superoxide dismutase was injected through a vein. It was not confirmed whether the oral administration of an antioxidant would be as effective as intraperitoneal administration. More recently, Bleich et al. have shown that 12-lipoxygenase gene knockout mice were highly resistant to STZ-dependent diabetes development in comparison with control mice and had a higher serum insulin level. This study suggests that the oral administration of a lipoxygenase inhibitor may be beneficial to produce

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Abbreviations: TBHQ, tert-butylhydroquinone; STZ, streptozotocin; SD, Sprague-Dawley; OFR, oxygen free radicals; AIN, American Institute of Nutrition; KRBB, Krebs-Ringer bicarbonate buffer
resistance to STZ.

The aim of this study was to determine whether the oral administration of an antioxidant would be beneficial to STZ-induced diabetic rats. In this study, tert-butyhydroquinone (TBHQ) and curcumin (the yellow pigment in turmeric and curry) were used as typical antioxidants. TBHQ is one of the most powerful antioxidants, having been synthesized by Ke et al., and is recommended by American Institute of Nutrition (AIN) as a supplement to the rodent diet. Curcumin possesses antioxidative activity by scavenging reactive free radicals and an inhibiting lipoygenase and cyclooxygenase. Sajithhal et al. have reported that orally administered curcumin (200 mg/kg of body weight) significantly reduced the antioxidant status of the serum in diabetic rats.

Materials and Methods

Animals and diets. Five-week-old male Sprague-Dawley (SD) rats were obtained from Seac Yoshitomi (Fukuoka, Japan) and maintained in a temperature-controlled room (23 ± 1°C) with 12-hour light (0800-2000) and 12-hour dark cycle. They were fed on a commercial non-purified diet (NMF; Oriental Yeast Co., Tokyo, Japan) with free access to deionized water, prior to feeding on a purified diet. The rats were divided into groups according to their treatment protocol. STZ (Sigma Chemicals, St. Louis, MO, U.S.A.), which was administered at a dose of 40 mg/kg of body weight, was dissolved in a 0.05 m citrate buffer at pH 4.5. The purified diet was formulated according to the AIN-76® formula (g/kg of diet) 100 dietary fat, 200 casein (Wako Pure Chemicals, Osaka, Japan), 150 corn starch (Nihon Shokuhin Kako, Aichi, Japan), 50 cellulose (Nihon Nosan Kogyo, Tokyo, Japan), 35 mineral mixture (AIN76®, Nihon Nosan Kogyo, Tokyo, Japan), 10 vitamin mixture (AIN76®, Nihon Nosan Kogyo, Tokyo, Japan), 3 dl-methionine (Nacalai Tesque, Kyoto, Japan), 2 choline bitartrate (Wako Pure Chemicals, Osaka, Japan) and sucrose (Nishinon Sugar Manufacturing, Fukuoka, Japan) to 1000. Dietary fat was prepared from palm oil, high-oleic safflower oil and safflower oil at equal rates of saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid, as described elsewhere.

Two separate studies were carried out with each group of rats, a group consisting of 6 or 7 rats. In the first experiment, the rats were fed on either the purified diet alone (control diet) or on the purified diet supplemented with TBHQ (Kanto Chemical, Tokyo, Japan) at the level of 0.028 g/kg of diet, which is equivalent to twice as much as that recommended for use in the AIN-93G formula, for 7 days each before and after the STZ administration. In the second experiment, both groups of rats were fed on the control diet, but daily intragastrically received curcumin (200 mg/kg of body weight, Nacalai Tesque, Kyoto, Japan) emulsified with 1.5% carboxymethylcellulose (Sigma Chemicals, St. Louis, MO, U.S.A.) or carboxymethylcellulose alone, as described elsewhere. The reason why curcumin was not supplemented to the diet was that the rats consumed less food when it contained curcumin. This manipulation was carried out before and after the STZ administration.

Following an overnight fast 16 hours, but with unlimited water, STZ was injected via a tail vein. Food and water were immediately returned to the rats after the injection. Seven days later, the rats were killed by withdrawing the aortic blood under diethyl ether anesthesia after a 7-hour fast, and the pancreatic islets were immediately excised. These experiments were carried out under the guidelines for animal experiments in the Faculty of Agriculture and Graduate Course of Kyushu University (Fukuoka, Japan) and Law No. 105 and Notification No. 6 of the government of Japan.

Purification of the rat islets and islet studies. Rat islets of Langerhans were isolated by a modification of the method previously described. After withdrawing the aortic blood under anesthesia, the intrathoracic aorta was transected to minimize interstitial hemorrhaging in the pancreas. The common bile duct was clamped at its entrance to the duodenum and cannulated with a polyethylene tube (Nippon Becton Dickinson Co., Tokyo, Japan) connected to an injection syringe. Approximately 7 ml of cold Hanks’ balanced salt solution containing collagenase (0.5 mg/ml; Nitta Gelatin Co., Osaka, Japan) was injected into the common bile duct. The distended pancreas was removed and incubated at 37°C for 20 min. A cold Krebs-Ringer bicarbonate buffer (KRB) containing 129.4 mM NaCl, 5.2 mM KCl, 2.7 mM CaCl2, 1.3 mM KH2PO4, 1.3 mM MgSO4, 24.8 mM NaHCO3, 10 mM HEPES and 3.3 mM glucose at pH 7.4 was added to stop the digestion, and the digested pancreas was dispersed into small fragments by pipetting into a centrifuge tube. The tube was then centrifuged at 400 g for 30 seconds. The resulting supernatant was discarded, and the pellet was washed twice under the same conditions with cold KRB. The pellet was resuspended in Ficoll (Pharmacia Biotech, Uppsala, Sweden)-Conray (Daichi Seiyaku Co., Tokyo, Japan) solution whose specific gravity was 1.100. The tissue suspension was layered under two discontinuous Ficoll-Conray gradient layers (specific gravities of 1.075 and 1.050) and then centrifuged at 600 g for 10 min. The tissue at the 1.075/1.050 interface was collected and washed three times with cold KRB. Five islets per tube were then handpicked and incubated for 30 min in 3.3, 8.3, 11.1 or 16.7 mM glucose in KRB containing 0.2% bovine serum albumin (Sigma Chemi-
cals, St. Louis, MO, U.S.A.). The concentration of glucose required for basal insulin secretion (3.3 mM glucose) or glucose-stimulated insulin secretion (16.7 mM) had been determined in a preliminary experiment as shown in Fig. 1. At the end of the incubation period, the KRBB incubation medium was stored at −30°C until needed for the assay.

Analysis. The released insulin was measured with a rat-specific insulin radioimmunoassay kit (Eiken Chemical Co., Tokyo, Japan). Serum glucose and triglycerides were determined with a glucose CIITest Wako kit and triglyceride G-test Wako kit (Wako Pure Chemicals Co., Tokyo, Japan).

Statistics. A statistical analysis was performed with the statistical program in Excel (Microsoft Co., Tokyo, Japan) for a personal computer. Data were compared by the two-tail Student’s t-test or by Duncan’s new multiple-range test. Each value is expressed as the mean ± SEM.

Results

Differences in the food and water intake and body weight before and after the streptozotocin injection

The STZ injection induced hyperphagia in experiments 1 and 2 (Fig. 2). The rats that received TBHQ tended to consume less food than those without TBHQ. The administration of curcumin appeared to have no effect on the hyperphagia after the STZ injection (Fig. 2, Experiment 2). Water consumption was increased in the rats after the STZ injection (Fig. 3). The rats that received TBHQ tended to consume less water after the STZ injection than those without TBHQ. The rats that received curcumin tended to consume more water than those without curcumin. The STZ injection resulted in a retarded body weight gain (Fig. 4). The rats that received TBHQ exhibited a greater body weight gain 2 days after the STZ injection than those without TBHQ (Fig. 4, Experiment 1), whereas the curcumin administration had no significant effect on the body weight gain (Fig. 4, Experiment 2).

Concentration of serum glucose, insulin and triacylglycerols after the streptozotocin injection

The rats fed on the TBHQ-containing diet had a
Table 1. Concentrations of Serum Glucose, Insulin and Triacylglycerols in Diabetic Rats that Received tert-Butylhydroquinone or Curcumin

<table>
<thead>
<tr>
<th>Group</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td></td>
<td>−TBHQ</td>
<td>+TBHQ</td>
</tr>
<tr>
<td>Serum glucose (mm)</td>
<td>24.0 ± 3.2</td>
<td>14.9 ± 0.6</td>
</tr>
<tr>
<td>Serum insulin (mU/l)</td>
<td>17.5 ± 5.1</td>
<td>27.4 ± 4.2</td>
</tr>
<tr>
<td>Serum triacylglycerol (mm)</td>
<td>37.0 ± 9.8</td>
<td>28.9 ± 8.5</td>
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<table>
<thead>
<tr>
<th></th>
<th>−Curcumin</th>
<th>+Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose (mm)</td>
<td>22.3 ± 3.4</td>
<td>23.8 ± 1.8</td>
</tr>
<tr>
<td>Serum insulin (mU/l)</td>
<td>20.7 ± 5.3</td>
<td>20.9 ± 5.5</td>
</tr>
<tr>
<td>Serum triacylglycerol (mm)</td>
<td>42.7 ± 12.8</td>
<td>69.8 ± 7.1</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM, n = 6 for experiment 1 and n = 7 for experiment 2.
*Significantly different from the corresponding group at P < 0.05.

Fig. 4. Body Weight of Diabetic Rats that Received tert-Butylhydroquinone or Curcumin.

Experiment 1: The rats received the purified diet without TBHQ (−TBHQ) or the purified diet supplemented with TBHQ (+TBHQ). Experiment 2: The rats received the purified diet and carboxymethylcellulose plus curcumin (+Curcumin) or the purified diet and carboxymethylcellulose alone (−Curcumin). Bars show the mean ± SEM, n = 6 for experiment 1 and n = 7 for experiment 2. *Significantly different from the corresponding values at each time point at P < 0.05.

Fig. 5. Insulin Release from Islets of Diabetic Rats that Received tert-Butylhydroquinone or Curcumin.

Experiment 1: The rats received the purified diet without TBHQ (−TBHQ) or the purified diet supplemented with TBHQ (+TBHQ). Experiment 2: The rats received the purified diet and carboxymethylcellulose plus curcumin (+Curcumin) or the purified diet and carboxymethylcellulose alone (−Curcumin). Islets were isolated from STZ-treated rats and incubated for 30 min with different concentrations of glucose (3.3 and 16.7 mM). Bars show the mean ± SEM, n = 6 for experiment 1 and n = 7 for experiment 2. *Different letters show significant difference at P < 0.05.

lower concentration of serum glucose than those fed on the control diet (Table 1). The TBHQ group tended to have a higher serum insulin concentration (P = 0.165) and lower serum triacylglycerol concentration (P = 0.088). The administration of curcumin had no significant effect on the serum concentration of glucose, insulin and triacylglycerols.

Secretion of insulin from the islets after the streptozotocin injection

The basal insulin secretion with 3.3 mM glucose was similar between the rats fed on the TBHQ-containing diet and the control rats (Fig. 5, Experiment 1). In contrast, the insulin secretion stimulated with 16.7 mM glucose was higher in the rats fed on the TBHQ-containing diet than in the control group. Curcumin administration had no significant effect on the basal or glucose-stimulated insulin secretion (Fig. 5, Experiment 2).

Discussion

STZ exerts its diabetogenic effect by acting as an oxidant or a free-radical producer.8-10 The present study has demonstrated for the first time that a small amount of synthetic TBHQ antioxidant supplemented to a purified diet prior to the administration of STZ resulted in partial prevention of the diabetogenic action of STZ, as reflected by the greater body weight gain and lower concentration of serum glucose. The intake of TBHQ also tended to improve the consumption of food and water, and the concentrations of serum insulin and triacylglycerol in the STZ-administered rats. These beneficial effects of TBHQ appear to be attributable to the protection of islet-β-cells from an insulin secretory dysfunction, because the glucose-stimulated insulin secretion from the islets was higher in TBHQ group than in the control group. These findings are in agreement with histological observations that have been made by Slonim et al.8 and Sandler and Andersson9 who demonstrated that the administration of vitamin E or dimethyl urea partially protected rat islets from STZ-induced destruction. Accordingly, pretreatment with TBHQ, vitamin E or dimethyl urea of STZ-dependent diabetic rats resulted in preventing free-radical mediated necrosis or apoptosis8-9 of the islets and consequently...
maintaining the capacity of the islets to secrete insulin.

In contrast to the oral administration of TBHQ, the administration of curcumin prior to the STZ treatment had no significant effect on diabeticogenic action of STZ. It has been reported that diabetic rats fed on 0.5% curcumin had less lipid peroxidation in the plasma.\textsuperscript{20} The inhibitory effect of curcumin on the \textit{in vitro} lipoxygenase activity in the mouse epidermis has been reported to be stronger than that of such structurally related dietary compounds as chlorogenic acid, caffeic acid and ferulic acid.\textsuperscript{13} Bleich et al.\textsuperscript{13} have recently reported that 12-lipoxygenase knockout mice showed resistance to the diabeticogenic action of a low dose of STZ. We therefore expected that orally administered curcumin prior to the STZ administration would be absorbed by the intestines and exert an antidiabeticogenic effect through both the antioxidative action and inhibitory action of lipoxycygenase. However, this was not the case in the present study. The negligible effect of curcumin on the diabeticogenic action of STZ might have been due to a poor absorption rate of curcumin\textsuperscript{24} and/or rapid degradation of the absorbed curcumin.\textsuperscript{25}

The present findings directly support the suggestion that dietary supplementation with an antioxidant may exert a preventive effect on the diabeticogenic action of free-radical producers. The present animal model would also be applicable to evaluating the role of antioxidants \textit{in vivo}.

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References


