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Short refereed paper

Astaxanthin protects β-cells against glucose toxicity in diabetic db/db mice

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Oxidative stress induced by hyperglycemia possibly causes the dysfunction of pancreatic β-cells and various forms of tissue damage in patients with diabetes mellitus. Astaxanthin, a carotenoid of marine microalgae, is reported as a strong anti-oxidant inhibiting lipid peroxidation and scavenging reactive oxygen species. The aim of the present study was to examine whether astaxanthin can elicit beneficial effects on the progressive destruction of pancreatic β-cells in db/db mice – a well-known obese model of type 2 diabetes. We used diabetic C57BL/KsJ-db/db mice and db/m for the control. Astaxanthin treatment was started at 6 weeks of age and its effects were evaluated at 10, 14, and 18 weeks of age by non-fasting blood glucose levels, intraperitoneal glucose tolerance test including insulin secretion, and β-cell histology. The non-fasting blood glucose level in db/db mice was significantly higher than that of db/m mice, and the higher level of blood glucose in db/db mice was significantly decreased after treatment with astaxanthin. The ability of islet cells to secrete insulin, as determined by the intraperitoneal glucose tolerance test, was preserved in the astaxanthin-treated group. Histology of the pancreas revealed no significant differences in the β-cell mass between astaxanthin-treated and -untreated db/db mice. In conclusion, these results indicate that astaxanthin can exert beneficial effects in diabetes, with preservation of β-cell function. This finding suggests that anti-oxidants may be potentially useful for reducing glucose toxicity.

INTRODUCTION

A number of experimental studies have suggested the participation of reactive oxygen species (ROS) in the onset of diabetes mellitus and development of diabetic complications.[1] By the development of useful oxidative stress markers, such as 4-hydroxy-2-nonenal (HNE), an index of oxidative injury of lipids, and 8-hydroxy-deoxyguanosine (8-OHdG), a marker of the DNA oxidation, the accumulation of these oxidative products in serum provides evidence of increased oxidative stress in patients with diabetes mellitus.[2–4] Under diabetic conditions, ROS are produced by the non-enzymatic glycation reaction of proteins, mitochondria, and protein kinase C (PKC)-dependent activation of NAD(P)H oxidase in both vascular smooth muscle cells and endothelial cells. Among these sources of ROS, advanced glycosylation end products (AGEs) are shown to be detectable in β-cells kept under high glucose concentrations. Under that condition, glycation-mediated ROS production reduces insulin gene transcription and apoptosis of β-cells.[5] This indicates that glycation and subsequent oxidative stress may in part mediate the toxic effect of hyperglycemia. Therefore, an agent having anti-oxidative actions might reduce glucose toxicity. Kaneto *et al.*[6] have recently demonstrated that antioxidant treatment (*N*-acetyl-L-cysteine and vitamins C plus E) can exert beneficial effects in diabetic C57BL/KsJ-db/db mice, with preservation of *in vivo* β-cell function. In this mouse, a well-known obese model of type 2 diabetes, hyperglycemia arises because of increasing insulin resistance and the

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subsequent insufficiency of the β-cell compensation. Astaxanthin, which is a carotenoid of marine microalgae, is reported to be a potent antioxidant and inhibits low-density lipoprotein oxidation induced by azo-compound,[7] peroxidation of liposomes induced by ADP and Fe^{2+},[8] and NADPH-dependent microsomal phospholipid peroxidation.[9] It also has direct scavenging activity against peroxyl radical,[10] and quenching singlet oxygen.[11] Astaxanthin's pharmacological effects in animal models are extensive, ranging from protection against *Helicobacter pylori*-induced gastric inflammation[12] and the protection from hepatoxicity by carbon tetrachloride[13] to increasing immune function.[14,15]

However, the effects of astaxanthin on glucose toxicity in a rodent model of type 2 diabetes have not been investigated. Therefore, we examined whether astaxanthin can elicit beneficial effects on the progressive destruction of pancreatic β-cells in db/db mice.

Materials and Methods

Female db/db mice, a rodent model of type 2 diabetes, and their non-diabetic db/m litter-mates were divided into three groups as follows: non-diabetic db/m; diabetic db/db; and diabetic db/db treated with astaxanthin. Each of the groups contained 8 mice. Astaxanthin was given orally mixed in chow (1.0 mg/mouse/day) from the age of 6–18 weeks. Body weights were determined at 6, 10, 14 and 18 weeks of age. Non-fasting blood glucose levels were measured at 6, 12 and 18 weeks of age. Urinary albumin levels were measured at 18 weeks of age. Intraperitoneal glucose tolerance test (IPGTT) was performed at 18 weeks of age as follows: a 20% glucose solution (1.0 g/kg body weight) was injected intraperitoneally into the animals in the fasting state. Blood samples were taken at various time points (0–120 min). Blood glucose and serum insulin concentrations were measured at each time point. To evaluate the β-cell damage, histological examination was performed.

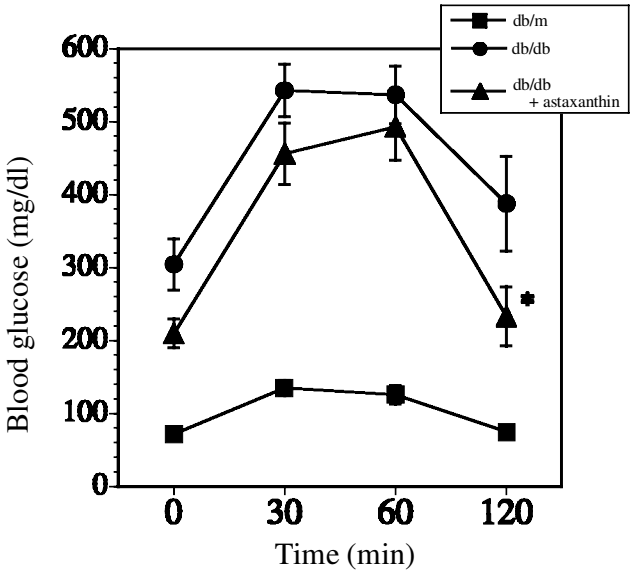

Fig. 1. Blood glucose level on IPGTT. The level in astaxanthin-treated db/db mice ($n = 8$) was lower than that of non-treated db/db mice ($n = 8$). *$P < 0.001$ *versus* non-treated group.

Table 1. Changes in body weight and non-fasting blood glucose levels in db/db mice treated with or without astaxanthin and db/m mice

Age (weeks)	Body weight (g)	Non-fasting blood glucose (mg/dl)
db/db ($n = 8$)		
12	40.3 ± 0.5	387.4 ± 15.0
18	47.6 ± 0.6	417.6 ± 13.7
db/db + astaxanthin ($n = 8$)		
12	43.3 ± 0.6	301.6 ± 41.5*
18	46.0 ± 1.7	338.0 ± 43.8**
db/m ($n = 8$)		
12	25.7 ± 0.5	118.1 ± 3.1~
18	29.5 ± 1.1	111.1 ± 3.4

*$P < 0.01$ *versus* db/db mice at the age of 12 weeks.
**$P < 0.01$ *versus* db/db mice at the age of 18 weeks.

Results

While there were no differences in food intake (data not shown) or body weight between the db/db groups (Table 1), body weight of db/db mice treated with or without astaxanthin were greater than db/m mice. The non-fasting blood glucose levels in db/db mice were significantly higher than those of db/m mice and, as expected due to progression of disease, the older animals had more glucose in blood than the younger animals (Table 1). Non-fasting blood glucose was slightly, but significantly ($P < 0.01$), decreased after treatment with astaxanthin. To determine the effect of astaxanthin on the function of the pancreatic β-cells in db/db mice, we carried out IPGTT by administration of glucose (Fig. 1). The treated db/db group showed significantly ($P < 0.001$) lower glucose levels than non-treated db/db group; for instance, the blood glucose level at 120 min after glucose injection was 144.9 ± 64.8 mg/dl in the treated group and 233.0 ± 40.6 mg/dl in the non-treated group (Fig. 1). At 120 min after glucose injection, the serum insulin level of the treated group was significantly ($P < 0.001$) higher than that of non-treated group (treated, 8425.0 ± 1509.1 pg/ml; non-treated, 2950.0 ± 560.5 pg/ml). Histological study of the pancreas revealed that islets were irregular in shape and islet cell numbers were decreased compared to db/m mice (Fig. 2). However, there were no significant differences in the β-cell mass between astaxanthin-treated and untreated db/db mice

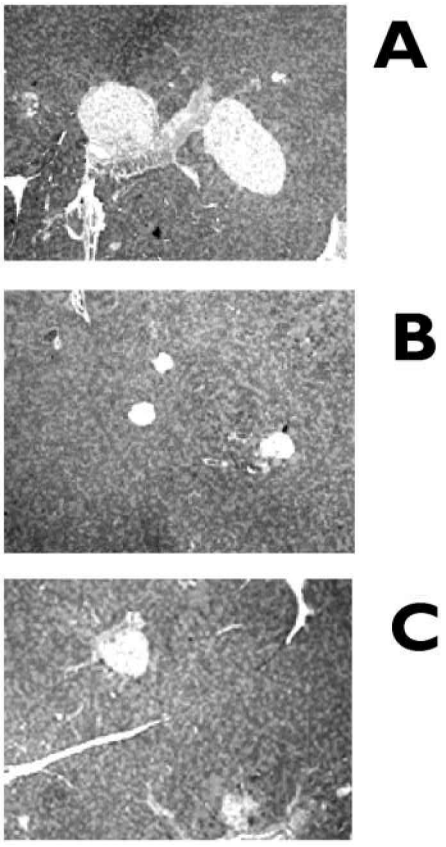

Fig. 2. The histology of pancreatic islet cells in db/m mice (A), non-treated mice (B), and astaxanthin-treated mice (C).

(Fig. 2). To determine the effect of astaxanthin on the renal complications in db/db mice, we compared urinary albumin levels and the mesangial cell proliferation between two groups. Urinary albumin level of the non-treated group of db/db mice was significantly ($P < 0.001$) increased. In contrast astaxanthin treatment significantly inhibited the increase in urinary albumin (data not shown).

Discussion

In this study, we have demonstrated that treatment with astaxanthin can improve glycemic control with preservation of pancreatic β-cell function in diabetic db/db mice. There is wide agreement that hyperglycemia leads to the chronic generation of ROS, and may also attenuate the antioxidant activity of scavenger enzymes by the glycation of these proteins.[16] Because expression of antioxidant enzymes in the pancreatic islets is reportedly very low,[17,18] the pancreatic β-cells are thought to be especially vulnerable to attack by ROS. Indeed, under high glucose concentrations, AGEs were shown to be detectable in β-cells,[5] and the levels of 8-OHdG (a marker for DNA oxidation) and 4-hydroxy-2-nonenal (an index of oxidative injury of lipids) are increased in β-cells of diabetic GK rats.[4] Antioxidants such as α-tocopherol or *N*-acetyl-L-cysteine (NAC) may prevent or delay β-cell dysfunction in diabetes by providing protection against glucose toxicity.[6,19] Together, these findings suggest that oxidative stress on the pancreatic β-cells induced by the chronic hyperglycemic state of type 2 diabetes causes cytotoxicity that might worsen the clinical diabetic state. It is possible that antioxidant supplementation in type 2 diabetes may effectively improve diabetic control.

In the present study, we found that glucose tolerance in astaxanthin-treated db/db mice was significantly improved compared to non-treated db/db mice. Blood glucose levels were significantly ($P < 0.001$) lower at 120 min in astaxanthin-treated mice and serum insulin levels were significantly ($P < 0.001$) higher at 120 min in astaxanthin-treated mice. In contrast, histological assessment of the pancreas showed no significant differences in the β-cell mass in astaxanthin-treated db/db mice and untreated db/db mice. These findings suggest that astaxanthin may diminish the oxidative stress caused by hyperglycemia in the pancreatic β-cells. In this study, we also evaluated the renal damage by measuring urinary albumin level at 18 weeks of age and, in astaxanthin-treated db/db mice, this parameter was significantly lower than in untreated mice. However, because the blood glucose level of astaxanthin-treated mice was also significantly lower than non-treated mice, it remains uncertain whether the antioxidant activity of astaxanthin was directly responsible for the lessened glomerular damage. Nonetheless, our present study does show that astaxanthin may exert beneficial effects on pancreatic β-cell function in diabetes.

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