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Hypoglycemic and Hepatoprotective Effects of Betaine on Streptozotocin-Induced Diabetic Rats

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This study was to investigate the effect of betaine on the hypoglycemia and hepatoprotection of streptozotocin (STZ)-induced diabetic rats. Male Sprague-Dawley rats weighing around 280 g were randomly assigned to the three experimental groups: a healthy normal group and two groups with STZ-induced diabetes and fed either control diet or betaine diet. Betaine given to the STZ-diabetic rats had significant effect in lowering the serum glucose concentrations compared to the STZ-diabetic rats. The alanine aminotransferase (AST) and aspartate aminotransferase (ALT) activities and triglyceride contents in serum were dramatically higher in the STZ-diabetic rats, but these increases in relation to diabetes also decreased in the STZ-diabetic rats fed betaine. However, the total-cholesterol concentration in the STZ-diabetic rats was even increased by betaine. The morphology of the pancreatic islets in the normal rats showed a typical round form, but most of the islets in the STZ-diabetic rats showed severe morphological alterations by being markedly destroyed. However, the islet morphology of STZ-diabetic rats given betaine mostly maintained a normal rounded appearance. The present study strongly suggests that the administration of betaine showed a moderate hypoglycemic effect by protecting the pancreatic beta-cells by morphological examination from STZ-induced destruction.

Key words – Betaine, streptozotocin, diabetes mellitus, hypoglycemia, hepatoprotection

Introduction

Betaine is an intermediate in an oxidative metabolite of choline, and is abundant in animal tissues as well as plants[9]. Betaine has been used a traditional remedy for hepatic disorders in Southeast Asia. Dietary intake of betaine is estimated at 0.5~2 g/d generally, and major food sources are wheat products, beets, spinach, Hamcho (*Saliconia herbacea* L.) and Boxthorn (*Lycium ohinense*)[2,19, 28]. Betaine has been found to protect the liver against carbon tetrachloride-induced lipidosis[5] and ethanol-induced hepatotoxicity[13]. Betaine is used as therapy to lower plasma homocysteine in hyperhomocysteinemic patients[3]. Recently studies showed that supplementation with 1.5 g/d of betaine, which is in the range of daily intake, lowers fasting plasma homocystein concentration in healthy humans[24], and this compound was approved for a drug in the treatment of homocystinuria by FDA[1].

Cardiovascular diseases are the major cause of death in diabetic patients, and there has recently been much interest in the possible role of homocysteine in the development of cardiovascular diseases in the diabetic patients[14,29]. Zied A *et al.* reported that cardiovascular complications in type 2 diabetes patients are known to be associated with high levels of homocysteine[8]. Insulin treatment prevents the increase in the plasma homocysteine concentrations in the diabetic rats induced by intravenous administration of streptozotocin. Betaine supplementation in humans has also been shown to lower plasma homocysteine concentrations in modestly hyperhomocysteinemic patients. In addition, betaine also plays an important role in lipid metabolism under ethanol or carbon tetrachloride-induced hyperlipidemic conditions[30]. However, the hypoglycemic effect of betaine in STZ-induced diabetic rats has not been studied. In the present study, we used a STZ-induced diabetic rat model to investigate the hypoglycemic and hepatoprotective effects of betaine.

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Materials and Methods

Animal and Experimental design

Seven-week-old male Sprague-Dawley rats were purchased from Hyochang Science (Daegu, Korea), and housed individually in suspended wire-mesh stainless cages in a temperature (21~24℃) and light (08:00~20:00) controlled animal room. The composition of a semi-synthetic diet was

as follows (g/kg); starch 400, casein 200, sucrose 200, corn oil 100, cellulose 50, mineral mixture (AIN 93) 35, vitamin mixture (AIN 93) 10, DL-methionine 3 and choline bitartrate 2. Betaine anhydrous (Danisco, Finland) supplementation in the STZ-betaine rats was replaced with starch at the level of 1% (w/w). The rats were divided into groups according to their treatment protocol. The rats were fed a semi-synthetic diet supplemented with or without betaine 10 g/kg body weight for 3 weeks. The streptozotocin solution was prepared in 0.05 M citrate buffer (pH 4.5), immediately prior to injection into intraperitoneal with dosage of 50 mg/kg body weight following overnight fasting. Diabetes was defined as a blood glucose concentration above 300 mg/dl 48 hr after the streptozotocin injection. The body weights were recorded every week, and the water and food intake were recorded by every other day.

Analytical procedure

In the end of the treatment period, after 12 hr of fasting, the rats were placed under light diethyl ether anesthesia and sacrificed by withdrawing blood from the abdominal aorta. The serum was separated by centrifuging the bloods at 1,026 *g* for 15 min. The pancreas were quickly removed and weighted, with the tissue weights onto the absolute (g) or relative weights (g/100 g body weight). The concentrations of total-cholesterol, HDL-cholesterol, triglyceride, glucose, fructosamine and the activities of AST, ALT and lactate dehydrogenase (LDH) in serum were measured in the clinical laboratory of the Neodin Medical Institute (Seoul, Korea).

Pancreatic histopathological examination

The pancreas were carefully removed for morphological examination, and immersed in 4% paraformaldehyde prepared 0.1 M phosphate buffered saline (pH 7.4), embedded in paraffin, and cut into 6-㎛ thick for Hematoxylin & Eosin staining as described previous[16,22].

Statistical analysis

The data from animal experiments are presented as the mean±S.E., and were analyzed using a one way analysis of variance (ANOVA), with the differences analyzed using the Duncan's new multiple-range test[7]. A p value <0.05 was accepted as being a statistically significant difference.

Results and Discussion

Body weights, pancreatic weights, and water and food intake

The body weight gains, water and food intake, and absolute and related pancreatic weights are presented in Table 1. Generally, the body weights are reduced in STZ-induced diabetic rats and recovered subjected to hypoglycemic treatment[4,5]. The body weight gain was significantly lower in the STZ-control rats compared to the normal rats, as expected[4,5]. However, the body weight reduction by STZ-treatment was markedly greater in the STZ-betaine rats than STZ-control rats. The water consumption and food intakes were also significantly increased in the diabetic animal groups compared to the normal rats, however, both parameters were markedly lower in the STZ-betaine rats than those of the STZ-control rats. The absolute and relative pancreatic weights were not significantly different.

Blood glucose and fructosamine concentrations

The fasting blood glucose and fructosamine concen-

Table 1. Body weight gain, pancreatic weights, food intake, and water intake in the STZ-induced diabetic rats

Ingredient	Normal	Control	Betaine
	Streptozotocin-induced diabetic rats		
Body weight			
Initial (g)	289.0 ± 6.40[a]	283.3 ± 2.03[a]	285.0 ± 2.52[a]
Gain (g/3 weeks)	47.13 ± 6.25[a]	-28 ± 2.52[b]	-5.8 ± 2.67[b]
Water intake (ml/day)	28.13 ± 2.25[a]	217.50 ± 25.36[b]	121.67 ± 30.27[c]
Food intake (g/day)	8.94 ± 1.82[a]	16.58 ± 1.72[b]	12.92 ± 2.05[ab]
Pancreas weight			
Absolute (g)	0.85 ± 0.06[a]	0.72 ± 0.12[a]	0.68 ± 0.05[a]
Relative (%)[1)]	0.25 ± 0.01[a]	0.28 ± 0.05[a]	0.25 ± 0.03[a]

[a,b,c]Values with different letters are significantly different at $p < 0.05$.
Values are means±SE of six rats per group.
[1)]Relative(%) = g/100 g body weight

trations were significantly increased in the STZ-control rats compared to the normal rats (Fig. 1), but these concentrations were slightly decreased in the STZ-betaine rats compared to the STZ-control rats. Fructosamine is a risk factor for the development of hyperinsulinemic insulin resistance and type 2 diabetes mellitus[16]. Fructosamine in diabetic patients is used to evaluate long-term control of diabetes mellitus, and most accurately reflects the previous 2~3 wk of glycemic control[23]. The correlation between fructosamine and plasma glucose levels was observed in this study.

AST, ALT and LDH activities

Liver disease is one of the leading causes of death in persons with diabetes mellitus. Diabetic patients can present abnormal liver chemistries, from benign nonalcoholic fatty liver disease to severe cirrhosis of the liver, because liver disease is associated with impaired glucose tolerance and diabetes mellitus[11,23]. The activities of AST and ALT are generally increased by metabolic changes in the liver due to the administration of toxins, such as diabetic-inducing STZ or alloxan[10,23]. Thus, the serum AST and ALT activities can be used as biomarker for monitoring the extent of hepatic injury in diabetic mellitus. The activities of ALT and AST have previously been reported to be significantly higher in the STZ-induced diabetic rats and genetically diabetic Zucker rats compared to the corresponding normal rats[10]. The AST and ALT activities were significantly higher in the STZ control rats than the normal rats, but the rise was significantly lowered in the STZ-betaine rats (Fig. 2). The re-

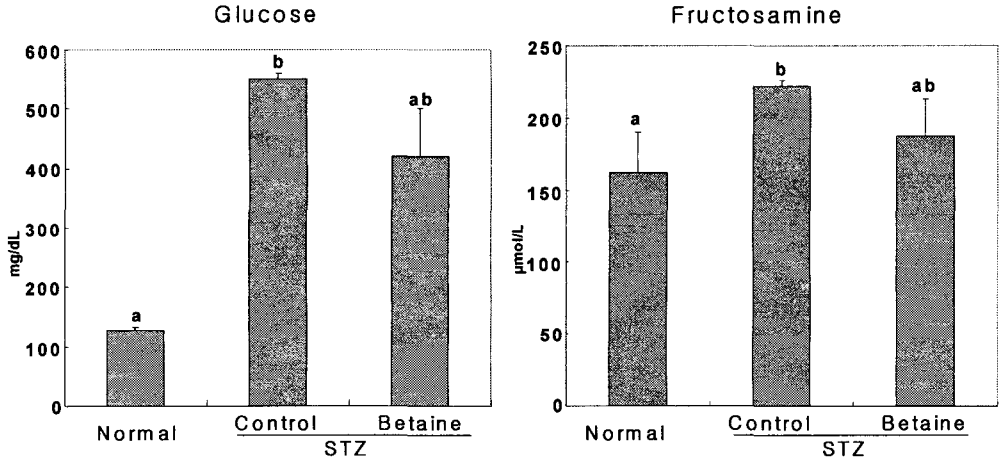

Fig. 1. The concentrations of serum glucose and fructosamine in the STZ-induced diabetic rats. [a,b,c]Values with different letters are significantly different at $p < 0.05$. (mean±S.E., n=6).

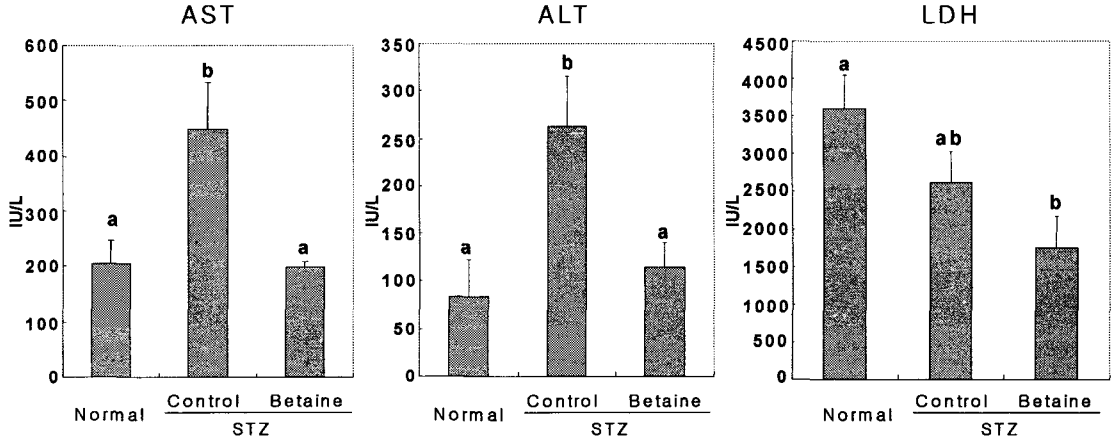

Fig. 2. The activities of ALT, AST and LDH in serum of the STZ-induced diabetic rats. [a,b,c]Values with different letters are significantly different at $p < 0.05$. (mean±S.E., n=6).
AST: alanine aminotransferase, ALT: aspartate aminotransferase, LDH: lactate dehydrogenase.

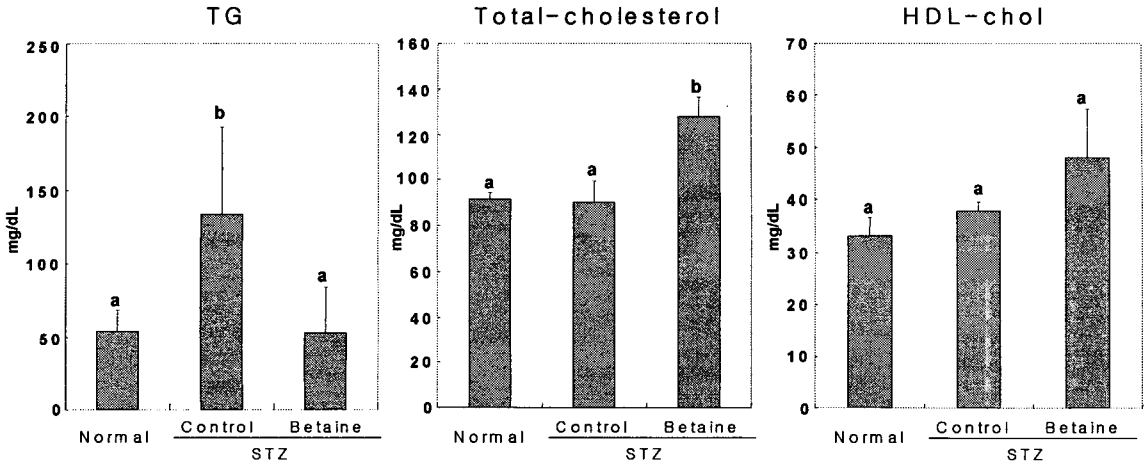

Fig. 3. The concentrations of serum triglyceride, total-cholesterol and HDL-cholesterol in the STZ-induced diabetic rats. [a,b,c]Values with different letters are significantly different at $p < 0.05$. (mean±S.E., n=6).
TG: triglycerid, HDL-chol: high-density lipoprotein-cholesterol.

duction in serum AST and ALT activity in the STZ-betaine rats returned to the levels of the normal rats. Thus, the present study confirmed the hepatoprotective effect by betaine on the hepatic injury in the STZ-induced diabetic rats. The LDH activity was slightly decreased in the STZ-control rats and significantly decreased the STZ-betaine rats compared to the normal rats (Fig. 2).

Serum concentrations of triglyceride and cholesterol

The prevention of common chronic disorders, such as arteriosclerosis, hyperlipidemia, hypertension and hepatic injury related to the development of diabetes mellitus, has become a topic of interest in recent years[6,25]. Hyperlipidemia is a major risk factor leading to lifestyle-related diseases, obesity, arteriosclerosis and hypertension, and much attention has focused on improving serum lipids by the intake of functional foods, and is the major risk factor leading to arteriosclerosis in diabetes mellitus[6,27]. Although these perturbations progress with the development of diabetes, it seems possible to delay and/or prevent these developments through improvements in the diet and nutritional factors[15,17]. The serum triglyceride concentration was significantly higher in the STZ-control rats than the normal rats (Fig. 3). However, this rise was significantly lowered in the STZ-betaine rats compared to the STZ-control rats (Fig. 3). The reduction in serum triglyceride concentration in the STZ-betaine rats returned to the levels of the normal rats. This result suggests that the betaine lowers elevated blood triglyceride concentrations in diabetes-related rats. There were no significant difference in the total-cholesterol and HDL-cholesterol concentrations in serum between the normal rats and the STZ-control rats, but these concentrations in the STZ-betaine rats were significantly increased compared to the normal rats. Previous studies also reported that betaine supplementation increased plasma low-density-lipoprotein(LDL)-cholesterol in modestly hyperhomocysteinemic patients[29] and in healthy humans[21].

Histopathological changes of the pancreatic islet cells

The typical arrangement and shape of pancreatic islets in the pathological experiment was observed in most of the pancreas of the normal rats (Fig. 4). As previously observed[18], the islet cells were markedly shrunken and rather irregular in shape, after STZ treatment in rats (Fig.

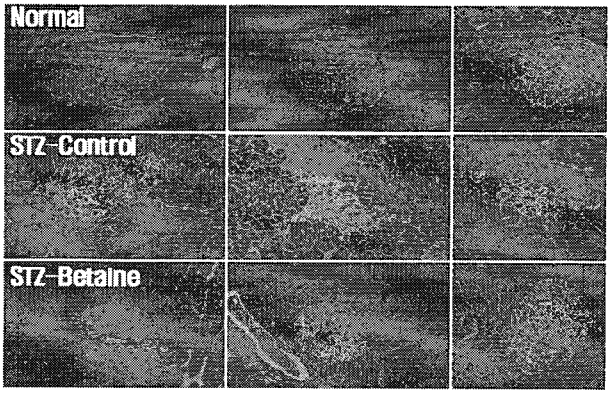

Fig. 4. Histopathology of pancreatic islets in the STZ-induced diabetic rats (200 × magnification). The sections were stained with hematoxylin and eosin to demonstrate the general islet morphology.

4). However, these morphological alterations were less severe in the STZ treatment rats given betaine, as the islets mostly maintained a normal rounded appearance. The present study strongly suggests that the administration of betaine plays important roles in protecting the pancreatic beta-cells from in STZ-induced destruction for normal glucose homeostasis.

Thus, the present study demonstrated that the administration of betaine in the STZ-diabetic rats almost results in the prevention of the diabetogenic action of STZ.

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초록 : Streptozotocin-유발 당뇨쥐의 베타인 첨가에 의한 항당뇨 및 간보호 효과

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Sprague-Dawley 수컷에 streptozotocin (50 mg/kg body weight)을 복강 주사하여 유발시킨 당뇨군에서 베타인(1%, w/w) 첨가에 의한 항당뇨 및 간보호 효과를 검토하였다. Streptozotocin-유발 당뇨군에서 혈당치 및 fructosamine 농도가 현저히 증가하였고, 이러한 증가는 베타인 투여로 감소하였다. 간 기능의 biomarker로 사용되는 alanine aminotransferase (AST) 및 aspartate aminotransferase (ALT) 활성은 당뇨군에서 현저히 증가한 반면 간보호 효과가 있는 베타인 투여 당뇨군에서는 정상군 수준으로 회복됨으로써 당뇨성 간 손상에 효과가 있는 것으로 나타났다. 혈중 중성지질 농도는 당뇨군에서 현저히 증가하였으나, 베타인 투여에 의해 정상군 수준까지 감소하였으나, 총 콜레스테롤 농도는 베타인 첨가에 의해 증가하였다. 췌장 조직의 조직검사에서 췌도 세포의 정상적인 타원형 모양이 정상군에서는 잘 유지되었으나, Streptozotocin-유발 당뇨군에서는 췌도 세포의 파괴가 일어났으며, 베타인 투여에 의해서는 회복된 것으로 나타났다. 이상의 결과로 볼 때 당뇨군에서 베타인 투여에 의한 혈당강하 효과는 췌장의 췌도세포 파괴 억제에 의한 것으로 시사되었다.