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Effect of Taurine Supplementation on Hepatic Lipid Peroxide Metabolism in Streptozotocin-induced Diabetic Rats*

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ABSTRACT

The purpose of this study was to examine the effect of taurine supplementation time on the activity of the enzymes metabolizing lipid peroxide in the liver of streptozotocin(STZ)-induced diabetic rats. Sprague-Dawley male rats were fed the purified diet for 7 weeks. They were supplemented with or without 1% taurine in drinking water before or after STZ injection or during all experimental procedure. In comparison to diabetic group without taurine, glucose-6-phsphatase(G6Pase) activity was decreased in diabetic group supplemented with taurine before STZ injection, and it was increased in diabetic group supplemented with taurine after STZ injection, but the difference was not significant. Glutathione S-transferase(GST) activity was significantly increased by the injection of STZ. However, the GST activities of diabetic groups exposed to taurine after STZ injection or during all experimental procedure were significantly decreased. Glutathione peroxidase(GPx) activities was significantly decreased by STZ injection. However, only in diabetic group supplemented with taurine before STZ injection, GPx activities was not decreased by the STZ injection. These results suggest that taurine supplementation may change the activities of GSH-related enzymes metabolizing lipid peroxide in the liver of streptozotocin(STZ)-induced diabetic rats and that may be helpful for the prevention of diabetic complication. (*J Community Nutrition* 2(2) : 164~169, 2000)

KEY WORDS : taurine · diabetes mellitus · glutathione S-transferase · glutathione peroxidase.

Introduction

Diabetes mellitus(DM) is a chronic metabolic disease that has been extensively studied throughout the entire life span of the disease. Streptozotocin(STZ), a nitrosourea derived from *streptomyces acromogenes*, causes diabetes in rats(Younes et al. 1980).

Free radicals may play an important role in causation and complications of DM. The result of Garg et al.(1996) showed that increased oxidative stress and accompanying decrease in antioxidants may be related to the causation of DM. In diabetics, ischemic heart disease occurs earlier an to a more marked degree than in nondiabetics and is a major cause of death (Kessler et al. 1971). The role of reactive oxygen radicals in determining myocardial ischemic/reperfusion injury seems firmly established(McCord 1985 ; Van der Vusse & Reneman 1985). Salch et al.(1987) supported the view that tissue antioxidant status may be an important factor in the etiology of diabetes and its complication. Also there has been interest in metabolism of glutathione(GSH) in diabetes ; abnormalities in the generation and disposal of free radicals have been postulated to play a pathogenic role in the chronic complications of diabetes. GSH is an organosulfur compound that is important in the regulation of the redox state, and a decline in its tissue level has often been considered to be an indicative of increased oxidative stress in diabetes(McLennan et al. 1991). Lipid peroxidase activity was increased and total GSH concentrations was declined in liver of STZ- or alloxan-induced diabetic rats(Matkovics et al. 1982). The glutathione S-transferase(GST) is an enzyme that functions in detoxification by conjugating GSH with many xenobiotics. STZ has been reported to increase hepatic 1-chloro-2,4-dinitrobenzene(CDNB)-con-

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jugated GST activity in the lives of mice(Rouer et al. 1981).

Taurine(2-aminoethane sulfonic acid) is a sulfur-containing -amino acid that is present in high concentrations in almost all tissue of mammals. It is available from dietary sources and can also be biosynthesized from cysteine. It has many physiological functions, such as bile acid conjugation(Monte et al. 1997), membrane protection(Emudianughe et al. 1983), antiox-idation(Cunningham et al. 1998 ; Raschke et al. 1995), enhancement of host defense systems(Redmond et al. 1998), and many others.

It has been reported that taurine can protect cells against damage by streptozotocin via membrane stabilization by binding to the membrane at or near the insulin receptor(Kulakowski & Matura 1984). Lim et al.(1996) reported that taurine supplementation protects type I diabetic mice from lipid peroxide formation.

We hypothesized that taurine may react as an antioxidant, cure complication or prevent diabetes affecting on the GSH-related antioxidant enzyme system. Therefore, we examined the effect of taurine supplementation time on the activity of GSH-related enzymes and glucose-6-phosphatase, an index of membrane stability, in the liver of streptozotocin-induced diabetic rats.

Materials and Method

1. Animals and diet

Sprague-Dawley male rats weighing 90 – 100g were acclimatized for 1 week prior to experiments. The rats were kept at 25 – 30℃ with a 12 hr light-dark cycle. For 7 weeks the rats were fed an experimental diet and were maintained *ad libitum* on drinking water with or without 1% taurine. The experimental diet was composed of 18% casein protein, 64.7% corn starch, 7% corn oil, 5% α- cellulose, 4% salt mixture, 1% vitamin mixture, and 0.3% DL-methionine, of which calorie density was 393.8 kcal per 100g diet(Table 1).

Three weeks after feeding rats the experimental diet, diabetes was induced by a single thigh subcutaneous injection of STZ(50mg/kg body weight).

The forty animals were randomly divided into 6 groups(Fig. 1) : CO(control, without taurine), CT(control, with taurine), DO(diabetes, without taurine), DB (diabetes, with taurine before the STZ injection), DA (diabetes, with taurine after the STZ injection), DT (diabetes, with taurine during all experimental procedure).

Table 1. Diet composition of experiment

Component	(g/100g diet)
Corn starch	64.7
Casein	18.0
Corn oil	7.0
α-Cellulose	5.0
Mineral mixture	4.0
Vitamin mixture	1.0
DL-methionine	0.3
Calories(kcal/100g)	393.8

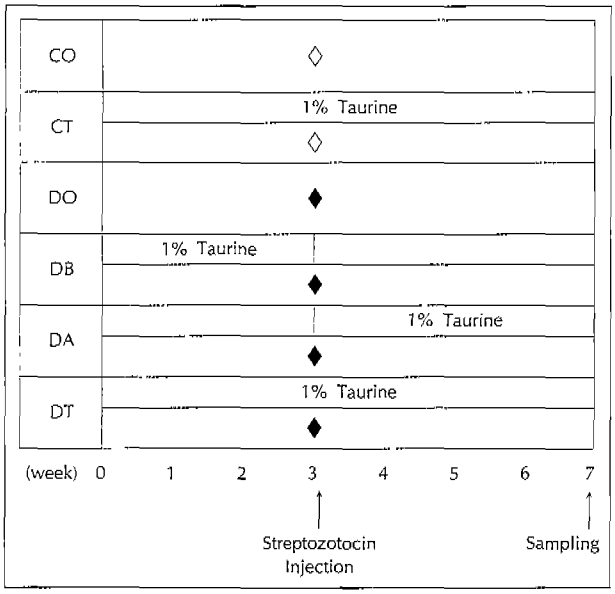

Fig. 1. Experimental design. CO : Control group, CT : Control group+1% taurine, DO : Diabetic group, DB : Diabetic group+pre 1% taurine, DA : Diabetic group+post 1% taurine, DT : Diabetic group+1% taurine, Diabetes was induced by thigh subcutaneous injection of STZ(♦), Instead of STZ, control group was treated with saline(◊).

2. Biochemical analysis

Four weeks after inducing diabetes, a determination of the blood glucose level was conducted. Two hours after an oral administration of glucose(0.1g/100g body weight), blood samples were collected. Blood glucose concentration was determined with a reagent strip and a blood glucose sensor(Exactech, Medisense,

Inc., U.S.A.).

The liver was homogenized upon removal on the next day to blood collection. Microsomal and cytosolic fractions were prepared by differential centrifugation and were stored in small aliquots at −70℃ until used.

Microsomal glucose 6-phosphatase(G6Pase) activity was determined by the measurement of the amount of inorganic phosphate liberated from glucose-6-phosphatate(Baginski et al. 1983).

Glutathione S-transferase(GST) activity was assayed in hepatic cytosolic fraction using the method of Habig et al.(1974). The conjugate of GSH with 1-chloro-2,4-dinitrobenzene(CDNB) was measured at 340nm using a dual beam spectrophotometer. Calculation was made using a molar extinction coefficient of $9.6mM^{-1}cm^{-1}$.

Activities of GSH peroxidase(GPx) in the hepatic cytosolic fraction were measured by monitoring the oxidation of NADPH at 340nm according to the method of Tappel(1978).

Protein concentrations in hepatic microsomal and cytosolic fractions were estimated by the Lowry method(Lowry et al. 1951) using bovine serum albumin as a standard.

3. Statistical analysis

All values present the mean S.E. All statistical analysis were carried out using SAS program with Duncan's multiple range test. Differences were considered statistically significant at $p<0.05$.

Results and discussion

In experimentally induced diabetes models, free radicals may play an important role in causation and complications. Increased oxidative stress and accompanying decrease in antioxidants may be related to the causation of DM(Garg et al. 1996). Kakkar et al.(1995) suggested that oxidative damage to tissue may be a contributory factor in complications associated with diabetes. GSH is an organosulfur compound that is important in the regulation of the redox state, and a decline in its tissue level has often been considered to be indicative of increased oxidative stress in diabetes(McLennan et al. 1991). Taurine is one of organosulfur compounds with membrane protection(Emudianughe et al. 1983), antioxidation(Cunningham et al. 1998 ; Raschke et al. 1995), and enhancement of host defense system(Redmond et al. 1998). Also it was reported that experimental diabetes induced by STZ, can produce biochemical changes not only in pancreas but also in liver tissue(Mukherjee et al. 1994). Our previous studies(You & Chang 1998 ; Chang 1999) reported that taurine supplementation inhibits the lipid peroxide formation and suggested that taurine supplementation is necessary for diabetes in order to prevent diabetic complications such as cardiac vascular diseases.

As shown in Table 2, final body weights were significantly lower in the diabetic group supplemented with taurine after the STZ injection compared to the control group($p<0.05$). Although final body weights of other diabetic groups were also lower than that of control groups, but the differences were not significant.

Two hours after an oral administration of glucose, blood glucose level was as follows. Among diabetic groups, only the group supplemented with taurine after STZ injection, blood glucose level was significantly increased(Table 2). From the our previous results concerning oral glucose tolerance test(OGTT), blood glucose concentration were significantly higher in diabetic group without taurine compared to control group

Table 2. Effects of taurine supplementation time on final body weight and blood glucose level

	Final body weight (g)	Blood glucose level (mg/dl)
CO	258.3 ± 11.7^{b}	67.0 ± 4.5^{b}
CT	331.3 ± 14.2^{a}	72.0 ± 3.2^{b}
DO	239.4 ± 15.8^{b}	122.3 ± 15.2^{b}
DB	202.4 ± 18.6^{b}	77.0 ± 16.2^{b}
DA	199.2 ± 16.1^{c}	236.5 ± 32.8^{a}
DT	238.9 ± 8.2^{b}	122.0 ± 22.7^{b}

CO : Control group
CT : Control group+1% taurine
DO : Diabetic group
DB : Diabetic group+pre 1% taurine
DA : Diabetic group+post 1% taurine
DT : Diabetic group+1% taurine
Values are mean S.E.
Means with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

at 30 minutes and 1 hour after an oral administration of glucose and there was no significant difference between two groups at 2 hours in OGTT(You & Chang 1998). Also our previous study had shown that blood glucose level of diabetic group supplemented with 1% taurine before STZ injection was significantly lower compared to that of diabetic group without taurine at I hour in OGTT(You & Chang 1998), which was confirmed in the other previous study(Chang 1999). Therefore, taurine supplementation before STZ injection may prevent or ameliorate experimentally induced diabetes by STZ injection.

It has been reported that STZ treatment generally induces an oxidative stress in tissue(Matkovics et al. 1997). Also it has been reported that the TBARS content of liver and pancreatic islets from type I diabetic rats was significantly increased compared to the control group, an effect which was significantly attenuated by taurine supplementation(Lim & Kim 1995). However, Yadav et al.(1997) reported that the heart tissue showed an increased lipid peroxidation in diabetic rats while no significant change was observed in the liver and kidney. Previously we reported that hepatic TBARS content was increased following the injection of STZ ; in the diabetic group supplemented with taurine before the STZ injection, hepatic TBARS content was significantly lower compared to the diabetic group without taurine supplementation and the hepatic contents of TBARS in the diabetic group supplemented with taurine after the STZ injection and during all experimental procedures were slightly decreased compared to the diabetic group, but the differences were not significant(You & Chang 1998). From this previous findings, we have suggested that taurine supplementation protect the liver against lipid peroxidation in the type I diabetic rat.

G6Pase activity was measured as an index of membrane stability of the microsomal fraction. In groups without taurine supplementation, there was no difference of G6Pase activity between control groups and diabetic groups(Table 3). In comparison to the diabetic group without taurine, G6Pase activity was decreased in the diabetic group supplemented with taurine before STZ injection, and was increased in the diabetic group supplemented with taurine after STZ injection, but the difference was not significant.

Hepatic GST activity of rat was significantly increased by the injection of STZ(Table 3). This result agrees with several previous reports on hepatic GST activity in experimentally induced diabetes(Mukherjee et al. 1994 ; Yadav et al. 1997). However, there are different results(Pachecka et al. 1993 ; Saito-Yamanaka et al. 1993 ; Kumar & Menon 1992) which showed that GST activity was not significantly affected in diabetic rat tissues. Hepatic GST activity of control group with taurine was higher than that of control group without taurine. In this study, the GST activities of groups exposed to taurine after STZ or during all experimental procedure were significantly decreased. However, Lim et al.(1998) reported that no effect on the activity of GST was observed in both types of diabetes(I and II) following taurine supplementation.

In agreement with previous studies(Altan et al. 1994 ; Saxena et al. 1993 ; Toleikis & Godin 1995), we fo-

Table 3. Effect of taurine supplementation time on glucose 6-phsphatase activities, glutathione S-transferase activities and glutathione peroxidase activities

	Glucose 6-phsphatase activities (nmole *Pi liberated*/ min/mg protein)	Glutathione S-transferase activities (nmole CDNB conjugated/ min/mg protein)	Glutathione peroxidase activities (nmole NADPH oxidized/ min/mg protein)
CO	757.5 ± 60.1^{ab}	149.9 ± 7.9^{c}	59.9 ± 3.1^{b}
CT	491.5 ± 49.1^{c}	198.4 ± 6.8^{a}	105.0 ± 4.5^{a}
DO	828.6 ± 57.0^{ab}	176.2 ± 4.3^{b}	45.3 ± 2.1^{c}
DB	664.4 ± 47.7^{bc}	166.9 ± 4.3^{b}	66.6 ± 2.4^{b}
DA	969.7 ± 61.3^{a}	146.4 ± 2.7^{c}	49.5 ± 1.7^{c}
DT	927.3 ± 46.8^{a}	9.4 ± 3.6^{d}	47.3 ± 2.9^{c}

CO : Control group CT : Control group+1% taurine DO : Diabetic group
DB : Diabetic group+pre 1% taurine DA : Diabetic group+post 1% taurine DT : Diabetic group+1% taurine
Values are mean S.E. Means with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

und that GPx activity of diabetes group was decreased compared to those of the control groups(Table 3). It was reported that total and se-dependent GPx activity in the heart was markedly lowered in diabetic rats which recovered with insulin as well as with Capparis decidua power(hypoglycaemic agent)(Yadav et al. 1997). Our study showed that only in diabetic group supplemented with taurine before STZ injection GPx activity was not decreased in the liver. In control group, taurine supplementation increased hepatic GPx activity.

Therefore, these results suggest that taurine may change the activities of GSH-related enzymes metabolizing lipid peroxide in the liver of streptozotocin(STZ)-induced diabetic rats and that may be helpful for prevention of diabetic complication.

Summary and Conclusion

The purpose of this study was to examine the effect of taurine supplementation time on the activity of the enzymes metabolizing lipid peroxide in the liver of streptozotocin(STZ)-induced diabetic rats. Sprague-Dawley male rats were fed the purified diet for 7 weeks. They were supplemented with or without 1% taurine in drinking water before or after STZ injection or duting all experimental procedure.

1) Final body weights were significantly lower in the diabetic group supplemented with taurine after the STZ injection compared to control group.

2) Two hours after an oral administration of glucose, blood glucose level in the diabetic group supplemented with taurine after STZ injection was significantly increased compared to other groups.

3) Hepatic GST activity significantly increased by the injection of STZ. Hepatic GST activity of control group with taurine was higher than that of control group without taurine. The GST activities of liver in diabetic groups exposed to taurine after STZ or during all experimental procedure were significantly decreased.

4) Hepatic GPx activity of diabetic group was decreased compared to the control groups. Hepatic GPx activity was not decreased only in diabetic group supplemented with taurine before STZ injection. In control group, taurine supplementation increased hepatic GPx activity.

Therefore, these results suggest that taurine may change the activities of GSH-related enzymes metabolizing lipid peroxide in the liver of streptozotocin(STZ)-induced diabetic rats and that may be helpful for the prevention of diabetic complication.

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