Taurine supplementation restores antioxidant status and hepatic membrane-bound enzymes in streptozotocin-induced diabetic rats

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ABSTRACT

Introduction and Aim: Chronic hyperglycemia in diabetes causes cellular damage through increased lipid peroxidation and reduced levels of antioxidants. The activities of membrane-bound enzymes are affected by oxidative stress. Taurine, a sulfur containing amino acid is shown to have hypoglycemic activity, antioxidant property and membrane stabilization. The aim of the study is to check the effect of supplementation of taurine on lipid peroxidation, antioxidant status and hepatic membrane-bound enzymes in streptozotocin-induced diabetic rats.

Materials and Methods: Thirty-two Wistar male albino rats of 19±1 weeks of age weighing 200-220 grams were randomly divided into four groups and each group consisted of eight animals. Group I (control) standard chow diet; Group II (chow diet with taurine); Group III (diabetes induced) and Group IV (diabetic receiving taurine). At the end of 45th day, all animals were sacrificed by cervical decapitation after overnight fasting. Blood and liver tissue samples were collected. The levels of glucose in plasma and lipid peroxidation, antioxidants and the activities of Na^+/ K^+, Ca^{2+} and Mg^{2+}-ATPases in liver homogenate were analyzed.

Results: Altered levels of antioxidants and activities of Na^+/ K^+, Ca^{2+} and Mg^{2+}-ATPases were restored to normal by taurine supplementation in diabetic rats.

Conclusion: The present study indicates that supplementation of taurine could protect liver plasma membrane against oxidative damage by acting as antioxidant and restoring the normal activities of Na^+/ K^+, Ca^{2+} and Mg^{2+}-ATPases.

Keywords: Taurine; lipid peroxidation; antioxidants; membrane-bound enzymes; ATPases.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia which causes cellular damage through several pathways (1). Free radicals are formed disproportionately in diabetes by auto-oxidationof glucose, non-enzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins (2). The free radicals are neutralized by the antioxidants present in the living system. However, excess production of free radicals exhausts antioxidants, which results in oxidative stress (3). Oxidative stress plays a major role in the development of complications in DM (4). ATPases are membrane-bound enzymes which

regulate movements of many different types of ions or molecules across biological membranes (5). They play a significant role in many metabolic pathways and in a variety of pathological processes. For instance, the activity of Na^+/ K^+-ATPase is impaired in the cell membrane of various tissues in diabetic subjects and this defect may play a role in the development of complications (6). Findings of many *in vivo* and *in vitro* studies indicated that the activities of these enzymes were altered due to oxidative stress in DM.

Taurine, 2-aminoethanesulfonic acid is present as a free amino acid in mammalian tissues like liver, heart, brain, and leukocytes. It is hypoglycemic, hypolipidemic and anti-atherosclerotic and an effective antioxidant (7). It plays a major role in the maintenance of various cellular functions like osmoregulation, neuromodulation, detoxification, bile acid conjugation, calcium homeostasis, and membrane stabilization (8). It is considered as semi essential nutrient but cells deficient with taurine show various pathologies (9).Various clinical complications observed due to altered metabolism of taurine in DM showed positive outcomes with taurine supplementation (10).The aim of the present study was to investigate the effect of taurine supplementation on lipid peroxidation and antioxidant status and the hepatic membrane-bound enzymes such as Na^+/ K^+,Ca^{2+} and Mg^{2+}-ATPases in streptozotocin (STZ) - induced diabetic rats.

MATERIALS AND METHODS

Wistar strain male albino rats of 19±1 weeks of age weighing 200-220 grams were obtained from the Central Animal House, Rajah Muthiah Medical College and Hospital (RMMC&H), Annamalai University. They were housed in standard conditions and maintained on a standard chow diet and water *ad libitum.* They were randomly divided into four groups and each group consisted of eight animals. Group I (control) standard chow diet; Group II (chow diet plus taurine) Group III (diabetes induced), Group IV (diabetic receiving taurine). After 24-hour fasting, the animals were injected intraperitoneally with STZ at a dose of 50 mg/kg body weight in 0.1 M citrate buffer (pH 4.5). The control animals received citrate buffer alone. Diabetes was confirmed by measuring the fasting plasma glucose concentration 48 hour after STZ injection. Taurine was administered orally once daily at a dose of 100 mg/kg body weight. At the end of 45^{th} day, all animals were sacrificed by cervical decapitation after overnight fasting. Blood samples were collected in heparinized tubes and liver specimens were collected in homogenizing buffer (0.1 M Tris-HCl, pH 7.4). The study was approved by the Institutional Animal Ethics Committee (IAEC).

Biochemical analyses

Plasma glucose was determined by glucose oxidase-peroxidase (GOD-POD) method by using Boehringer Mannheim reagent kit in Erba Smart Lab analyzer, USA. A portion of the liver was homogenized, and the homogenate was used for the estimation of lipid peroxidation as thiobarbituric acid reactive substances (TBARS), antioxidants, and membrane-bound enzymes. TBARS was estimated by the methods of Yagi (11) and Ohkawa *et al.*, (12). The activities of superoxide dismutase (SOD) by the method of Kakkar *et al.*, (13) Catalase (CAT) by Sinha (14) Glutathione peroxidase (GPX) by Rotruck (15) and the concentration of reduced glutathione (GSH) by Beutler (16). Na^+/ K^+-ATPase activity was assayed by the method of Bonting (17), Mg^{2+} -ATPase by the method of Ohnishi et al. (18) and Ca^{2+}-ATPase by the method of Hjerten and Pan (19).

Statistical Analysis

One-way analysis of variance (ANOVA) test was applied in order to evaluate any significant difference in the mean values. All values used in analysis represent the mean ± SD of eight rats in each group. The results were considered

statistically significant if the *p* values were 0.05 or less.

RESULTS

Table 1 shows the status of plasma glucose in control and experimental animals in each group. An increase in plasma glucose was noticed in diabetic animals. Supplementation of taurine slightly lowered the plasma glucose levels but that was not statistically significant.

Table 1: Food intake, body weight and plasma glucose levels in experimental animals

Groups	Food Intake (gm)	Body weight (gm)	Plasma glucose(mg/dl)
Control	18.22 ± 0.05	244.1 ± 8.94	103.75 ± 4.77
Control + Taurine	18.35 ± 0.06*	241.5 ± l0.86*!	100.25 ± 2.92*
Diabetic	20.82 ± 0.14**	202.75 ± 13.35**!	344.13 ± 41.71**#
Diabetic +Taurine	19.53 ± 0.16***	221.00 ± 11.01***#	329.52 ±2 7.4***

Values are expressed as mean ± SD. *Group 2 compared with Group 1; ** Group 3 compared with Group 1 ***Group 4 compared with Group 3; ! p <0.05; # p<0.001.

Table 2 shows the hepatic lipid peroxidation and antioxidant status. The levels of TBARS were significantly raised in diabetic animals. Supplementation of taurine reduced their levels significantly. The activities of antioxidants-SOD, CAT and concentration of GSH were significantly reduced while the activities of GPX were markedly increased in diabetic rats. Taurine administration restored to their normal status.

Table 2: Hepatic lipid peroxidation and antioxidant status

Groups	TBARS	SOD	Catalase	GPX	GSH
Control	0.34 ± 0.40	5.87± 0.13	52.5 ± 0.55	3.66 ± 0.37	5.01± 0.11
Control + Taurine	0.28 ± 0.42*	5.51 ± 0.06*#	53.29 ± 0.56*!	3.81 ± 0.09*#	5.22 ± 0.14*!
Diabetic	0.49 ± 0.06**	3.92 ± 0.68**#	34.88 ± 0.55** #	6.8 ± 0.06**#	2.83 ± 0.39**#
Diabetic + Taurine	0.37 ± 0.02***#	5.78 ± 0.70***#	41.13 ± 1.16***#	4.14 ± 0.08***#	4.28 ± 0.62***#

Values are expressed as mean ± SD. *Group 2 compared with Group 1; ** Group 3 compared with Group 1 ***Group 4 compared with Group 3; !p <0.05; # p<0.001.
TBARS – nmoles /mg of protein; Catalase- µ moles of H_2O_2 utilized/min/ mg of protein; GPX – µg of GSH consumed / min/ mg of protein; SOD- 50% inhibition of NBT reduction/min / mg of protein, GSH- mg/ 100g tissue.

Table 3 shows the activities of hepatic membrane-bound enzymes. The activities of Na^+/ K^+, and Mg^{2+}-ATPases were profoundly reduced while the activities Ca^{2+}-ATPase were overexpressed in diabetic rats. These activities were restored to normal with taurine supplementation.

Table 3: Hepatic membrane-bound enzymes

Groups	Na^+/ K^+-ATPase U/mg of protein	Mg^{2+}-ATPase U/mg of protein	Ca^{2+}-ATPase U/mg of protein
Control	1.845 ± 0.056	2.575 ± 0.406	1.79 ± 0.067
Control + Taurine	1. 25 ± 0.057*	2.297 ± .84*	1.783 ± 0.069*
Diabetic	0.798 ± 0.05**#	1.537 ± 0.287**!	4.74 ± 0.142**#
Diabetic + Taurine	1.562 ± 0.424***#	2.187 ± 0.454***!	2.415 ± 0.384***#

Values are expressed as mean ± SD. *Group 2 compared with Group 1; ** Group 3 compared with Group 1 ***Group 4 compared with Group 3; !p <0.05; # p<0.001.

DISCUSSION

The present study found that taurine supplementation did not improve the glycemic status in diabetic rats. This is in contrast to earlier studies which reported that taurine was effective in reducing hyperglycemia (20, 21). This could be

attributed to the low dosage used in the present study. Hyperglycemia is the most important factor in the onset and progress of diabetic complications. Diabetes is always associated with over generation of free radicals and depletion of antioxidants with concomitant oxidative stress (2, 3, 8). In hyperglycemia, the excess polyol pathway causes depletion of NADPH which is required by glutathione reductase for regeneration of GSH from glutathione disulfide (GSSG). This explains the decreased concentration of GSH and overexpression of GPX. This indicates the oxidative stress in the background of hyperglycemia. Many animal studies showed that taurine supplementation was beneficial to diabetes and its complications (20, 21). The present study observed that taurine administration restored the hepatic antioxidant enzyme activities and reduced glutathione. This could be due to to the action of taurine by preventing the generation of oxidants (22).

ATPases are very sensitive to oxidative stress which inactivates the enzyme by modifying the active site (7). The present study found that the activities Na^+/ K^+, and Mg^{2+}-ATPases in liver homogenates were significantly decreased while the activities of Ca^{2+}-ATPase was found to be increased. Taurine supplementation restored the normal activities of these ATPases. The sulfonic acid group of taurine plays a major role in preventing a direct attack by oxidants on cell membranes (23). These calcium pumps are the major targets that are readily affected in diabetes (24). The over expression of Ca^{2+}-ATPase may be due to the energy imbalance because of impairment of Na^+/ K^+, and Mg^{2+}-ATPases. The present study strengthens the findings of the various animal studies. As some of the findings of the human studies are inconclusive, more studies on human trials will be of highly useful. If an effective dose of taurine for human beings is established, that could be used in the prevention of diabetic complication.

CONCLUSION

The present study found that lowering of glucose induced lipid peroxidation and enhancement of the antioxidant status. The activities of hepatic Na^+/ K^+, Ca^{2+} and Mg^{2+}-ATPases are preserved by taurine supplementation, implicating the inhibition of development of complication in diabetes mellitus.

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