Research Journal of Biological Sciences 3 (11): 1271-1275, 2008
ISSN: 1815-8846

Effect of Dietary Taurine on Lipid Profile and Oxidative Stress in Tissues of Homocystein-Treated Rats

[1]Zakaria Vahabzade, [1]Aliakbar Abolfathi,
[2]Mohammadhasan K. Ansari and [3]Abdolrasul Safaiyan
[1]Department of Biochemistry, Medicine College,
Tabriz University of Medical Sciences, Tabriz, Iran
[2]Department of Clinical Biochemistry, Urmia Medical School, Urmia, Iran
[3]Health and Nutrition College, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract: It has been suggested that taurine as intracellular non-protein amino acid has hypolipidemic and antiatherosclerotic effects. To study the beneficial effect of taurine on serum lipids and markers of oxidative stress in tissues of rats treated with low oral dose of Homocysteinethiolacton hydrochloride, 32 Male adult Wistar rats of body weight 212±12 g were divided in to the 4 groups consisting of 8 rats each i.e., Control, Control + Homocysteine, Control + Taurine and Control + Homocysteine + Taurine, for a 6 weeks period. Food intake, fluid intake and body weight changes were monitored weekly. At the end of the experimental period, the rats were anesthetized, plasma was collected by direct cardiac puncture in heparinated tube and were used for measurement of HDL-C, LDL-C, total cholesterol, triglyceride (TG) and total antioxidant capacity. The heart and kidney were removed immediately and homogenized in phosphate-buffered saline (pH 7.4). Supernatants were separated in tree aliquots; Glutathione peroxidase (GP_X), superoxide dismutase (SOD) and malondialdehayde (MDA) were measured in tissue homogenate supernatants. There were no significantly differences in lipid profile of homocystein-treated rats with control rats and other experimental groups. The levels of MDA of Control+Homocysteine rats were significantly higher in compare with Control + Taurine group. The activity of SOD in Control + Homocysteine group, were significantly lower in compare to rats received 2% taurine in Control + Taurine group. In conclusion, increase in TG and MDA levels and decrease in SOD activity of rats treated with Homocysteine were observed and beneficial effect of taurine in treated rats in our study are obvious.

Key words: Homocystein thiolacton hydrochloride, taurine, oxidative stress, total antioxidant capacity, reactive oxygen species (ROS), rat

INTRODUCTION

Taurine (2-amino ethane sulphonic acid) is an intracellular sulphur-containing non-protein amino acid that is the most abundant free amino acid (millimolar concentration) in excitable tissues and Cells including nervous tissues, heart, liver, kidney and retina (Nandhini *et al*., 2005a, 2004b). It serves several physiological and metabolic functions and has been reviewed extensively (Nandhini *et al*., 2005c; Yoshibumi *et al*., 2003). Taurine comprises over 50% of the total free amino acid pool of the heart and has a positive inotropic action on cardiac tissue. Taurine with its ability to stabilize biomembranes and to scavenge reactive oxygen species can reduce the peroxidation of unsaturated membrane lipids and oxidative stress Taurine may also inhibit lipid peroxidation by inducing Glutathione peroxidase (GPx) and superoxide dismutase (SOD) (Hisashi *et al*., 2004). Taurine has hypolipidemic and antiatherosclerotic effects and has been to be hepatoprotective and cytoprotective agents (Nandhini *et al*., 2005c; Jale *et al*., 2002). Taurine significantly increased serum HDL-C, without effect on LDL-C and VLDL-C (Nandhini *et al*., 2005a).

Homocystein is non-protein forming amino acid, not codified in DNA that is characterized by containing sulphur and binge intermediary in metabolism of methionin and cysteine. Genetic defects of some enzymes, nutritional deficiencies such vitamins, demographic factor and overloading methionin and homocysteine are some

Corresponding Author: Zakaria Vahabzade, Department of Biochemistry, Medicine College,
Tabriz University of Medical Sciences, Tabriz, Iran

causes of high plasma homocysteine, elevation in cells and concentrated in blood that provokes direct and indirect endothelial damage, promoting a procoagulant and proinflammatory status in blood. Homocystein rapidly auto-oxidizes when it enters plasma and produces highly reactive oxygen molecules such as superoxide onion radicals, hydrogen peroxides and hydroxyl radicals. The underlying mechanisms of homocysteine toxicity to vessel wall, however, have not been fully elucidated yet (Frauscher *et al.,* 1995). Feeding low dose Homocystein thiolacton hydrochloride to rats makes significant increase of plasma triglycerides and induces oxidative stress (Frauscher *et al.,* 1995). This made us investigate the beneficial effect of taurine on serum lipids and markers of oxidative stress in kidney and heart of rats treated with low oral dose of Homocystein.

MATERIALS AND METHODS

All experimental on rats carried out according to the principles of laboratory Animal Care (NIH publication no. 85-23, revised 1985). Thirty two adult Male Wister rats aged 45 day with the initial body weight 212±12 g used for the study. After acclimatization, the animals were divided into the 4 groups consisting of 8 rats each that housed 4 per cage under controlled conditions on a 12 h light/12 h dark cycle: Group control (CON) received the control diet contained starch (65.1%) as the sole source of carbohydrate, 20% crude protein, 6% crude fiber, 6% crude oil, 0.6% lysine, 0.8% methionin and homocystein, 0.4% absorbable phosphor, 0.5% NaCL, 0.6% Ca^{+2} and water *Ad libitum.* Group control and homocysteine (CON + HCY) received control diet and were allowed to drink 50 mg kg^{-1} body weight/day Homocystein thiolacton hydrochloride solution *Ad libitum.* Group control and taurine (CON + TAU) animals were given control diet and 2% taurine solution *Ad libitum.* Group control, homocysteine and taurine (CON + HCY + TAU) animals received the control diet, 50 mg kg^{-1} body weight/day Homocystein thiolacton hydrochloride and 2% taurine solution *Ad libitum.* The animals were maintained in their respective groups for a period of 6 weeks. Food and fluid intake and body weight were measured weekly. At the end of the experimental period, the rats were anesthetized by 0.2cc ketamine 2% and 0.3cc xylazin 10%. Blood was collected by direct cardiac puncture and heparinated plasma was separated in 2 aliquots after centrifugation in 5000 rpm for 2 min at 4°C (Sigma 2 K 15 C, Nr 12139-H; D-37520, Germany) then stored at -80°C for biochemical analysis. The heart and kidney were removed and immediately rinsed in ice-cold saline, weighted and then placed in liquid N_2 tanks and then stored at -80°C until the test were carried out. Homogenates were prepared from kidney and heart of rats for tissues analysis. Tissues of kidney and heart were homogenized in 5 parts of phosphate-buffered saline (pH = 7.4) with Homogenizer (Heidolph DAIX 900, 6F, Germany) for 2 min. Then, homogenates were centrifuged at 10000 g for 15 min at 4°C. Supernatants were separated, divided in to tree Portions; one part was directly used for immediate malondialdehyde (MDA) measurement and other parts kept at -80°C for superoxide dismutase (SOD) and glutathione peroxidase activities measurements.

Blood assay: Heparinated plasma obtained form experimental rats were used for measurement of High Density Lipoproteins Cholesterol (HDL-C), Low Density Lipoproteins Cholesterol (LDL-C), Total Cholesterol (TC) and triglycerides (TG) with ELITECH kits (Seppim SAS zone Industrielle 61500 Sees, FRANCE) in automated analyzer (RIA-1000).

Tissues assay: Protein concentration was determined by the method of Bradford, using bovine serum albumin as standard (Bradford *et al.*, 1976). Glutathione peroxidase (GP_X) activity in kidney and heart were measured using ransol kit (Randox laboratories, Crumlin, UK), based on that of Paglia and valentine. Briefly Glutathione peroxidase (GP_X) in supernatants of homogenates catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the Presence of Glutathione Reductase (GR) and NADPH the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP +. The decrease in absorbance at 340 nm is measured and Glutathione peroxidase (GP_X) activities were expressed as umoles of GSH oxidized/min/mg protein. superoxide dismutase (SOD) activities in homogenates of kidney and heart were measured using ransod kit (Randox laboratories, Crumlin, UK). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2- (4-iodophenyl)-3- (4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay. The superoxide dismutase (SOD) activities were expressed as units per milligram of protein (U/mg protein).

The level of malondialdehayde (MDA) in tissue homogenate was determined using the method of Mihara and Uchiyama (1978). Half a milliliter of supernatants of kidney and heart was mixed with 3 mL H_3PO_4 solution (1% v v^{-1}) followed by addition of 1 mL thiobarbituric

acid solution (0.67% w v^{-1}). Then the mixture was heated in water bath for 45 min. The colored complex was extracted with 3 mL n-butanol and absorption at 532 nm was measured using tetramethoxypropane as standard. MDA levels were expressed as a nanomol per milligram of protein (nm/mg protein).

Statistical analysis: Statistical analysis was carried out using the SPSS 11.9 statistical program. All data were expressed as mean±SE. $p<0.05$ was considered statistically significant.

RESULTS

The body weights, Gain of weights, food and fluid intakes of rats have no significantly difference between groups of study during period of sex weeks that are shown in Table 1. The levels of plasma triglycerides (TG), High Density Lipoproteins Cholesterol (HDL-C), Low Density Lipoproteins Cholesterol (LDL-C) and Total Cholesterol (TC) are listed in Table 2. The levels of triglycerides in control and homocysteine (CON+HCY) group are higher than other groups, but this increase was not statistically significant ($p>0.05$). In control and taurine (CON+TAU) group, the levels of triglycerides, was lower in compare with other groups of study ($p>0.05$).

Table 3 shows the oxidative stress biomarkers in heart and kidney homogenates of control and experimental animals. The levels of malondialdehayde (MDA) of control and homocysteine group (CON + HCY) were higher than control and taurine (CON + TAU) group ($p<0.05$) and other groups of study ($p>0.05$). The levels of malondialdehayde (MDA) of rats received 2% taurine in control and taurine (CON + TAU) group were lower than control and homocysteine (CON + HCY) group ($p<0.05$) and other experimental groups of study ($p>0.05$). The activities of superoxide dismutase (SOD) in heart and kidney homogenates of control and homocysteine group (CON + HCY) were lower than control and taurine (CON + TAU) group ($p<0.05$) and other groups of study ($p>0.05$). The activities of superoxide dismutase (SOD) in

Table 1: Body weight (g), food intake (g/day) and fluid intake (mL/day)

Groups	CON (Means±SE)	CON+HCY (Means±SE)	CON+TAU (Means±SE)	CON+HCY+TAU (Means±SE)
Initial body weight	218.12±6.33^{a}	204.37±14.10^{a}	198.75±8.00^{a}	225±8.29^{a}
Last body weight	288.75±7.02^{a}	284±8.83^{a}	254.62±11.02^{a}	293.87±13.44^{a}
Initial food intake	115.66±22.28^{a}	102.88±16.10^{a}	137.73±20.83^{a}	131.59±5.40^{a}
Last food intake	216.04±2.04^{a}	218.58±2.02^{a}	225.85±2.57^{a}	208.78±0.25^{a}
Initial fluid intake	123±10.00^{a}	116.5±35.50^{a}	113±5.00^{a}	129.5±9.50^{a}
Last fluid intake	155.5±5.50^{a}	144.5±9.50^{a}	229±48.00^{a}	261±26.00^{a}

CON: Control group, CON+HCY: Control + Homocysteine group, CON+TAU: Control + Taurine group, CON+HCY+TAU: Control + Homocysteine + Taurine group. Values with different superscript letter (a, b) considered significant ($p<0.05$). Values are Mean±SE from 8 animals in each group. SE: Standard Error

Table 2: Plasma Lipid profile

Groups	CON (Means±SE)	CON+HCY (Means±SE)	CON+TAU (Means±SE)	CON+HCY+TAU (Means±SE)
TG (mg dL^{-1})	52.87±2.89^{a}	68.75±5.68^{a}	46.16±2.83^{a}	68.71±9.91^{a}
TC (mg dL^{-1})	97.95±2.32^{a}	100.25±1.82^{a}	93.9±4.17^{a}	103.65±5.48^{a}
LDL-C (mg dL^{-1})	30.62±1.79^{a}	30.12±1.15^{a}	29.5±1.56^{a}	33.75±3.10^{a}
HDL-C (mg dL^{-1})	56.75±0.81^{a}	56.37±0.37^{a}	55.167±2.52^{a}	57.87±1.77^{a}

CON: Control group, CON+HCY: Control+ Homocysteine group, CON+TAU: Control+ Taurine group, CON+HCY+TAU: Control+Homocysteine+Taurine group, TG: Triglyceride, TC: Total Cholesterol, HDL-C: high density lipoproteins cholesterol, LDL-C: low density lipoproteins cholesterol. Values with different superscript letter (a, b) considered significant ($p<0.05$). Values are Mean±SE from 8 animals in each group. SE: Standard Error

Table 3: Oxidative stress data from homogenates of kidney and heart

Groups	CON (Means±SE)	CON+HCY (Means±SE)	CON+TAU (Means±SE)	CON+HCY+TAU (Means±SE)
SOD (Units/mg protein)				
Heart	6.18±0.46^{ab}	5.8±0.10^{a}	6.87±0.08b	6.11±0.14^{ab}
Kidney	7.02±0.17^{ab}	6.83±0.14^{a}	7.78±0.35^{b}	6.87±0.12^{ab}
GPX (umoles/mg protein)				
Heart	8.07±0.41^{a}	7.44±0.33^{a}	8.09±0.29^{a}	7.90±0.39^{a}
Kidney	6.98±0.92^{a}	4.12±0.91^{a}	7.74±1.71^{a}	6.48±1.00^{a}
MDA (nmoles/mg protein)				
Heart	5.39±0.35^{ab}	5.95±1.00^{a}	4.82±0.26^{b}	5.45±0.46^{ab}
Kidney	10.79±1.02^{ab}	12.42±0.84^{a}	8.01±0.78^{b}	10.92±0.93^{ab}
Plasma	0.89±0.02^{a}	0.88±0.01^{a}	0.90±0.02^{a}	0.96±0.01^{a}

CON: control group, CON+HCY: Control+ Homocysteine group, CON+TAU: Control+ Taurine group, CON+HCY+TAU: Control+Homocysteine+Taurine group, GP_X: Glutathione peroxidase, SOD: superoxide Dismutase, MDA: malondialdehayde. Values with different superscript letter (a, b) considered significant ($p<0.05$). Values are Mean±SE from 8 animals in each group. SE: Standard Error

heart and kidney of rats received 2% taurine in control and taurine (CON + TAU) group were higher than control and homocysteine (CON + HCY) group ($p<0.05$) and other experimental groups of study is not statistically significant. Activities of glutathione peroxidase (GP_X) in heart and kidney of control and homocysteine group (CON + HCY) were lower than other groups of study ($p>0.05$) and in control and taurine (CON + TAU) group were higher than other groups of study ($p>0.05$). The levels of total antioxidant in plasma have no significantly differences between groups of study ($p>0.05$).

DISCUSSION

Homocysteinethiolacton hydrochloride is cleaved rapidly to homocysteine at physiological conditions (alkaline) and readily absorbed (Frauscher *et al*., 1995). When it enters plasma rapidly auto-oxidizes and produces reactive oxygen molecules. There are some reports, describing that homocysteine derivatives (by parenteral administration) increase dietary atherogenesis (Takashi and Ontkao, 1981), cholesterol and triglyceride in man (Andrzej *et al*., 1989) and triglyceride in rats (Frauscher *et al*., 1995). Inhibition of fatty acid oxidation is proposed to be underlying mechanism as reflected by decrease of beta-hydroxybutirate/acetoacetate ratio (Frauscher *et al*., 1995; Takashi and Ontkao, 1981). The involvement of free oxygen species, interaction of homocysteine with nitrite oxide (NO) system and increased plasma triglyceride and homocysteic acid are concepts for the interpretation of homocysteine induced oxidative damages (Frauscher *et al*., 1995; Stanley *et al*., 2004).

In our study the level of TG in homocysteine-treated group (CON + HCY) was higher in compare with CON and CON + TAU groups that is in agreement with previous study (Jale *et al*., 2002a; Zhang *et al*., 2004). Accordingly, SOD activities were lower and malondialdehayde were higher significantly in homocysteine-treated group that can be supported by other studies (Frauscher *et al*., 1995; Hidemi *et al*., 2001; Stanley *et al*., 2004).

Taurine attenuate lipid peroxidation in tissue either by inhibition of reactive oxygen species (ROS) formation, scavenging of ROS or by its sulphonic group binding to Fe^{+2}, Cu^{+2} and oxidant metalloproteines, like a chelator (Wu *et al*., 1999; Trachtman *et al*., 1992). Also, Taurine stimulates SOD and GP_X activities as another mechanism for inhibition of lipid peroxidation (Nandhini *et al*., 2005a).

In our study taurine was significantly decreased MDA and SOD activities, both in heart and kidney and non-significantly increased total antioxidant capacity. In the Previous studies, have shown that taurine supplementation elevates or restores serum HDL-C levels by stimulation of apoA-1 production, although it is unclear, in spontaneously Hyperlipidemic (SHL) mice (Yoshibumi *et al*., 2003), rats fed a normal chow (Trachtman *et al*., 1992) and high cholesterol diet (Ogasawara *et al*., 1994) without affecting VLDL-C and LDL-C levels. Taurine in Ethanol-treated rats and in overweight or obese non-diabetic subjects caused significant decrease in serum triglycerides (Jale *et al*., 2002a; Zhang *et al*., 2004). In present study we found no significant differences in lipid profiles, although the level of TG was lower in taurine received group. Some studies have shown that taurine has no significant effects on body weight, food and fluid intake that are in agreement with our results (Nandhini *et al*., 2004b; Frauscher *et al*., 1995; Pushpakiran *et al*., 2004). However, in another studies taurine decreased body weight (Zhang *et al*., 2004; Fujihira *et al*., 1970; Nakaya *et al*., 2000) and increased fluid intake in fructose-fed rats due to diuretic effect of taurine (Nandhini *et al*., 2004b; Kohashi *et al*., 1990).

CONCLUSION

In conclusion, our study show that low oral dose homocysteinethiolacton hydrochloride slightly increases plasma triglycerides, markedly increases MDA and decreases SOD activities in tissues of rats and beneficial effect of taurine adverse these effects.

ACKNOWLEDGEMENT

This research was supported financially by Tabriz University of medical sciences. The authors thank Dr. Ahamadi head of Animal House Center for medicine collage of Tabriz University, MSc. Amir. M Vatankhah technical staff of biochemistry lab from Tabriz University of medical sciences, Dr. Naser Agh director of Artemia and Aquatic Animal Research Institute (AAARI) and Dr. Adel Saberivand and Dr. Reza Sabzi, heads of biotechnology and biosciences Institute, Dr. Eshagh Karimi and Dr. Naser Mahmoodiaghdam of Urmia University for providing their assistances for this study.

REFERENCES

Andrzej, J. *et al*., 1989. Reduction of plasma lipid and homocysteine levels by pyridoxine, folate, cobalamin, choline, riboflavin and troxerutin in atherosclerosis, 75: 1-6.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the protein-day binding. Analy. Biochem., 72: 248-254.

Frauscher, G. *et al*., 1995. Oral administration of homocysteine leads to increased plasma triglyceride and homocysteic acid-additional mechanism in homocysteine induced endothelial damage? Life Sci., 57: 813-817.

Fujihira, E. *et al*., 1970. Effect of long-term feeding of taurine in hereditary hyperglycemic obese mice. Chem. Pharm Bull., 18: 1636-1642.

Gurer, H. *et al*., 2001. Antioxidant effect of taurine against lead-induced oxidative stress. Arch. Environ. Contam. Toxicol., 41: 397-402.

Hidemi, N. *et al*., 2001. Taurine Prevents the Decrease in Expressionand Secretion of Extracellular Superoxide DismutaseInduced by HomocysteineAmelioration of Homocysteine-Induced Endoplasmic Reticulum Stress by Taurine. Circulation, 104: 1165-1170.

Hisashi, H. *et al*., 2004. Oral taurine supplementation prevents fructose-induced hypertension in rats. Heart Vessels, 19: 132-136.

Jale, B. et al., 2002a. Taurine treatment reduces hepatic lipids and oxidative stress in chronically ethanol-treated rats. Biol. Pharm. Bull., 25: 1231-1233.

Jale, B. *et al*., 2002b. Effect of taurine on erythrocyte lipids and oxidative stress in rabbit fed a high cholestrol diet. Biosci. Biotechnol. Biochem., 66: 2701-2707.

Kohashi, N. *et al*., 1990. Mechanism of taurine natriuresis in rats. Adv. Exp. Med. Biol., 247: 635-640.

Mihara, M. and M. Uchiyama, 1978. Determination of malonaldehyde precursor in tissues by hiobarbituric acid test. Anal. Biochem., 86: 271-278.

Nakaya, Y. *et al*., 2000. Taurine improves insulin sensitivity in the Otsuka Long-Evans Tokushima Fatty rat, a model of spontaneous type 2 diabetes. Am. J. Clin. Nutr., 71: 54-58.

Nandhini, A.T.A. *et al*., 2004b. Hoe 140 abolish the blood pressure lowering effect of taurine in high fructose-fed rats. Amino Acids, 26: 299-303.

Nandhini, A.T.A. *et al*., 2005a. Effect of taurine on biomarkers of oxidative stress in tissues of fructose-fed insulin-resistant rats. Singapore Med. J., 46: 82-87.

Nandhini, A.T.A. *et al*., 2005c. Taurine prevents collagen abnormalities in high fructose-fed rats. Indian J. Med. Res., 122: 171-177.

Ogasawara, M. *et al*., 1994. Reactivity of taurine with aldehydes and its physiological role. Adv. Exp. Med. Biol., 359: 71-8.

Pushpakiran, G. et al., 2004. Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissues. Amino Acids, 27: 91-96.

Stanley, J.H. et al., 2004. L-homocysteine and L-homocystine streospecifically induce endothelial nitric oxide synthase-dependent lipid peroxidation in endothelial cells. Free Radical Biol. Med., 36: 632-640.

Takashi, I. J.A. and Ontkao, 1981. Increased secretion of very low density lipoprotein triglyceride Following inhibition of long chain fatty acid oxidation in isolated liver. J. Biol. Chem., 256: 10247-10255.

Trachtman, H. *et al*., 1992. Taurine attenuates renal disease in chronic puromycin aminonucleoside nephropathy. Am. J. Physiol., 262: 117-23.

Wu, Q.D. *et al*., 1999. Taurine prevents high glucose induced human vascular endothelial cell apoptosis. Am. J. Physiol., 277: 1229-38.

Yoshibumi, M. *et al*., 2003. Effects of taurine on serum cholesterol levels and development of atherosclerosis in spontaneously hyperlipidaemic mice. Clin. Exp. Pharmacol. Physiol., 30: 295.

Zhang, M. *et al*., 2004. Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. Amino Acids, 26: 267-271.