Research Article

Improvement in Renal Function of Hyperhomocysteinemic Rats by Co-Enzyme Q10

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ABSTRACT

The aim of the study was to evaluate the potential role of Coenzyme Q10, a lipophilic moiety against renal impairment in hyperhomocysteinemic rat. Twenty Wistar albino rats were divided into four groups. Each group had five animals. Group 1 served as control group who received normal diet (chow feed) and water *ad libitum*. Group 2 hyperhomocysteinemia (HHCY Control) were fed on L-methionine (1.7g/kg/day, *p.o.*) once a day. The third group (test drug 1) was treated with Coenzyme Q10 at a low dose of (50 mg/kg body weight) + L-methionine (1.7g/kg/day, *p.o.*) through oral gavage. The fourth group (test drug 2), received high dose of Coenzyme Q10 (100 mg/kg body weight) + L-methionine (1.7g/kg/day, *p.o.*) through same route. Additionally, doxorubicin injections at a dose of 5 mg/kg was given through intraperitoneal route after 1 hour of L-methionine dosing at an interval of 15 days to second, third and fourth groups of animals to induce hyperhomocysteinemia mediated nephrotoxicity. The experiment was terminated after 28 days, animals were killed and homocysteine, creatinine and urea concentration in the serum were determined. The serum homocysteine, creatinine and urea levels were determined. These levels in HHCY group were significantly elevated with respect to normal group of animals and were characterized with severe hyperhomocysteinemia. The levels were reduced in the Coenzyme Q10 (50 and 100 mg/kg, p.o) treated groups in dose dependent manner when compared to the HHCY group. Coenzyme Q10, fat soluble moiety can be considered as a feasible candidate for nephroprotection in rats with hyperhomocysteinemia.

Keywords: Hyperhomocysteinemia, Coenzyme Q10, doxorubicin, methionine, creatinine, urea.

INTRODUCTION

Hyperhomocysteinemia (HHCY) is a biochemical alteration characterized by an abnormal increased level of homocysteine (Hcy) in the blood; above 15µmol/L and a second most frequent metabolic disorder of amino acid. Liver and kidney eliminate excess homocysteine from the blood. Hyperhomocysteinemia induces blood clots in veins and arteries[1]. Jukes *et al* reported that Homocysteine is a sulphur containing amino acid, isolated from a urinary bladder stone in 1933 by Vincent du Vigneaud[2]. It is metabolized either by remethylation pathway to methionine or the transsulfuration pathway to cysteine. The former pathway is dependent on the proper functioning of methionine synthetase, methylene tetrahydrofolatereductase enzyme, vitamin B12 and folic acid. The later pathway is dependent on the enzymes cysthathionine beta synthetase and methyl tetrahydrofolatereductase[3]. Intracellular homocysteine is also released into blood and urine. Hyperhomocysteinemia is a rare autosomal recessive disorder with serious elevations of homocysteine in urine and plasma[4]. The increased homocysteine synthesis and its decreased intracellular utilization increase its flux into the blood. Hyperhomocysteinemia is caused by the excess deficiencies of the vitamins like pyridoxine (B6), folic acid (B9), or cynocobalamin (B12). High protein consumers are usually at risk for hyperhomocysteinemia because of low plasma B12 levels. It is approximated that mild hyperhomocysteinemia occurs in 5-7% of the general population and 40% in patients with vascular disease. Normal plasma homocysteine level in the blood is 5 to 15 µmol/L. Mild hyperhomocysteinemia ranges from 31 to 100 µmol/L and severe above 100 µmol/L[5].

The relationship between hyperhomocysteinemia and atherosclerosis was proposed by Mc Cully in 1969. It is now well recognized that hyperhomocysteinemia is a strong, independent risk factor for stroke, myocardial infarction and other vascular events. Bright A reported that homocysteine influence on atherosclerosis is multidirectional. One includes generation of free oxygen radicals which converts low density lipoproteins of sub endothelial tissues to oxidized low density lipoproteins. Oxidized LDL further acts as a key mediator of inflammatory process in atherosclerosis. Oxidized LDL releases vascular cell adhesion molecule and monocyte chemo attractant protein. The monocytes then get reformed to macrophages, which take up Oxidized LDL to get converted to foam cells. The foam cells get settled below the endothelium to form fatty streak. The other includes the suppression of nitric oxide activation by free radicals, which results in endothelial dysfunction and contributed to atherosclerosis. Hyperhomocysteinemia favors the formation of reactive oxygen species, causes lipid peroxidation and develop inflammation in the vascular endothelium[6,7]. Patients with kidney disease, exhibit excessively elevated plasma Hcy levels. Hcy levels enhances as renal function declines. The function of the kidney in the management of homocysteine level is an area of current research. It has been reported that the healthy kidney plays a major role in the clearance of excessive homocysteine, as it does with other amino

acids. Renal homocysteine clearance and filtration is affected by dietary protein intake[8].

Coenzyme Q10 is a fat soluble drug, commonly known as ubiquinone. It is a naturally occurring lipophilic antioxidants consisting of benzoquinone ring. It is biosynthesized in all tissues of the body and has a major role in mitochondrial respiration. It prevents lipid peroxidation by inhibiting the propagation of lipid peroxyradicals. Coenzyme Q10 also protects protein and lipoproteins from oxidation by the same mechanism. Current evidences suggest that Coenzyme Q10 has many anti-inflammatory effects that reduce the secretion of proinflammatory cytokines in lymphocytes and monocytes. It has been reported that Coenzyme Q10 improves endothelial dysfunction in diabetic patients[9,10].

MATERIALS AND METHODS

Methionine manufactured by Sigma Company and supplied by Praveen Chemicals Mathura, India. Doxorubicin hydrochloride (DOX) supplied by Sun pharma Ltd., India was used in this experiment. Studies were conducted in Wistar albino rats of either sex, weighing 200-300 g. They were maintained to the environment for 4 weeks before experimentation and accommodated individually in stainless steel wire bottomed cages under 12-h light/12-h dark cycles 50% humidity at a room temperature of 25±2°C. Animals were fed standard pellets diet. They had free access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee in conformity with the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) Guidelines for handling of laboratory animals.

Experimental Design

Induction of Hyperhomocysteinemia

Animals were randomly assigned to four groups follows: Normal control (n=5) was given typical chow feed and distilled water throughout the experimental protocol and served as a untreated group. The second group (HHCY Control, n=5) were fed on L-methionine (1.7g/kg/day, p.o) via oral gavage. The aim of such a diet was to cause hyperhomocysteinemia. The third group (Test drug 1, n=5) was treated with Coenzyme Q10 at a low dose of 50 mg/kg body weight + L-methionine (1.7g/kg/day, p.o) via oral gavage. The fourth group (Test drug 2, n=5), received high dose of Coenzyme Q10 (dose: 100 mg /kg body weight) + L-methionine (1.7g/kg/day, p.o) via oral gavage. Additionally, doxorubicin injections i.p. after 1 hour was given at a dose of 5 mg/kg bodyweight at 2 week interval to second, third and fourth group in order to induce nephrotoxicity. The experiment was terminated after 28 days, animals were killed and homocysteine, creatinine and urea concentration in the serum were determined.

Statistical Analysis

All data are presented as mean ± SEM. Difference between groups are evaluated by one-way ANOVA followed by Dunnett's test using Graph pad Instat version 3.Values of P<0.05 were considered significant.

RESULTS

Serum homocysteine concentration in second group of animals (HHCY Control), fed L-methionine (1.7g/kg/day, p.o) were significantly higher than in first group of rats (Normal control) fed chow diet (25.28±0.21 versus 4.33±0.28 µmol/L). Still higher homocysteine level were observed in third group of animals (test drug 1), fed low dose (50 mg/kg body weight) of Coenzyme Q10 compared to group 2 (25.28±0.21 versus 24.11±0.25 µmol/L). There was significant difference in homocysteine level of group 2 and rats fed high dose (100 mg /kg body weight) of Coenzyme Q10 in group 4 (25.28±0.21 versus 11.50±0.29 µmol/L). Similarly, there was a significant decrease in the blood urea level in the test rats (test drug 1) when compared to the group 2 (33.74±3.50 versus 47.12±3.71). Major reduction in urea level in (Test drug 2) compared with group 2 is observed (22.32±1.29 versus 47.12±3.71). Further, the serum level of creatinine also showed a significant reduction in the group 3 (Test drug 1) and group 4 (test drug 2) when compared to the group 2 (0.54±0.04 versus 1.15±0.19), (0.68±0.04 versus 1.15±0.19).

Table 1: Effect of Co-enzyme Q10 in renal function of hyperhomocysteinemic rats.

S. No.	Groups	Serum Level		
		Homocysteine (µmol/L)	Creatinine (mg/dL)	Urea (mg/dL)
1	Control	10.10±0.43	0.54±0.01	17.60±0.70
2	HHCY	27.30±0.80	1.15±0.19	47.12±3.71
3	Test 1	16.10±0.43**	0.68±0.04*	33.74±3.50**
4	Test 2	10.24±0.73**	0.54±0.04**	22.32±1.29**

Values are expressed as Mean ± SEM. (One-Way ANOVA followed by Dunnett's test).*Significant difference at P<0.05 as compared with HHCY group, **Significant difference at P<0.01 as compared with HHCY group.

CONCLUSION

The present analysis yield major finding that Coenzyme Q10 has dose dependent effect on renal dysfunction in hyperhomocysteinemic rats.

Coenzyme Q10 at a low dose of 50 mg/kg body weight had less effect on serum homocysteine, creatinine and urea level whereas the same drug at a dose of 100 mg /kg body weight significantly reduced increased homocysteine level in dose dependent manner. Hyperhomocysteinemia is a pathological condition with plasma levels of homocysteine higher than 15µmol/L, associated with atherosclerosis, and with venous and arterial thrombosis by damaging endothelial cells. It is approximated that mild hyperhomocysteinemia occurs in 5-7% of the general population and 40% in patients with vascular disease[11,12]. Patients with heart failure, impaired renal function, and diabetes should be screened since the

prevalence of hyperhomocysteinemia in these patients appears to be quite high. Hyperhomocysteinemia is caused by the excess deficiencies of the vitamins like pyridoxine (B6), folic acid (B9), or cynocobalamin (B12). The Internationally confirmed treatment for hyperhomocysteinemia involves the use of folic acid, vitamin B12 and pyridoxine.

Patients with renal disorders, who exhibit abnormal high rates of cardiovascular morbidity, tend to be hyperhomocysteinemic, predominantly as renal function declines.

The relationship between homocysteine levels and glomerular filtration rate (GFR) involves the kidney as an important participant in homocysteine handling. The kidney plays an important role in plasma amino acid clearance and metabolism. Homocysteine level is augmented as renal function declines. Renal dysfunction progresses to end stage renal disease, ultimately causing hyperhomocysteinemia[13].

Over the past decade, homocysteine related research provoke a huge amount of scientific literature and sparked a vigorous debate, as an emerging risk factor for neural tube defects (NTD) and non-communicable disease (NCDs), including type 2 diabetes and cancer[14-16].

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REFERENCES

1. Guilliams TG. Homocysteine: A Risk Factor worth Treating, The Standard, 6(1), 2004, 1-7.
2. Jukes TH. Vincent du Vigneaud (1901-1978). A Biographical sketch, American Institute of Nutrition, 112(8), 1982, 1468.
3. Barnabe Folate, Vitamin B12 and Homocysteine status in the post-folic acid fortification era in different subgroups of the Brazilian population attended to at a public health care center, Nutrition Journal, 14(19), 2015, 2-10.
4. Cattaneo M. Hyperhomocysteinemia and Thrombosis, Lipids, 36(1), 2001, S13-S26.
5. Lawrence de Koning AB, Werstuck Geoff H, Zhou Ji, Austin Richard C. Hyperhomocysteinemia and its role in the development of atherosclerosis, Clinical Biochemistry, 36, 2003, 431.
6. Qureshi SS, Gupta JK, Upmanyu N. A review on Hyperhomocysteinemia and its risk factors, Innovare Journal of Medical Sciences, 4(1), 2016, 1-3.
7. Bright A, Renuga Devi TS, Gunase karan S. Plasma Homocysteine Levels and efficacy of Vitamin Supplementation among patients with atherosclerosis – a spectral and clinical follow up, International Journal of Pharma and Bio Sciences, 2(3), 2011, B347.
8. Gupta JK, Qureshi SS. Potential benefits of Methylcobalamin; A Review, Austin Journal of Pharmacology and Therapeutics, 3(3), 2015, 1-4.
9. Friedman AN. The Kidney and Homocysteine Metaboism, J Am Soc Nephrol, 12, 2001, 2181-89.
10. Botham KM, Napolitano M, Bravo E. The emerging role of disturbed CoQ metabolism in Nonalcoholic fatty liver disease development and progression, Nutrients, 7, 2015, 9834-46.
11. Ahmed HH. Potential role of some nutraceuticals in the regression of Alzheimer's disease in an experimental animal model, Turk J Med Sci, 41(3), 2011, 455-66.
12. Sato Y, Kaji M, Kondo I, Yoshida H, Satoh K, Metoki N. Hyperhomocysteinemia in Japanese patients with convalescent stage ischemic stroke: Effect of combined therapy with folic acid and mecobalamine, J NeurolSci, 202, 2002, 65-8.
13. Clark WM, Wechsler LR, Sabounjian LA, Schwiderski UE. A phase III randomized efficacy trial of 2000 mg citicoline in acute ischemic stroke patients, Neurology, 57(9), 2001, 1595-1602.
14. Makoff R. Folic acid, pyridoxine, cobalamin, and homocysteine and their relationship to cardiovascular disease in end-stage renal disease, Journal of Renal Nutrition, 6(1), 1996, 2-11.
15. Misra A. Hyperhomocysteinemia and low intake of folic acid and vitamin B12 in urban North India, Eur J Nutr, 41, 2002, 68-77.
16. Handy DE, Loscalzo J. Homocysteine and Atherothrombosis: Diagnosis and Treatment, Current Atherosclerosis Reports, 5, 2003, 276-283.

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