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Research Article

EFFECT OF NEBIVOLOL ON LIPID PROFILE AND OXIDATIVE STRESS IN HYPERCHOLESTEREMIC RATS

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ABSTRACT

This study was performed to estimate effect of Nebivolol (NV), a novel antihypertensive drug, on lipid profile and oxidative stress in hypercholesteremic rats. Hypercholesteremia was induced in normal rats by including 0.75 gm% cholesterol and 1.5 gm% bile salt in normal diet and these rats were used for the experiments. NV was administered as 5 mg/kg/day and 10 mg/kg/day to the hypercholesteremic rats. Plasma lipid profile parameters, ascorbic acid, catalase activity, malondialdehyde and superoxide dismutase activity were estimated by using standard methods. Statistical analysis was done by one way analysis of variance (ANOVA). Treatment with NV resulted in no significant changes in serum cholesterol, low density lipoproteins, triglycerides, very low density lipoproteins and high density lipoproteins in both groups. NV as a 10mg/kg/day increased activities of catalase enzyme, superoxide dismutase activity and ascorbic acid concentration, but there was no significant change in the concentration of malondialdehyde. Therefore, the present study concluded that NV does not change the plasma lipid profile, but causes reduction in oxidative stress in hypercholesteremic rats only in 10 mg/kg/day NV treated group.

Key Words: *Antioxidant, Nebivolol, Hypercholesteremia, Hypertension*

INTRODUCTION

Hypertension (HT) has been, evidently, the most important contributing factor to cardiovascular diseases, the leading cause of morbidity and ultimately death. Sustained HT damages heart, kidney, blood vessels and brain which lead to ischaemic heart disease, congestive cardiac failure, renal failure and stroke. HT, therefore, is one of the most serious concerns of modern medical practice. Most of the hypertensive patients are associated with abnormal lipid levels such as increased cholesterol, triglycerides levels and/or decreased high density lipoproteins (Lamina, 2012). Also oxidative stress has emerged as an important pathogenic factor in the development of HT and most of the complications related to HT are associated with oxidative stress, induced by the generation of free radicals (Banappa, 2009). Therefore, it is important to find antihypertensive drug that improves lipid profile and also reduces oxidative stress in hypertensive patient. But among the currently available antihypertensive drugs, a very few drugs are available which are having hypolipidemic and antioxidant properties.

Beta blockers have been used as a first line treatment of HT, since last four to five decades. Apart from anti-hypertensive action, they also have anti-anginal and anti-arrythmic actions which effectively reduce coronary artery disease and ultimately death (Gielen, 2006). NV, a novel third generation beta blocker, is used for the treatment of HT and it lowers blood pressure by reducing peripheral vascular resistance, and also significantly increases stroke volume with preservation of cardiac output (Kamp, 2003). As very few studies were conducted in past to evaluate effect of NV on lipid profile and oxidative stress, this study was undertaken to estimate effect of NV on lipid profile and oxidative stress in hypercholesteremic rats.

MATERIALS AND METHODS

Animals

Male albino rats weighing 200-250 gm were used for this experiment. They were kept on balanced diet and water *ad libitum* in a well-ventilated animal unit. Permission for conduction of study was taken from Institutional Animal Ethics Committee.

Drugs

NV hydrochloride drug was obtained as a gift sample from Abbott Healthcare Pvt. Ltd., India. Cholesterol and bile salt were purchased in pure form from Yucca Enterprises, Wadala (E) Mumbai, India. All other chemicals and reagents used for the biochemical tests were of analytical grade.

Study Design

Study was conducted as follows:

After 10 days adaptation period, 24 animals were divided into four groups, each containing six animals (n=6). The groups were treated as follows for **four weeks**: **Group I:** Control group (Only standard diet is given). **Group II:** Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt of the weight of the total diet to induce hypercholesteremia (Visavadiya, 2005). **Group III:** Standard diet mixed with 0.75gm% cholesterol and 1.5 gm% bile salt to induce hypercholesteremia, along with NV 5mg/kg/day orally (Ma L, 2012). **Group IV:** Standard diet mixed with 0.75gm% cholesterol and 1.5 gm% bile salt to induce hypercholesteremia, along with NV 10mg/kg/day orally (Ma L, 2012).

Collection of Blood Samples

On 30^{th} day, after overnight fasting, blood was collected directly from heart of rat anaesthetized with ether. Abdomen was opened by taking a midline incision. Blood was sent to biochemistry laboratory; plasma was separated by centrifugation. Liver was excised and, both plasma and liver were kept frozen until analyzed. After biochemical tests, remaining part of liver sample was sent to pathology for histo-pathological examination.

Biochemical Analysis

Plasma lipid profile was assessed by following parameters by standard methods: serum total cholesterol by Modified Roeschlau`s Method (Roeschlau, 1974), serum total triglycerides (TG) by method of Wako, modified by McGowan and Fossati (McGowan, 1983), serum total high density lipoproteins (HDL) by Phosphotungstic Acid method (Klaus Loreniz, 1979), serum total low density lipoproteins (LDL) and serum total very low density lipoproteins (VLDL) by Friedewald formula (Chatterji, 2007).

Antioxidant potential was assessed by following parameters: Hepatic ascorbic acid by Schaffert RR et al method (Schaffert, 1955), catalase activity in liver by Cohen G. et al method (Cohen, 1970), serum malondialdehyde (MDA) by Pasha and Sadasivadu method (Pasha, 1984), serum superoxide dismutase activity (SOD) by Marklund and Marklund method (Marklund, 1974).

Statistical Evaluation

The results are expressed as means ± SD (standard deviation). Significant differences among groups were determined by one way analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered significant if $P < 0.05$.

RESULTS

Plasma Lipid Profile

NV 5mg/kg/day and 10mg/kg/day as drug treatments to hypercholesteremic rats resulted in no significant changes in total serum cholesterol, serum LDL and serum HDL. The cholesterol level decreased (in group IV) from 233.62±11.35mg% to 230.97 ± 12.28mg% (Table No.1).

There were no significant decreases in serum triglyceride and serum VLDL level in NV treated groups. The values were changed from 56.98±4.08 mg% to 52.98 ± 3.79 mg% and from 11.39±0.86 mg% to 10.59 ±0.81 mg% in case of triglyceride and VLDL, respectively, in NV (10mg/kg/day) treated rats (Table No.2).

Table No. 1: Effect of Nebivolol on serum cholesterol, LDL and HDL level in male Albino rats.

Groups (n=6)	Treatment given	Sr. TC (mg/dl)	Sr. .LDL (mg/dl)	Sr. HDL (mg/dl)
Group I	Control	128.19 ± 6.11	50.94 ± 5.81	66.78 ± 2.24
Group II	HC	233.62 ± 11.35	180.45 ± 10.07	42.95 ±1.94
Group III	HC+5NV	223.73 ± 10.57 ^{NS}	168.98 ± 12.35 ^{NS}	45.22 ± 3.01^{NS}
Group IV	HC+10NV	230.97 ± 12.28 ^{NS}	174.29 ± 11.75 ^{NS}	46.41 ± 3.15 ^{NS}

(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC + 5NV = Hypercholesteremic group+ 5mg/kg/day Nebivolol, HC + 10NV = Hypercholesteremic group+ 10mg/kg/day Nebivolol, TC = Total Cholesterol, LDL = low density lipoproteins, HDL = high density lipoproteins, NS= Non-significant, as compared to group II (ANOVA followed by Dunnett's test).

Table No. 2: Effect of Nebivolol on serum TG and serum VLDL level in male Albino rats.

Groups (n=6)	Treatment given	Sr. TG (mg/dl)	Sr. VLDL (mg/dl)
Group I	Control	51.43 ± 2.75	10.36 ± 0.55
Group II	HC	56.98 ±4.08	11.39 ± 0.86
Group III	HC+5NV	54.77 ± 3.68 ^{NS}	10.95 ±0.79 ^{NS}
Group IV	HC+10NV	52.98 ± 3.79 ^{NS}	10.59 ±0.81 ^{NS}

(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC + 5NV = Hypercholesteremic group+ 5mg/kg/day Nebivolol, HC + 10NV = Hypercholesteremic group+ 10mg/kg/day Nebivolol, TG = Total triglycerides, VLDL = very low density lipoproteins, NS= Non-significant as compared to group II (ANOVA followed by Dunnett's test).

Antioxidant potential

There were no significant increase in total ascorbic acid and catalase activity in NV (5mg/kg/day) treated rats. But these values were increased from 44.67 ±3.61 to 49.98 ± 3.17mc/g and 13.81 ± 0.64 to 16.26 ±0.75nm, respectively, in NV (10mg/kg/day) treated rats (Table No.3). These reductions were statistically significant ($P < 0.05$).

Table No. 3: Effect of Nebivolol on total ascorbic acid and activities of catalase in liver of male Albino rats.

Groups (n=6)	Treatment given	Total ascorbic acid (mc/g)	Catalase nm H_2O_2 decomposed/sec/gm
Group I	Control	55.93 ± 2.85	21.01 ± 0.57
Group II	HC	44.67 ±3.61	13.81 ± 0.64
Group III	HC+5NV	46.87 ± 3.91^{NS}	14.01 ±0.82 ^{NS}
Group IV	HC+10NV	49.98 ± 3.17*	16.26 ±0.75*

*(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC + 5NV = Hypercholesteremic group+ 5mg/kg/day Nebivolol, HC + 10NV = Hypercholesteremic group+ 10mg/kg/day Nebivolol, NS= Non-significant, *P < 0.05 as compared to group II (ANOVA followed by Dunnett's test).*

Table No. 4: Effect of Nebivolol on serum MDA and serum SOD level in male Albino rats.

Groups (n=6)	Treatment given	Sr. MDA (nmol/ml)	Sr. SOD (U/ml)
Group I	Control	1.44 ± 0.28	11.99 ± 0.54
Group II	HC	3.57 ±0.43	5.98 ± 0.83
Group III	HC+5NV	3.99 ± 0.58 ^{NS}	6.10 ± 0.98 ^{NS}
Group IV	HC+10NV	3.43 ± 0.49 ^{NS}	7.82 ± 0.83*

(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC + 5NV = Hypercholesteremic group+ 5mg/kg/day Nebivolol, HC + 10NV = Hypercholesteremic group+ 10mg/kg/day Nebivolol, MDA = Malondialdehyde, SOD = Superoxide dismutase. NS = Non significant, P < 0.05 as compared to group II (ANOVA followed by Dunnett's test).*

The lipid peroxidation product, malondialdehyde, in serum decreased in 10mg/kg/day NV treated rats as compared to hypercholesteremic group i.e .from 3.57±0.43 nmol/ml to 3.43±0.49 nmol/ml (Table No. 4). But this reduction was not statistically significant (P = 0.168).

The activity of superoxide dismutase enzyme increased in NV treated rats (group IV) as compared to hypercholesteremic group i.e. from 5.98±0.83 U/ml to 7.82 ± 0.83 U/ml (Table No. 4). This increase in superoxide dismutase activity was statistically significant (P < 0.05).

DISCUSSION

Hypercholesteremia and oxidative stress have emerged as important pathogenic factors in the development of HT and also most of the complications related to HT are associated with increased lipid levels and oxidative stress (Soanker, 2012). Therefore, drugs having both lipid lowering and antioxidant properties would be useful as anti-hypertensive agents. Hence, the present study was conducted to study effect of NV, a third generation beta blocker, on lipid profile and oxidative stress in hypercholesteremic rats. The effect of NV on lipid profile in hypercholesteremic rats, demonstrated in the present investigation, was related primarily to no significant changes in total serum cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and VLDL-cholesterol.

Increase in the levels of serum cholesterol, serum-triglycerides and lowered values of HDL-cholesterol adversely affect the process of atherosclerosis, increasing the risk of coronary artery disease. (Durrington, 2003). High level of HDL-C is associated with fewer problems with cardiovascular diseases and vice versa. It is very clear that an increase in HDL-C level could potentially contribute to reversal of process of atherosclerosis. This is because high level of HDL-C protects endothelial cells from the cytotoxic effects of oxidized LDL (Assmann, 2003). Generally beta blockers have adverse effects on lipid profile in hypertensive patients (Gielen, 2006). In the present study, there were no significant increase in total serum cholesterol, LDL-cholesterol and no significant decrease in serum HDL-C level, which definitely indicate the beneficial role of NV administration to hypercholesteremic animals.

Free radical-mediated oxidative stress has been implicated in the etiology and pathogenesis of several diseases such as neurodegenerative disorders, cancer, cardiovascular diseases and inflammation. Thus, oxidative stress is a cardinal in the pathogenesis of HT and atherosclerosis. Understanding the mechanisms of oxidative stress and the means of suppressing it are important in controlling complications related to atherogenesis and HT as well (Singh, 2008). Drugs with multiple protective mechanisms, including antioxidant activity, may be one way of minimizing complications of such type of oxidative stress related diseases.

Presently noted increased levels of catalase and superoxide dismutase enzyme activities and also increase in ascorbic acid level in NV treated group indicate the possible role of NV as an antioxidant. In past, many studies were conducted with NV to confirm its antioxidant activity. In one study, NV decreased oxidative stress in essential hypertensive patients and increases nitric oxide by reducing its oxidative

inactivation (Fratta Pasini, 2005). In another study, Eradamar studied antioxidant activity of NV in patients with cardiac syndrome-X (Eradamar, 2009). Collectively, these observations indicate that NV administration to hypercholesteremic animals can improve lipid profile and also antioxidant activities.

There were certain drawbacks of this study. Sample size was small; duration of therapy was also short. We investigated antioxidant activities of NV in hypercholesteremic condition, instead of hypertensive condition in which this drug is mainly used. All biochemical tests of antioxidant property were not performed due to unavailability of agents.

Conclusion

Thus, we conclude that Nebivolol could improve lipid profile as well as decrease oxidative stress in hypercholesteremic conditions which suggest that Nebivolol may reduce certain hypercholesteremia and oxidative stress related complications in patients of hypertension.

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