International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2013 Vol. 3 (2) May-August, pp.367-371/Bhosale
Research Article

COMPARATIVE EFFICACY OF CARVEDILOL VERSUS NEBIVOLOL ON LIPID PROFILE AND OXIDATIVE STRESS IN HYPERCHOLESTEREMIC RATS

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ABSTRACT
The present study was conducted to compare efficacy of Carvedilol (CV) versus Nebivolol (NV) on lipid profile parameters and oxidative stress in hypercholesteremic rats. Hypercholesteremic condition in normal rat was induced by including 0.75 gm% cholesterol and 1.5 gm% bile salt powder in normal diet. CV and NV were administered as 20 mg/kg/day and 10 mg/kg/day dose levels, respectively, to the hypercholesteremic rats. Plasma lipid profile parameters and antioxidant properties were estimated by using standard methods. Statistical analysis was done by one way analysis of variance (ANOVA). Treatment with CV resulted in significant increase in only serum HDL while NV did not change any lipid profile parameter. Both CV and NV increased activities of catalase and superoxide dismutase enzymes and also ascorbic acid concentration, but there was no significant change in malondialdehyde concentration. Thus, the present study demonstrated that treatment with only CV improves the plasma lipid profile while both CV and NV reduce oxidative stress in hypercholesteremic animals.

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

INTRODUCTION
Hypertension is a major public health problem worldwide and is a major risk factor for cardiovascular and cerebro-vascular diseases. It is well established that hypertension and hyperlipidemia are the two major contributing risk factors for cardiovascular diseases. Most of the hypertensive patients are having abnormal lipid levels (Lamina, 2012). Also oxidative stress plays an important pathological role in the development of hypertension and also most of the complications related to hypertension are due to oxidative stress, induced by the generation of free radicals (Banappa, 2009).
Beta blockers are particularly used as first line drugs for management of hypertension, since last four-five decades. These beta blockers are also having anti-anginal and anti-arrythmic actions which effectively reduce coronary artery disease and ultimately death (Gielen, 2006). Carvedilol (CV) and Nebivolol (NV), third generation beta blockers, are used for the treatment of hypertension. As very few studies were conducted in past to evaluate effects of CV and NV on lipid profile and oxidative stress, this study was undertaken to compare efficacy of CV and NV on lipid profile parameters and oxidative stress in hypercholesteremic rats.

MATERIALS AND METHODS
Animals
Healthy male adult albino rats of Wistar strain weighing 200-250 gm were used for this study. They were kept on standard balanced diet and water *ad libitum* in a well-ventilated animal unit. The care and procedures, adopted for the present investigation, were in accordance with the approval of Institutional Animal Ethics Committee.
Drugs
Powdered salt forms of CV and NV were obtained as gift samples from Dr. Reddy's laboratories Ltd., India and Abbott Healthcare Pvt. Ltd., India, respectively. Cholesterol and bile salt were purchased in

pure and edible powder form from Yucca Enterprises, Wadala (E) Mumbai, India. All other chemicals and reagents used in the investigations of present study were of analytical grade.

Study Design

Study was conducted as follows: After ten days adaptation period, 24 animals were divided into four groups, each group 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 0.75 gm% cholesterol and 1.5 gm% bile salt to induce hypercholesteremia, along with CV (20mg/kg/day p.o.) as a suspension (Rodríguez, 2001). **Group IV:** Standard diet mixed with 0.75 gm% cholesterol and 1.5 gm% bile salt to induce hypercholesteremia, along with NV (10mg/kg/day p.o.) as a suspension (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; plasma was separated by centrifugation. Liver was excised and, both plasma and liver were kept frozen until analyzed.

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 HDL by Phosphotungstic Acid method (Klaus Loreniz, 1979), serum total 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). Post hoc t-test analysis was done by using software StatPac. Differences were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Results

Plasma Lipid Profile

CV as 20mg/kg/day treatment to hypercholesteremic rats resulted in no significant decrease in total serum cholesterol and serum LDL-C as well, but serum HDL-C level increased significantly ($P < 0.05$) in this group. There were no significant changes in all these parameters in NV treated group (Table 1).

Table 1: Effects of Carvedilol and 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+20CV	243.23 ± 10.23 ^{NS}	183.23 ± 12.82 ^{NS}	48.56 ± 3.21*
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 + 20CV = Hypercholesteremic group+ 20mg/kg/day Carvedilol, 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, *P < 0.05 as compared to group II and group IV (ANOVA followed by Dunnett's test).*

Table 2: Effect of Carvedilol and 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+20CV	53.93 ± 3.87 ^{NS}	10.76 ±0.76 ^{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 + 20CV = Hypercholesteremic group+ 20mg/kg/day Carvedilol, 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).

There were no significant decreases in serum triglyceride ($P > 0.05$) and serum VLDL ($P > 0.05$) level in both CV and NV treated group (Table 2).

Antioxidant Activities

There were significant increase in total ascorbic acid and catalase activity in liver in both CV and NV treated groups ($P < 0.05$) (Table 3).

Table 3: Effect of Carvedilol and 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+20CV	50.93 ± 3.59*	15.07 ±0.87*
Group IV	HC+10NV	49.98 ± 3.17*	16.26 ±0.75*

*(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC + 20CV = Hypercholesteremic group+ 20mg/kg/day Carvedilol, HC + 10NV = Hypercholesteremic group+ 10mg/kg/day Nebivolol, *P < 0.05 as compared to group II (ANOVA followed by Dunnett's test).*

Table 4: Effect of Carvedilol and 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 IV	HC+20CV	3.13 ± 0.41 ^{NS}	7.35 ± 0.87*
Group IV	HC+10NV	3.43 ± 0.49 ^{NS}	7.82 ± 0.83*

(All values are Mean ±Standard Deviation). HC = Hypercholesteremic group, HC + 20CV = Hypercholesteremic group+ 20mg/kg/day Carvedilol, 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).*

Serum MDA was changed in both CV and NV treated groups as compared to hypercholesteremic group (Table No. 4). But the reductions of both treated group were not significant ($P > 0.05$). The activity of SOD changed in both experimental CV and NV treated groups as compared to hypercholesteremic group i.e. from 5.98±0.83 U/ml to 7.35±0.87 U/ml and from 5.98±0.83 U/ml to 7.82±0.83 U/ml, respectively, in group III and IV. (Table No. 4). These changes in SOD activity were statistically significant ($P < 0.05$).

Discussion

The present study was conducted to compare efficacy of CV versus NV on different lipid profile parameters and oxidative stress in hypercholesteremic rats. In present study, treatment with CV resulted in significant increase in only serum HDL while NV did not improve any lipid profile parameter. Most of the older beta blockers adversely affect the lipid profile parameters (Gielen, 2006). Therefore, both CV and NV, newer beta blockers, can be better choice in hypertensive patients associated with abnormal lipid profile.

In past, one study was conducted to compare the effects of CV and captopril on serum lipid concentrations in patients with mild to moderate essential hypertension and dyslipidaemia. In this study, CV improved all the parameters of lipid profile (Hauf-Zachariou, 1993). Sharp RP et al, in their study, studied the impact of CV on the serum lipid profile and concluded that CV had a potentially negative effect on high-density lipoprotein cholesterol (Sharp, 2008).

In present study, increased levels of catalase and SOD enzyme activities and also increase in ascorbic acid level in CV treated group indicate the possible role of CV as an antioxidant. In past, many studies were conducted with CV to confirm its antioxidant activity (Noguchi, 2000; Dandona, 2007). The antioxidant property of CV could be explained by a greater degree of lipophilicity and also the molecular structure of CV favors redox recycling. Therefore, CV could have additional pharmacologic effects that are favorable for long-term therapy (Lysko, 2000).

Presently noted increased levels of catalase and SOD 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).

There were certain drawbacks of this study. Sample size was small; duration of therapy was also short. We investigated antioxidant properties of CV and NV in hypercholesteremic condition, instead of hypertensive condition in which these drugs are mainly used. All biochemical tests of antioxidant property were not performed due to unavailability of chemical reagents.

Thus, the present study demonstrated that treatment with only CV improves the plasma lipid profile while both CV and NV reduce oxidative stress in hypercholesteremic animals.

Conclusion

Thus, we conclude that CV could improve lipid profile and decrease oxidative stress while NV did not improve any lipid profile parameter but decrease oxidative stress in hypercholesteremic conditions. Thus, both CV and NV may reduce cardiovascular risk by their hypolipidemic and antioxidant actions in hypertensive patients.

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