Bangladesh Med Res Counc Bull 2009; 35: 105-109
DOI: 10.3329/bmrcb.v35i3.4116

Effect of carvedilol on adrenaline-induced changes in serum electrolytes in rat

Nurun Nahar and Nargis Akhter

Department of Pharmacology, Faculty of Basic Medical Sciences, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh.

Abstract

Circulating catecholamine that is increased in early phase of myocardial infarction alters serum electrolyte levels which might predispose to serious ventricular arrhythmias. In this study the effect of pretreatment of carvedilol on adrenaline-induced changes in the serum electrolytes (Mg^{2+}, K^{+}, Ca^{2+}, Na^{+}) was evaluated in rats. Adrenaline was administered at a dose of 2 mg/kg body weight subcutaneously 2 injections 24 hours apart and serum electrolytes were estimated at 12 hours, 24 hours and 7 days after the 2nd injection of adrenaline. Adrenaline administration initially caused hypomagnesemia, hypokalemia, hypocalcemia and hyponatremia, which were restored to normal spontaneously within 7 days. Pretreatment of carvedilol orally at a dose of 1 mg/kg body weight for 2 weeks significantly prevented initial reduction in serum electrolyte levels induced by adrenaline. It was concluded that prophylactic use of carvedilol might prevent the serious consequences of myocardial infarction as sudden cardiac death due to arrhythmia caused by electrolyte changes.

Introduction

Ischemic heart disease is an emerging health problem in Bangladesh[1]. Sudden cardiac death is the most common fatal outcome of ischemic heart disease. Inspite of major advances in the prevention and medical treatment of cardiac disease, more than 60% of cardiac disease deaths remain sudden[2]. Ventricular fibrillation is a potentially fatal cardiac arrhythmia during the acute stage of myocardial infarction and is the most common cause of sudden death resulting from sudden cardiac syncope[3]. Patients with higher plasma catecholamine level within the 1st few hours of acute myocardial infarction subsequently appear to have greater myocardial damage and higher mortality rate. This evidence supports the concept that catecholamine may exert a deleterious effect[4] and higher catecholamine level leads to myocardial ischemia, which may contribute to the genesis of ventricular fibrillation[5].

Heart is essentially an aerobic organ, a reduction in the availability of oxygen as occurs during severe ischemia results in a rapid decline in the tissue reserve of ATP[6]. The membrane function of heart cells is dependent on difference in the distribution of certain ions between external medium and interior of the cell and tissue reserve of ATP. Mg^{2+}, K^{+}, Ca^{2+} and Na^{+} are the major ions responsible for the normal electrical activity of the heart. During ischemic period ionic imbalance or changes in the serum electrolytes play a role in the genesis of arrhythmia[6].

An increased circulating catecholamine levels in the early phase of acute myocardial infarction is a crucial factor for the development of major ventricular arrhythmia through its direct effect on myocardial tissue or by alteration of major electrolyte levels or tissue reserve of ATP. So in this context a decrease in circulating catecholamine concentration or blockade of their effects appears to be a rational therapy to prevent early death from ischemic heart disease.

The cardioprotective effect of beta blocker in reducing mortality after acute myocardial infarction has been well documented with different β blockers[7]. Both cardioselective and nonselective beta-blockers have reduced the hospital mortality of patients suffering from myocardial infarction. Cardioselectivity seems to have only a limited role to play in the overall cardioprotective action of beta blocker[8]. It has been suggested that non-selective beta blocking agents may be more effective in the reduction of arrhythmia than β_1 selective drug because of their ability to prevent stress induced

increase in catecholamine level and hypokalemia[9]. Among the β-blockers, carvedilol provides a more comprehensive anti-adrenergic effect than do β_1 selective compound[10]. The α_1 blocking capacity of carvedilol may provide further antiarrhythmic effect over and above that is provided by β-blockade[11].

Investigators have studied the major electrolyte (Mg^{2+}, K^+, Ca^{2+}, Na^+) levels in both experimental[12] and clinical[13] myocardial infarction and there have been many conflicting reports regarding electrolyte changes. Prophylactic use of carvedilol may attenuate the electrolyte changes in the early phase of myocardial infarction and therefore may prevent sudden cardiac death in patients who are at risk of myocardial infarction. Animal model of myocardial infarction and effect of drug can be studied by administering adrenaline, an established regimen to induce experimental myocardial infarction[14]. Therefore the present study has been undertaken to demonstrate the adrenaline-induced serum electrolyte changes and the protective role of carvedilol.

Materials and Methods

Chemicals and Reagents: Reagent kits for estimation of serum magnesium were purchased from Techworth. Reagent kit for estimation of serum calcium was purchased from Biomedia. Adrenaline (Gaco Bangladesh) was purchased from local market and carvedilol was provided by incepta pharmaceutical.

Animals: The adult rats (Long Evans Norwegian strain) of either sex, 3-5 months old and weighing between 200-300 gms were included in the study. They were kept in medium sized plastic cages and allowed to live at room temperature. They were fed on standard laboratory diet and allowed to drink water *ad libitum*.

Experimental design: The study was conducted on 78 rats. They were divided into 3 groups. The group I (control) consisted of 18 rats and received vehicle (distilled water) 1 ml subcutaneously 2 injections 24 hours apart. The group II consisted of 30 rats, which received adrenaline (2 mg/kg body weight) subcutaneously 2 injections 24 hours apart that is an established regimen to induce experimental myocardial infarction[14]. The group III consisted of 30 rats that received carvedilol (1 mg/kg body weight)[15] orally through intragastric tube once daily for 2 weeks followed by 2 injections of adrenaline on 15th and 16th day (24 hours apart). All the animals were sacrificed under light anesthesia (chloroform). In Group I among 18 rats 6 were sacrificed after 12 hours, 6 after 24 hours and 6 after 7 days of 2nd injection of vehicle. In Group II among 30 rats 10 were sacrificed after 12 hours, 10 after 24 hours and 10 after 7 days of 2nd injection of adrenaline. In Group III among 30 rats 10 were sacrificed after 12 hours, 10 after 24 hours and 10 after 7 days of 2nd injection of adrenaline. Blood was collected by cutting the carotid artery and serum was separated by centrifugation, 2,500 rpm, for estimation of serum Mg^{2+}, K^+, Ca^{2+} and Na^+ levels.

Estimation of serum electrolytes: Serum magnesium was estimated by using kit (Human, Germany)[16]. Absorbance was taken by a photometric colorimeter (AE-11M) at 520 nm. Serum calcium was measured by photometric colorimeter by using specific reagent kits manufactured by Chronolab, Switzerland[17] and absorbance was taken at 520 nm. Serum potassium and sodium estimation were done by Flame photometer (PFP7)[18] after diluting the serum with deionized water. Statistical analysis of data was carried out manually by unpaired student's t-test. The level of significance was set at P value of 0.05 to 0.001.

Results

Serum Mg^{2+} level was significantly ($p<0.001$) reduced in 12 hours and 24 hours after treatment of adrenaline as compared to control. However the value became normal after 7 days. In carvedilol pretreated group there were less reduction in serum Mg^{2+} level after 12 hours and 24 hours as compared to only adrenaline treated group and the change was highly significant ($p<0.001$). Pretreatment of carvedilol prevented the fall in serum Mg^{2+} level by 83.33% in 12 hours and 89.28% in 24 hours groups respectively (Table I).

In adrenaline treated group there was highly significant ($p<0.001$) reduction in serum K^+ level after 12 hours and significant ($p<0.01$) reduction after 24 hours as compared to control. The serum K^+ level that was measured after 7 days was within normal range. There was less reduction in serum K^+ level in carvedilol pre-treated group as compared to only adrenaline treated group and the change was significant ($p<0.01$). Pretreatment of carvedilol prevented the initial reduction in serum K^+ level by 66.66% after 12 hours and 72.22% after 24 hours of adrenaline treatment respectively (Table I).

Serum Ca^{2+} level was significantly ($p<0.05$) reduced in 12 and 24 hours after injection of adrenaline as compared to control and no significant change was noted after 7 days. In

carvedilol pretreatment group there was less reduction in serum Ca^{2+} level as compared to only adrenaline treated group but the change was not significant. However, the initial reduction in serum Ca^{2+} was prevented by carvedilol pretreatment by 42.84% after 12 hours and by 52.63% after 24 hours (Table I).

Adrenaline administration caused significant ($p<0.001$) reduction in serum Na^+ level after 12 hours as compared to control. No significant change was observed after 24 hours or 7 days. Carvedilol pretreatment caused less reduction in serum Na^+ level as compared to adrenaline treated group and the change was significant ($p<0.01$). Pretreatment of carvedilol thus prevented the initial reduction of serum Na^+ level by 56% (Table I).

Table I: Effect of pretreatment of carvedilol on serum electrolytes in adrenaline treated rats

Variable		Vehicle (Group I)	Adrenaline (Group II)	Carvedilol + Adrenaline (Group III)	%prevention
Serum Mg^{2+} (mmol/L)	After 12 hours	0.98 ± 0.02	0.74 ± 0.02 ***	0.94 ± 0.02 ***	83.33
	After 24 hours	0.96 ± 0.02	0.68 ± 0.03 ***	0.93 ± 0.01 ***	89.28
	After 7 days	0.97 ± 0.03	0.91 ± 0.02 ^{NS}	0.95 ± 0.01 ^{NS}	----
Serum K^+ (mmol/L)	After 12 hours	7.0 ± 0.44	4.6 ± 0.16 ***	6.2 ± 0.39 **	66.66
	After 24 hours	7.0 ± 0.36	5.2 ± 0.32 **	6.5 ± 0.30 **	72.22
	After 7 days	6.8 ± 0.47	6.0 ± 0.33 ^{NS}	6.5 ± 0.34 ^{NS}	----
Serum Ca^{2+} (mmol/L)	After 12 hours	2.38 ± 0.03	2.24 ± 0.04 *	2.30 ± 0.03 ^{NS}	42.85
	After 24 hours	2.37 ± 0.04	2.18 ± 0.06 *	2.28 ± 0.04 ^{NS}	52.63
	After 7 days	2.40 ± 0.04	2.31 ± 0.04 ^{NS}	2.33 ± 0.05 ^{NS}	----
Serum Na^+ (mmol/L)	After 12 hours	140 ± 1.29	130 ± 1.00 ***	135.6 ± 1.21 **	56.00
	After 24 hours	137 ± 1.41	135 ± 1.18 ^{NS}	136.6 ± 1.55 ^{NS}	----
	After 7 days	138 ± 1.27	136 ± 1.27 ^{NS}	137 ± 1.01 ^{NS}	----

Data expressed as mean ± SE; Comparison was made between Group I & Group II and Group II & Group III; *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$, NS = Not significant

Discussion

Acute myocardial infarction is associated with profound alteration in the sympathetic nervous activity. In early phase of myocardial infarction circulating catecholamine level is strikingly increased and a higher catecholamine level leads to myocardial ischemia[4]. Ischemia results in rapid decline in the tissue reserve of ATP[6]. Mg^{2+}, K^+, Ca^{2+} and Na^+ are the major ions responsible for the normal electrical activity of the heart. During ischemic period following myocardial infarction changes in serum electrolytes and decline in ATP reserve plays an important role in the genesis of cardiac arrhythmias[6]. Since the magnitude of sympathoadrenal activation early in the course of acute myocardial infarction is related to the extent of myocardial damage and mortality, beta adrenergic receptor blockade may provide a rationale therapy to curtail or, minimize the extent of myocardial damage.

The purpose of this study was to evaluate the protective effect of carvedilol on adrenaline-induced electrolyte changes and to explore the possible mechanism of this protective effect. In the present study administration of adrenaline produced hypomagnesemia, hypokalemia, hypocalcemia and hyponatremia.

Hypomagnesemia in early phase of myocardial infarction was observed in previous study[13,19]. Hypomagnesemia is known to produce myocardial irritability and frequently associated with increased risk of arrhythmia, sudden cardiac death and mortality[20]. Hypomagnesemia might be due to catecholamine-induced lipolysis (β effects)[19,21]. Two weeks pretreatment with carvedilol significantly ($p<0.001$) prevented the hypomagnesemia which might be the contribution of beta blocking effects[11].

Adrenaline administration caused significant reduction in serum K^+ level. Similar alteration in serum K^+ level in early myocardial infarction was observed by couple of researchers[13,22], however some others[23,24] found no change in serum K^+ level in myocardial infarction. This decrease in serum K^+ appears to be mediated primarily by the β_2–adrenoceptors due to increased catecholamine level[22] or could be a consequence of hypomagnesemia[25] and the same mechanism probably accounts for hypokalemia that occurs in early phase of acute myocardial infarction. In

clinical and experimental myocardial infarction hypokalemia lowers the thresholds for stimulated ventricular fibrillation and may increase the risk of spontaneous ventricular fibrillation[13]. Pretreatment with carvedilol significantly ($p<0.01$) prevented the initial hypokalemia and this may be due to its beta blocking effect, which correlates with another observation earlier[11].

In this study hypocalcemia occurred after administration of adrenaline in the early phase, though one study found no change in serum Ca^{2+} after myocardial infarction[26] and others found hypocalcemia[13,24]. Hypocalcemia after adrenaline administration might be due to accumulation of this ion in the irreversibly injured cardiac cell or may be due to increased catecholamine level or may be a consequence of hypomagnesemia[13,24]. Hypocalcemia in ischemic heart disease has been related to cardiac arrhythmia[24]. However, carvedilol pretreatment prevented the initial hypocalcemia and this may be due to its antagonistic effect on increased catecholamine during ischemia. This finding partly corresponds to the finding of other investigators[11].

In the present study, hyponatremia was observed only in early phase (after 12 hours) of experimental myocardial infarction. This observation correlates to the findings of one study[12] but not to another[24]. Hyponatremia in early phase may be due to increased catecholamine or a consequence of hypomagnesemia which causes inadequate activity of Na^+K^+ATPase resulting in intracellular accumulation of sodium[3]. Carvedilol pretreatment prevented the adrenaline induced initial hyponatremia and this protective effect may be due to its antagonistic effects on increased catecholamine during ischemia.

It may be concluded that carvedilol prevented adrenaline-induced electrolyte changes that are observed in acute myocardial infarction and this effect might be due to its non-selective adrenoceptor blocking property[27]. This effect on electrolyte changes contributes to its anti-arrhythmic and anti-fibrillatory potential, which might help to explain cardio-protective role of carvedilol by preventing sudden cardiac death in patients suffering from acute myocardial infarction.

References

1. Mohibullah AKM, Hossain MFA, Chowdhury FI, Islam MN. Pattern of cardiovascular diseases in Sir Salimullah Medical College and Mitford Hospital, Dhaka a six year study. (Abstract). SSMC J. 1998; 6: 13-16.
2. Zheng Z, Croft JB, Giles WH, Mensah GA. Sudden cardiac death in the United States, 1989 to 1998. Circulation 2001; 104: 2158-63.
3. Hulting J. In-hospital ventricular fibrillation and its relation to serum potassium. Acta Med Scand. 1981; 647: 109-16.
4. Karlsberg RP, Cryer PE, Roberts R. Serial plasma catecholamine response early in the course of clinical acute myocardial infarction: Relationship to infarct extent and mortality. Am Heart J. 1981; 102: 24-29.
5. Opie LH, Nathan D, Lubbe WF. Biochemical aspects of arrhythmogenesis and ventricular fibrillation. Am J Cardiol. 1979; 43: 131-48.
6. Nayler WG. The heart cell: Some metabolic aspects of cardiac arrhythmias. Acta Med Scand. 1981; 647 Suppl.: 17-30.
7. Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta blockade during and after myocardial infarction: An overview of the randomized trials. Prog Cardiovasc Dis. 1985; 27: 335-71.
8. Bhalla S, Barar FS, Sharma ML. Isoprenaline induced myocardial damage and the cardioprotective action of some beta-adrenoceptor blocker in albino rats. Indian J Med Res. 1987; 85: 77-84.
9. Vincent HH, Man in't Veld AJ, Boomsma F, Derkx FH, Wenting GJ, Schalekamp MA. Cardioprotection by blockade of β_2 adrenoceptors. Eur Heart J. 1983; 4 Suppl.: 109-15.
10. Bristow MR. β-Adrenergic receptor blockade in chronic heart failure. Circulation 2000; 101: 558-69.
11. Hansen O, Johansson BW, Nilsson-Ehle P, Eklund B, Ohlsson I, Palenmark E, Pauler AM, Svensson K. Effect of carvedilol on the metabolic, hemodynamic and electrocardiographic responses to increased plasma epinephrine in normal subjects. J Cardiovasc Pharmacol. 1994; 24: 853-59.
12. Cummings JR. Electrolyte changes in heart tissue and coronary arterial and venous plasma following coronary occlusion. Circ Res. 1960; 8: 865-70.
13. Abraham AS, Rosenman D, Meshulam Z, Zion M, Eylath U. Serum, lymphocyte and erythrocyte potassium, magnesium, and calcium concentrations and their relation to tachyarrhythmia in patients with acute myocardial infarction. Am J Med. 1986; 81: 983-88.
14. Nahar N, Akhter N, Rahman MS. Protective role of carvedilol in experimental myocardial infarction. Bangladesh J Physiol Pharmacol. 2004; 20: 9-12.
15. Feuerstein GZ, Ruffolo RR Jr. Carvedilol, a novel vasodilating beta-blocker with the potential for cardiovascular organ protection. Eur Heart J. 1996; 17 Suppl B: 24-29.
16. Mann CK, Yoe JH. Spectrophotometric determination of magnesium with 1-azo-2-hydroxy-3-(2.4-dimethyl-carboxanilido)-naphthalene-1-(2-hydroxybenzene). Anal Chim Acta. 1957; 16: 155-60.

17. Stern J, Lewis WH. The colorimetric estimation of calcium in serum with Ocresophthalein complexone. Clin Chem Acta. 1957; 2: 576-80.

18. Fall MJ. Models PFP7 & PFP7/C Flame Photometer. Operating and service manual. 1994; 500095. pp 1-16.

19. Flink EB, Brick JE, Shane SR. Alteration of long chain free fatty acid and magnesium concentration in acute myocardial infarction. Arch Intern Med. 1981; 141: 441-43.

20. Eisenberg MJ. Magnesium deficiency and sudden death. Am Heart J. 1992; 124: 544-49.

21. Brown DF, McGaudry RB, Gillie E, Doyle JT. Magnesium-lipid relations in health and in patients with myocardial infarction. Lancet 1958; 2: 933-35.

22. Brown MJ, Brown DC, Murphy MB. Hypokalemia from $beta_2$ receptor stimulation by circulating epinephrine. N Engl J Med. 1983; 309: 1414-19.

23. Rasmussen HS, Aurup P, Hojberg S, Jensen EK, McNair P. Magnesium and acute myocardial infarction. Arch Intern Med. 1986; 146: 872-74.

24. Khan SN, Rahman MA, Samad A. Trace elements in serum from Pakistani patients with acute and chronic ischemic heart disease and hypertension. Clin Chem. 1984; 30: 644-48

25. Kafka H, Langevin L, Armstrong PW. Serum magnesium and potassium in acute myocardial infarction. Arch Intern Med. 1987; 147: 465-69.

26. Abraham AS, Eylath U, Weinstein M, Czaczkes E. Serum magnesium levels in patients with acute myocardial infarction. N Engl J Med. 1977; 296: 862-63.

27. Landmark K, Urdal P. Serum magnesium and potassium in acute myocardial infarction: relationship to existing β-blockade and infarct size. Angiology 1993; 347-52.