Original Article

Role of Atenolol and Carvedilol in Prevention of Adrenaline Induced Myocardial Infarction: A Comparative Study on Experimental Animal

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Abstract

Introduction: In some trials in United States of America (USA) and Bangladesh, vasodilating non selective β blockers (e.g. carvedilol, propranolol etc.) have been shown to be better tolerated than non-vasodilating β_1 selective blocker (e.g. atenolol, metoprolol etc.) to prevent cardiovascular diseases (Coronary Heart Disease, Ischemic Heart Disease and other cardiovascular conditions)

Objective: To compare the role of atenolol and carvedilol in the prevention of adrenaline induced myocardial infarction (MI) in experimental animal (rats).

Materials and Methods: This experimental study was carried out in the Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka for a period of one year (from July 2014 to June 2015). Seventy two (72) healthy rats of Long Evan Norwegian strains, 3-4 months of ages of both sexes, weight between 180-220g were used. These rats were randomly selected and ethical issues were addressed. In this study cardio-protective effect was assessed by measuring the serum enzymes (CK-MB and AST) levels and antiperoxidative action was estimated by the hepatic and cardiac reduced glutathione (GSH) contents in experimentally (adrenaline) induced myocardial infarction.

Results: Adrenaline (2mg/kg) induced myocardial damage in rat model was evaluated biochemically by significant ($P<0.001$) increase in CK-MB and AST levels. Free radical production following adrenaline induced myocardial infarction was indirectly reflected by significant ($P<0.001$) depletion in hepatic & cardiac GSH contents. Cardio protection provided by atenolol and carvedilol pretreatment in adrenaline induced myocardial infraction was assessed by significant prevention of increase in serum CK-MB and AST levels. Antioxidant properties of carvedilol & atenolol were evaluated by significantly ($P<0.001$) increase in and significantly no (NS) change in GSH (hepatic & cardiac) contents respectively.

Conclusion: The study indicated that carvedilol (nonselective β blocker) through their antioxidant property in addition to α and β-blocking effect afforded more cardio protection than atenolol (selective $\beta_1$adrenoceptor blocker) in experimental MI.

Key-words: Acute myocardial infarction, coronary arterial disease, adrenaline induced cardiac damage, atenolol, carvedilol, reduced glutathione (GSH).

Introduction

During recent decades Bangladesh has experienced a rapid epidemiological transition from communicable to non-communicable diseases. Of these, being the fourth leading cause of death in Bangladesh, ischemic heart disease claimed 50,700 deaths in 2012[1,2]. European Society of Cardiology (ESC) issued its guideline which suggested β-blocker as the first line of therapy for indications such as heart failure, hypertension with angina, hypertension with MI[3,4]. Isoproteronol, a potent synthetic catecholamines (like adrenaline, noradrenaline) when administered to animals at high doses, produces infarct like lesions in the heart, which are similar to those found in myocardial infarction (MI) in humans[5].

Diagnosis of MI is dependent on an elevation of serum levels of cardiac biomarkers (cardiac specific troponins and the CK-MB, AST, LDH isoenzymes). However, CK-MB, AST,LDH will often not be detectable before 8 to 24 hours after the first symptoms of MI occur[6,7]. GSH (reduced glutathione) scavenges the free radicals after MI. The level of GSH was decreased in isoproterenol (like adrenaline, noadrenaline) induced myocardial necrosis. Thus, the reduction of content of GSH is an indirect evidence of antioxidant properties[8,9].

A comparative study was done in a dog model of experimental MI and investigators showed that carvedilol (non- selective β-blocker) may protect against reactive oxygen species (ROS) though scavenging of the free radicals, suppression of free radical generation and prevention of ferric-ion-induced oxidation after AMI. But atenolol (cardioelective β-blocker) does not provide any antioxidative effect like carvedilol after AMI[10]. Carvedilol has better evidence than atenolol for reducing morbidity in patients with heart failure (HF) and those who have experienced an acute myocardial infarction (AMI)[11]. Atenolol is selective β_1-blocker preferentially inhibiting cardiac β_1-receptors,but not β_2-receptors.Carvedilol, in contrast, inhibits β_1,β_2 (postsynaptic and

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presynaptic) and α_1 receptors, upregulates cardiac muscarinic M_2(muscarinic)receptors and possesses antioxidant effects and anti-inflammatory effects[12,13]. This experimental study was conducted in rats with an aim to compare cardioprotective role and antioxidant property of atenolol (β_1 selective blocker) and carvedilol (nonselective β blocker) in experimental MI (induced by injecting adrenaline subcutaneously in rats).

Materials and Methods

This experimental study was carried out in the department of pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka, during the period from July 2014 to June 2015. Seventy two (72) healthy rats of Long Evan Norwegian strains, 3-4 months of ages of both sexes, weighed between 180-220g were used and were obtained from the animal house of BSMMU. For this experimental study all rats were randomly selected and ethical issues were addressed. Cardio-protective effect and antiperoxidative action was assessed by measuring the serum enzymes (CK-MB and AST) released from necrotic myocardial tissues and the hepatic and cardiac reduced glutathione (GSH) contents in experimentally induced myocardial infarction respectively.

Experimental Design

The experiment was divided into two parts, Part-I and Part-II. PART-I Experiment (Table-I) was carried out to demonstrate the effect of adrenaline on serum CK-MB, AST levels and hepatic and cardiac GSH contents from 32 rats which are arranged into two groups: Group-I & II. Group-I was served as control and consisted of 12 rats and divided into two sub-groups (Group-Ia & Ib). They received vehicle i.e.1 ml of distilled water (D/W) s.c. for two consecutive days in 24 hours apart. Serum CK-MB and GSH contents (hepatic and cardiac) 12 hours after (Group-Ia, n=6) and serum AST level and GSH contents (hepatic and cardiac) 24 hours after (Group-Ib, n=6) the 2nd injection of vehicle was measured. Group-II consisted of 20 rats which received inj adrenaline at a dose of 2 mg/kg body weight s.c. for 2 consecutive days in 24 hours apart and served as experimental group. Group-II was again divided into two sub groups: Group-IIa (n=10 rats) and Group-IIb (n=10 rats). We measured serum CK-MB level and GSH contents (hepatic and cardiac) 12 hours after (Group-IIa, n=10) and serum AST level and GSH contents (hepatic hand cardiac) 24 hours after (Group-IIb, n=10) the 2nd injection of adrenaline.

Table-I: Showing the experimental design (Part-I& II) (n=72)

Part of Experiments	Group	Sub Group	No of Rats	Treatment Schedule	Sacrificing Schedule	Parameter Studied
Part –I (32 rats)	I	I(a)	6	Distilled water 1 ml s.c. (1st inj on the 1st day of experiment, 2nd inj after 24 hours)	12 hours after 2nd inj	CK-MB, & GSH contents (hepatic & cardiac)
		I(b)	6		24 hours after 2nd inj	AST & GSH contents(hepatic and cardiac)
	II	II(a)	10	Inj Adrenaline 2mg/kg s.c. (1st inj on the 1st day of experiment, 2nd inj after 24 hours)	12 hours after 2nd inj	CK-MB & GSH contents (hepatic and cardiac)
		II(b)	10		24 hours after 2nd inj	AST & GSH contents(hepatic and cardiac)
Part –II (40 rats)	III	III(a)		Atenolol 2mg/kg,1 ml orally(daily for 14 consecutive days) +Inj Adrenaline 2 mg/kg s.c. (1st inj Adrenaline on 15th day and 2nd inj after 24 hours)	12 hours after 2nd inj	CK-MB & GSH contents (hepatic & cardiac)
		III(b)			24 hours after 2nd inj	AST& GSH contents(hepatic & cardiac)
	IV	IV(a)		Carvedilol 1mg/kg,1 ml orally(daily for 14 consecutive days) +Inj Adrenaline 2 mg/kg s.c. (1st inj Adrenaline on 15th day and 2nd inj after 24 hours)	12 hours after 2nd inj	CK-MB & GSH contents (hepatic & cardiac)
		IV(b)			24 hours after 2nd inj	AST & GSH contents (hepatic & cardiac)

PART-II Experiment (Table-I) was done to demonstrate the effect of pretreatment of atenolol and carvedilol on serum enzymes (CK-MB and AST) levels and GSH contents (hepatic and cardiac) on adrenaline treated rats and 40 rats which were taken into two groups: Group-III (n=20 rats) and Group-IV (n=20 rats).Group-III (n=20 rats)received atenolol at a dose of 2mg/kg body weight, 1 ml containing 400 microgram orally daily through Ryles tube (size 5G) for 14 consecutive days starting from the 1st day of experiment. On the 15th day they received 1st injection of adrenaline at a dose of 2 mg/kg body weight s.c. and after 24 hours 2nd injection of adrenaline was given. Serum CK-MB level and hepatic and cardiac GSH contents 12 hours after (Group-IIIa, 10 rats) and serum AST level and GSH contents (hepatic and cardiac) 24 hours after (Group-IIIb, n=10) the 2nd injection of adrenaline was estimated. 20 rats of Group-IV were again divided into two subgroups, such as, Group-IVa (n=10 rats) and Group-IVb (n=10 rats) and received carvedilol at a dose of 1mg/kg body weight, 1 ml containing 200 microgram orally daily through Ryles tube (size 5G) for 14 consecutive days starting from the 1st day of experiment. On the 15th day they received 1st injection of adrenaline at a dose of 2 mg/kg body weight s.c. and after 24 hours 2nd injection of adrenaline was given. We measured serum CK-MB level and hepatic and cardiac GSH contents 12 hours (Group-IVa, n=10 rats) after and serum AST level and GSH contents (hepatic and cardiac) 24 hours(Group-IVb, n=10 rats) after the 2nd injection of adrenaline.

Statistical analyses were carried out using computer based programme Statistical Package for Social Science (SPSS) for windows version 10. Data obtained from the findings of the above experiments were analyzed by student's unpaired 't' test.

Results

Hepatic & cardiac GSH contents, 12 hours and 24 hours after the 2nd injection of adrenaline and distilled water treatment were measured and there was a marked decrease in hepatic & cardiac GSH contents in adrenaline treated group as compared to control and both the changes were highly significant (P<0.001). These results of the Part-I experiment (Table-II) of this study indicated that adrenaline caused oxidative stress on heart and liver.

Table-II: Effect of Adrenaline on serum CK-MB and AST levels and GSH contents (hepatic and cardiac)

Variable	Group-I(a) (n=6) Control (D/W) (mean±SE)	Group-II(a) (n=10) 12 hours after 2nd inj of Adrenaline (mean±SE)	Group-I(b) (n=6) Control (D/W) (mean±SE)	Group-II(b) (n=10) 24 hours after 2nd inj of Adrenaline (mean±SE)
Serum CK-MB level (U/L)	9.9± 1.1	48.3±1.2***		
Serum AST level (U/L)			192.6±4.2	429±4.9***
Hepatic GSH content (mg/gm of protein)	6.1±0.4	2.1±0.2***	6.1±0.6	2.2±0.1***
Cardiac GSH content (mg/gm of protein)	1.8±0.2	0.4±0.04***	1.7±0.2	0.5 ± 0.1***

***= *Highly significant (P<0.001)*

Table-III: Preventive effect of Atenolol and Carvedilol pretreatment on serum CK-MB and AST level

Drug treatment	Serum CK-MB level (U/L) (mean±SE) (12 hours after 2nd inj of Adrenaline)	Prevention by drug treatment	Serum AST level (U/L) (mean±SE) (24 hours after 2nd inj of Adrenaline)	Prevention by drug treatment
Group-I(a), (n=6) Control (D/W)	9.9 ± 1.1		192.6 ±4.2	
Group-II(a), (n=10) Adrenaline(2 mg/kg)	48.25 ±1.21		429 ± 4.9	
Group-III(a), (n=10) Atenolol (2 mg/kg) + Adrenaline (2mg/kg)	25.1 ±1.7**	60.4%		
Group-III(b), (n=10) Atenolol (2 mg/kg) + Adrenaline (2mg/kg)			338.4 ± 10.8**	38.3%
Group-IV(a), (n=10) Carvedilol (1mg/kg) + Adrenaline (2mg/kg)	17.6 ±1.2	79.9%		
Group-IV(b), (n=10) Carvedilol (1mg/kg) + Adrenaline (2mg/kg)			293.4 ± 4.5	57.4%

***= *Highly significant (P<0.001), ** =Significant (P<0.01)*

It was observed that 02 weeks pretreatment with atenolol & carvedilol in adrenaline treated rats caused highly significant (P<0.001) decrease in serum CK-MB and AST levels 12 hours & 24 hours after adrenaline administration. But carvedilol pretreatment prevented the adrenaline induced rise in serum CK-MB level by 79.9% & AST level by 57.4% and atenolol pretreatment by 60.4% (CK-MB level) and 38.3 % (AST level). Carvedilol decreased serum CK-MB & AST levels significantly (P<0.01) as compared to atenolol pretreated group in experimental MI (Table-III).

Table-IV: Preventive effect of Atenolol and Carvedilol pretreatment on Hepatic GSH & on Cardiac GSH contents

Drug Treatment	Hepatic GSH content (mg/gm of protein) (mean±SE) (12 hours after 2nd inj of Adrenaline)	Prevention by drug treatment	Hepatic GSH content (mg/gm of protein) (mean±SE) (24 hours after 2nd inj of Adrenaline)	Prevention by drug treatment	Cardiac GSH content (mg/gm of protein) (mean±SE) (12 hours after 2nd inj of Adrenaline)	Prevention by drug treatment	Cardiac GSH content (mg/gm of protein) (mean±SE) (24 hours after 2nd inj of Adrenaline)	Prevention by drug treatment
Group-I(a), (n=6), Control (D/W)	6.1±0.4		6.1±0.6		1.8±0.2		1.7±0.2	
Group-II(a), (n=10), Adrenaline (2 mg/kg)	2.1±0.2***		2.2±0.1		0.4±0.04***		0.5 ± 0.1	
Group-III(a), (n=10) Atenolol (2 mg/kg) + Adrenaline (2mg/kg)	2.7±0.3**	13.3%			0.6 ±0.1**	12.5%		
Group-III(b), (n=10) Atenolol (2 mg/kg) + Adrenaline (2mg/kg)			3.0±0.4***	20.5%			0.7±0.1***	13.2%
Group-IV(a), (n=10), Carvedilol (1mg/kg) + Adrenaline (2mg/kg)	5.3 ±0.6	80.8%			1.2±0.1	59.6%		
Group-IV(b), (n=10), Carvedilol (1mg/kg) + Adrenaline (2mg/kg)			5.5 ± 0.2	84.6%			1.4 ± 0.1	74.4%

***= *Highly significant (P<0.001), ** =Significant (P<0.01)*

This study showed that carvedilol pretreated rats had highly significant (P<0.001) increase in hepatic &cardiac GSH contents 12 hours and 24 hours after adrenaline administration. But no significant (NS) changes in hepatic & cardiac GSH contents was found in atenolol pretreated rats.

Carvedilol pretreatment prevented the adrenaline induced decrease in hepatic GSH contents by 80.8% and 84.6% and cardiac GSH contents 59.6% and 74.4% after 12 hours and 24 hours adrenaline administration respectively. On the contrary atenolol pretreatment prevented adrenaline induced decrease in hepatic GSH contents 13.3% & 20.5% and cardiac GSH content was 12.5% and 13.2% after 12 hours and 24 hours adrenaline administration respectively (Table-IV). The results of this study indicated that carvedilol pretreatment provided effective antioxidative action and atenolol provided no antioxidative action.

Discussion

The present study was aimed to evaluate the comparative study of cardioprotective role of atenolol and carvedilol in rat model of MI. For this purpose, experimental MI was induced by injecting adrenaline subcutaneously in rats[14]. In this investigation the evidence of experimental MI was assessed by estimation of serum CK-MB and AST levels like Nahar et al & Khatun M et al[14,15].

After MI, released catecholamines undergo auto-oxidation and generate free radicals which causes further cardiotoxicity[16,17]. We also measured hepatic and cardiac GSH contents for indirect evidence of free radical induced myocardial damage in experimental MI as per few scientists (Vennila L, Pugalendi KV)[8,18].

In the present investigation, it was investigated that 02 weeks pretreatment with atenolol & carvedilol in adrenaline treated rats caused highly significant (P<0.001) decrease in serum CK-MB level 12 hours after & serum AST levels 24 hours after adrenaline administration. The reduction of serum CK-MB & AST levels by carvedilol pretreatment was significant (P<0.01) as compared to atenolol pretreated group. Hampton C et al cited quite similar results in their studies[19]. So, in present study we found that carvedilol provided more cardio protection than atenolol[11,13].

In this study antiperoxidative effects were measured indirectly by estimation of hepatic & cardiac GSH contents. It was found that in carvedilol pretreated rats caused highly significant (P<0.001) increase in hepatic & cardiac GSH contents 12 hours and 24 hours after adrenaline administration. Similarly, the investigators (Yue TL et al and Ratore N et al) evaluated the free radical scavenging activity of carvedilol in rabbit model of MI and showed that carvedilol provided antioxidant effect (by preventing reduction of hepatic & cardiac GSH content) and endothelial protective effect[20,21].

But atenolol pretreatment prevented the change in hepatic & cardiac GSH contents in atenolol pretreated group as compared to only adrenaline treated group was found not significant (NS)[22]. Atenolol and metoprolol had no significant effect on free radical induced myocardial damage in ischemic-reperfusion injury [23,24,25].

A few reports (Ozaydin M et al and Jocelyn PC)[11,24] were available to compare the findings of this study and compared the antioxidant effect of carvedilol with atenolol and they showed that atenolol did not prevent the reduction of hepatic GSH content in experimental MI[25,26].

Zaca V and Jarmila L compared the antiperoxidative action of carvedilol with atenolol and reported that atenolol was ineffective in providing significant protection against oxygen radical mediated injury to canine myocyte in experimental MI[23].

It was concluded that non selective β-blockers through their antioxidant property in addition to their β-blocking effect and vasodilating (α-blocking effect) prevent free radical mediated injury to catecholamine assault following myocardial infarction[24]. Both cardio selective (atenolol) and nonselective β-blockers (carvedilol) provided cardioprotective effect in experimental MI in rat model. But carvedilol afforded more protection than atenolol[27,28].

Conclusion

Carvedilol provided cardioprotection by blocking both α, β_1 & β_2 adrenoceptors. Atenolol blocks only β_1 adrenoceptor and provided less cardio protection than carvedililol. Carvedilol also has free-radical scavenging activity (antioxidant property) which reduces oxidative stress induced further myocardial necrosis. But atenolol does not have any antioxidant property. In this study cardio protective role of carvedilol was compared to atenolol in adrenaline induced MI in rat model and proved that carvedilol (nonselective β-blocker) provided more cardio protection than atenolol (cardio selective β-blocker).

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