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Original Article

Cardioprotective Effects of Mebudipine in a Rat Model of Doxorubicin-Induced Heart Failure

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What's Known

- Among calcium-channel blockers, dihydropyridines are extensively used due to their potent vasodilatory and weak cardiodepressant properties.
- Mebudipine is a newly synthesized dihydropyridine calcium-channel blocker that has significant negative chronotropic effects but without considerable negative inotropic properties.

What's New

- In an animal model, mebudipine reversed the increased plasma levels of biochemical markers, which act as the prognostic and diagnostic indicators of heart failure.
- Administration of mebudipine to animals with doxorubicin -induced heart failure palliated the clinical and biochemical signs of the disease.

Abstract

Background: Mebudipine, a dihydropyridine calcium-channel blocker (CCB), shows greater time- and voltage-dependent inhibitory effects than nifedipine. Its significant negative chronotropic effects without having considerable negative inotropic properties may make it a suitable candidate for the pharmacotherapy of heart failure (HF). This study aimed to investigate the possible beneficial action of mebudipine in a rat model of HF.

Methods: The present study carried out in the Department of Pharmacology at the Iran University of Medical Sciences during the years of 2009-2011. An experimental model of HF was induced in male Wistar rats using doxorubicin (DOX). The rats were divided into five groups with seven animals in each group: normal control group, DOX-induced HF control groups, and treatment groups. The animals were administered DOX for 15 days. A consistent deterioration occurred after a four-week rest period. The animals were then treated with intraperitoneal mebudipine (0.5 mg/kg) and intraperitoneal amlodipine (0.35 mg/kg), as well as an equal volume of distilled water for 15 days. The plasma levels of big endothelin-1 (BET-1), creatine kinase-myocardial band (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), as well as the clinical status (heart rate and blood pressure), were assessed before and after treatment. Statistical analysis was performed with SPSS software using parametric and nonparametric ANOVA.

Results: Mebudipine and amlodipine reversed the increased plasma BET-1 values in the treated animals when compared with the HF control group (0.103 and 0.112 vs 0.231 pg/mL, respectively). The increased plasma levels of AST, ALT, CK-MB, and LDH were also reversed in the HF animals that received mebudipine or amlodipine.

Conclusion: The administration of mebudipine to HF animals, akin to amlodipine, palliated the clinical and biochemical signs of the disease in the present study.

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Introduction

Heart failure (HF) is a chronic condition that results from any structural or functional cardiac disorders, leading to reduced cardiac output.[1, 2]

The prevalence of HF is increasing and despite advances in pharmacotherapy, the disease is still associated with high morbidity and mortality.[3, 4] A developing body of evidence suggests that mediators involved in the control of myocardial function and vascular tone may contribute to the pathophysiology of HF. A potential role for the endothelin (ET) system in the disease progression has been proposed by some recent studies.[5, 6] The ET family consists of four closely related peptides, namely ET-1, ET-2, ET-3, and ET-4, which are produced from the biologically inactive "big ET" by ET-converting enzymes. ET-1 is the principal isoform of the ET family.[7] Many studies have found that the plasma level of ET-1 is increased in symptomatic patients with congestive HF and its concentration increases correlatively with the severity of HF.[8-10] The plasma levels of ET-1 and its precursor, big endothelin-1 (BET-1), have been shown to predict mortality in patients with moderate-to-severe HF.[11]

Activated ET system has gained special interests in terms of its prognostic value along with various clinically applied tests to evaluate the impact of numerous therapeutic efforts and changes in extracardiac neurohormonal systems such as sympathetic and renin-angiotensin systems, which are upregulated in HF. Furthermore, the correlation between the increased plasma level of BET and the severity of the disease (left ventricular ejection fraction <20%) is well established. Therefore, the measurement of the plasma level of BET-1 is an appropriate method to determine the severity of doxorubicin (DOX)-induced HF and to evaluate the subsequent deterioration.[12, 13]

Calcium-channel blockers (CCBs) are drugs used in treatment of hypertension, angina pectoris, and other cardiovascular as well as non-cardiovascular diseases.[2] Among CCBs, dihydropyridines (DHPs) are extensively used due to their potent vasodilatory and weak cardiodepressant properties.[14, 15] Since their introduction in the 1960s, the members of DHPs have been subjected to several changes to optimize their safety and efficacy.

Among DHPs, amlodipine exhibits more stable pharmacokinetic and less cardioselectivity than the previously introduced congeners; thus, it is well tolerated in patients with HF.[16] Many studies have reported that amlodipine prolongs survival and reduces myocardial damage in patients with myocardial infarction.[17, 18] Since the beneficial effects of amlodipine have been clearly demonstrated in some patients with HF, especially in a subgroup of non-ischemic cardiomyopathy,[19] the synthesis of a new CCB with less cardioselective and more vasoselective properties has been on demand.

Mebudipine ([±]-t-butyl, methyl-1, 4-dihydro-2, 6-dimethyl-4-[3-nitrophenyl]-3, 5-pyridine dicarboxylate) is a 1, 4-DHP derivative developed in our lab.[14] Compared with nifedipine, mebudipine has shown high potency in inhibiting calcium-evoked spikes in *Helix aspersa*.[20] Moreover, it has been demonstrated that the potency of mebudipine in inhibiting potassium chloride (KCl)-induced contractions of the isolated rat aorta and its time- and voltage-dependent actions are significantly greater than those of nifedipine.[14] It has also been shown that, compared with nifedipine, mebudipine possesses a negligible cardiodepressant activity, while its negative chronotropic property is markedly pronounced.[21] Furthermore, it appears that mebudipine offers some other advantages over nifedipine such as a longer biological half-life, a longer time to reach the maximum effect,[22] and higher vasoselectivity.[21]

In contrast to the beneficial effects seen with amlodipine, some other DHPs have demonstrated deleterious effects in HF.[23] Therefore, any information regarding the safety and efficacy profile of any other CCBs in cardiac failure would be helpful. Since the effect of mebudipine in HF has not yet been investigated, the present study aimed to assess the potential therapeutic effects of mebudipine in an animal model of HF. In this study, the plasma levels of big BET-1, as a prognostic marker, as well as cardiac marker enzymes including creatine kinase-myocardial band (CK-MB) and lactate dehydrogenase (LDH), were determined. Additionally, the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. These parameters were assessed in a rat model of DOX-induced HF treated with mebudipine, compared with amlodipine.

Materials and Methods

Drugs

Amlodipine was purchased from Razak Pharmaceutical Company (Tehran, Iran). Mebudipine was provided by Pars Biopharmacy Research Co (Tehran, Iran). DOX was also commercially purchased (Adriblastina®, Pfizer Inc, NY, USA). Polyethylene glycol 400 was used as a solvent.

Animals

In this study, 35 adult male Wistar albino rats (weight=200–250 g) were purchased from *the Razi Vaccine and Serum Research Institute* (Karaj, Iran). The rats were randomly divided

into five groups (seven animals per group). The sample size was calculated using the following formula:[24]

$n=z^2p\ (1-p)\ (d^2)^{-1}$

where n represents the sample size, z the level of confidence, p the estimated proportion of the population that presents the characteristic, and d the tolerated margin of error.

All the rats were housed in a controlled environment with an approximate room temperature of 25±2 °C in a 12 hour/12 hour day-night cycle and were given ad libitum access to food and water.

Experimental Protocol

The present study carried out in the Department of Pharmacology at the Iran University of Medical Sciences during the years of 2009-2011. All the applied protocols were approved by the Research Ethics Committee of Iran University of Medical Sciences (Code of Ethics: IR.IUMS.FMD.REC.1387.87005). The rats were randomly divided into five groups with seven animals in each group: Group I consisted of distilled water-treated animals serving as normal controls; Group II and Group III were comprised of DOX-induced HF animals serving as HF control groups I and II, respectively; Group IV comprised DOX-induced HF animals treated with mebudipine; and Group V contained DOX-induced HF animals treated with amlodipine. The induction of HF was in keeping with a previously described procedure.[25] Briefly, DOX was intraperitoneally (ip) administered at a total dose of 15 mg/kg, at six-time points, every three days for a duration of 15 days followed by four weeks of rest. At the end of week four, the animals of the normal control group (Group I) and HF control$_1$ (Group II) were euthanized following ketamine (100 mg/kg; Alfasan, Worden, Netherlands)/xylazine (10 mg/kg; Alfasan, Worden, Netherlands) anesthesia to evaluate the baseline values in the DOX-induced HF animals. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes (Sigma, St. Louis, USA). Subsequently, all the remaining animals (Groups III, IV, and V) were assigned to a following treatment for the next 14 days: Group III equivolume of distilled water, Group IV mebudipine (0.5 mg/kg; ip), and Group V amlodipine (0.35 mg/kg; ip), once daily. Finally, 24 hours after the last treatment, blood samples were taken from the euthanized animals, via cardiac puncture, and collected in EDTA tubes.

Biochemical Analysis

The plasma level of BET-1 was measured using the ELISA kit (IBL, Gunma, Japan). The plasma levels of marker enzymes, namely AST, ALT, CK-MB, and LDH, were also assessed using a commercially available kit (Pars Azmoon Kits, Tehran, Iran). The clinical signs of HF, namely the systolic blood pressure and the heart rate, were assessed by monitoring the animals before and after treatment using a non-invasive tail-cuff method (Power Lab, Australia).

Statistical Analysis

The data were presented as mean±SEM. The statistical analyses were carried out using the one-way analysis of variance (ANOVA), followed by the Tukey *post hoc* test, via SPSS software, version 18, (SPSS Inc, Chicago, IL, USA). A P value of less than 0.05 was considered statistically significant.

Results

Four weeks following the DOX regimen, all the rats in Group II presented elevated plasma BET-1 (P<0.001) (table 1) and marker enzymes, comprised of LDH (P=0.001), AST (P=0.005), ALT (P<0.001), and CK-MB (P<0.001) (table 2), compared with the normal control rats (Group I), which was indicative of pronounced HF signs, comprising a decreased blood pressure

Table 1: Plasma big endothelin-1 values (pg/mL) in the normal control and heart failure control$_1$ groups four weeks following the doxorubicin regimen and the subsequent changes following mebudipine and amlodipine treatment, compared with the heart failure control$_2$ group, at the end of the 2-week medication

Animal Groups (each group [n=7])	Plasma Big Endothelin-1 Values (pg/mL) mean±SEM	P value
Four weeks after DOX regimen		
Group I	0.10±0.08	
Group II	0.22±0.02*	<0.001
At the end of the 2-week treatment		
Group III	0.23±0.02	
Group IV	0.10±0.00#	<0.001
Group V	0.11±0.04#	<0.001

Group I: Normal control; Group II: HF control$_1$; Group III: HF control$_2$; Group IV: Mebudipine-treated; Group V: Amlodipine-treated; asterisk (*P) vs normal control, number sign (#P) vs HF control$_2$; Statistical analysis was carried out using the one-way analysis of variance, followed by the Tukey *post hoc* test. DOX: Doxorubicin; HF: Heart failure

Table 2: Plasma marker enzyme values in the normal control and heart failure control$_1$ groups four weeks following the Doxorubicin regimen and the subsequent changes following mebudipine and amlodipine treatment, compared with the heart failure control$_2$ group, at the end of the 2-week medication

	Plasma Marker Enzyme Values (IU/L) mean±SEM							
Animal Groups (each group [n=7])	**LDH**	**P value**	**AST**	**P value**	**ALT**	**P value**	**CK-MB**	**P value**
Four weeks after the DOX regimen								
Group I	510.80±11.10		130.40±9.10		76.30±8.62		120.90±10.30	
Group II	1113.00±139.80*	0.001	218.70±30.50*	0.005	148.50±15.21*	<0.001	195.41±9.47*	<0.001
At the end of the 2-week treatment								
Group III	279.92±135.61		192.40±6.74		176.41±20.22		192.40±6.70	
Group IV	470.41±28.10#	<0.001	131.71±7.10#	0.022	93.00±12.50#	<0.001	136.41±8.35#	<0.001
Group V	16.70±61.33#	<0.001	123.42±18.60#	0.012	71.73±8.31#	<0.001	141.82±8.86#	<0.001

Group I: Normal control; Group II: HF control$_1$; Group III: HF control$_2$; Group IV: Mebudipine-treated; Group V: Amlodipine-treated; Asterisk (*P) vs Group I, number sign (#P) vs Group III; LDH: Lactate dehydrogenase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; CK-MB: Creatine kinase-myocardial band; HF: Heart failure; DOX: Doxorubicin

Table 3: Clinical values (heart rate and blood pressure) in the normal control and heart failure control$_1$ groups four weeks following the Doxorubicin regimen and the subsequent changes following mebudipine and amlodipine treatment, compared with the heart failure control$_2$ group, at the end of the 2-week medication

	Clinical values			
Animal Groups (each group [n=7])	**Heart Rate (beat/min) mean±SEM**	**P value**	**Systolic Blood Pressure (mm Hg) mean±SEM**	**P value**
Four weeks after the DOX regimen				
Group I	227.21±19.60		119.62±2.90	
Group II	281.60±7.00*	<0.001	115.81±1.80	0.077
At the end of the 2-week treatment				
Group III	282.60±11.61		110.21±3.00*	0.022
Group IV	241.10±28.80#	0.001	110.40±3.00*	0.023
Group V	244.92±24.70#	0.001	109.21±2.40*	0.013

Group I: Normal control; Group II: HF control$_1$; Group III: HF control$_2$; Group IV: Mebudipine-treated; Group V: Amlodipine-treated; Asterisk (*P) vs Group I, number sign (#P) vs Group III; HF: Heart failure; DOX: Doxorubicin

(P=0.077) and an increased heart rate (P<0.001) (table 3), as well as edema, dyspnea, and acrocyanosis.

The increased plasma level of BET-1, in parallel with worsening HF, a statistically significantly decreased blood pressure, and almost unchanged initial increased enzyme values, demonstrated a correlation with clinical deterioration in HF. The treatment groups, consisting of Group V (amlodipine-treated animals; P<0.001) and Group IV (mebudipine-treated rats; P<0.001), unlike the HF group, which received distilled water (Group III), counteracted further increases in the plasma concentrations of BET-1 (table 1). Further, the previously elevated enzyme values and most of the HF signs such as edema, dyspnea, and acrocyanosis were ameliorated in the groups treated with mebudipine or amlodipine compared with the HF control animals. The rats treated with CCBs for two weeks showed a significant decrement in plasma enzyme values (AST, P=0.022 and P=0.012 in Group IV and Group V, respectively; LDH, ALT, and CK-MB, P<0.001 in both groups) by comparison with the group receiving distilled water (table 2).

The increased heart rate in the animals with HF was lowered significantly after treatment with mebudipine (Group IV) (P=0.001) or amlodipine (Group V) (P=0.001); nonetheless, there was no statistically significant difference in the blood pressure of amlodipine- and mebudipine-treated groups compared with the HF-control group (table 3).

Discussion

In the present study, a 2-week DOX regimen caused a significant elevation in plasma BET-1 levels in all the animals of Group II (HF group) compared with those in the normal control group (Group I). Four weeks following the DOX administration, the elevated plasma levels of BET-1 were correlated with pronounced HF signs including edema, dyspnea, acrocyanosis, tachycardia, and hypotension in the HF group. Our results showed that a two-week treatment with mebudipine and amlodipine successfully

reversed the increased plasma BET-1 values. In addition, the elevated plasma enzyme levels of AST, ALT, CK-MB, and LDH were significantly declined in the groups treated with mebudipine or amlodipine compared with those in the rats receiving distilled water. Amelioration in most of the HF manifestations was observed in both amlodipine- and mebudipine-treated groups. Nevertheless, the decreased systolic blood pressure remained unchanged in both mebudipine- and amlodipine-treated groups. In our study, the systolic blood pressure was decreased, as anticipated, in the animals with HF, indicating impairment in cardiac output. The persistence of this decrease in blood pressure after the administration of CCBs could be attributed to their antihypertensive action. The effect of mebudipine on blood pressure has been previously reported.[26] We, however, could not truly evaluate it in the present study, since blood pressure was already decreased by DOX-induced HF.

Amlodipine and mebudipine induced comparable effects on the cardiovascular system. A study on the inhibitory effects of mebudipine, compared with amlodipine, on calcium currents have reported their equipotent actions in differentiated PC12 cells.[27] The similar effects of mebudipine and amlodipine on BET-1 plasma levels, enzyme values, and cardiovascular signs, which were detected in this study may be attributed to their selectivity for vascular calcium channels and long half-lives.

We measured BET-1 levels in our study to evaluate disease severity in a rat model of DOX-induced HF. Several studies have confirmed that BET-1 is a potent prognostic marker in patients with HF.[28, 29] It has also been reported that plasma ET concentrations are closely related to the outcome after myocardial infarction and supply prognostic information independent of biochemical and clinical variables.[30]

Hence, elevated BET-1 values and the subsequent increase in the ET-1 synthesis rate can be used for a better evaluation of the exacerbation in DOX-induced HF. In the present study, four weeks following the DOX regimen, the elevated plasma BET-1 level, which was associated with increased serum enzymes levels, was parallel with such pronounced signs of HF as edema, dyspnea, acrocyanosis, hypotension, and increased heart rate, as reported previously.[31, 32]

With references to studies regarding the beneficial effect of amlodipine on BET-1,[33] it appears that mebudipine, similar to amlodipine, has a consistent lowering effect on BET-1 concentrations in treated HF animals. Regarding the mechanism involved in increasing BET-1, several lines of evidence have indicated a relationship between nitric oxide (NO) and ET, whereby NO impairs the ET production. Therefore, increased serum BET values represent NO-failure. The reversal of otherwise increased serum BET values, thereby, likely indicates the restored balance and NO function.[34, 35] For instance, in a study by Champagne, it was demonstrated that amlodipine strongly increased NO in the coronary vessels in control animals.[36] Moreover, it releases NO even after HF, which may be partly responsible for its favorable effect in the treatment of HF.[33, 37] The NO-dependent action has been reported for other CCBs such as nicardipine, benidipine, and nifedipine.[38] Still, further studies are required to clarify whether mebudipine has such similar effects on vessels via NO production.

It has been demonstrated that mebudipine is a chiral molecule, in which the (+) isomer has mineralocorticoid receptor (MR) antagonist properties, while the (-) isomer has a CCB activity even more potent than the racemic form.[39] Both the MR antagonist and CCB properties could be beneficial in the treatment of HF.

The therapeutic effects of CCBs in HF are controversial. It has been reported that diltiazem, a first-generation CCB, is associated with an increased number of cardiac events and an increased risk of HF. The Multicenter Diltiazem Postinfarction Trials also conclusively demonstrated that diltiazem was involved in congestive HF risk.[40] The administration of CCBs, except for amlodipine, should be avoided in patients with congestive HF. Amlodipine, a third-generation CCB, has been shown to improve the exercise capacity of patients with mild-to-moderate HF in a double-blind, placebo-controlled clinical trial. It also produces a favorable effect on the survival of patients with HF resulting from non-ischemic dilated cardiomyopathy.[19, 41]

Mirkhani and others showed that in comparison with first-generation CCBs, the newly synthesized CCB, mebudipine, exhibited significant vasoselectivity. They also reported that, in comparison with nifedipine, mebudipine elicited a strong negative chronotropic effect but an insignificant negative inotropic property. Consequently, they posited that mebudipine had the potential to be used in cardiovascular disorders without producing adverse effects such as reflex tachycardia in HF, which had sometimes been observed with older medicine.[21]

Bohlooli and others reported that mebudipine showed a longer half-life than nifedipine and thus, suggested it as a potential therapeutic

alternative to the existing 1, 4-DHP CCBs.[22]

The results of the present study should be interpreted in light of some limitations, the most notable of which is our inability to use echocardiography to assess cardiac performance. In addition, we did not have access to a suitable physiograph to obtain electrocardiograms.

Conclusion

The results of the current investigation showed the cardioprotective action of mebudipine in an experimental model of DOX-induced HF. Mebudipine reduced BET-1 and other biochemical parameters, as well as clinical signs, in our HF animals, indicating its potential benefit for the management of HF. Further investigations are required to clarify whether mebudipine can ameliorate HF in some other animal models of HF. Echocardiographic assessment is also strongly recommended.

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Conflict of Interest: None declared.

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