

Original Article

Antihypertensive Effects of Some New Nitroxyalkyl 1,4-Dihydropyridine Derivatives in Rat Model of Two-kidney, One-clip Hypertension

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Abstract

Dihydropyridine calcium channel blockers consist one of the widely-used groups of drugs for the management of hypertension. In this study, antihypertensive effects of 5 newly synthesized derivatives of DHP was examined the in rat model of two-kidney, one-clip renal hypertension. The results showed that those compounds containing two nitroxy groups had less decreasing effect on MAP (Men Arterial Pressure) compared to those having one nitroxy group in 5 position. On the other hand, conformational analysis indicated that in two compounds with two chains (A and E), a great steric hindrance existed; therefore, steric hindrance is the most important factor for interaction of compounds with their receptors.

Keywords: two-kidney; one-clip hypertension; 1,4-dihydropyridines; MAP.

Introduction

Since the introduction of dihydropyridine calcium channel blockers in 1970s (1), their clinical uses have been increasing. They have been widely used for various cardiovascular disorders including hypertension, angina pectoris, heart failure and Raynaud's disease (2, 3).

Dihydropyridine calcium channel blockers are mainly vascular specific and act by inhibiting the influx of calcium ion into the vascular smooth muscle cells via L-type calcium channels (4). Their beneficial effects in cardiovascular disorders are due to their ability to relax vascular smooth muscles. In angina pectoris, such drugs

produce resistance in systemic and coronary arterial beds thereby reducing cardiac oxygen requirement and increasing cardiac oxygen supply, respectively (5).

The uses of dihydropyridine calcium channel blockers in hypertension were the subjects of a number of clinical trials. Dihydropyridine calcium channel blockers caused an equal incidence of fatal coronary heart diseases and nonfatal myocardial infarction, a lower incidence of stroke and a higher incidence of heart failure compared to those caused by angiotensin converting enzyme inhibitors (6). Meta-analysis of a number of clinical trials showed that the use of calcium channel blockers including dihydropyridine ones was associated with significant reductions in the risk of stroke, major cardiovascular events, and cardiovascular disease-related mortalities, but no significant

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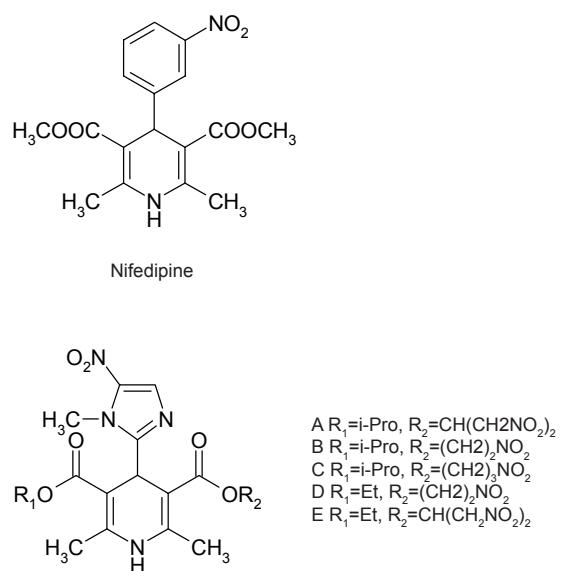


Figure 1. Chemical structure of nifedipine and 5 DHP derivatives used in this study.

reductions in coronary heart disease, heart failure or total mortality (7). The findings of clinical trials such as that of ALLHAT led to the establishment of dihydropyridine calcium channel blockers as one of the main groups of drugs used for the treatment of hypertension.

In our previous studies, we combined two antihypertensive approaches, Ca channel blocking using DHP derivatives fused to nitroxy group to 3,5 positions as NO donor (8). The *in vitro* evaluation showed that they are more potent Ca channel blockers.

Therefore, the objective of the present study was to examine the antihypertensive effects of a number of 5 synthesized derivatives of DHP in rat model of two-kidney, one-clip renal hypertension.

Experimental

Drugs

Xylosine and ketamine were obtained from Alfasan Co, Holland. Sodium thiopental was obtained from Biochem GmbH, Austria. Nifedipine was purchased from Sigma, USA. Compound A, B, C, D, and E were synthesized in the department of Medicinal Chemistry, School

of Pharmacy, Shiraz University of Medical Sciences (Figure 1). Ketamine, xylosine and sodium thiopental were dissolved in normal saline. Nifedipine and compounds A, B, C, D, and E were dissolved in DMSO.

Induction of renal hypertension

Male Sprague-Dawley rats (200-250 g) were subjected to sham operation (n=5) or placement of a solid plexiglass clips (9) around left renal artery (n=5). Animals were anesthetized with intraperitoneal injections of 8 mg/kg xylosine (Alfasan, Holland) and 60 mg/kg ketamine (Alfasan, Holland). Through a left flank incision, the left kidneys were exposed, and left renal arteries were separated from renal vein and surrounding tissues. Afterwards, plexiglass clips were placed applied on the exposed renal arteries as close as possible to the aorta. Once placed over renal artery, the clip was turned so that the slit opening faced the abdomen to ensure that the renal artery remains within the clip. Care was taken that the renal artery was placed deep in the clip slit, and that the flow of blood was visible down in the clip. The right kidney was not disturbed. Then antibiotic powder (Penicillin G) was applied to the site of incision, and abdominal wall and skin were sutured by absorbable chromic catgut and silk suture materials (Supa, Iran), respectively. Sham-operated animals were subjected to the same procedure, but clip was applied to the left renal artery. Animals were then recovered from anesthesia and kept two in a cage at 22-25°C and a light cycle of 12 h light/12 h darkness with food and water ad libitum.

Experimental design

Animals were divided into sham and one renal artery clipped group. Then renal artery clipped rats were further divided into 7 groups (n=5), and assigned to receive dimethylsulfoxide (DMSO, 0.05 mL) as vehicle, nifedipine, A, B, C, D, or E at 100, 300, or 1000 $\mu\text{g}/\text{kg}$.

Experimental protocol

Four weeks after the operation, animals were anesthetized with diazepam (3 mg/kg) and sodium thiopental (70 mg/kg, ip). The right jugular veins were cannulated with PE50

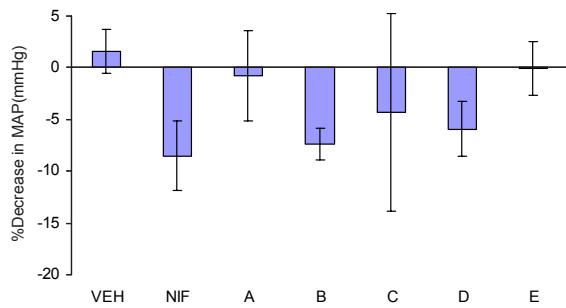


Figure 2. Decrease in mean arterial pressure (Mean \pm SEM) by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 100 μ g/kg.

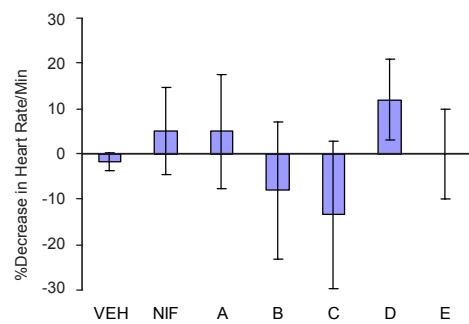


Figure 3. Decrease in Heart rate (Mean \pm SEM) by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 100 μ g/kg.

catheters (Polyethylene Tubing, Becton Dickinson, USA) for supplying anesthetic as needed and injection of vehicle or drugs. The left external carotid arteries were cannulated for the measurement of blood pressure using a Gould Statham pressure transducer (Model P23D), connected to a Grass polygraph (Model 7P1D). Animals were then allowed to recover for 30 min, and a measurement of blood pressure and heart rate was performed. The renal artery clipped rats were assigned to receive an intravenous injection of dimethylsulfoxide vehicle, nifedipine, or compounds A, B, C, D, or E. Twenty five min after administration of the vehicle or drugs, another measurement of blood pressure and heart rate was made. Animals were then sacrificed by a bolus of anesthetic, and heart as well as left and right kidney were removed and weighed.

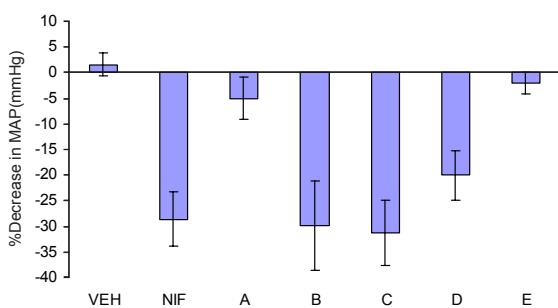


Figure 4. Decrease in mean arterial pressure (Mean \pm SEM) by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 300 μ g/kg.

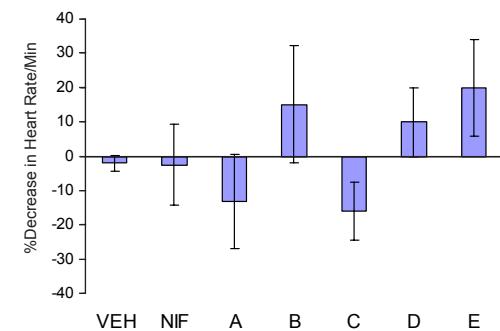


Figure 5. Decrease in Heart rate (Mean \pm SEM) by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 300 μ g/kg.

Calculations and statistical analysis

Mean arterial pressure was calculated as diastolic arterial pressure plus one third of pulse pressure. Heart rate was counted from arterial pulse pressure upstroke. The changes in mean arterial pressure and heart rate were calculated as the percentages of their values prior to administration of vehicle or drugs. The weights of heart and left and right kidneys were calculated as percentages of body weight. The data, presented as mean \pm SEM, were analyzed using one way analysis of variance (ANOVA). Where a significant difference was found with ANOVA, the source of difference was located using Duncan multiple range test. In cases that the data were not normally distributed, statistical analysis was performed using Kruskal-Wallis test followed by Dunn's procedure for multiple comparisons. A P value of ≤ 0.05 was considered

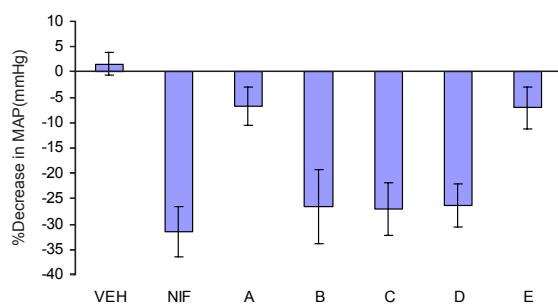


Figure 6. Decrease in mean arterial pressure (Mean \pm SEM) by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 300 μ g/kg.

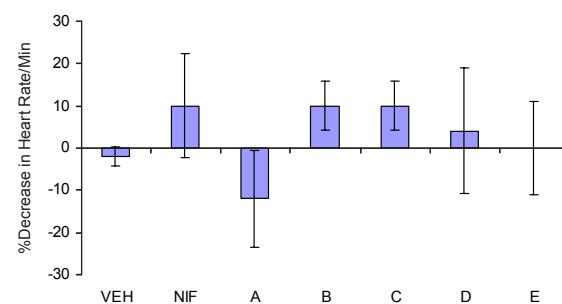


Figure 7. Decrease in Heart rate (Mean \pm SEM) by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 1000 μ g/kg.

statistically significant.

Conformation analysis

Chemical structure of each molecule was built by Hyperchem software for the structural chemistry (10). Gaussian98 program was operated to optimize the molecular structure (11). The structures were optimized by the RHF method of ab initio at the level of STO-3G. No molecular symmetry constraint was applied; rather full optimization of all bond lengths and angles was carried out. The root mean square of 0.1 kcal mol⁻¹ was used as ending criteria in geometry optimization. Then, the molecules were reloaded to Hyperchem (12).

Results

The renal artery clipped groups assigned to receive vehicle, nifedipine or new calcium

channel blocker had significantly higher baseline mean arterial pressures compared to that of sham-operated group (Table 1). However, there was no significant difference in the heart rate between such groups. Moreover, renal artery clipped groups had significantly higher right kidney and heart weights, and significantly lower left kidney weights compared to those of sham-operated group. In addition, there was no significant difference in the animals' body weights on operation or experiment days between renal artery clipped or sham-operated rats (Table 1).

Administration of vehicle (DMSO) did not make a significant difference in mean arterial pressure (169 \pm 8 vs 171 \pm 7 mmHg) or heart rate (420 \pm 10 vs 424 \pm 13 bpm).

compared to the changes induced by the administration of DMSO; administration of nifedipine, or compounds B or A' at 100 μ g/kg caused a significant reduction in mean arterial

Table 1. Animal's body weights on operation or experiment days from renal artery clipped or sham-operated rats.

Group name	100 μ g/kg administration day	300 μ g/kg administration day	1000 μ g/kg administration day
	Mean weight \pm SD		
Sham	209.2 \pm 10.7	209.2 \pm 10.7	209.2 \pm 10.7
Vehicle	218.3 \pm 12.9	218.3 \pm 12.9	218.3 \pm 12.9
Nifedipine	200.0 \pm 11.5	211.2 \pm 10.3	208.3 \pm 6.8
A	199.2 \pm 4.9	210.0 \pm 14.1	205.0 \pm 7.1
B	199.6 \pm 9.9	217.5 \pm 16.5	217.0 \pm 15.2
C	207.8 \pm 15.2	216.6 \pm 18.0	211.0 \pm 7.4
D	213.3 \pm 16.6	213.7 \pm 17.5	206.2 \pm 4.8
E	207.5 \pm 8.8	206.2 \pm 7.5	219.0 \pm 18.1

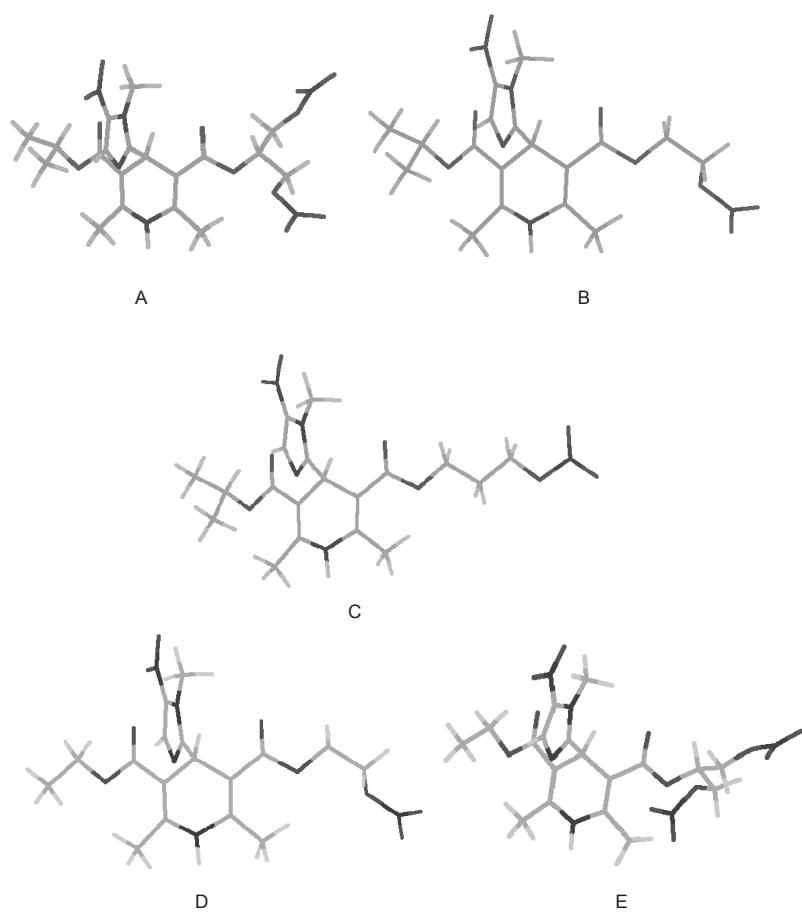


Figure 8. Optimized structures of DHP derivatives.

pressure. However, compounds A, C, or E did not significantly change the mean arterial pressure from that of DMSO. There was no significant difference between the changes induced in mean arterial pressure by nifedipine, or compounds B, C, D whereas the reduction in mean arterial pressure by nifedipine was significantly more than those of compounds A or E (Figure 2). Moreover, there was no significant difference among the changes induced in the heart rate by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 100 μ g/kg (Figure 3).

Compared to the changes induced by the administration of DMSO, administration of nifedipine, or compounds B or D at 300 μ g/kg caused a significant reduction in mean arterial

pressure. However, compounds A, C, and D did not significantly change the mean arterial pressure from that of DMSO. There was no significant difference between the changes induced in mean arterial pressure by nifedipine and compounds B, C, or D whereas the reduction in mean arterial pressure by nifedipine was significantly more than those of compounds A or D (Figure 4). Moreover, there was no significant difference among the changes induced in the heart rate by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 300 μ g/kg (Figure 5).

Relative to the changes induced by the administration of DMSO, administration of nifedipine, or compounds B, C, or D 1000 μ g/kg caused a significant reduction in mean arterial

pressure. However, compounds A and E did not change the mean arterial pressure significantly different from that of DMSO. There was no significant difference between the changes induced in mean arterial pressure by nifedipine and compounds B, C or D. the reduction in mean arterial pressure by compounds A, E and F were significantly less than that of nifedipine (Figure 6). Moreover, there was no significant difference among the changes induced in the heart rate by the administration of DMSO, nifedipine, or compound A, B, C, D, or E at 1000 μ g/kg. (Figure 7).

Discussion

The findings of the present study showed that the placement of renal arterial clips around left renal artery, while leaving the right kidney intact, did result in hypertension characterized by increased mean arterial pressure as well as increased heart and right kidney weights and decreased left kidney weight. Two-kidney, one-clip or Goldblatt renal hypertension has been produced using silver clips. We showed that the placement of solid plexiglass clips around left kidney was associated with a hypertension comparable to that induced by silver clips (9). The values of blood pressure in hypertensive rats in the present study were within the range of those induced by silver clips. Moreover, the values of heart and left and right kidney weights were comparable to those reported previously (9).

From the SAR viewpoint, we expected that those compounds containing two nitroxy groups had more decreasing effect on MAP, since it was supposed that nitroxy group can act as NO donor which resulted in more decrease in MAP and also the in vitro results suggest that the presence of nitroxy group can result in greater Ca channel blocking effect. However, % decrease in MAP showed the exact opposite results i.e. the one chain compounds (B, C and D) in 5 position had more decreasing effect.

On the other hand, conformation analysis (Figure 8) indicated that in two compounds with two chains (A and E), a great steric hindrance existed, since there was an angle of more than 90° between two chains while in other compounds,

which have one chain, such hindrance did not occur.

Therefore, it can be concluded that 5 position had a great impact on antihypertensive ability of these DHP derivatives and also the possible interaction with Ca channels. Furthermore, presence of nitroxy group does not affect the activity, since there is no sign of decrease in MAP compared to reference drug (Nifedipine) which has not any nitroxy group.

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