Original Article

Cardiodepressant activity of newer dihydropyrimidine derivative in comparison to Nifedipine on perfused Rabbits heart

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ABSTRACT

Background: *Substituted dihydropyridine calcium channel blockers are used in the treatment of cardiovascular diseases. Dihydropyridines are considered as analogue of dihydropyrimidines.*
Objectives: *In present study newly synthesized test compound 5-Acyl-6-methyl-4(2',3'-methylenedioxy) phenyl - 2 - S - ethyl - 1, 4-dihydropyrimidine, a dihydropyrimidine derivative was investigated with an aim to get valuable substitute for the well known dihydropyridine, Nifedipine.*
Material & Methods: *The Calcium Channel blocking actiuity of test compound was studied on Rabbit's Heart and its effects were compared with Nifedipine used as control.*
Results: *Test compound has dose-dependent negative chronotropic and negative inotropic effect on Rabbit's heart but these effects appeared at doses higher than those of Nifedipine. Test compound had no significant change in coronary flow however Nifedipine show significant increase in coronary flow at lower doses.*
Conclusion: *Test compound appears to be less potent myocardial depressant compared to Nifedipine. Test compound produced calcium channel blocking activity which was dose related and in order to ascertain the status of this compound as a drug, further studies are needed not only in other animals and tissue models but also in various pathophysiological models.*

Key Words: *Test compound (5-Acyl-6-methyl-4(2',3'-methylenedioxy) phenyl - 2 - S - ethyl - 1, 4-dihydropyrimidine), DMSO (Dimethylsulphoxide),nifedipine*

Introduction

Calcium ion is a simple, evolutionarily ancient, and universal second messenger. Ca^{2+} enters cell through many different Ca^{2+} channels. Some of these are ligand gated and other is voltage gated. There appears to be Stretch activated channel as well. [1] The voltage dependent calcium channels include L-type, N-type, T-type and P-type. The work in the 1960s of Fleckenstein, Godfraind, and their colleagues led to the concept that drugs can alter cardiac and smooth muscle contraction by blocking the entry of Ca^{2+} into myocytes. Calcium entry blockers or Calcium channels blockers have been classified as dihydropyridines or nondihydropyridines. Dihydropyridines

include Amlodipine, Felodipine, Nicardipine, and Nifedipine, whereas nondihydropyridines comprise agents such as Diltiazem and Verapamil. Dihydropyridines are among the most widely used drugs for the management of cardiovascular disease. These drugs are used for the treatment of Hypertension, PSVT, Prinzemetal's angina, Hypertrophic cardiomyopathy, Migraine, Raynaud's phenomenon and Atherosclerosis. [2] Introduced in the 1960s, dihydropyridines have undergone several changes to optimize their efficacy and safety. Dihydropyrimidines are commonly described as potent mimics of dihydropyridine calcium channel blockers. The synthesis of 6-methyl-4-substituted-phenyl-2-oxo-1,2,3,4-tetrahydropyrimidine - 5-carboxylic acid ethyl esters by condensation of an aldehyde, urea and ethylacetoacetate was first described by Bignelli in 1893. In recent years there has been an increasing interest in the design of alkyl-1,4-dihydropyrimide-5-carboxylates and related Bignelli-like compounds, which are presented as valuable substitutes for the well known nifedipine and other 1,4 dihydropyridine drugs. Some of these compounds have exhibited not only more potent and longer lasting vasodilator action but also a hypotensive activity with slow onset as compared to dihydropyridines. Moreover some dihydropyrimidines are found to be weaker in blocking atrioventricular conduction and less toxic than the dihydropyridines. [3, 4]

In view of the wide range of biological activity associated with 1-4-dihydropyrimidines, Department of Chemistry, Punjabi University, Patiala has synthesized analogues with the aim of getting biologically active molecules with improved activity, lesser toxicity and least undesirable effects compared with other Calcium Channel Blockers in clinical use. One such compound 5-Acyl-6-methyl-4(2',3'-methylenedioxy) phenyl - 2 - S - ethyl - 1, 4-dihydropyrimidine, a dihydropyrimidine derivative, has been taken up for the present study to find out its calcium channel blocking activity in comparison to Nifedipine on Rabbit's Heart.

Material and methods

After getting the approval from the Institutional animal ethics committee the present study was conducted. Following materials were used for conducting this study.

- Test compound 5-Acyl-6-methyl-4(2, 3'-methylenedioxy)-phenyl-2-S-ethyl-1,4-dihydropyrimidine (Molecular weight = 318).
- Solvent: Dimethylsulphoxide (DMSO)
- Nifedipine
- Ringer Locke solution
- Equipment: Langendorff's assembly, Kymographs, Starling heart lever.
- Animals: Rabbit (either sex) weight b/w 1.5-2.5 kg

The test compound 5 - Acyl - 6 - methyl - 4 (2, 3' - methylenedioxy) phenyl – 2 – S – ethyl - 1, 4 - dihydropyrimidine was synthesized as follows:

Step I

Synthesis of 5 - acyl - 6 - methyl - 4 (2, 3' – methylenedioxy) phenyl – 2 - thioxo - 1, 2, 3, 4 – tetrahydropyrimidine

A mixture of 2,3-methylenedioxy benzaldehyde (0.01 mole, 1.5 gm), thiourea (0.01 mole, 0.76 gm), acetyl acetone (0.015 mole, 1.5 ml) and conc. Hcl (3-4 drops) in absolute alcohol (10 ml) taken in a borosil beaker (100 ml) were irradiated at 30% microwave power level in domestic microwave oven for 4.5

minute. The reaction mixture was allowed to stand at room temperature and the product separated out was filtered at reduced pressure, washed with ethanol, then with water. It was dried and recrystallised from methanol as light brown crystals in 87% yield. Melting point of this is 165ºC.

Step II

Synthesis of 5 - acyl - 6 - methyl - 4 (2, 3' – methylenedioxy) Phenyl – 2 – S – ethyl - 1, 4 – dihydropyrimidine

To the 5 - acyl - 6 - methyl - 4 (2, 3' – methylenedioxy) phenyl – 2 - thioxo - 1, 2, 3, 4 –tetrahydropyrimidine (0.004 mole, 1.16gm) dissolved in methanol was added NaOH solution which was prepared by dissolving NaOH (0.160 gm) in water (2 ml). The mixture was cooled. To this mixture diethyl sulfate (0.4 ml, 0.004 moles) was added drop wise while stirring the mixture continuously. Then the mixture was refluxed for 3 hours in order to complete ethylation. The mixture was cooled and poured over ice. Solid separated was filtered under reduced pressure and dried. It was recrystallised from methanol. Melting point of this is 196ºC. The compound was confirmed by taking its IR (Infra-red), NMR (Nuclear magnetic resonance) and Mass spectra.

Test compound was found to be soluble in dimethylsulphoxide (DMSO) and this solvent was used in present study.

With the purpose of evaluation of pharmacological activity of the newly synthesized test compound the experiments were conducted on isolated perfused Rabbit Heart and Heart rate, Amplitude, Coronary flow were noted.

Adult healthy rabbits of either sex of weight between 1.5 to 2.5 kg were used in this study. They were given uniform experimental conditions and care was taken about the environment and diet. The diet comprised of green leafy vegetables, grass, soaked grams and milk given at libidum.

Ringer Locke solution was used for mounting the heart preparation. The Composition (per litre) of fluid used was as follows [5].

Sodium chloride	9.0 g
Potassium chloride	0.42 g
Calcium chloride	0.24 g
Sodium bicarbonate	0.50 g
Glucose	1.0 g
Distilled water to make	1.0 L

The experiments were performed as per following methods:

Study on Isolated Perfused Rabbit's Heart

The rabbit heart was mounted as per the methods described by Burn and Perry. [5, 6] The animals were stunned and bled through carotid arteries. The chest was opened and heart along with an inch of ascending aorta was cut and transferred to a petri-dish containing oxygenated Ringer Locke solution at 37ºC. The ventricles were squeezed to remove all blood in order to prevent development of thrombi in the vessels.

The heart was mounted in the Langendorff's assembly. The drugs solutions were injected through a polyethylene tube inserted into the rubber tube perfusing the heart. The apex of the heart was attached to the Starling's heart lever with the help of a bent pin passed through the apex and connected to a thread. Baseline recordings were taken after giving a stabilisation time of around 15 minutes. The drugs were administered through the polyethylene tube and each time it was followed by injection of 0.2 ml of Ringer Locke solution to ensure complete displacement of the drug. The sensitivity of the heart was tested by administration of Adrenaline 2

µg. The effect of the test compound was observed and compared with vehicle (Dimethylsulphoxide 50%) alone and Nifedipine used as control regarding:

Heart Rate: Heart rate was counted for 1 minute after the injection of each drug including the vehicle and effect observed.

Amplitude: The amplitude of heart contractions was observed for one minute after injection of each drug including the vehicle.

Coronary Flow: The rabbit heart was mounted as described above in the Langendorff's assembly. After injection of each drug and control, coronary flow was estimated for one minute. Six such experiments were conducted with the test compound and six with Nifedipine as control and mean value calculated. Statistical significance of the difference between various groups, were analyzed by using Student 't' test. The P value of ≤ 0.05 was considered statistically significant.

Results

Heart Rate

DMSO (0.2 ml) given alone before administration of drug, led to mean percentage decrease in heart rate by 0.52% (P > 0.05) in one minute. With test compound there was mean percentage decrease 2.14% (P > 0.05), decrease 5.29% (P > 0.05), decrease 3.98% (P > 0.05), decrease 29.10% (P ≤ 0.05) and decrease 92.57% (P < 0.001) in heart rate in one min. in doses of 25µg, 50µg, 100µg, 200µg, 400µg respectively (Table 2). With Nifedipine, there was mean percentage decrease 3.54% (P ≤ 0.05), decrease 4.31% (P < 0.01), decrease 20.75% (P < 0.001), decrease 25.71% (P < 0.001) and decrease 100% (P < 0.001) in heart rate in one min. in doses of 1µg, 2µg, 4µg, 8µg, 16µg respectively (Table 3).

Amplitude

The mean percentage increase in amplitude was caused by DMSO 32.98% (P < 0.02) With test compound there was mean percentage decrease 26.2% (P ≤ 0.05), decrease 26.15% (P < 0.001), decrease 26.08% (P > 0.05), decrease 12.14% (P > 0.05) and decrease 67.1% (P < 0.001) in amplitude in one min. in doses 25µg, 50µg, 100µg, 200µg, 400µg respectively (Table 2). With Nifedipine there was mean percentage decrease 7.69% (P < 0.01), decrease 17.97% (P < 0.001), decrease 57.39% (P < 0.001), decrease 91.08% (P < 0.001) and decrease 100% (P < 0.001) in amplitude in one min. in doses of 1µg, 2µg, 4µg, 8µg, 16µg respectively (Table 3).

Coronary Flow

Dimethylsulphoxide given alone in the dose of 0.2 ml led to mean percentage increase in coronary flow 8.25% (P < 0.001) in one minute. With test compound there was mean percentage increase 1.13% (P > 0.05), increase 5.58% (P < 0.01), increase 1.74% (P > 0.05), decrease 1.66% (P > 0.05) and decrease 21.22% (P < 0.01) in coronary flow in one minute in doses 25µg, 50µg, 100µg, 200µg, 400µg respectively (Table 2). With Nifedipine there was mean percentage increase 6.36% (P < 0.001), increase 9.40% (P < 0.001), increase 13% (P < 0.001), decrease 4.19 (P < 0.001) and decrease 13.09% (P < 0.001) in coronary flow in doses of 1µg, 2µg, 4µg, 8µg, 16µg respectively (Table 3).

Table 1: Comparison Of Mean Effect Of Increasing Molar Doses Of Test Compound And DMSO (Solvent) On Heart Rate(Beats/Min), Amplitude (mm) And Coronary Flow (ml/min) Of Isolated Perfused Rabbit Heart (n=6)

DRUG & DOSE µg (molar dose)	MEAN CHANGE IN HEART RATE (beats/min) MEAN± SE	MEAN CHANGE IN AMPLITUDE (mm) MEAN± SE	MEAN CHANGE IN CORONARY FLOW (ml/min) MEAN± SE
Test Compd. 25 (7.86X10-5)	-1 ± 0.8 (P > 0.05)	-7.84 ± 2.83 (P ≤ 0.05)	-0.04 ± 0.02 (P > 0.05)
Test Compd. 50 (1.57X10-4)	-5.0 ±3.38 (P > 0.05)	-6.34 ± 0.03 (P < 0.001)	0.23 ±0.07 (P < 0.001)
Test Compd. 100 (3.14X10-4)	-3.83±2.64 (P > 0.05)	-6.0 ± 2.80 (P > 0.05)	0.03 ±0.02 (P > 0.05)
Test Compd. 200 (6.28X10-4)	-27.5 ± 6.79 (P ≤ 0.05)	-12.6 ± 3.98 (P > 0.05)	-0.24 ± 0.08 (P > 0.05)
Test Compd. 400 (1.25X10-3)	-75.33 ±5.35 (P < 0.001)	-18.0 ± 0.60 (P < 0.001)	-0.43 ±0.03 (P < 0.001)

DMSO 0.2ml	**0.5 ± 0.34 (P > 0.05)**	**9.00 ± 2.06 (P < 0.02)**	**0.36 ± 0.061 (P < 0.001)**

Table 2: Mean Percentage Change in Heart Rate, Amplitude And Coronary Flow Of Isolated Perfused Rabbit Heart With Test Compound And DMSO (Solvent) (n=6)

DRUG & DOSE µg (molar dose)	MEAN %age CHANGE IN HEART RATE	MEAN %age CHANGE IN AMPLITUDE	MEAN %age CHANGE IN CORONARY FLOW
Test Compd. 25 (7.86X10-5)	**↓ 2.14 (P > 0.05)**	**↓ 26.2 (P ≤ 0.05)**	**↑ 1.13 (P > 0.05)**
Test Compd. 50 (1.57X10-4)	**↓ 5.29 (P > 0.05)**	**↓ 26.15 (P < 0.001)**	**↑ 5.58 (P > 0.05)**
Test Compd. 100 (3.14X10-4)	**↓ 3.98 (P > 0.05)**	**↓ 26.08 (P > 0.05)**	**↑ 1.74 (P > 0.05)**
Test Compd. 200 (6.28X10-4)	**↓ 29.10 (P ≤ 0.05)**	**↓ 12.14 (P > 0.05)**	**↓ 4.54 (P > 0.05)**
Test Compd. 400 (1.25X10-3)	**↓ 92.57 (P ≤ 0.05)**	**↓ 67.1 (P < 0.001)**	**↓ 21.22 (P < 0.001)**
DMSO 0.2ml	**↓ 0.52 (P > 0.05)**	**↑ 32.98 (P < 0.02)**	**↑ 8.25 (P < 0.001)**

Table 3: Mean Percentage Change in Heart Rate, Amplitude And Coronary Flow Of Isolated Perfused Rabbit Heart With Nifedipine (n=6)

DOSE OF NIFEDIPINE µg	MEAN %age CHANGE IN HEART RATE	MEAN %age CHANGE IN AMPLITUDE	MEAN %age CHANGE IN CORONARY FLOW
1 µg	↓ 3.54 (P ≤ 0.05)	↓ 7.69 (P < 0.01)	↑ 6.36 (P < 0.001)
2 µg	↓ 4.31 (P < 0.01)	↓ 17.97 (P < 0.001)	↑ 9.40 (P < 0.001)
4 µg	↓ 20.75 (P < 0.001)	↓ 57.39 (P < 0.001)	↑ 13.0 (P < 0.001)
8 µg	↓ 25.17 (P < 0.001)	↓ 91.08 (P < 0.001)	↓ 4.19 (P < 0.001)
16 µg	↓ 100.00 (P < 0.001)	↓ 100.00 (P < 0.001)	↓ 13.09 (P < 0.001)

Discussion

In recent years, there has been increasing interest in the design of 1,4-dihydropyrimidine-5, carboxylate which are presented as valuable substitutes for the well known Nifedipine and other dihydropyridine drugs clinically used in the treatment of cardiovascular diseases. [3, 4, 7]

In the present study the pharmacological actions, of a newly synthesized dihydro- pyrimidine derivartive 5-Acyl-6-methyl-4(2',3'-methylenedioxy)phenyl-2-S-ethyl-1,4-dihydropyrimidine were studied on cardiovascular system and compared with Nifedipine used as control. Test compound produces significant negative chronotropic effect in higher doses and produces significant dose dependent negative inotropic effect but shows no significant change in coronary blood flow. Nifedipine has negative inotropic and negative chronotropic effect and increases coronary blood flow in lower doses. Calcium channel blockers have negative inotropic and negative chronotropic effect on experimental animals as shown by Morgan and Coworkers, and Fleckenstein X. [8] Similar results were obtained by Hess, Hartfelder and Kerins. [9] Thus from the above cited study, it can be concluded that the test compound has negative inotropic and negative chronotropic effect but no significant effect on coronary blood flow. Hence, test compound appears to be less potent myocardial depressant compared to Nifedipine. In order to ascertain the status of this compound as a drug, further studies are needed not only in other animals and tissue models but also in various pathophysiological models, since some drugs show more pronounced effect in disease and in pathophysiological models than in physiological conditions. [8]

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