Calcium channel blocking activity of a dihydropyrimidine derivative (BKVIII) on rabbit's aortic strip

Sir,

Calcium channel blockers are mainly used in hypertension, angina and cardiac arrhythmias.[1] It had been known since the late 1800s that calcium influx was necessary for the contraction of smooth and cardiac muscle. Calcium channels blockers are classified as dihydropyridines or nondihydropyridines. Dihydropyridines include amlodipine, felodipine, nicardipine, and nifedipine, whereas nondihydropyridines comprise agents such as diltiazem and verapamil. Dihydropyrimidines are commonly described as potent mimics of dihydropyridine calcium channel blockers. 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 (Code BK VIII), a dihydropyrimidine derivative, has been taken up for the present study to find out its biological effects and calcium channel blocking activity in Rabbit's Aortic strip, after taking approval from the institutional animal ethics committee. In order to carry out preliminary pharmacological investigations following materials were used for conducting this study.

1. Test compound 5-Acyl-6-methyl-4 (2′, 3′-methylenedioxy) phenyl-2-S-ethyl-1,4-dihydropyrimidine (Code BK VIII) (Molecular weight = 318).
2. Drugs and chemicals: $CaCl_2$, Kcl, EDTA
3. Physiological solutions: Krebs solutions and oxygen gas
4. Equipment: Dale's organ bath, Kymographs, Frontal writing levers, smoked drum.
5. Animals: Rabbit (either sex)

With the purpose of evaluation of pharmacological activity of the newly synthesized compound BK VIII experiments were conducted on Aortic strip of Rabbit. Adult healthy rabbits of either sex of weight between 1.5 to 2.5 kg were used in this study. Isolated rabbit aortic from the descending thoracic aorta was mounted in modified Kreb's solution initially for relaxation and then later on, deplolarized in the absence of free calcium

Table 1: Mean effect of increasing doses of compound BK VIII on height of calcium induced contraction of depolarized isolated rabbit aortic strip (*n*=6)

Bath conc. of BK VIII µg/ml	Mean±SE height of contraction induced by $CaCl_2$ before compound	Mean±SE height of contraction induced by $CaCl_2$ after the compound BK VIII	Mean change	Mean % age inhibition
5 (1.57×10^{-5} M)	6.33±0.80	6.16±0.79	−0.16±0.16	2.68
10 (3.14×10^{-5} M)	6.33±0.55	5.16±0.47	−1.16±0.16***	18.48
20 (6.28×10^{-5} M)	5.33±0.33	3.16±0.40	−2.16±0.16***	40.71
40 (1.25×10^{-4} M)	6.16±0.30	2.83±0.40	−3.33±0.55**	54.0
80 (2.5×10^{-4} M)	7.33±0.21	2.16±0.16	−5.16±0.16***	81.17

**P<0.01, ***P<0.001. Statistical significance of the difference between various groups, were analysed by using student's '*t*' test

Table 2: Mean effect of increasing doses of Nifedipine on height of calcium induced contraction of depolarized isolated rabbit aortic strip (*n*=6)

Bath conc. of Nifedipine µg/ml	Mean±SE height of contraction induced by $CaCl_2$ before Nifedipine	Mean±SE height of contraction induced by $CaCl_2$ after the Nifedipine	Mean change and *P* value	Mean % age Inhibition
0.25 (7.21×10^{-7} M)	5.5±1.11	3.5±0.79	−2.0±0.36**	36.3
0.5 (1.44×10^{-6} M)	5.58±0.43	3.08±0.59	−2.83±0.47**	44.8
1 (2.88×10^{-6} M)	7.16±1.13	2±0.44	−5.16±0.69***	72.06
2 (5.77×10^{-6} M)	7.43±1.13	0.50±0.22	−7.0±0.77***	92.35

**P<0.01, ***P<0.001. Statistical significance of the difference between various groups, were analysed by using student's '*t*' test

ions and the contraction was evoked by subsequent addition of calcium.[2] Descending thoracic aortas were excised and trimmed free of the adhering fat and connective tissue. Aortic strips 4cm long and about 2-3mm wide were prepared by spiral section.[3,4] For removal of endothelial lining, procedure described by Furchgott and Zawadzki was adopted. Aortic strips were dragged slowly for one minute, with the intimal surface down, over a sheet of filter paper wetted with Kreb's solution. Aortic strips were then suspended in a 25ml organ bath containing modified Kreb's solution at 37°C, which was continuously oxygenated by using oxygen gas. A tension load of 3.0g was applied to each preparation for relaxation and kept so for 90 minutes, while changing the bath fluid every 10 minutes. Further, incubations were done in Ca^{2+} free Kreb's solution containing EDTA for 10 minutes. Next, the preparation were depolarised in Ca^{2+} free - K^{+} rich Kreb's solution. Then 10 millimoles of calcium chloride was added and response was taken for 10 minutes.[5] Then preparations were washed with modified Kreb's solution and the whole procedure was repeated as stated above in the presence of compound under test. Six such experiments were conducted with the test compound and six with nifedipine used as control. Mean value and standard error for all parameters were determined separately and shown in tables as Mean ± SE. Statistical significance of the difference between various groups, were analysed by using student's '*t*' test.

Results obtained from six experiments with each concentration of compound BK VIII and Nifedipine are depicted in tables. BK VIII was given in different doses so as to give the final bath concentrations of 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. The mean percentage inhibition brought about with the above doses was 2.68% ($P > 0.05$), 18.48% ($P < 0.001$), 40.71% ($P < 0.001$), 54% ($P < 0.01$) and 81.17% ($P < 0.001$) respectively [Table 1]. Nifedipine used as control was given in different doses so as to give the final bath concentrations of 0.25 µg/ml, 0.5 µg/ml, 1 µg/ml, 2 µg/ml. The mean percentage inhibition brought about with the above doses was 36.3% ($P < 0.01$), 44.8% ($P < 0.01$), 72.06% ($P < 0.001$) and 92.35% ($P < 0.001$) respectively [Table 2].

Test compound, 5-Acyl-6-methyl-4 (2′,3′-methylenedioxy) phenyl-2-S-ethyl-1,4-dihydropyrimidine have a calcium channel blocking activity and showed relaxant effect on aortic smooth muscle in higher doses as compared with 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.

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DOI: 10.4103/0976-500X.107688