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STUDY OF SYNERGISTIC EFFECT OF THEOPHYLLINE WITH MILRINONE IN THE TREATMENT OF CONGESTIVE HEART FAILURE

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ABSTRACT

Aim of the present work was to evaluate the effect of theophylline on the positive inotropic effect of milrinone used in the treatment of congestive heart failure. The positive inotropic effect of milrinone alone and in presence of theophylline was evaluated in frog heart and guinea pig left atria. Cumulative dose response study of milrinone alone and along with theophylline was performed. The results of the study demonstrated an enhancement in the inotropic effect of milrinone when given along with theophylline. The mechanism of positive inotropic effect of milrinone involves increase in intracellular cyclic AMP by inhibiting phosphodiesterase enzyme III while theophylline is non-specific phosphodiesterase enzyme inhibitor and produces weak positive inotropic effect. The study revealed that theophyline produces synergism with milrinone in producing positive inotropic effect.

Keywords: Milrinone, theophylline, positive inotropic effect, congestive heart failure and phosphodiesterase enzyme

INTRODUCTION

Congestive heart failure is the complex clinical syndrome characterized by the inability of the heart to pump an adequate amount of blood to achieve the demand of the different organ systems **[1]**. In the congestive heart failure heart cannot pump enough oxygen and nutrients to meet the body's needs because of its weaker pumping power results in slow movement of blood through the heart and body. Congestive heart

failure is a major health problem in developed countries with increased incidence and prevalence in the aging population **[2, 3]**. Milrinone is a new inotropic agent for the treatment of acute and chronic heart failure as it causes direct stimulation of myocardial contractility and acceleration of myocardial relaxation. It increases cardiac output by stimulating myocardium and the decrease in left ventricular afterload **[4]**. Additionally it acts as vasodilator causing balanced venous and arterial dilation with a consequent fall in pulmonary and systemic vascular resistances. Milrinone produces different adverse effects like bronchospasm, chest pain, hypokalemia and headache **[5]**.

Theophylline is a derivative of xanthine alkaloid. It is used as bronchodilator. It reduces the asthma by relaxing the smooth muscle of airway and inhibiting the mediators release from mast cells. It antagonizes adenosine which is a potent bronchoconstrictor **[6]**. It is non-specific phosphodiesterase enzyme inhibitor and produces weak positive inotropic effect **[7]**. The present study was carried out to evaluate the effect of theophylline on the positive inotropic effect of milrinone used in the treatment of congestive heart failure.

MATERIALS AND METHODS

Drugs and chemicals

Milrinone (Hochest Pharmaceuticals), Theophylline (Unilab Chemicals and Pharmaceuticals). All other reagents and chemicals used in the experiments were of analytical grade.

Preparation of milrinone solution: A fresh solution of milrinone, 1 mg/ml in 0.05 M HCl was diluted to 75, 150 and 300 µg/ml and administered as a bolus injection in the isolated frog heart. A stock solution of 1 mM of milrinone prepared in 0.05 M HCL was added to the Chenoweth-Koelle solution to obtain the final concentration of 1µM, 2 µM and 4 µM for the experiment on isolated guinea pig left atria

Preparation of theophylline solution: A stock solution of 1 mM theophylline prepared in distilled water was added to the Chenoweth-Koelle solution and frog ringer solution to obtain the concentration of 1 µM.

Animals

Frogs of either sex were used in the study. They were kept in tank filled with water. Guinea pigs of either sex (300 - 400 gm each) were used throughout the study. Animals were housed under standard environmental condition, fed standard laboratory diet and water ad libitum. The protocol was approved by the institutional animal ethical committee (GCPA/IAEC/2011/235).

Experimental procedures for measurement of inotropic effect in isolated frog heart:

Frog heart was dissected out and perfused with frogs ringer solution having a composition (mM): NaCl- 111.11, $CaCl_2$- 1.08, KCl- 1.88, NaH_2PO_4- 0.07 $NaHCO_3$- 2.38, and glucose 11.1 (pH 7.8). Ringer solution was kept at room temperature and constantly bubbled with air. 0.1 ml of milrinone was administered as a bolus injection. Theophylline was administered as a part of ringer solution. Student's Physiograph was used to record contractile force developed by the heart through force displacement transducer. It was expressed in gram **[8, 9]**.

Experimental procedures for Measurement of inotropic effect in isolated guinea pig left atria:

Guinea pig heart was dissected out and kept in physiological salt solution bubbled with carbogen. Left atrium was rapidly isolated from the heart of animal and secured to bipolar platinum electrode through apical margin and basal end of the atrium was attached to a force displacement transducer with resting tension of 1g. It was suspended in a 20 ml jacketed organ bath containing Chenoweth-Koelle solution of composition (mM): NaCl: 135, $CaCl_2$: 2.18, KCl: 5, $NaHCO_3$: 19 $MgCl_2$: 2, and glucose: 9.9 (pH 7.4). Milrinone was administered by adding to the Chenoweth-Koelle solution containing theophylline. The atrium was stimulated electrically by bipolar platinum electrode connected to a stimulator. Student's Physiograph was used to record contractile force developed by the heart through force displacement transducer and expressed in gram **[10]**.

Statistics

The results of all experiments were reported as mean ± S.E.M. Statistical analysis was carried out using Student's 't'-test. A significance level of $P < 0.05$ was regarded as statistically significant. Percent of Synergistic effect = 100 x [(FCMT-FCM)/FCM] Where, FCM = Force of contraction (g) produced by milrinone, FCMT = Force of contraction (g) produced by milrinone with theophylline.

RESULTS

Inotropic effect of milrinone and milrinone with theophylline in isolated frog heart

Milrinone produced dose dependent positive inotropic effect in the isolated frog heart. Milrinone showed increase in magnitude of inotropic effect in the dose range of 75 µg to 300 µg. Theophylline 1 µM produced increase in the inotropic effect of milrinone

by 66.66%, 39.39% and 34.14% at the dose of 75 µg, 150 µg and 300 µg respectively as shown in **Table 1**.

Inotropic effect of milrinone and milrinone with theophylline in isolated guinea pig left atria

Milrinone produced dose dependent positive inotropic effect in the isolated guinea pig left atria. Milrinone showed increase in magnitude of inotropic effect in the dose range of 1 µM to 4 µM. Theophylline 1 µM produced increase in the inotropic effect of milrinone by 68.42%, 50.00% and 27.90% at the dose of 1 µM, 2 µM and 4 µM respectively as shown in **Table 2**.

Table 1: Positive inotropic effect of milrinone and milrinone with theophylline in isolated frog heart

Sr. No.	Group	Dose	Force of contraction (g)	Synergistic effect (%)
1	Control		1.1 ± 0.21	
2	Milrinone	75 µg	2.1 ± 0.26	
3	Milrinone + Theophylline	75 µg 1 µM	3.5 ± 0.27	66.66
4	Milrinone	150 µg	3.3 ± 0.29	
5	Milrinone + Theophylline	150 µg 1 µM	4.6 ± 0.32	39.39
6	Milrinone	300 µg	4.7 ± 0.29	
7	Milrinone + Theophylline	300 µg 1 µM	6.1 ± 0.34	34.14

Values are mean ± standard error of mean. $P < 0.05$ vs. control, student's '*t*' test

Table 2: Positive inotropic effect of milrinone and milrinone with theophylline in isolated guinea pig left atria

Sr. No.	Group	Dose (M)	Force of contraction (g)	Synergistic effect (%)
1	Control		0.7 ± 0.19	
2	Milrinone	1 µM	1.9 ± 0.24	
3	Milrinone + Theophylline	1 µM 1 µM	3.2 ± 0.22	68.42
4	Milrinone	2 µM	3.0 ± 0.26	
5	Milrinone + Theophylline	2 µM 1 µM	4.5 ± 0.27	50.00
6	Milrinone	4 µM	4.3 ± 0.31	
7	Milrinone + Theophylline	4 µM 1 µM	5.5 ± 0.37	27.90

Values are mean ± standard error of mean. $P < 0.05$ vs. control, student's '*t*' test

DISCUSSION

Milrinone is Phosphodiesterase III enzyme inhibitor used in the therapy of acute heart failure. It decreases the degradation of cyclic adenosine monophosphate (cAMP) to 5 AMP causing an increase in intracellular concentrations of cyclic AMP **[11]**. Elevated level of cellular cyclic AMP mediates the phosphorylation of protein kinases which stimulates cardiac calcium channels. Increase level of calcium in the cytosol trigger calcium induces calcium release from the sarcoplasmic reticulum which further enhances calcium concentration in the

cytosol leads to actin and myosin interaction through calcium binding to troponin C producing myocardial contraction. Thus increased influx of calcium from the sarcoplasmic reticulum during the cardiac action potential produces positive inotropic effect by increase in the force of cardiac contraction **[12, 13]**.

Theophylline belongs to a class of xanthenes drugs used to treat lung diseases such as asthma and chronic obstructive pulmonary disease **[14]**. It is a bronchodilator, central nervous system stimulant and cardiac stimulant. It causes airway smooth muscle relaxation by nonselective inhibition of phosphodiesterases enzymes causing increase in the intracellular cyclic AMP concentrations and activation of protein kinases. It act as nonselective adenosine receptor antagonist and produces its cardiac effects **[15]**.

In present study milrinone produced dose dependent positive inotropic effect in both the amphibian and mammalian myocardia. Positive inotropic effect of milrinone was increased significantly when given with theophylline. Thus theophylline produced synergistic effect in the positive inotropic response of milrinone. Hence Synergistic composition of milrinone and theophylline can minimize the effective dose of milrinone in the treatment of congestive heart failure will results in reduction of dose dependent adverse effect of milrinone. Theopylline will also be helpful in the reduction of the bronchospasam produced by milrinone.

CONCLUSION

These results indicate that theophylline produces synergistic effect on the cardiac response to milrinone by enhancement in the positive inotropic effect. Synergistic composition of milrinone and theophylline will be more beneficial than milrinone alone in the treatment of congestive heart failure.

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