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SHORT COMMUNICATION

COMPARATIVE STUDY OF BETA-ADRENOCEPTOR BLOCKING EFFICACY OF TWO FORMULATIONS OF ESMOLOL *IN VIVO* AND *IN VITRO*

C. B. TRIPATHI, P. BHATTACHARYA, J. D. BHATT*
AND K. G. HEMAVATHI

Department of Pharmacology,
Medical College,
Baroda – 390 001

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Abstract : The objective of the present study was to compare the cardiovascular beta-blocking activity of two different formulations of esmolol. Spontaneously beating guiea-pig isolated atria and the heart rate and blood pressure of anaesthetized cat were employed in the study to compare the beta-blocking efficacy of the two formulations of esmolol using isoprenaline as an agonist. In guinea-pig isolated atria the standard esmolol formulation (Brevibloc) reduced basal atrial rate more significantly than the indigenously formulated esmolol (test formulation). Both the formulations produced similar parallel rightward shift of cumulative concentration response curves of isoprenaline with closely comparable pA_2 values. In anaesthetized cats, only indigenous esmolol formulation significantly decreased basal heart rate. Both the formulations did not modify the basal blood pressure and isoprenaline-induced fall in blood pressure, despite significantly blocking isoprenaline-induced tachycardia. It is suggested that both the formulations produced similar degree of beta-1 adrenoceptor blocking activity.

Key words : beta blocker, guiea-pig isolated atria, cat blood pressure, esmolol

INTRODUCTION

The relatively long duration of action of currently available intravenous beta-adrenoceptor blockers limits their usefulness in patients with acute myocardial ischaemia or infarction because of their potential for adverse effects which may not be rapidly reversible in these critically ill patients (1, 2). Thus in these acute care settings, short acting titrable beta-adrenoceptor blockers like esmolol with half life of about 9 min could be extremely useful since they permit precise control over the magnitude and duration of beta-blockade.

Recently an indigenously formulated (test) preparation of esmolol has been introduced, in the market for the clinical application. The objectives of the present

*Corresponding Author

study were to evaluate the beta-1 adrenoceptor blocking efficacy of the indigenous (test) formulation of esmolol in-vivo and also to compare its beta-1 adrenoceptor blocking efficacy with an imported (standard) formulation of esmolol.

METHODS

Guinea-pigs (350–450 g) and cats (3–4.5 kg) of either sex were used in the study.

In-vitro studies :

Guinea-pigs were sacrificed by stunning followed by exsanguination. The heart was quickly removed and placed in a Petri-dish containing warm oxygenated modified Hukovic-Krebs physiological salt solution of the following composition (In mM) :NaCl-118.4; KCl-4.7; $MgSO_4$, $7H_2O$; $CaCl_2$-2.5; $NaHCO_3$-12.5; glucose-11.7 and Na_2 EDTA-0.03. The two atria were dissected free and mounted in a 40ml organ bath containing the physiological salt solution maintained at 37 ± 0.5°C and continuously oxygenated. The tissue was allowed to stabilize over one hr with regular washes at every 15 minutes. Spontaneous atrial beats were recorded isotonically on smoked kymograph drum with the help of a Starling's heart lever. After initial equilibration, the preparation was exposed to graded concentrations of isoprenaline (0.5–32 ng/ml) added cumulatively, after each cumulative concentraton-response study with isoprenaline the preparation was allowed to recover to near basal atrial rate over 45–60 min with regular washes every 10 minutes. The preparation was then exposed to different concentrations (3.01×10^{-7}M; 3.01×10^{-6}M; 9.03×10^{-6}M; 3.01×10^{-5}M) of either standard or test esmolol formulation, for 30 min and the cumulative concentration-response curves to isoprenaline were re-elicited. Separate preparations were used for each concentration of standard and test esmolol formulations. The EC_{50} values of isoprenaline were determined in the absence and presence of different concentrations of either the standard or the test formulation. The EC_{50} concentration ratios were computed for different concentrations of each formulation and pA_2 values were derived by the graphical method described by Arunalakshana and Schild (3).

In-vivo studies :

The cats were anaesthetized with sodium pentobarbitone (40 mg/kg, iv) and ventilated with room air on a respirator when required. The vagus nerves were severed and right carotid artery as well as femoral vein were cannulated for the measurement of blood pressure and for administration of drugs, respectively. Responses to isoprenaline (0.5 µg/kg, iv bolus) were elicited before and after infusion of esmolol at different infusion rates (infusion rates 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25 µg/kg/min for 10 minutes). Separate groups of cats were used for the two formulations of esmolol. Degree of beta-adrenoceptor blockade was determined by calculation of percentage of inhibition of the isoprenaline-induced tachycardia and hypotension during the infusion of the blockers.

Statistical analysis :

Regression analysis was performed to draw the graph for the calculation of pA_2 value. Analysis of variance was performed to compare the differences among the

different groups. Paired 't' test was applied to compare the level of significance between two means. $P<0.05$ considered statistically significant.

Drugs :

Isoprenaline sulphate, indigenous formulation of esmolol [M/s USV (India) Ltd., Mumbai] and imported formulation of esmolol (Brevibloc; M/s Dupont Puerto Roco, USA). Each ml of the indigenous and the imported formulations of esmolol contains 250 mg of esmolol in 25% propylene glycol, aUSP; 25% alcohol, USP and water for injection USP. Buffered with sodium acetate and glacial acetic acid USP. Fresh solution of esmolol was prepared in normal saline for intravenous administration.

RESULTS

(a) In-vitro studies :

In atrial preparations both the standard and the test formulations of esmolol produced concentration-dependent decrease in basal atrial rate (144 ± 7 beats/min) except at the concentration of 9.03×10^{-6} M the inhibition produced by the standard formulation was less of than anticipated. However, at concentration of 3.01×10^{-6}M the standard formulation of esmolol produced greater inhibition of basal atrial rate than that produced by the test formulation (Table I).

Both the formulations of esmolol produced a concentration-dependent parallel rightward shift of cumulative concentration-response curve to isoprenaline in isolated artial preparation with nearly identical pA_2 (viz. Standard : 6.58 vs Test : 6.53).

TABLE I : Effect of the standard and the test formulations of esmolol on the change in basal atrial rate of guinea-pig isolated atria.

Concentrations of esmolol	*% decrease in basal atrial rate (Mean ± SEM)*	
	Standard	*Test*
3.01×10^{-7}M	3.0±5.8	2.6±3.1
3.01×10^{-6}M	23.3±5.4	6.0±1.1*
9.03×10^{-6}M	12.8±2.6	11.4±12.2
3.01×10^{-5}M	53.0±14.6	28.5±3.4

n = 6 for each concentration
*P<0.05 significantly different from the corresponding value of the standard formulation.

(b) In-vivo studies :

Mean basal heart rate and blood pressure of anaesthetized cat were 208 ± 8 beats/min and 102 ± 9 mmHg respectively. Repeated intravenous administration of isoprenaline (0.5 µg/kg) at every 10 min interval produced increase in heart rate (22 ± 3 beats/min) and decrease in blood pressure (33 ± 4 mmHg) in anaesthetized cats. These responses were reproducible for a 5 hour period. The standard formulation of esmolol did not alter the basal heart rate significantly at any of the infusion rate; however, the test formulation of esmolol significantly ($P<0.1$) decreased the basal heart rate (180 ± 5 beats/min) at doses of 1.6 µg/kg/min and above. Intravenous infusion of both the standard and the test formulations of esmolol significantly blocked isoprenaline-induced tachycardia in a dose-dependent manner (Fig. 1). Both the formulations of esmolol neither altered the basal blood pressure nor the hypotensive response to isoprenaline significantly at any of the infusion rate studied.

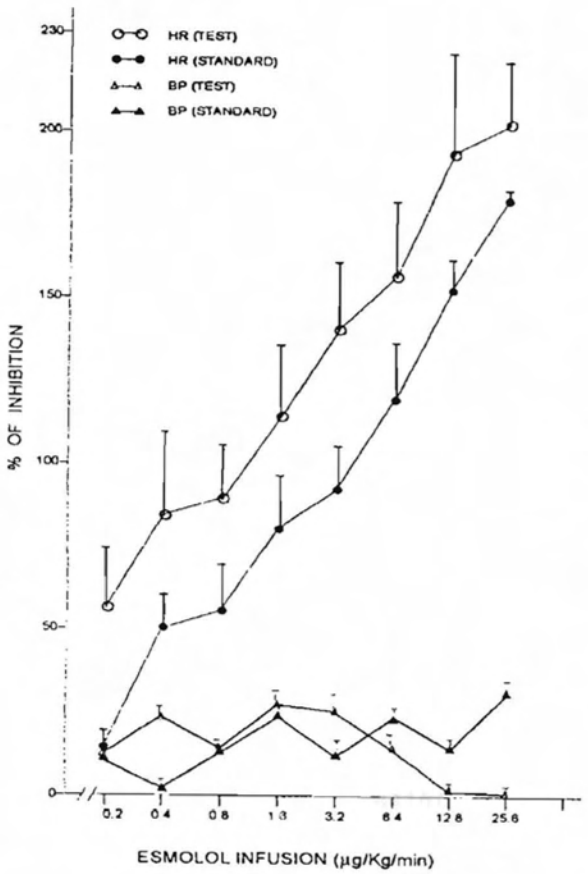

Fig. 1 : Effect of esmolol infusion on % of inhibition of isoprenaline induced decrease in blood pressure (mmHg) and increase in heart rate (beats/min) in anaesthetized cats. Esmolol was infused continuously at different rates for 10 minutes. Vertical lines indicate SEM (n=6)

DISCUSSION

The results of the present study suggest that the test formulation possesses cardioselective beta-adrenoceptor blocking activity comparable to that of the standard formulation. The pA_2 values of both the formulations of esmolol were nearly similar and was consistent with that reported in the literature (4) suggesting competitive antagonism at the same receptor site. The dose dependent inhibition of isoprenaline-induced tachycardia by the standard and the test formulations substantiated the beta-adrenoceptor blocking property of these formulations in our *in-vitro* study. Both the formulations exhibited cardioselective properties as prolonged infusion of these formulations did not modify the basal blood pressure as well as isoprenaline-induced reduction of blood pressure despite producing marked blockade of isoprenaline-induced tachycardia.

In summary the test formulation of esmolol possesses comparable cardioselective beta-blocking property to that of the standard formulation.

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