Comparison between the effects of Captopril and Lisinopril on Bradykinin-induced contraction in tracheal smooth muscle of guinea pig

JAVARIA ARSHAD MALIK¹, WAQAR AHMED SIDDIQUI², SEHRISH ZAFAR³

ABSTRACT

Background: ACE inhibitors are used for the treatment of hypertension and congestive heart disease may induce hyper-reactivity of the airways which may be associated with the accumulation of bradykinin and other inflammatory mediators present in the airways.

Aim: To compare the effect of captopril with lisinopril on bradykinin-induced tracheal smooth muscle contraction of guinea pig trachea.

Methods: A Comparative controlled in-vitro experimental study was carried out on the tracheal smooth muscle strips of guinea pig pretreated with indomethacin (10⁻⁵M), phentolamine (10⁻⁶M) and propranolol (10⁻⁶M) to remove the effect of endogenous Catecholamin and prostaglandins. The activity of trachealis smooth muscle was noted through the Isometric Force Displacement Transducer on a Four Channel Oscillograph. Cumulative concentration-response relationship was displayed by adding successive concentrations of bradykinin on the tracheal strips starting with 22µg to 132 µg/dl.

Results: Bradykinin produced concentration-dependent reversible contraction of isolated tracheal smooth muscle. The mean value of response achieved with 132 µg/dl of bradykinin in the presence of captopril was 51.33±2.79 and in the presence of lisinopril was 38.17±2.94. These ACE inhibitors shifted the concentration response curves of bradykinin to left and upward. On comparison among themselves it was observed that lisinopril as compared to captopril produced less enhancement of bradykinin-induced contraction of guinea pig trachea.

Conclusion: Lisinopril produce less enhancement of bradykinin induced tracheal smooth muscle contraction then captopril.

Keywords: ACE inhibitors, cough, Bradykinin, Captopril, lisinopril, guinea pig trachea

INTRODUCTION

Angiotensin-converting-enzyme inhibitor (ACE inhibitor) are used for the treatment of hypertension and congestive heart failure. These drug cause vasodilatation, hypovolemia, results in decreasing blood pressure and decreased myocardial oxygen demand. Their major action is to inhibit the angiotensin-converting enzyme, of the renin–angiotensin–aldosterone system¹. It is proposed that ACE inhibitor through blockade of angiotensin II, causes the reduction of vasoconstriction and water and sodium retention². Commonly used ACE inhibitors are captopril, lisinopril, enalapril and ramipril. Captopril is a Sulfhydryl-containing agent and Lisinopril is a Dicarboxylate-containing agent. Studies suggest that these ACE inhibitors may be useful not only in the treatment of essential hypertension, chronic heart failure but also in nephropathy³.⁴. Their adverse effect include hypotension, fatigue, headache, cough, hyperkalemia and renal impairment⁵. ACE inhibitors might increase inflammation-related pain, due to increase of bradykinin (a vasodilator peptide)⁶. Dry, irritant cough is reported in about 15% hypertensive patients due to accumulation of bradykinin and its degradation by ACE inhibitor⁷.

The exact mechanism of ACE inhibitor-induced cough is not known. However, it is proposed that bradykinin (BK) is degraded by ACE drugs and precipitated in the upper respiratory tract and bring airway smooth muscle contraction carried out via tachykinins released from C-fiber endings⁸. Lately it is thought that bradykinin is converted to inactive metabolites by ACE, therefore inhibition of this ACE enzyme leads to raised levels of bradykinin, which causes cough via bronchoconstriction. The incidence of ACE inhibitor-induced cough has been reported to be in the range of 5 to 35% among patients treated with these agents⁹.

This fact has been now proved in many aspects that all ACE Inhibitors are not equivalent¹⁰. ACE inhibitors differ in chemical structure¹¹. The structures of captopril, enalapril and lisinopril were compared in
MATERIALS AND METHODS

Chemicals: Bradykinin acetate and Phentolamine Hydrochloride was purchased from Sigma Chemical Co, USA. Captopril Disulfide and Lisinopril Dihydrate was kindly provided by Chemo S.A Lugano Brach, Hetero Drug Limited and Tanabe/Selikaku Japan respectively. Indomethacin Acetate by Shanghai-Chang-Hua industry limited China, and Propranalol Hydrochloride by Changzhou Yabang Pharmaceutical Company. All other chemicals used were purchased from local commercial sources. Solutions and dilutions of all drugs were prepared in the distilled water.

Experimental Procedure: Experiments performed were compiled with the rulings of the Institute of Laboratory Animal Resources Commission on Life Sciences National Research Council, and were approved by the PCGS committee for research, the National University of Science and Technology Islamabad Pakistan (NUST). Guinea pigs of either sex, of the Dunkin Hartley variety (500 to 600g) were housed at the animal house of the Army Medical College, Rawalpindi, NUST University, at room temperature. They were given tap water ad libitum and a standard diet. The guinea pigs were killed by cervical dislocation after approval of method by ethical committee. The tracheal tube was taken out and cut into rings 2–3 mm wide, each containing about two cartilages. The tissue preparation was mounted to an isolated tissue bath of 50 ml, capacity containing Kreb’s Henseleit solution at 37º C and was aerated with oxygen continuously. The tissue was allowed a period of equilibration for 45 minutes against an imposed tension of two grams. A tension of one gram was applied to the tracheal strips continuously throughout the experiments. The tracheal smooth muscle activity was measured with an Isometric Force Displacement transducer (Harvard model no 72-4494) and was recorded on Four Channel Oscillograph (Harvard model no 50-9307). After the equilibration period tracheal muscle preparation was incubated for 15 minutes with indomethacin (10⁻⁶M), with phentolamine (10⁻⁵M) and with propranolol (10⁻⁶M) to eliminate the effect of endogenous prostaglandins and catecholamines. These drugs were added simultaneously in all the experiments and after 15 minutes experiments were started with this preparation.

In group I, after the preincubation period with the baseline tension of 1 gram cumulative concentration-response curves of bradykinin was obtained using concentrations 22, 44, 66, 88, 110 and 132μg/dl. When the plateau was achieved with the first concentration of bradykinin, then the subsequent dose was added to the tissue bath without washing the previous concentration.

In group II, cumulative concentration-response curve of bradykinin was obtained using the same concentrations of bradykinin as in the previous experiments in the presence of captopril 10⁻⁵ concentration.

In group III, cumulative concentration-response curve of bradykinin was obtained using the same concentrations of bradykinin as in the previous experiments in the presence of lisinopril 10⁻⁵ concentration.

In group IV, cumulative concentration-response curve of captopril was obtained using concentrations 1, 1.5, 2, 2.5 and 3μM of captopril in the presence of fixed concentration of bradykinin 66μg/dl. This concentration of bradykinin has been chosen which causes consistent and submaximal effects, enabling us to observe potentiation or inhibition of contraction. Maximum response of smooth muscle contraction with captopril 3 µM concentration was taken as hundred percent and effects with lisinopril were compared to that.
In group V, cumulative concentration-response curve of lisinopril was obtained using concentrations 1, 1.5, 2, 2.5 and 3 µM of lisinopril in the presence of fixed concentration of bradykinin 66µg/dl. Six experiments were performed in the same way to get six recordings in all the five groups.

Statistical analysis
The results were expressed as Means±Standard deviation. The arithmetic means of amplitudes of contractions and S.D were calculated using SPSS version 18. In order to find the significance of the difference between two observations ‘student t test’ was used. P value <0.05 was considered significant.

RESULTS
Captopril enhanced the amplitude of tracheal contraction from mean value of 7.7mm to 35.6mm. Semi logarithm concentration-response curve of bradykinin in the presence of captopril shifted to the left and upwards.

Lisinopril at $10^{-5}$ M concentration enhances the amplitude of tracheal contraction from mean value of 7.7mm to 29.1mm. The concentration response curve of bradykinin in the presence of lisinopril was shifted to the left and upwards.

In comparison of Control Group I (Bradykinin) and Group II (Captopril+Bradykinin) The mean values of response produced by each concentration of bradykinin used, compared between Group I and Group II were found statistically significant ($P<0.05$). In comparison of Control Group I (Bradykinin) and Group III (Lisinopril+Bradykinin) The mean values of response produced by each concentration of bradykinin used compared between Group I and Group III were found statistically significant showing $P$ values of 0.002, 0.002, 0.00, 0.00 and 0.001 ($P<0.05$). Comparison of concentration response curves of two drugs are shown in figure I. Figure I: Cumulative log concentration-response curves of bradykinin in the presence of fixed concentrations of captopril ($10^{-5}$ M) and lisinopril ($10^{-5}$ M).

In the second set of experiments, bradykinin in a fixed concentration of 66µg/dl was added in the organ bath and then concentration-response curve was obtained by increasing concentration of captopril. Same procedure was repeated with lisinopril. This was carried out to determine the concentration-dependent effects of these ACE inhibitors on bradykinin induced contraction. The concentration of bradykinin (66µg/dl) was chosen because it produced consistent and submaximal effects enabling us to observe potentiating or inhibition of contraction. Results were similar to first set of experiments in which lisinopril had produced less bradykinin-induced contraction than captopril. Enhancement of the contraction produced by lisinopril was near to the effect produced with captopril. Cumulative concentration-response curve with captopril has been taken as the control and curves with lisinopril was compared to that. The shift of the curve is statistically significant ($P<0.05$) (figure II).

Table 1: Comparison of responses to bradikinin between group 11 (captopril + bradykinin) and group 111 (lisinopril +bradykinin) with a drug dose of 22, 44 and 66 µg

<table>
<thead>
<tr>
<th>Tissue 1-6</th>
<th>Group 11</th>
<th>Group 111</th>
<th>Group 11</th>
<th>Group 111</th>
<th>Group 11</th>
<th>Group 111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of drug</td>
<td>22 µg</td>
<td>22 µg</td>
<td>44 µg</td>
<td>44 µg</td>
<td>66 µg</td>
<td>66 µg</td>
</tr>
<tr>
<td>Mean Response(mm)</td>
<td>13.00</td>
<td>12.83</td>
<td>26.5</td>
<td>23.00</td>
<td>35.00</td>
<td>29.50</td>
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<tr>
<td>SD</td>
<td>10.47</td>
<td>8.86</td>
<td>4.37</td>
<td>5.40</td>
<td>3.16</td>
<td>4.64</td>
</tr>
<tr>
<td>P value</td>
<td>0.03</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 2: Comparison of responses to bradikinin between group 11 (captopril + bradykinin) and group 111 (lisinopril +bradykinin) with a drug dose of 88, 110 and 132 µg

<table>
<thead>
<tr>
<th>Tissue 1-6</th>
<th>Group 11</th>
<th>Group 111</th>
<th>Group 11</th>
<th>Group 111</th>
<th>Group 11</th>
<th>Group 111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of drug</td>
<td>88 µg</td>
<td>88 µg</td>
<td>110 µg</td>
<td>110 µg</td>
<td>132 µg</td>
<td>132 µg</td>
</tr>
<tr>
<td>Mean Response(mm)</td>
<td>41.67</td>
<td>35.0</td>
<td>46.5</td>
<td>36.67</td>
<td>51.33</td>
<td>38.17</td>
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<tr>
<td>SD</td>
<td>3.33</td>
<td>6.36</td>
<td>3.94</td>
<td>6.53</td>
<td>6.83</td>
<td>7.19</td>
</tr>
<tr>
<td>P value</td>
<td>0.00</td>
<td>0.038</td>
<td>0.009</td>
<td>0.009</td>
<td>0.009</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Comparison between Captopril and Lisinopril on Bradykinin-induced contraction

Fig. I: Cumulative log concentration-response curves of Captopril and lisinopril in the presence of fixed concentration of bradykinin (66 µg/dl).

DISCUSSION

ACE inhibitors are used for the treatment of hypertension and congestive heart disease may induce hyperreactivity of the airways with occurrence of a persistent dry cough, wheezing and dyspnoe in some of the patients. It is supposed that the hyperreactivity is associated with the accumulation of tachykinins, bradykinin and other inflammatory mediators present in the airways.

According to our study, captopril enhanced the amplitude of tracheal contraction from mean value of 7.7 mm to 35.6 mm. Our study is in line with number of studies. One of the study reported that extreme contraction of tracheal smooth muscle is responsible for hyperresponsiveness of airway. This hyperresponsive airways show increased sensitivity to certain bronchoconstrictors, and the resulting airway obstruction is highly responsive to bronchodilators. Another study stated that ACE inhibitors are often linked with an increased occurrence of bronchial responsiveness and cough that may cause more deterioration of patients with altered pulmonary function.

Present study has observed that lisinopril at $10^{-5}$ M concentration enhances the amplitude of tracheal contraction from mean value of 7.7 mm to 29.1 mm. A study found that lisinopril enhanced the cough response persuaded by both chemical and mechanical stimulation due to increase in the numeral of coughs induced by each stimulation.

The results of a study stated the central role of lisinopril mediated by an accumulation of substance P and bradykinin.

We also compared the response of bradykinin alone with the response produced by the combination of bradykinin with captopril. This shows an increased effect with a P value is <0.05. A study is experimentally proved that ACE inhibitors give the effects on the vascular system and increase the indirect effects of bradykinin on B2 receptors. ACE inhibitors directly activate B1 receptors via the zinc-binding present in B1 receptor.

According to a study bradykinin has been associated as a mediator of the acute pathophysiological and inflammatory outcomes of respiratory tract infections and may be responsible for chronic diseases of lung. Study experimentally proved that Bradykinin may stimulate cough and other conditions. It is found that these cough responses quickly desensitized, with desensitization of B2 receptor. Bradykinin-evoked cough was made effective by inhibition of both angiotensin-converting enzyme and neutral endopeptidase.

Mean dose of BK is needed to produce 100% increase in airway pressure. The dose-response
curve for the effect of BK was significantly shifted to the left by the captopril. Data suggest that ACE degrade BK in the airway lumen without the involvement of kininase II26.

In comparison of Control Group I (Bradykinin) and Group III (Lisinopril +Bradykinin) The mean values of response produced by each concentration of bradykinin used compared between Group I and Group III were found statistically significant (P<0.05).

A study found that bradykinin (BK) causes sensitization of airway sensory neurons and an increase in the cough reflex of experimental animal. It is suggested that BK trigger the production of prostaglandin synthesis and increase the release of pro-inflammatory neuropeptides from neurons, a system that may be observed during inflammation, and this can be stopped by a bradykinin B2 receptor antagonist27.

Bradykinin (BK) has many effects on airway function which may be applicable in obstructive airways disease in both animal and human. These effects are carried out via B2-receptors. Bradykinin is a effective bronchial vasodilator, increases microvascular leakage, triggers mucus secretion and epithelial cells to release bronchodilators and also triggers mucus secretion leading to response of bronchoconstriction, neurogenic inflammation and coughing via release of neuropeptides from sensory nerves28.

REFERENCES