Role of liquid membrane phenomenon in biological actions of ACE inhibitors, captopril and lisinopril

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Received 3 September 2000; accepted 2 February 2001

The liquid membrane phenomenon in angiotensin converting enzyme (ACE) inhibitors namely, captopril and lisinopril has been studied. Hydraulic permeability data have been obtained to demonstrate the existence of the liquid membrane in series with a supporting membrane generated by the ACE inhibitors. Data on the transport of the relevant permeants in presence of the liquid membrane formed by ACE inhibitors indicate that liquid membrane phenomenon is likely to play a significant role in the action of ACE inhibitors.

In the present study, a cellulose acetate micro filtration membrane/water interface has been chosen as the site for the liquid membrane, so that the specific and active interaction of the drug with the components of the biological membranes is ruled out and the data on the passive transport alone are obtained.

Materials and Methods
Captopril (Wokhard Ltd., Aurangabad), Lisinopril (Lupin Lab.Ltd., Aurangabad), calcium chloride A.R. (Loba Chemie Pvt Ltd., Mumbai) were obtained. Potassium chloride A.R. and sodium chloride A.R. (Ranbaxy Lab. Ltd., Nagar), D-glucose (Qualigens Fine Chemicals, Mumbai), sterile nor adrenaline concentrate I.P (Samarth Pharm Pvt., Mumbai), dopamine hydrochloride injection U.S.P. (Neon Antibiotics Pvt. Ltd., Mumbai), adrenaline tartarate injection I.P. (Harson Lab., Baroda), and distilled water, distilled once over the potassium permanganate in an all pyrex glass still were used.

![Captopril](https://example.com/captopril.png)

![Lisinopril](https://example.com/lisinopril.png)

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The critical micelle concentration (CMC) of aqueous solutions of captopril and lisinopril were determined from the variation of the surface tension with concentrations. The CMC values derived were $6 \times 10^{-1}$ M and $7 \times 10^{-4}$ M for captopril and lisinopril, respectively. The surface tension was measured using a Du Nouy surface tensiometer (Komal Scientific Co., Mumbai).

An all glass cell described earlier was used for the transport studies. A sartorius cellulose acetate micro filtration membrane (Catalog No. 11107, pore size 0.2 μm of thickness $1 \times 10^{-4}$ m and area $2.55 \times 10^{-5}$ m$^2$) acted as a support for the liquid membrane and separated the transport cell into compartments C and D (Fig. 2). For measurements of hydraulic permeability, aqueous solutions of captopril and lisinopril of various concentrations, ranging from 0 to $18 \times 10^{-4}$ M and 0 to $21 \times 10^{-4}$ M respectively, were placed in compartment C and compartment D was filled with distilled water. The concentration ranges of the drug were so chosen to obtain data both above and below the CMC of the respective drug. Known pressures were applied on compartment C by adjusting the pressure head and the consequent volume flux was measured by noting the rate of advancement of liquid meniscus in the capillary with a cathetometer of least count 0.001 cm and a stopwatch reading up to 0.1 sec. Magnitude of the applied pressure was also measured by noting the position of the pressure head with the Cathetometer. During the volume flux measurements, the solution in compartment C was well stirred and the electrodes E$_1$ and E$_2$ were electrically short-circuited. The method of measurement has been described earlier.

For measurement of solute permeability ($\omega$) of the relevant permeants, the equation was used:

$$\omega = \frac{J_s}{\Delta \pi} J_s = 0$$

where $J_s$ and $J_c$ stand for the solute flux and volume flux per unit area of the membrane, respectively and $\Delta \pi$ is the osmotic pressure difference across the membrane. The method of measurement has been described earlier. Compartment C of the transport cell was filled with solution of known concentration of the permeants in the aqueous solution of captopril concentration, $12 \times 10^{-4}$ M (2 CMC) and lisinopril concentration, $14 \times 10^{-4}$ M (2 CMC). Compartment D was filled with distilled water. In control experiments, no ACE inhibitor was used. Concentration of the drugs in these experiments was always kept higher than their CMC to ensure complete coverage of the supporting membrane with the liquid membrane generated by the drugs. All measurements were made constant temperature using a thermostat set at 37 ± 0.1°C.

The amounts of the various permeants transported in compartment D in a known period of time were estimated as follows:

(i). Cations—The amounts of sodium, potassium and calcium were estimated using flame photometer (Model CL 22A, Elico-India); (ii). Biogenic amines—The amounts of adrenaline, noradrenaline and dopamine were estimated using UV VIS spectrophotometer (SL 159, Elico India) by measuring absorbance at 282.4 nm ($\lambda_{max}$); (iii). Glucose was estimated using UV VIS spectrophotometer (SL 159 Elico India) by GOD/POD method.

Results and Discussion

The Hydraulic permeability data at all concentrations of captopril and lisinopril were found to be in accordance with the linear relationship

$$J_s = L_p \cdot \Delta \pi$$

where $J_s$ represents the volume flux per unit area of the membrane, $\Delta \pi$ the applied pressure difference, and $L_p$ the hydraulic conductivity coefficient. The values of $L_p$ estimated from the slopes of the $J_s$ versus $\Delta \pi$
plots, show a progressive decrease up to its CMC beyond which, it becomes more or less constant (Tables 1 and 2). This is indicative of progressive coverage of the supporting membrane with the liquid membrane generated by the drug in accordance with the kesting's hypothesis. At the CMC, coverage of the supporting membrane with the drug liquid membrane is complete. The values of \( L_p \) are expressed as arithmetic mean ± standard deviation.

Analysis of the hydraulic permeability data in the light of the mosaic membrane model further supports the existence of the liquid membrane in series with the supporting membrane. Following the arguments given earlier, it can be shown that when the concentration of the surfactant is \( n \) times it’s CMC, \( n \) being less than or equal to 1, the values of \( L_p \) would be equal to \( \left[ \left( \frac{n}{L_{sp}} + \frac{1}{L_p} \right) \right] \), where \( L_{sp} \) and \( L_p \) represent the values of \( L_p \) at 0 and the CMC of the surfactant respectively. The values of \( L_p \) thus computed for 0.25 CMC, 0.5 CMC and 0.75C M of captopril and lisinopril are in good agreement with the experimentally determined values (Tables 1 and 2).

**Solute permeability data and biological actions**

The solute permeability for sodium ions enhanced in the presence of liquid membrane generated by captopril and lisinopril (Table 3). These observations are consistent with the reports that ACE inhibitors produce natriuresis (excretion of abnormal amounts of sodium in the urine). So, the liquid membrane generated by the captopril and lisinopril in kidney may also be contributing by increasing the transport of sodium that leads to natriuresis. The observed trend is also in correlation with the substantial lowering of blood pressure in sodium depleted (hyponatremic) patients than the sodium replete patients with single dose of captopril.

ACE inhibitors may rarely cause hyperkalemia in patients with renal insufficiency or in patient taking potassium-sparing diuretics, potassium supplements, \( \beta \)-adrenoceptor blockers or NSAID. Also, significant retention of potassium is encountered with ACE inhibitors in patients with normal renal function who are not taking other drugs that cause potassium retention. So, retention of potassium ion in the blood that leads to hyperkalemia is in consistent with solute permeability observations (Table 3) that transport of potassium ions is reduced when compared to control. So, the liquid membrane generated by both the ACE inhibitors captopril and lisinopril may reduce the transport of potassium into the urine by kidney, which may lead to hyperkalemia.

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**Table 1—Values of \( L_p \) at various concentrations of captopril**

<table>
<thead>
<tr>
<th>Captopril concentration ((\times 10^3 \text{ M}))</th>
<th>( L_p \times 10^5 ) ((\text{m}^{-1} \text{s}^{-1} \text{N}^{-1}))</th>
<th>( L_p \times 10^5 ) ((\text{m}^{-1} \text{s}^{-1} \text{N}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0)</td>
<td>0.875±0.042</td>
<td>0.715±0.032</td>
</tr>
<tr>
<td>1.5</td>
<td>0.714±0.045</td>
<td>0.556±0.038</td>
</tr>
<tr>
<td>3.0</td>
<td>0.545±0.028</td>
<td>0.397±0.018</td>
</tr>
<tr>
<td>4.5</td>
<td>0.380±0.017</td>
<td>0.238±0.018</td>
</tr>
<tr>
<td>6.0</td>
<td>0.227±0.016</td>
<td>0.200±0.014</td>
</tr>
<tr>
<td>12.0</td>
<td>0.227±0.016</td>
<td>0.200±0.014</td>
</tr>
<tr>
<td>18.0</td>
<td>0.227±0.016</td>
<td>0.200±0.014</td>
</tr>
</tbody>
</table>

**Table 2—Values of \( L_p \) at various concentrations of lisinopril**

<table>
<thead>
<tr>
<th>Lisinopril concentration ((\times 10^3 \text{ M}))</th>
<th>( L_p \times 10^5 ) ((\text{m}^{-1} \text{s}^{-1} \text{N}^{-1}))</th>
<th>( L_p \times 10^5 ) ((\text{m}^{-1} \text{s}^{-1} \text{N}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0)</td>
<td>1.166±0.085</td>
<td>1.025±0.086</td>
</tr>
<tr>
<td>1.75</td>
<td>1.002±0.078</td>
<td>0.828±0.052</td>
</tr>
<tr>
<td>3.5</td>
<td>0.894±0.054</td>
<td>0.742±0.054</td>
</tr>
<tr>
<td>5.25</td>
<td>0.744±0.022</td>
<td>0.601±0.032</td>
</tr>
<tr>
<td>7.0</td>
<td>0.601±0.032</td>
<td>0.532±0.028</td>
</tr>
<tr>
<td>14.0</td>
<td>0.412±0.019</td>
<td>0.412±0.019</td>
</tr>
</tbody>
</table>
The depolarization of vascular smooth muscle is primarily dependent on the influx of calcium ions. An increase in cytosolic calcium results in the constriction of smooth muscle through myosin light chain. ACE inhibitors lower systemic vascular resistance and mean diastolic and systolic blood pressure in various hypertensive states. They dilate both veins and arterioles. These reports are consistent with the observation (Table 3) that both captopril and lisinopril reduce the transport of calcium ion compared to control. So, by reducing the transport of calcium ions to the vascular smooth muscle by the liquid membrane generated by captopril and lisinopril may also contribute to the vasodilatation effect of the drugs.

A reversible side effect of ACE inhibitors is spillage of glucose into the urine in the absence of hypoglycemia, the mechanism of which is unknown. Our studies have shown enhanced transport of glucose across the liquid membrane generated by captopril and lisinopril (Table 3) compared to control. The observed glycosuria may have bearing with the liquid membrane phenomenon of the drugs. It has already been shown that lisinopril treatment is not associated with an increased incidence of hypoglycemic events in diabetic patients and it does not affect glycemic control. Also, it has been shown in a few case reports that hypoglycemia result from the combination of an ACE inhibitor and an oral hypoglycemic agent. In these cases, an enhanced transport of the liquid membrane may be influencing the glucose influx into the cell.

Dopamine is the immediate metabolic precursor of the noradrenaline and adrenaline. The cardiovascular effects of dopamine are mediated by several distinct types of receptors that vary in their affinity for catecholamine. At high concentrations, dopamine activates vascular alpha-one adrenergic receptors, leading to vasoconstriction and increase in systolic blood pressure. Although the transport of noradrenaline and adrenaline are not affected much, dopamine transport is highly affected in presence of liquid membranes generated by the captopril and lisinopril (Table 3). These trends of catecholamine transport are consistent with antihypertensive effect of ACE inhibitors. The impendence in catecholamine transport may be contributing to the transport of circulating catecholamine especially dopamine and observed antihypertensive effect may be due to the combination of ACE inhibition and decreased dopaminergic activity.

Acknowledgement
Thanks are due to All India Council for Technical Education, New Delhi for their financial support (grant No. 8017/RDII/BOR/95), Sri Sha Bra Chandramoulsvara Swamiji, President, TMAE Society, Harapanahalli, and to Wockhardt Ltd. and Lupin Laboratories Ltd., Aurangabad for gift samples of captopril and lisinopril and to Mr Subhajit Pual for assistance in preparation of revised manuscript.

References


