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Role of liquid membrane hypothesis in the mechanism of action of Cefuroxime sodium

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ABSTRACT

The role of liquid membrane hypothesis has been studied in the mechanism of action of cephalosporins like cefuroxime sodium. In the present study transport of selected permeants (glucose, p-amino benzoic acid and ions like magnesium, ammonium, phosphate, calcium, sodium, potassium and chloride) through liquid membrane generated by cefuroxime sodium in series with supporting membrane has been studied. The results indicate that the liquid membrane generated by cefuroxime sodium inhibit the transport of various essential bio-molecules and permeants into the cell. The data indicate that modification in permeability of different permeants in the presence of the liquid membrane is likely to play significant role in the biological actions of cefuroxime sodium. It seems that the formation of liquid membrane by cefuroxime may also contribute for the bactericidal activity of the drug, in addition to its conventionally established mechanism i.e. inhibition of cell wall synthesis.

Key words: Cefuroxime sodium, liquid membrane, altered permeability, antimicrobial activity.

INTRODUCTION

Many Pharmacologically active compounds are amphiphillic in nature, which may undergo different types of association, and whose site of action is frequently, the Plasma membrane. In many cases excellent correlation between surface activity of drugs and their biological actions was demonstrated [1-6]. Earlier reports show that the wide variety of drugs acts by common mechanism i.e due to their surface activity, which governs their action [7-11]. It has been shown that the surface-active drugs at the site of their action generate liquid membranes, which act as barrier to the transport of essential permeants. There are reports that many antmicrobial agents like norfloxacin, ciproflaxacin, etc are amphiphillic in nature and generate the liquid membrane at the site of their action. In addition reports are indicating that this phenomenon also contributes for their anti-microbial action[12].

In the present study Cefuroxime sodium, (Fig. 1) a second generation broad-spectrum cephalosporin was used, which is having both hydrophilic and hydrophobic domains in its structure. Cefuroxime sodium is therefore expected to generate a liquid membrane at the interface. The cytoplasmic membrane consists of Phospholipids and Proteins. The phospholipid

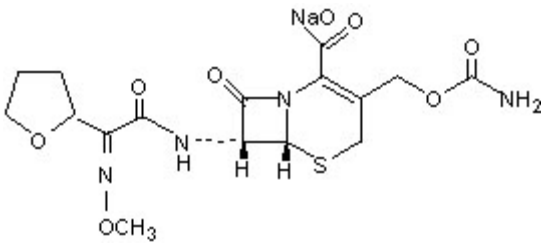

Fig. 1: Cefuroxime sodium

molecules are arranged in a bimolecular layer with polar groups directed towards both sides. The non-polar part of Cefuroxime sodium is likely to be placed across the hydrophobic core of the membrane at critical micellar concentration (CMC) of Cefuroxime sodium. The Cefuroxime sodium may form the liquid membrane over the cytoplasmic membrane. Because of the liquid membrane formed by Cefuroxime sodium the transport of essential ions, glucose and p-amino benzoic acid may alter. The present study is designed so as to assess this hypothesis and its role in the anti-microbial activity of the study drug. Cellulose Acetate Microfiltration membrane has been specifically chosen as the site for formation of liquid membrane.

MATERIAL AND METHODS

Materials

Cefuroxime sodium (KAPL Bangalore), Glucose , Calcium chloride , Sodium chloride, Ammonium chloride, Magnesium sulphate, Potassium dihydrogen phosphate. Para amino benzoic acid, Lecithin, Triple distilled water, Calcium diagnostic kit (ERBA), Sodium, potassium and chloride, diagnostic kit (ERBA), Glucose diagnostic kit (Agappe Diagnostics). The chemicals used were of Qualigens Chemicals Pvt Ltd. of AR grade.

Methods

The Critical Micellar Concentration (CMC) of aqueous Cefuroxime sodium was determined from the variation of surface tension with the concentration at 37 ± 0.1° C and was found to be 5×10^{-4}. Surface tensions were measured using a model–144 Du Nouy surface tensiomat (Komal scientific Co., Bombay–47). The all glass transport cell as shown in fig 2 [12] was used to obtain hydraulic permeability and solute permeability data. It essentially consists of two components, C and D separated by sartorius cellulose acetate micro filtration membrane (Cat.No.11107, pore size 0.2µm,

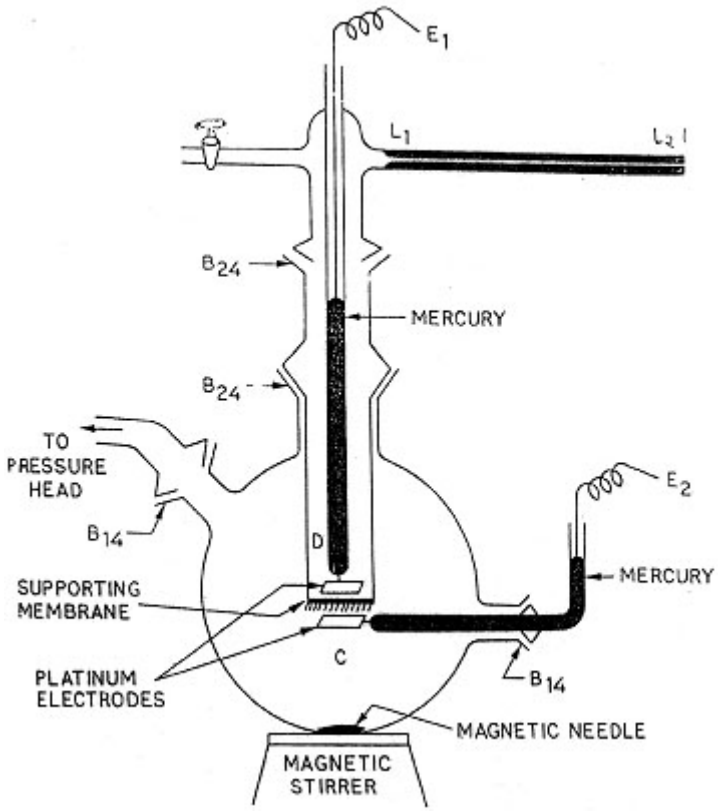

Fig. 2: All glass Transport Cell

thickness 1×10^{-4}m, area $2.55\times10^{-5}m^2$), which acts as a supporting membrane for the liquid membrane. The cellulose acetate membrane was coated with lecithin (1.919×10^{-5}M) and lecithin-cholesterol (1.919×10^{-5}M lecithin and 1.175×10^{-6}M cholesterol) so as to simulate the bacterial and human cell membranes respectively. For the measurement of hydraulic permeability data, aqueous solution of Cefuroxime sodium at various concentrations were placed in compartment C and compartment D was filled with de-ionized water. The concentration ranges of Cefuroxime sodium were so chosen that covers both above and below CMC. The hydraulic permeability was determined separately for Sartorius cellulose acetate micro filtration membrane coated with lecithin alone and cholesterol + lecithin. The procedure described in the earlier publications was adopted for obtaining the hydraulic permeability data.

For measurement of solute permeability (ω) of the relevant permeants, the equation [13-15] was used.

$$\omega = [Js / \Delta\pi]_{\underline{Jv=0}} \qquad ...(1)$$

Where Js and Jv are the solute flux and volume flux per unit area of the membrane, respectively. Dp is the Osmotic pressure difference across the membrane. For the measurement of w, one compartment of the transport cell (2CMC) was filled with aqueous solution of Cefuroxime sodium along with permeant. Other compartment was filled with de-ionized water. In control experiments, no drug was used; concentration of the drug in these experiments was always kept higher than its CMC to ensure complete coverage of the supporting membrane with the liquid membrane generated by Cefuroxime sodium. All measurements were made at constant temperature (37 ± 0.1°C) using a thermostat.

Estimation of permeants transported through liquid membrane generated by Cefuroxime sodium

The amounts of Sodium, Potassium, Chloride, Calcium and D-Glucose, were estimated using Star-21 semi-auto analyzer by utilizing diagnostic kits. The amount of p-amino benzoic acid, ammonium and Phosphate were estimated by using UV-VIS Spectrophotometer (Elico, SL - 164 India), amount of magnesium was estimated by using atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Hydraulic permeability data at various concentrations of Cefuroxime sodium were found to obey the linear relationship i.e.

$$Jv = Lp.\ \Delta p. \qquad ...(2)$$

Where Jv represents the volume flux per unit area of the membrane, Dp is the applied pressure difference. And Lp is the hydraulic conductivity coefficient. Values of Lp at various concentrations of Cefuroxime sodium were obtained

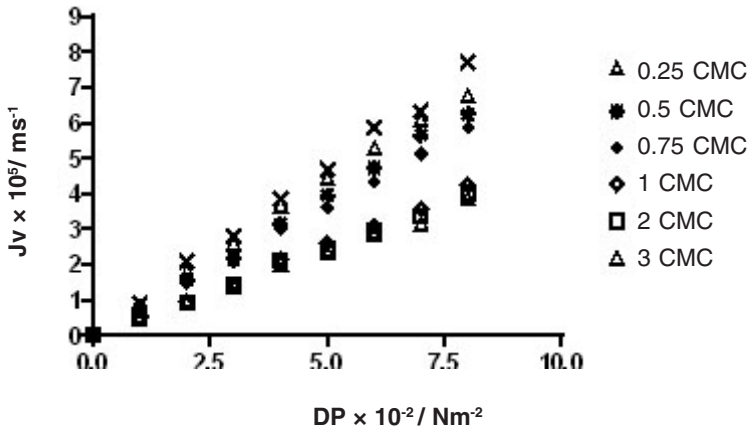

Fig. 3: The Hydraulic Permeability Data

from a plot Jv Vs Dp (slope of the plot is Lp) (Table.1) (Fig 3). The value shows decreasing trend with increasing concentration of Cefuroxime sodium up to CMC. Beyond which it becomes more or less constant. This is indicative of progressive coverage of the supporting membrane with liquid membrane generated by the drug in accordance with the Kesting hypothesis [16].

Table 1: Values of Lp at various concentrations of cefuroxime sodium

Critical Micellar Concentration CMC	Experimental Lp X 10^5 $m^3 S^{-1}N^{-1}$	Calculated Lp$X10^5$, $m^3 S^{-1}N^{-1}$
0	0.962± 0.300	
0.25	0.843±0.141	0.845±0.234
0.5	0.779±0.138	0.738±0.175
0.75	0.732± 0.122	0.615±0.142
1	0.533 ±0.096	
2	0.502±0.123	
3	0.486±0.162	
Lecithin	0.308±0.080	
Lecithin+1CMC drug	0.267±0.126	
L + C	0.380± 0.101	
L+C+1CMC Drug	0.306 ± 0.142	

The values are presented as arithmetic mean ±standard deviation of 10 determinations.
L + C = Lecithin + Cholesterol mixture

Analysis of hydraulic permeability data in the light of mosaic membrane model [17-18] further supports the existence of the liquid membrane in series with supporting membrane. Following the arguments given earlier[3] it can show that concentration of the surfactant is n' times its CMC, n being less than or equal to 1, the value of Lp would be equal to [(1-n) $L^s p + n L^c p$], where $L^s p$ and $L^c p$ represents the value of Lp at 0 and the CMC of the surfactant respectively. The values of Lp thus computed for 0.25CMC, 0.5CMC and 0.75CMC of Cefuroxime sodium are in good agreement with the experimentally determined values.

The hydraulic permeability data using aqueous mixtures of lecithin alone and lecithin with drug are given in table 1. It was earlier reported that 1.919×10^{-5}M lecithin aqueous solution form liquid membrane, which completely covers the supporting membrane indicate the fall in Lp values when 1 CMC solution was added to the compartment C, which provides additional evidence regarding incorporation of Cefuroxime sodium in lecithin membrane[19].

From the solute permeability (w) data (Table 2) it can be observed that Cefuroxime sodium

Table 2: Solute permeability (ω) of various permeants in presence of liquid membrane generated by Cefuroxime sodium in presence of lecithin – cholesterol mixtures

Permeant	Initial concentration	ω_0 (X 10^6) (moles $s^{-1}N^{-1}$)	ω_1 (X 10^6) (moles $s^{-1}N^{-1}$)	ω_2 (× 10^6) (moles $s^{-1}N^{-1}$)	ω_3 (× 10^6) (moles $s^{-1}N^{-1}$)
Chloride	500mg/Lt	0.102±0.086	2.028±0.059	2.043±0.048	1.721±0.035
Glucose	20mg/mL	29.380±0.627	19.044±0.095	17.950±0.334	15.848±0.132
PABA	01mg/mL	0.217±0.008	0.175±0.006	0.150±0.004	0.131±0.006
Sodium	5.382mg/mL	23.772±0.558	19.266±0.397	13.949±0.414	12.579±0.367
Potassium	10.43mg/mL	8.619±0.226	7.897±0.159	5.499±0.119	0.032±0.10
Calcium	10mg/mL	18.439±0.255	16.899±0.764	13.046±0.410	12.203±0.096
Phosphate	10mg/mL	0.680±0.011	0.506±0.026	0.546±0.017	0.342±0.004
Ammonium	10mg/mL	2.940±0.066	2.1228±0.048	2.5153±0.0315	1.857±0.066
Magnesium	10mg/mL	22.611±0.650	15.180±0.864	18.524±0.859	3.470±0.135

The values of ù mole s^{-1} N^{-1} are reported as ± S.D of 10 repeats.

reduce the permeation of D-Glucose, PABA and ions like magnesium, phosphate, ammonium, Sodium, Potassium, Calcium and Chloride. It seems Cefuroxime sodium forms the liquid membrane over the cell membrane and inhibits the transport of the essential ions and bio-molecules and thereby inhibits the normal functioning of cell. This may also contribute for the bactericidal effect of the Cefuroxime sodium. There are reports that MIC of Cefuroxime sodium is 4,16,>32,32 µg/ml against *E.coli, H.influenzae, E.faecalis, B.fragilis* organisms[20]. CMC of Cefuroxime sodium was found

Table 3: Solute permeability (ω) of various permeants in presence of liquid membrane generated by cefuroxime sodium in presence of lecithin mixtures

Permeant	Initial concentration	ω_0 (X 10^6) (moles $s^{-1}N^{-1}$)	ω_1 (X 10^6) (moles $s^{-1}N^{-1}$)	ω_2 (× 10^6) (moles $s^{-1}N^{-1}$)	ω_3 (× 10^6) (moles $s^{-1}N^{-1}$)
Chloride	500mg/Lt	0.102±0.086	2.039±0.063	2.043±0.048	1.661±0.007
Glucose	20mg/mL	29.380±0.627	21.623±0.607	17.950±0.334	13.258±0.278
PABA	01mg/mL	0.217±0.008	0.165±0.004	0.150±0.004	0.137±0.005
Sodium	5.382mg/mL	23.772±0.558	5.698±0.957	13.949±0.414	3.736±0.223
Potassium	10.43mg/mL	8.619±0.226	5.742±0.175	5.499±0.119	2.960±0.160
Calcium	10mg/mL	18.439±0.255	16.071±0.018	13.046±0.410	12.144±0.102
Phosphate	10mg/mL	0.680±0.011	0.450±0.009	0.546±0.017	0.288±0.004
Ammonium	10mg/mL	2.940±0.066	2.186±0.048	2.515±0.031	2.038±0.019
Magnesium	10mg/mL	22.611±0.650	13.821±0.821	18.524±0.859	7.430±1.306

The values of ω mole s^{-1} N^{-1} are reported as ± S.D of 10 repeats.

to be 2.33 µg/ml . Since the MIC of Cefuroxime sodium is greater than CMC of the drug, it may be concluded that, surface activity of the Cefuroxime sodium may also contributing to the antibacterial activity. In addition to this the permeability of various solutes through lecithin + cholesterol membrane is also reduced significantly (Table 3). This may contribute for the side effects associated with the drug. However, further studies are needed to confirm this. From the present study it may be concluded that the proposed hypothesis (i.e. the capability of formation of liquid membrane over the bacterial cell wall by Cefuroxime sodium may also contribute for the bactericidal effect of the drug in addition to its conventional mechanism, which involves the inhibition of cell wall synthesis) is justifiable.

CONCLUSION

It is apparent from the results that the generation of liquid membrane over the cell membrane may also contribute for the bactericidal effect of Cefuroxime sodium. Even it may be further concluded that formation of liquid membrane may also contribute to some of the adverse effects associated with the drug. However further studies are needed to confirm the same.

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