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ROLE OF LIQUID MEMBRANE HYPOTHESIS IN THE MECHANISM OF ACTION OF ERTAPENEM

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ABSTRACT: The role of liquid membrane hypothesis has been studied in the mechanism of action of Ertapenem. 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 Ertapenem in series with supporting membrane has been studied. The results indicate that the liquid membrane generated by Ertapenem 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 Ertapenem. It seems that the formation of liquid membrane by Ertapenem may also contribute for the bactericidal activity of the drug, in addition to its conventionally established mechanism i.e. inhibition of cell wall synthesis.

INTRODUCTION: Many Pharmacologically active compounds are amphiphilic 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 [1-12]. Earlier reports show that the wide variety of drugs acts by common mechanism i.e. due to their surface activity, which governs their action [13-20]. 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.

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There are reports that many antimicrobial agents like norfloxacin, ciprofloxacin, etc. are amphiphilic 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 [21]. In the present study Ertapenem, **(Fig. 1)** a second generation broad-spectrum was used, which is having both hydrophilic and hydrophobic domains in its structure.

Ertapenem is therefore expected to generate a liquid membrane at the interface. The cytoplasm membrane consists of Phospholipids and Proteins. The phospholipids molecules are arranged in a bimolecular layer with polar groups directed towards both sides. The nonpolar part of Ertapenem is likely to be placed across the hydrophobic core of the membrane at critical micellar concentration (CMC) of Ertapenem. The Ertapenem may form the liquid membrane over the cytoplasm membrane. Because of the liquid membrane

formed by Ertapenem 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: Glucose, Calcium chloride, Sodium chloride, Ammonium chloride, Magnesium sulphate, Potassium dihydrogen phosphate. Para aminobenzoic acid, Lecithin, ertapenem (Merck), 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.

The Critical Micelle Concentration (CMC) of aqueous Ertapenem was determined from the variation of surface tension with the concentration at 37 ± 0.1^{o}C and was found to be 5×10^{-4}. Surface tensions were measured using amodel–144 Du Nouy surface tensiomat (Komal scientific Co., Bombay–47). The all glass transport cell as shown in **Fig. 2** [21] was used to obtain hydraulic permeability and solute permeability.

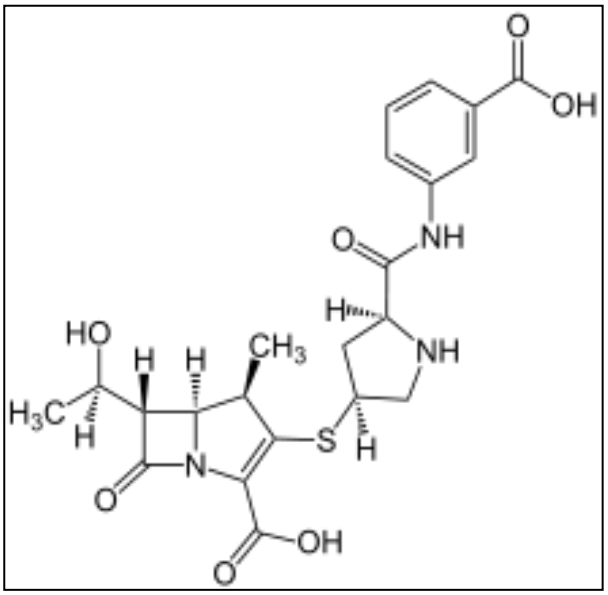

FIG. 1: ERTAPENEM

Data it essentially consists of two components, C and D separated by Sartorius cellulose acetate microfiltration membrane (Cat.No.11107, pore size 0.2µm, thickness 1×10^{-4}m, area 2.55×10^{-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 Ertapenem at various concentrations were placed in compartment C and compartment D was filled with de-ionized water. The concentration ranges of Ertapenem 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 [22-27] was used.

$$\omega = [Js/\Delta\pi]_{Jv=0} \quad \ldots\ldots (1)$$

Where Js and J_V are the solute flux and volume flux per unit area of the membrane, respectively. Δp is the Osmotic pressure difference across the membrane. For the measurement of ω one compartment of the transport cell (2CMC) was filled with aqueous solution of Ertapenem along with permeants. 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 Ertapenem. All measurements were made at constant temperature (37±0.1°C) using a thermostat.

Estimation of permeants transported through liquid membrane generated by Ertapenem: 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 - 164India), amount of magnesium was estimated by using atomic absorption spectrophotometer.

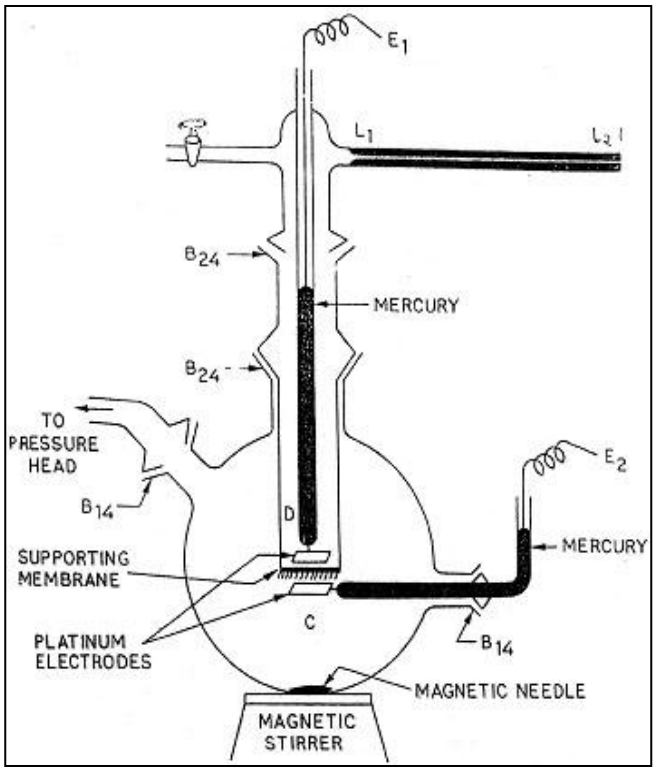

FIG. 2: ALL GLASS TRANSPORT CELL

RESULTS AND DISCUSSION: Hydraulic permeability data at various concentrations of Ertapenem were found to obey the linear relationship i.e.

$$Jv = Lp.\Delta p$$

Where Jv represents the volume flux per unit area of the membrane, Δp is the applied pressure difference. And Lp is the hydraulic conductivity coefficient. Values of Lp at various concentrations of Ertapenem were obtained from a plot Jv Vs Δp (slope of the plot is Lp) **(Table.1) (Fig 3).** The value shows decreasing trend with increasing concentration of Ertapenem up to CMC. Beyond which it becomes more or less constant.

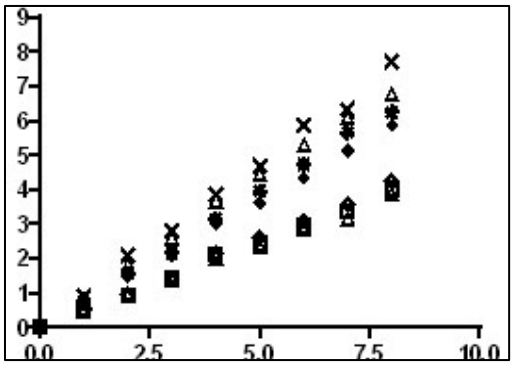

FIG. 3: THE HYDRAULIC PERMEABILITY DATA

TABLE 1: VALUES OF LP AT VARIOUS CONCENTRATIONS OF ERTAPENEM

CMC	**Experimental** Lp X10^6 m^{3}S^{-1}N^{-1}	**Calculated** Lp X10^6 m^{3}S^{-1}N^{-1}
0.00	0.942±0.248	
0.25	0.849±0.171	0.854±0.254
0.50	0.769±0.128	0.748±0.165
0.75	0.782±0.143	0.625±0.156
1.00	0.563±0.086	
2.00	0.519±0.132	
3.00	0.467±0.142	

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

TABLE 2: SOLUTE PERMEABILITY (Ω) OF VARIOUS PERMEANTS IN PRESENCE OF LIQUID MEMBRANE GENERATED BY ERTAPENEM IN PRESENCE OF LECITHIN–CHOLESTEROL MIXTURES

Permeants	**Initial Concentration**	**ω_0(X10^6) (moles s^{-1}N^{-1})**	**ω_1(X10^6) (moles s^{-1}N^{-1})**	**ω_2(X10^6) (moles s^{-1}N^{-1})**	**ω_3(X10^6) (moles s^{-1}N^{-1})**
Chloride	500mg	0.102±0.096	2.036±0.063	2.043±0.059	1.631±0.045
Glucose	20mg/mL	28.370±0.527	18.044±0.093	17.590±0.230	14.948±0.122
PABA	01mg/mL	0.215±0.007	0.185±0.016	0.190±0.005	0.151±0.009
Sodium	5.4mg/mL	22.673±0.547	19.436±0.397	13.746±0.439	12.635±0.365
Potassium	11.mg/mL	8.769±0.215	7.697±0.139	5.679±0.129	0.036±0.106
Calcium	10mg/mL	17.459±0.235	15.787±0.467	12.036±0.390	12.303±0.096
Phosphate	10mg/mL	0.779±0.013	0.603±0.024	0.534±0.032	0.352±0.004
Ammonium	10mg/mL	2.870±0.063	2.139±0.057	2.415±0.031	50.577±0.067
Magnesium	10mg/mL	21.511±0.749	15.190±0.864	18.524±0.895	3.470±0.135

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

TABLE 3: SOLUTE PERMEABILITY (Ω) OF VARIOUS PERMEANTS IN PRESENCE OF LIQUID MEMBRANE GENERATED BY ERTAPENEM IN PRESENCE OF LECITHIN MIXTURES.

Permeants	Initial Concentration	$\omega_0(X10^6)$ (moles $s^{-1}N^{-1}$)	$\omega_1(X10^6)$ (moles $s^{-1}N^{-1}$)	$\omega_2(X10^6)$ (moles $s^{-1}N^{-1}$)	$\omega_3(X10^6)$ (moles $s^{-1}N^{-1}$)
Chloride	500mg	0.103±0.076	2.043±0.053	2.039±0.054	1.671±0.009
Glucose	20mg/mL	28.377±0.657	21.674±0.619	17.850±0.363	13.239±0.276
PABA	01mg/mL	0.219±0.009	0.154±0.014	0.170±0.003	0.147±0.008
Sodium	5.4mg/mL	22.772±0.458	5.898±0.789	12.849±0.424	3.776±0.273
Potassium	11.mg/mL	8.539±0.356	5.742±0.175	5.489±0.179	2.930±0.170
Calcium	10mg/mL	18.529±0.263	15.081±0.016	13.046±0.419	12.134±0.102
Phosphate	10mg/mL	0.730±0.019	0.480±0.009	0.646±0.017	0.278±0.004
Ammonium	10mg/mL	2.879±0.063	2.216±0.043	2.415±0.029	2.038±0.019
Magnesium	10mg/mL	22.599±0.650	13.791±0.911	18.524±0.859	7.530±1.206

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

This is indicative of progressive coverage of the supporting membrane with liquid membrane generated by the drug in accordance with the Kesting hypothesis [28]. Analysis of hydraulic permeability data in the light of mosaic membrane model [29-30] 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) Lsp + n Lcp], where Lsp and Lcp represents the value of Lp at 0 and the CMC of the surfactant respectively.

The values of Lp thus computed for 0.25 CMC, 0.5 CMC and 0.75 CMC of Ertapenem 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 Ertapenem in lecithin membrane [31].

From the solute permeability (w) data **(Table 2)** it can be observed that Ertapenem reduce the permeation of D-Glucose, PABA and ions like magnesium, phosphate, and ammonium, Sodium, Potassium, Calcium and Chloride. It seems Ertapenem 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 Ertapenem. There are reports that MIC of Ertapenem is 4, 16,>32, 32 µg/ml against *E. coli*, *H. influenzae, E. faecalis, B. fragilis* organisms [32-33]. CMC of Ertapenem was found to be 2.33 µg/ml. Since the MIC of Ertapenem is greater than CMC of the drug, it may be concluded that, surface activity of the Ertapenem 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 Ertapenem 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 Ertapenem. 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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