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Transport studies through liquid membranes of ciprofloxacin and norfloxacin

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Ciprofloxacin and norfloxacin, the widely used drugs have been shown to generate liquid membranes in series with a supporting membrane (Sartorius celluose acetate microfiltration membrane). Transport of dextrose and ions, such as NH_4^+, Mg^{2+}, Ca^{2+}, K^+ and PO_4^{3-} has been studied in the presence of liquid membranes generated by these drugs. The data obtained on the modification in the permeability of dextrose and ions in the presence of liquid membrane indicate the significance of liquid membranes in passive transport.

Keywords: Ciprofloxacin, norfloxacin, liquid membrane, fluoroquinolones antibiotics, permeants, permeability, transport study

Many pharmacologically active compounds are amphiphilic in nature. These drugs tend to self-associate and interact with biological membranes. Amphiphilic compounds bear an ionic (zwitterionic, anionic, or cationic) or non-ionic polar head group and hydrophobic portion. In aqueous medium, they are able to organize themselves as micelles, bilayers, monolayers, and hexagonal or cubic phases. The spatial separation between polar and non-polar moieties, molecular shape[1] and hydrophilic and hydrophobic balance[2] determine the tendency to form different structures. A wide variety of drugs are, in fact, known to be surface-active in nature[3-4]. In a number of cases, excellent correlation between surface activity and biological effects has been demonstrated[5-7]. An amphiphilic substance when added to aqueous phase generates a liquid membrane at the interface[8]. As concentration of surfactant is increased, the interface gets progressively saturated by the liquid membrane and at critical micelle concentration (CMC), it is completely covered. The liquid membrane generated at the site of action of the respective drugs and acting as a barrier to the transport of relevant permeants might be an important step common to the mechanism of action of surface-active drugs[9]. Prompted by this concept, a number of investigations on the role of liquid membrane phenomenon in the mechanism of action of surface-active drugs have been carried out[9-16]. In view of their ability to alter the permeability of cells, surface-active drugs display anti-bacterial properties by acting on bacterial cell walls[17]. Surface-active long-chain containing quaternary ammonium or pyridinium ions as head groups have been used as bactericidal or bacteriostatic agents[17].

Fluoroquinolones are a group of antibacterial agents derived by systematic modification of nalidixic acid. The 6-fluoroquinolones (6-FQs) have been the focus of research in a variety of fields due to their therapeutic efficiency and clinical importance[18]. Their physico-chemical properties like solubility[19], ionisation-structure relationships[20], microspeciation[21], partitioning using octanol-buffer model[22], and complexation[23] have been widely studied. The main mechanism of action of 6-FQs which involves inhibition of intracellular enzymes DNA-gyrase and topoisomerase IV is well established[24-25]. However, their broad spectrum of action appears to be due to their ability to cross bacterial envelopes and cytoplasmic membranes. Besides, a hydrophobic pathway is also postulated. However, most of the studies devised to elucidate their mechanism suggest 6-FQs main entry into the cytoplasm is through the porins[26]. On the other hand, the emergence of 6-FQs resistant strains is becoming an increasing problem, which jeopardizes future application of these drugs[26]. The resistance may occur by the activity and/or over-expression of protein efflux pumps. The activity of these efflux proteins appears to depend on the ability of the surrounding inner monolayers of cytoplasmic membrane to capture the drug[27]. Once the drug is inserted at some level of bilayer, the molecule reaches by an unknown mechanism to the vicinity of the efflux protein and then it is expelled to the extracellular space. Thus, influx through lipid domains or lipid-mediated efflux through membrane proteins; the interaction between 6-FQs and lipid membranes is a crucial point to understand their antimicrobial activities.

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Several lines of evidence suggest that 6-FQs could have, as other amphiphilic compounds, surface-active properties. It appears that they might induce changes on neutral phospholipid[28-29] and can self-aggregate. In the present communication, studies on two representative drugs of this class ciprofloxacin and norfloxacin were undertaken for the study to evaluate their effect of surface activity on the transport of the ions and dextrose. These drugs have both hydrophilic and hydrophobic groups in its structure and hence they are likely to be surface-active[30].

Materials and Methods

Norfloxacin I. P. (gift from Cyper Drugs and Pharmaceutical Pvt. Ltd., New Delhi), ciprofloxacin I. P. (gift from Core Health Care Ltd., Ahmedabad), dextrose (Qualigens Fine Chemicals, Mumbai), glucose estimation kit (Span Diagnostic Ltd., Surat, India), magnesium sulphate, calcium chloride, potassium dihydrogen ortho phosphate (Loba Chemie, Mumbai), ammonium chloride and deionised water (Nice, Cochin) were used. Other chemicals used were of analytical grade.

CMC of aqueous solutions of the drugs were determined from the variation of surface tension with the concentration at 37± 0.1°C. CMC values derived were $3\times10^{-4}M$ and $3\times10^{-4}M$ for ciprofloxacin and norfloxacin, respectively. Surface tension was measured, using Du-Nouy surface tensiomat Model 144 (Komal Scientific Co., Bombay).

Transport studies

The all-glass transport cell described earlier[11] (Fig.1) was used for obtaining hydraulic and solute permeability data. It essentially consists of two compartments C and D, separated by sartorious cellulose acetate micro-filtration membrane (cat. no. 11107, pore size 0.2 μm, thickness of 1×10^{-6} m and area 2.55×10^{-5} m^2), which acts as supporting membrane for liquid membrane. For measurement of hydraulic permeability data, aqueous solution of ciprofloxacin/norfloxacin of various concentrations were placed in compartment C, while compartment D was filled with deionised water. Details of the method used for hydraulic permeability measurements were the same as used in earlier studies[9-12]. The range concentration chosen for the drug was of both above and below of CMC values. Known pressures were applied in 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. The magnitude of applied pressure was also measured by noting the position of pressure head with cathetometer. During volume flux measurement, the solution in compartment C was well stirred and the electrodes E1 and E2 were electrically short-circuited.

The solute permeability (ω) of the relevant permeants in the presence of the liquid membrane generated by ciproflaxacin and norfloxacin was determined using the equation:

$$(J_s / \Delta\pi)_{Jv=0} = \omega \qquad \ldots (1)$$

where J_s and J_v are the solute flux and volume flux per unit area of the membrane, respectively and $\Delta\pi$ is the osmotic pressure difference. For solute perme-ability measurements, the concentration of drugs chosen was the one at which the liquid membrane generated by them completely covered the supporting membrane and was saturated with the drugs. This concentration was derived from the present data on hydraulic permeability in the presence of the varying concentrations of the drugs. For measurement of ω, compartment C of transport cell was filled with aqueous solution of ciprofloxacin/norfloxacin, along

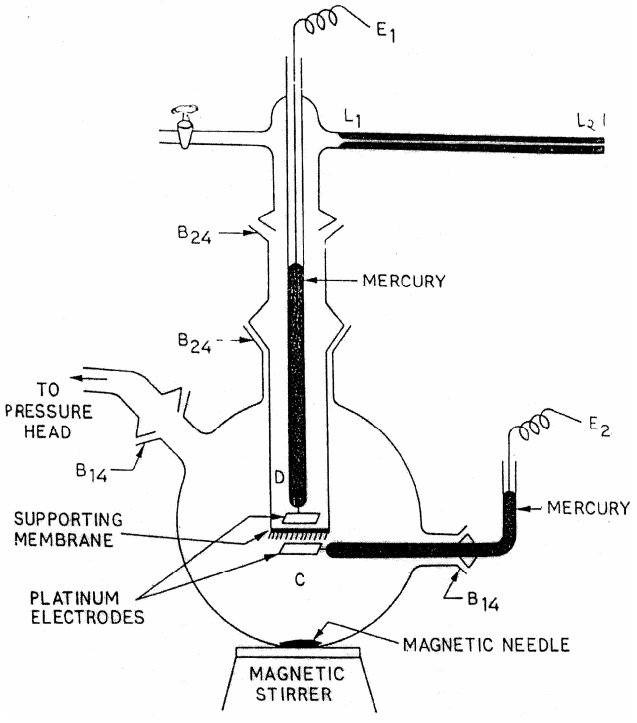

Fig. 1 — All glass transport cell [E1 E2, Electrode terminals, L1 L2, capillary]

with a permeant, and compartment D was filled with deionised water. The condition J_v=0 was imposed on the system and the amount of a permeant transported to the compartment filled with distilled water in a known period of time was estimated. Details of the method of measurement of ω were same as described earlier[9,12]. All measurements were performed at constant temperature, using a thermostat setting of 37± 0.1°C.

Estimations

The amounts of various permeants transported to the compartment filled with distilled water were estimated as follows: Dextrose was estimated *in vitro* by enzymatic colorimetric method[31]. The amount of NH_4^+ ions was estimated from the amount of their reaction products with Nessler's reagent[32] at 525 nm. The amount of Mg^{2+} ions was estimated from the concentration of their reaction products (equimolar) with solo chrome black solution[33] at 520 nm. The amount of PO_4^{3-} ions was estimated from the reaction products with ammonium molybdate reagent and reducing agent[33] at 660 nm. The UV-visible, Spectrophotometer model SL-159, Elico, India was used for all estimations. K^+ and Ca^{2+} ions were determined[32] by using flame photometer model CL-22A, Elico, India.

Results and Discussion

The hydraulic permeability data at various concentrations of ciprofloxacin and norfloxacin were found to obey the proportional relationship:

$$J_v = L_p . \Delta P \qquad \ldots(2)$$

where J_v is the volume flux per unit area of the membrane, ΔP is the applied pressure difference and L_p is the hydraulic conductivity coefficient. The values of L_p at various concentrations of the drugs, estimated from the slopes of J_v *vs* ΔP plots, are given in Table 1. They show a decreasing trend with the increase in concentrations of drug up to CMC, beyond which they become more or less constant. This trend in values of L_p is consistent with the Kesting's liquid membrane hypothesis[8].

Analysis of L_p values in light of mosaic model[34-35] furnishes further evidence in favour of formation of a liquid membrane in series with the supporting membrane. When concentration of the surfactant is 'n' times, its CMC, n ≤ 1, value of L_p should be equal to [(1-n) L_p^s + n L_p^c], where superscripts 's' and 'c', respectively, represent values for the bare supporting membrane and the supporting membrane completely covered with surfactant layer liquid membrane. L_p^s and L_p^c represent the values of L_p, when surfactant concentrations are equal to zero and the CMC, respectively. The values of L_p thus computed as several concentrations of the drugs below their CMC match with the experimentally determined values (Table 1), lending additional support to formation of liquid membranes in series with the supporting membrane. The measurements of *p*H at the constant temperature of various compositions of the drugs have remained more or less constant.

Data on the solute permeability (ω) of all permeants such as dextrose, K^+, Ca^{2+}, Mg^{2+}, NH_4^+ and PO_4^{3-} ions in the presence of liquid membranes likely to be generated by the drugs in series with the supporting membrane are shown in Table 2. The permeability of all the permeants is diminished in the presence of ciprofloxacin and norfloxacin liquid membranes. Ciprofloxacin and norfloxacin, which contain carboxylic acid group at 3rd position, in

Table 1 — Values of L_p at various concentrations of norfloxacin and ciprofloxacin
[Values are arithmetic mean ± SD of 10 repeats]

Critical micellar conc. (CMC)	$L_p \times 10^6$ ($m^3 s^{-1} N^{-1}$)			
	Norfloxacin [3×10^{-4} *M* (1 CMC)]		Ciprofloxacin [3×10^{-4} *M* (1 CMC)]	
	Experimental	Calculated	Experimental	Calculated
0	3.521 ± 0.025	-	1.495 ± 0.014	-
0.25	3.025 ± 0.079	3.088 ± 0.170	1.308 ± 0.043	1.313 ± 0.013
0.50	2.540 ± 0.063	2.656 ± 0.117	1.122 ± 0.012	1.132 ± 0.012
0.75	2.131 ± 0.030	2.224 ± 0.063	0.911 ± 0.023	0.951 ± 0.010
1.0	1.792 ± 0.010	–	0.770 ± 0.010	–
2.0	1.679 ± 0.046	–	0.752 ± 0.005	–
3.0	1.675 ± 0.067	–	0.734 ± 0.030	–

Table 2 — Solute permeability (ω) of various permeants in the presence of liquid membranes generated by norfloxacin and ciprofloxacin [Values of ω (moles $s^{-1}N^{-1}$) are reported as arithmetic mean ± SD of 10 repeats]

Permeant	Initial conc. (g/l)	Norfloxacin (6×10^{-4} *M*)		Ciprofloxacin (6×10^{-4} *M*)	
		$\omega_0^* \times 10^6$	$\omega_1^\# \times 10^6$	$\omega_0^* \times 10^6$	$\omega_1^\# \times 10^6$
Dextrose	10.0	10.29 ± 0.02	5.18 ± 0.06	14.84 ± 0.11	6.71 ± 0.04
K^+	0.02	27.93 ± 0.31	5.56 ± 0.30	28.20 ± 0.28	5.09 ± 0.13
Ca^{2+}	0.02	31.82 ± 0.93	14.32 ± 0.96	28.64 ± 0.23	10.91 ± 0.16
Mg^{2+}	0.2	49.44 ± 0.62	26.99 ± 0.85	54.17 ± 0.65	28.03 ± 0.56
NH_4^+	2.5	38.93 ± 0.96	12.43 ± 0.14	32.57 ± 0.49	6.70 ± 0.05
PO_4^{3-}	0.05	7.35 ± 0.04	2.74 ± 0.02	6.60 ± 0.05	2.33 ± 0.02

*$\omega_{0,}$ when no drug was used; and #$\omega_{1,}$ in the presence of norfloxacin/ciprofloxacin

aqueous medium are dissociated into an anion (carboxylate ion), a surface-active ion. Anionic surfactants are electrolytes and a surface ion is an anion, when surfactants dissociates in water[36]. Hence, these molecules may act as an anionic surface-active molecule. Due to their surface activity, molecules may self-aggregate or bind with the supporting membrane. The non-polar part of these drugs is likely to be binding with non-polar part of cellulose acetate membrane, a supporting membrane. In such an event, the polar part (-anionic) is likely to be facing towards outside of the supporting membrane. In the present study, transport of both anions and cations are impeded (Table 2), which may be due to repulsion between anions and the surface anionic charge and attraction between cations and surface anionic charge. So, in both cases, the ions cannot permeate the supporting membrane freely. Likewise, dextrose molecules permeation is also impeded in presence of liquid membrane formed by these drugs.

In an earlier study, alteration of the hydrophobicity of bacterial membrane by antibiotics, such as ciprofloxacin and norfloxacin was reported[37]. The most significant reduction of bacterial cell surface hydrophobicity was found after treatment at 1/16 of MICs (to 20.3% for both the drugs, compared with control values)[38]. The reduction in hydrophobicity may be due to accumulation of these drugs on bacterial membranes[39]. In our study, these antibiotics are found to interact with the hydrophobic surfaces of cellulose acetate supporting membrane and form liquid membrane with hydrophilic surface.

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