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Liquid membrane phenomena in anticancer drugs. Studies on 5-fluorouracil and its derivatives

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Summary

Liquid membrane phenomena in 5-fluorouracil and its two derivatives: 1-(2-tetrahydrofuryl)-5-fluorouracil and 1-hexylcarbamoyl-5-fluorouracil have been studied. Modification in the transport of folic acid, vitamin B_{12} and a few relevant amino acids in the presence of the liquid membrane generated by these drugs in series with a supporting membrane has been studied. The data indicate that modification in the transport of the relevant permeants due to the liquid membranes likely to be generated by these drugs at their respective sites of action may also contribute to their anticancer activity.

Introduction

Recent studies (Bhise et al., 1984a and b, 1985a and b; Srivastava et al., 1984, 1985, 1986; Tandon et al., 1986) on a wide variety of structurally different surface-active drugs belonging to different pharmacological categories have revealed that liquid membranes likely to be generated at the site of action of the respective drugs, acting as a barrier modifying the transport of relevant permeants to these sites, might be an important step common to the mechanism of action of all surface-active drugs. It has been shown in the same studies that the modification in the transport of the relevant permeants to the site of action, due to the liquid membrane generated by the drugs, makes a significant contribution to their biological action. A detailed discussion of this point of view and its implications has been presented by Srivastava, et al. (1984). One of the important implications is that in a series of structurally related drugs which are congeners of a common chemical moiety and which act by altering the permeability of cell membranes, any structural variation which increases the hydrophobicity of the compound will increase the potency of the drug while any alteration of the hydrophilic moieties of the drug may change the nature of its action qualitatively (Srivastava et al., 1984).

It has been shown by Iigo et al. (1978) that the newly synthesized (Ozaki et al. 1977) 1-hexylcarbamoyl-5-fluorouracil (HCFU) is more active against various tumors in mice and less toxic to host animals than its parent drug 5-fluorouracil (5FU). Iigo et al. (1978) have tested the activities of these drugs on Lewis lung carcinoma and B16

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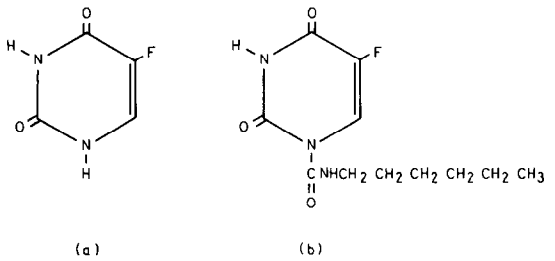

Fig. 1. Chemical structures of (a) 5-fluorouracil and (b) 1-hexylcarbamoyl-5-fluorouracil.

melanoma. It is evident from the structure of two drugs (Fig. 1) that HCFU will be more hydrophobic and more surface active than its parent compound 5FU. Prompted by this clue, 5FU and two of its derivatives, HCFU and 1-(2-tetrahydrofuryl) 5-fluorouracil (FT), have been investigated for the contribution of liquid membrane phenomena to their action, the results are reported in this communication.

All the 3 drugs, 5FU, HCFU and FT, have been shown to generate liquid membranes in series with a supporting membrane. Transport of relevant permeants through the liquid membranes generated by these drugs in series with the supporting membrane has been studied. The data obtained from these model experiments indicate that the modification in the transport of relevant permeants due to the drug liquid membranes likely to be generated at the sites of action may also make a significant contribution to the biological actions of these drugs. In these studies a non-specific non-living membrane has been chosen deliberately as the supporting membrane for the liquid membranes. Thus the possibility of active and specific interactions of these drugs with the constituents of biomembranes as cause for modification in the transport of relevant permeants is totally ruled out. The role of passive transport through the liquid membranes in the action of these drugs is highlighted.

Materials and Methods

Materials

HCFU, 5FU and FT, (all from Mitsui Pharmaceuticals, Inc., Tokyo, Japan); aspartic acid, folic acid, glutamine and glycine (all from Loba-Chemie) and cyanocobalamin (Sigma V-2876) were used in the present experiments. All other chemicals were analytical grade reagents. Distilled water, distilled twice in an all-pyrex glass still, was used for preparing the solutions. Aqueous solutions of HCFU and FT, which are not so easily soluble in water, were prepared by adding to the aqueous phase, with vigorous stirring, the necessary volume of ethanolic stock solution of known concentration of these substances. In the aqueous solutions of HCFU and FT thus prepared the final concentration of ethanol was never allowed to exceed 0.1% (v/v). It was experimentally found that 0.1% solution of ethanol in water did not lower the surface tension of water to any measurable extent.

Methods

The critical micelle concentrations (CMCs) of 5FU, HCFU and FT were estimated from the variation of surface tension with concentration and are recorded in Table 1. The surface tensions were measured using a Fisher tensiomat model 21.

The all glass cell described earlier (Tandon et al., 1986) was used for the transport studies. It is reproduced in Fig. 2 for ready reference. It essentially consisted of two compartments C and D separated by a Sartorius cellulose acetate microfiltration membrane (Cat no. 11107, pore size 0.2 μm) of thickness 1×10^{-4} m and area 2.55×10^{-5} m^2 which acted as a supporting membrane for the liquid membranes. To obtain the hydraulic permeability data which were utilised to demonstrate the formation of liquid membrane in series with the supporting membrane, aqueous solutions of varying concentrations of the drugs were filled in

TABLE 1

Critical micelle concentrations (CMC) of anticancer drugs

Drugs	CMC (M)
5FU	8.0×10^{-10}
FT	7.5×10^{-11}
HCFU	6.1×10^{-11}

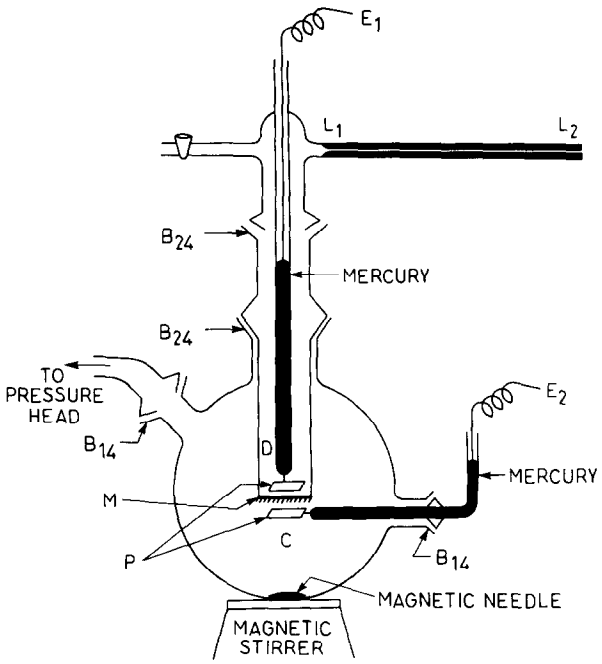

Fig. 2. Transport cell. M, supporting membrane, P, bright platinum electrodes, E_1 and E_2 electrode terminals, L_1L_2, capillary.

compartment C of the transport cell (Fig. 2) and compartment D was filled with distilled water. The concentrations of the drugs chosen were such that the hydraulic permeability data were obtained both below and above the CMCs of the drugs. The details of the method used for the hydraulic permeability measurements have been described earlier (Bhise et al., 1984a and b, 1985a and b; Srivastava et al. 1984, 1985, 1986; Tandon et al. 1986).

The solute permeability (ω) of the relevant permeants in the presence of the liquid membranes generated by the drugs were estimated using the equation (Katchalsky and Curran, 1967)

$$\left(\frac{J_s}{\Delta\pi}\right)_{J_v=0} = \omega \qquad (1)$$

where J_s stands for the solute flux per unit area of the membrane, $\Delta\pi$ is the osmotic pressure difference across the membrane and J_v is the volume flux. The method of measurement was the same as described in the publications cited above. For solute permeability measurements two sets of experiments were performed. In the first set of experiments an aqueous solution of the drug was filled in compartment C along with the permeant and compartment D was filled with distilled water (Fig. 2). In the second set of experiments the aqueous solution of the drug was filled in compartment D of the transport cell and compartment C was filled with the aqueous solution of the permeant. The concentrations of the drugs used in the ω measurements were always higher than their CMCs.

All measurements were made at constant temperature using a thermostat set at $37 \pm 0.1°$ C.

Estimations

The amounts of the various permeants transported to compartment D were estimated as follows:

Folic Acid. The amount of folic acid was estimated using a Cary 17-D spectrophotometer by measurement of ultraviolet absorption at 283 nm –the absorption maximum of folic acid in 0.1 M sodium hydroxide solution (Hashmi, 1973a).

Cyanocobalamin. The amount of cyanocobalamin was estimated spectrophotometrically in an aqueous solution by measurement of absorbance at 361 nm (Hashmi, 1973b; Heller, 1980).

Amino acids. The amounts of glycine, glutamine and aspartic acid were estimated from the amount of their reaction products with ninhydrin measured at 570 nm (Moore and Stien, 1954).

Results and Discussion

The hydraulic permeability data at various drug concentrations in the case of all three drugs, were found to be in accordance with the proportional relationship

$$J_v = L_p \, \Delta p \qquad (2)$$

where J_v represents the volume flux per unit area of the membrane, L_p the hydraulic conductivity

TABLE 2

Values of L_p at various concentrations of 5FU, FT and HCFU

	Conc. ($\times 10^{11}$ M)	$L_p \times 10^8$ ($m^3 s^{-1} N^{-1}$) *	$L_p \times 10^8$ ($m^3 s^{-1} N^{-1}$) **
5FU	0.000	–	2.162 ± 0.064–
	20.00 (0.25 CMC)	1.930 ± 0.058	1.944 ± 0.056
	40.00 (0.5 CMC)	1.720 ± 0.054	1.726 ± 0.059
	60.00 (0.75 CMC)	1.573 ± 0.074	1.508 ± 0.041
	80.00 (CMC)	1.290 ± 0.034	–
	160.00	1.260 ± 0.061	–
	240.00	1.266 ± 0.064	–
FT	0.000	2.162 ± 0.064	–
	1.875 (0.25 CMC)	1.778 ± 0.086	1.805 ± 0.081
	3.750 (0.5 CMC)	1.418 ± 0.049	1.406 ± 0.115
	5.625 (0.75 CMC)	1.095 ± 0.059	1.106 ± 0.039
	7.500 (CMC)	0.755 ± 0.017	–
	15.000	0.751 ± 0.025	–
	22.500	0.761 ± 0.031	–
HCFU	0.000	2.162 ± 0.064	–
	1.525 (0.25 CMC)	1.795 ± 0.041	1.770 ± 0.049
	3.050 (0.5 CMC)	1.422 ± 0.030	1.377 ± 0.036
	4.575 (0.75 CMC)	0.999 ± 0.018	0.985 ± 0.023
	6.100 (CMC)	0.592 ± 0.010	–
	12.200	0.594 ± 0.006	–
	18.300	0.582 ± 0.018	–

The values reported for L_p are arithmetic mean of 10 repeats ± S.D.

* Experimental values.

** Calculated values using mosaic model.

coefficient and Δp the applied pressure difference across the membrane. The values of L_p estimated from the slopes of J_v versus Δp plots, in the case of all 3 drugs, show a progressive decrease with increase in the concentrations of the drugs (Table 2) upto the CMCs of the drugs beyond which they become more or less constant. This trend in the values of L_p is in keeping with Kesting's liquid membrane hypothesis (Kesting et al., 1968) according to which, when a surfactant is added to an aqueous phase, the surfactant layer which forms spontaneously at the interface acts as a liquid membrane and modifies the mass transfer across the interface. As the concentration of the surfactant is increased the interface becomes progressively covered with the surfactant layer liquid membrane and at the CMC it is completely covered. Analysis of the values of L_p in the light of the mosaic model (Spiegler and Kedem, 1966; Sherwood et al., 1967; Harris et al., 1976) furnishes additional evidence in favour of the formation of liquid membranes by the drugs in series with the supporting membrane. Following the arguments, based on the concept of the progressive coverage, given in earlier publications (Bhise et al. 1984a and b, 1985a and b; Srivastava et al., 1985, 1986; Tandon et al. 1986), it can be shown that at concentrations less than the CMCs of the surfactants the value of L_p should be equal to $[(1-n)L_p^c + nL_p^s]$ where $n \leqslant 1$ and the superscripts c and s represent the values of L_p at 0 and the CMC of the surfactant, respectively. The values of L_p thus computed at several concentrations of the drugs below their CMCs compare favourably with corresponding experimental values in the case of all the 3 fluorouracils (Table 2).

Solute permeability data

Since all 3 drugs, being surface-active in nature, have both hydrophilic and hydrophobic parts in

their structure, it is expected that the hydrophobic ends of the drugs molecules in the liquid membrane would be preferentially oriented towards the hydrophobic supporting membrane and the hydrophilic moieties would be drawn outwards away from it. Thus in the first set of solute permeability experiments the permeants would face the hydrophilic surface of the drug liquid membrane generated in series with the supporting membrane while in the second set of experiments they would face the hydrophobic surface. The data on the solute permeability of relevant permeants in the two orientations of the drug molecules in the liquid membranes are recorded in Table 3 along with the corresponding values from control experiments where no drug was used.

Antimetabolites in general are known to act by impairing the synthesis of purine and pyrimidine bases by interfering with folic acid metabolism or prevent the incorporation of the bases into nucleic acids (Bowman and Rand, 1980a). The steps involved are known to be enzyme-catalysed. For example 5FU is ultimately converted enzymatically into 5-fluorodeoxyuridine-5-phosphate which inhibits the thymidylate synthetase enzyme system resulting in the blockade of DNA synthesis (Calabresi and Parks, 1980a). The present data (Table 3), however, indicate that the passive transport through the liquid membranes likely to be generated by the fluorouracils (5FU, HCFU and FT) at the respective sites of action may also contribute to their action.

Vitamin B_{12} and folic acid, which are dietary essentials for man, are required for the synthesis of purine and pyrimidine bases and their incorporation into DNA. Their deficiency may result in defective synthesis of DNA in any cell that attempts chromosomal replication and division (Hilman, 1980). The present data indicate that the transport of both, vitamin B_{12} and folic acid, are impeded by the liquid membranes generated by all 3 drugs presently studied (Table 3). This impediment in the transport may contribute to the deficiency of vitamin B_{12} and folic acid inside the cells resulting in the defective synthesis of DNA. Thus it appears that the phenomenon of liquid membrane formation may also contribute to the anticancer activities of 5FU and its derivatives. A perusal of Table 3 further reveals that inhibition in the transport vitamin B_{12} and folic acid, is more when the permeants face the hydrophilic surface of the liquid membranes than when they face the hydrophobic surface. This observation indicates that the specific orientation of the drug molecules in the liquid membranes with their hydrophilic ends facing the permeants may be necessary on cancerous cells while the drug molecules in the liquid membranes on the normal cells may have

TABLE 3

Solute permeability (ω) of various permeants in the presence of 5FU, FT and HCFU

Permeant	Initial concentration (mg/liter)	Control $\omega \times 10^9$	5FU (1×10^{-9} M)		FT (1×10^{-10} M)		HCFU (1×10^{-10} M)	
			$\omega \times 10^9$ C	$\omega \times 10^9$ D	$\omega \times 10^9$ C	$\omega \times 10^9$ D	$\omega \times 10^9$ C	$\omega \times 10^9$ D
Aspartic acid	150	0.856 ±0.011	0.628 ±0.004	0.688 ±0.006	0.475 ±0.020	0.715 ±0.062	0.398 ±0.008	0.568 ±0.042
Cyanocobalamin	30	0.488 ±0.018	0.281 ±0.024	0.365 ±0.021	0.316 ±0.015	0.379 ±0.018	0.282 ±0.026	0.347 ±0.037
Folic acid	0.05	8.715 ±0.266	6.013 ±0.557	7.541 ±0.316	6.631 ±0.496	7.590 ±0.010	4.406 ±0.220	5.743 ±0.334
Glutamine	500	0.474 ±0.031	0.759 ±0.065	0.694 ±0.052	0.160 ±0.011	0.363 ±0.014	0.417 ±0.013	0.399 ±0.008
Glycine	100	0.265 ±0.010	0.412 ±0.055	0.644 ±0.168	0.151 ±0.002	0.182 ±0.003	0.181 ±0.001	0.195 ±0.004

Values of ω are repored as arithmetic mean of 10 repeats ±S.D., in $mol \cdot s^{-1} N^{-1}$. C, drug in compartment C; D, drug in compartment D.

the other orientation - hydrophobic ends facing the permeants. This inference in turn implies that surface of the membranes of the cancerous cells should be less hydrophilic than those of the normal cells. Though there are some indications in literature (Bowman and Rand, 1980b; Burger 1971; Emmelot, 1964a) that the neoplastic state may also arise through an alteration in the surface properties of the cells, a thorough probe in terms of hydrophilicity of the cell surface is called for to substantiate this conjecture.

Amino acids like glycine, glutamine and aspartic acid are also required, in addition to folic acid, for the purine ring synthesis (Bowman and Rand, 1980c; Emmelot, 1964b). Compounds from which the atoms of the purine ring are derived in the biosynthetic pathway are depicted in Fig. 3. The present data (Table 3) indicate that except in the case of 5FU, the transport of glycine, glutamine and aspartic acid is also impeded in addition to folic acid and vitamin B_{12}, by the liquid membranes generated by both FT and HCFU. In the case of 5FU the transport of glycine and glutamine was enhanced. The impediment in the transport of the amino acids also in the case of HCFU and FT, was more in the specific orientation of the drug molecules in the liquid membrane with their hydrophilic ends facing the permeants. This impediment in the transport observed in the case of FT and HCFU may also be a factor responsible for the impairment of the synthesis of purine bases contributing to the anticancer activity of these drugs.

It has been reported by Iigo et al. (1978) that of the 3 drugs, HCFU, FT and 5FU, HCFU is most

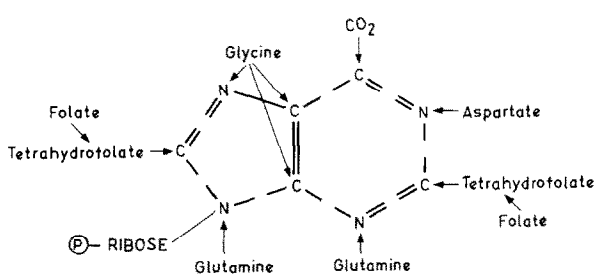

Fig. 3. Compounds from which the atoms of the purine ring are derived in the biosynthetic pathway. The breaks in the bonds separate the groups of atoms derived from each source (Bowman and Rand, 1980c).

potent. This finding is consistent with the liquid membrane hypothesis for drug action (Srivastava et al., 1984). The CMC of HCFU is the lowest (Table 1). As CMC is the concentration at which a complete liquid membrane is generated at the interface, it would appear that of the three drugs, HCFU would require the lowest concentration for the development of a complete liquid membrane at the site of action. Since modification of the transport of the relevant permeants, which affects the biological effect, is maximum when a complete liquid membrane is generated, the concentration of HCFU required to produce the maximum biological effect would be the lowest amongst the 3 drugs, making HCFU the most potent drug.

Some of the adverse side effects of cytotoxic drugs include megaloblastic anaemia (Horler, 1981), neurological disorders relating to spinal column and cerebral cortex (Calabresi and Parks, 1980b), ineffective haematopoiesis and pancytopenia (Bowman and Rand, 1980d). These symptoms are also the symptoms of deficiencies of vitamin B_{12} or folic acid or both (Hilman, 1980; Finch et al. 1956; Stebbins et al. 1973; Stebbins and Bertino, 1976). The impediment in the transport of vitamin B_{12} and folic acid in the specific orientation of the drug molecules in the liquid membrane with their hydrophobic ends facing the permeants, which may be the orientation on the normal cells, could also be a plausible explanation for the reported side-effects.

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