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Effect of propylene glycol, Azone and *n*-decylmethyl sulphoxide on skin permeation kinetics of 5-fluorouracil

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Summary

The effect of propylene glycol (PG), azone (LDA) and *n*-decylmethyl sulfoxide (LDB) on the permeation course of fluorouracil through the hairless mouse skin was studied. Steady-state fluxes and permeability coefficients were measured in buffer solutions and in systems containing the enhancing agents. The permeation rates of fluorouracil have been shown to be highly pH dependent in the pH range of 5–9, the rate decreases with an increase in pH. The solubility of fluorouracil in pure propylene glycol at equilibrium measured by the solubility method was found to be 2.2 mg · ml^{-1} at 25°C which is a relatively low value as compared to the solubility in water. The effect of various concentrations of propylene glycol in aqueous donor solutions on the drug permeation rate was examined at pH's 5.7 and 9.0. It was found that propylene glycol decreases the permeation flux when increasing concentrations are added to the aqueous pH 5.7 system; however, at pH 9, a strong enhancement effect was shown. PG was also found to decrease the drug reservoir in the hairless mouse skin, e.g. 8.4 and 2.8 mg · (mg skin)$^{-1}$ for buffer pH 9 and PG/aqueous solution pH 9 systems, respectively. The concentration dependent enhancement effects of azone and *n*-decylmethyl sulfoxide have been measured. Both have been found to be potent enhancing agents. However, at relatively low

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concentrations such as 5%, Azone induced a 50-fold and *n*-decylmethyl sulfoxide only a two-fold enhancement of the drug steady-state flux. At high concentrations as much as 40%, *n*-decylmethyl sulfoxide appears to be more effective than Azone. The fluxes measured with these systems were 0.21, 0.17 and 0.003 $mg \cdot cm^{-2} \cdot h^{-1}$ for the *n*-decylmethyl sulphoxide, Azone and PG/H_2O systems, respectively.

Introduction

Fluorouracil (5-FU) is a cytotoxic agent used topically on the skin in actinic keratosis and various epithelial neoplasma (Goodman and Gilman, 1980). The need for an improved therapy brought into use various ways towards increased fluorouracil skin permeation such as occlusivity (Martindale, 1982), prodrugs (Mollgaard et al., 1982) and chemical enhancing agents (Chien, 1982); based on the reports that propylene glycol is an effective enhancing permeation agent (Ostrenga et al., 1971; Lorenzetti, 1979) the most popular topical dosage forms of fluorouracil are propylene glycol solutions. Azone was found to give enhanced fluorouracil penetration through the skin mounted in vertical diffusion cells (Stoughton, 1982). However, the skin steady-state permeation profiles and the major parameters involved in the fluorouracil permeation process have not been studied systematically; the lack of such data makes it difficult to quantitate the effect of various agents on the fluorouracil skin permeation.

The present study was undertaken to measure the permeation parameters and to evaluate the enhancing effect of three chemical agents: propylene glycol, Azone and *n*-decylmethyl sulfoxide on the permeation rate and on the permeability coefficients of fluorouracil. The drug chosen (fluorouracil) represents a model for highly polar molecules with poor skin permeability.

Materials and Methods

Materials

The materials used were 5-fluorouracil (Sigma), 5-fluoro-[6-^{3}H]uracil with a specific activity of 800 $mCi \cdot mmol^{-1}$ (Amersham), propylene glycol (Sigma), 1-dodecylazacycloheptan-2-one (Azone) (Nelson) and *n*-decylmethyl sulfoxide (Cyclo). Instagel (Packard) was used as cocktail solution for scintillation counting. Solvents and buffer substances were of reagent grade.

Composition of solutions containing fluorouracil and either Azone or n-decylmethyl sulfoxide

Fluorouracil	5.0 mg
NaOH solution 1 N	0.075 ml
Azone or *n*-decylmethyl sulfoxide	q.s. % v/v
Propylene glycol to	1.0 ml

The solutions have been used immediately after preparation.

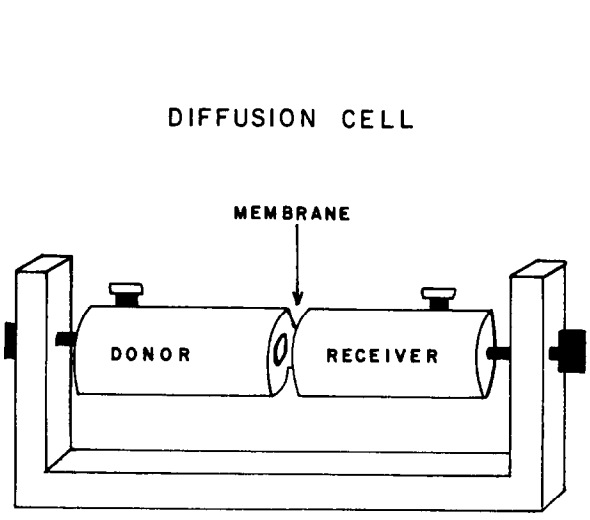


Fig. 1. Scheme of horizontal diffusion cell used.

Skin

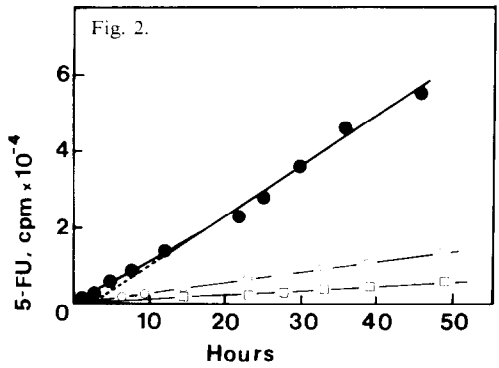
Full-thickness skin was excised from the abdomen of 6–8-week-old male hairless mice (Hadassah Hospital, Jerusalem) just before each experiment. The skin was examined for its integrity, rinsed with distilled water, cut and mounted on horizontal diffusion cells.

Permeation experiments

The permeation experiments have been assessed in a horizontal diffusion cell assembly designed in our laboratory and reported earlier (Touitou and Abed, 1985). The skin was mounted between the cell compartments with the stratum corneum towards the donor containing the drug solution (Fig. 1). The donor compartment was filled with 3 ml of fluorouracil solutions of various compositions containing [^{3}H]5-fluorouracil (20 μCi/cell) diluted with unlabeled drug. Freshly prepared solutions were used. The receiver compartment contained 3 ml of pH 7.2 buffer solution (KH_2PO_4/NaOH). Pseudo-sink conditions were kept in the system. The effective diffusion surface area was 0.78 cm^2. The experiments have been run for 24–30 h at 22 ± 1°C. 100 μl samples were withdrawn from the receiver at 0, 1, 2, 4, 6 up to about 30 h and replaced by 100 μl of buffer solutions. Correction was made in calculating the cumulative amount of drug that permeated the skin using the following expression:

$$C_n = C_{n1} + \frac{0.1}{3} \cdot \sum_{s=1}^{s=n-1} C_p \qquad (1)$$

where C_n is the corrected concentration of n^{th} sampling, C_{n1} is the measured concentration and C_p is the measured concentration of preceeding samples. 10 μl samples were taken at time 0 and at the end of the experiment from the donors. The drug concentration was measured by radioactive counting using the Kontron Be-



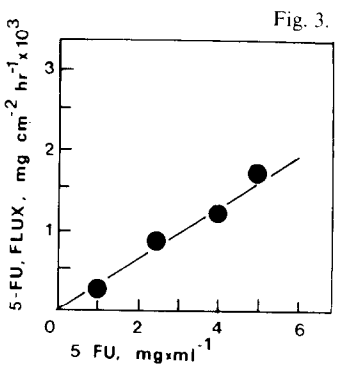


Fig. 2. Permeation profile of 5-FU through hairless mouse skin from various solutions: ● 5 mg·ml^{-1} in water (pH 5.7); ○ 2 mg·ml^{-1} in propylene glycol; and □ 1 mg·ml^{-1} in propylene glycol.

Fig. 3. Effect of drug concentration on the permeation flux of 5-FU from buffer pH 5.7 solution.

tamatic liquid scintillation counter (Lumitron Scientific Instruments). Each experiment was replicated 2–4 times.

Measurement of the amount of fluorouracil dissolved in the skin

Each piece of skin was weighed before the experiment. At the end of each experiment the surface of the skin was wiped off carefully with a tissue, and the effective permeation area of the skin was cut and solubilized in 1 ml tissue solubilizer (Packard) left for 24 h at room temperature. The solution was mixed with 5 ml Instagel and the amount of drug was measured by scintillation counting.

Results and Discussion

The permeation course of fluorouracil at 22 ± 1°C through hairless mouse skin from a buffer pH 5.7 solution at a concentration of 5 mg · ml^{-1} is shown in Fig. 2, where the cumulative amount of permeant was plotted as a function of time. It is important to observe that although the donor contained an unsaturated solution, a classic skin transport profile was obtained which indicates that after a lag time of about 3 h the process reaches a steady-state phase. The steady-state flux, F, may be calculated from the slope of the plot divided by the effective diffusion area. The permeability coefficient, K_p is obtained from the relationship given in Eqn. 2, where ΔC is the concentration gradient between the donor and the receiver compartments (Scheuplein and Blank, 1971; Dugard, 1983).

$$F = K_p \cdot \Delta C \qquad (2)$$

To test if this relationship is viable for our unsaturated systems, the effect of drug concentration on the permeation flux was tested. The linear plot drawn in Fig. 3

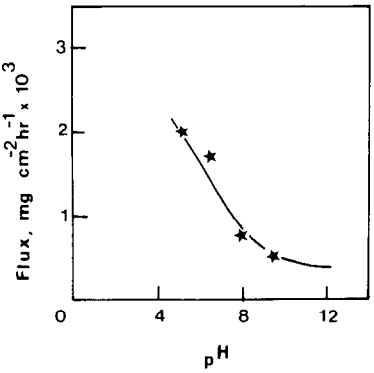


Fig. 4. Effect of pH on permeation rate of 5-FU from solutions containing 5 mg · ml^{-1} drug.

indicates that the concentration gradient across the skin remained practically constant for a certain donor concentration during the experiment time. Thus, the K_p value can be calculated either from the slope of the linear regression in Fig. 3 or by dividing the fluorouracil flux by the initial drug concentration in the system.

The aqueous solubility of fluorouracil is 12 mg · ml^{-1} at pH 7 and increases at higher pH's as a result of salt formation (pK_a 7.7 at 25°C) (Rork and Pitman, 1975). In examining the pH effect on the rate of fluorouracil transport through the skin, a marked pH-permeation rate dependency was observed. The results obtained with 5 mg · ml^{-1} solutions tested in the pH range of 5–9 show that the rate of drug permeation decreases as the pH increases according to the plot presented in Fig. 4. The observations indicate that a pH partitioning mechanism is involved in the transport of fluorouracil through the skin.

Effect of propylene glycol on skin permeation of fluorouracil

The saturation solubility of fluorouracil in propylene glycol (PG) was found to be much lower than in water. Measured by the solubility method at 25°C, the saturation value at equilibrium was 2.2 mg · ml^{-1}. Higher concentrations of drug may be obtained by adding alkaline solutions to propylene glycol. The preparations of fluorouracil in propylene glycol currently used for clinical applications contain an alkali, and their pH ranges between 8.5 and 9. In this investigation the permeation rate of the drug from both saturated and unsaturated solutions of fluorouracil in pure propylene glycol was measured. Results presented in Fig. 2 show that although lower in comparison to the aqueous solutions, constant skin permeation profiles were obtained for each solution. Thus for these systems, the rate of skin permeation at steady-state may be calculated from the slope of the plot of cumulative amount of permeant versus time. The effect of various concentrations of propylene glycol in the donor on permeation rate of the drug from unsaturated solutions at two pH's was also examined. These systems contained a constant 5 mg · ml^{-1} concentration of drug. The cumulative plot of the amount of permeated drug versus time exhibits a similar pattern to the previous systems tested where after a certain lag time, a

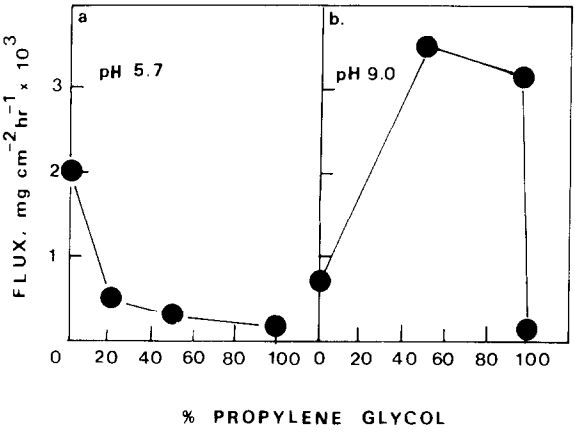
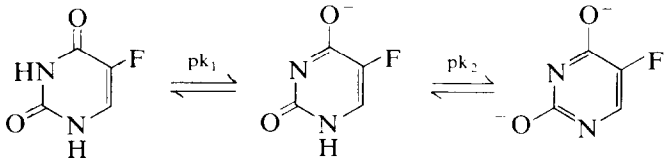


Fig. 5. Effect of various concentrations of propylene glycol on the flux of 5-FU from solutions at two pH's.

constant rate of skin permeation was obtained. However, it is interesting to observe that the concentration of propylene glycol at pH 5.7 affected the drug permeation fluxes in a different way than the pH 9 system. The concentration-dependent effect of PG on these systems is shown in Fig. 5. The flux of fluorouracil from PG/buffer solution pH 5.7 (Fig. 5a) was found to decrease from 2 to 0.36×10^{-3} mg · cm^{-2} · h^{-1} as the concentration of PG in the buffer solution was increased from 0 to 50% w/w. The results in Fig. 5b indicate that at pH 9 the permeation fluxes increase in the presence of PG, and penetration enhancement appeared to be dependent on the PG concentration. A maximal five-fold increase in flux from 0.7 to 3.5×10^{-3} mg · cm^{-2} · h^{-1} as compared to the buffer pH 9 solution. This pH-dependent behaviour may be explained by the chemical nature of fluorouracil and its potential binding to PG. One tautometric form of the acid–base dissociation is given in Eqn. 3:



(3)

The data obtained with pH 5.7 systems suggests that in the acid medium the diffusivity of the drug is reduced with increasing PG concentrations because of an increase in viscosity. At pH 9 the preferred tautomeric form of the drug is the anion which undergoes hydrogen bonding with PG. Based on the finding that PG is highly permeating through hairless mouse skin (Turi et al, 1979), it can be reasonably assumed that the fluorouracil anion is carried through the skin by propylene glycol. The decrease in fluxes occurring at PG concentrations higher than 50% may be explained by the effect of higher viscosities on lowering the diffusivities at higher PG ratios (Fig. 5b).

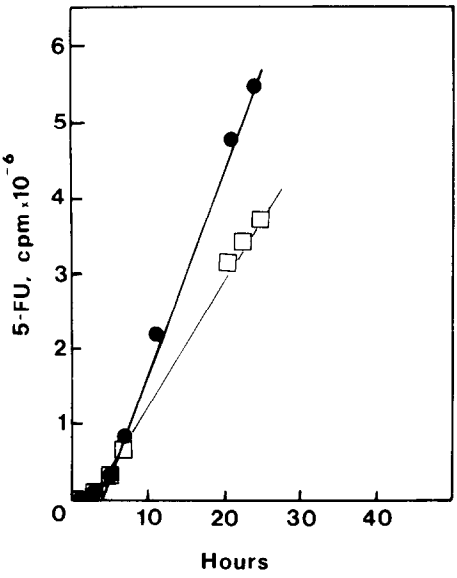


Fig. 6. Permeation profile of 5-FU from a solution containing 5 mg·ml^{-1} drug and: □ 1% LDA; or ● 40% LDB.

Effect of Azone and n-decylmethyl sulphoxide on kinetic parameters of fluorouracil penetration

For brevity, Azone and *n*-decylmethyl sulphoxide will be referred to as LDA and LDB, respectively, in the following discussion. The permeation course for 24 h of fluorouracil with LDA and LDB presented in Fig. 6 shows that the profile always consisted of a lag phase followed by a steady-state linear dependency. To evaluate the concentration-dependent enhancement effects, various concentrations of LDA and LDB were used. The kinetic parameters calculated by means of Eqn. 2 and presented in Table 1 show that the concentration dependence obtained with LDA was different from that with LDB; LDA effectively increased the flux of fluorouracil through the skin with increasing the steady-state concentrations of LDA from 1 to 40%. Penetration rates increased up to 56-fold over the control value. However, a 44-fold enhancement was already achieved at 1% LDA, and further increased concentrations determine only a relatively moderate promotion. With LDB systems the enhancement effect was observed to occur only at concentrations higher than 5%, and at 15% only a two-fold increase was measured; these results indicate that at the same concentration level, in the range of lower concentrations (1–15%), the LDA is evidently more potent than LDB (53 versus 2 times increase, respectively). However, it is interesting to note that at a higher concentration such as 40%, LDB was the more active enhancing agent which gave a 72-fold increase versus the 57-fold increase in the steady-state flux with LDA. This behaviour is well illustrated in Fig. 7.

Taking into account that LDB is a surface-active agent that undergoes micelliza-

TABLE 1

EFFECT OF VARIOUS CONCENTRATIONS OF AZONE AND *n*-DECYLMETHYL SULPHOXIDE (LDB) ON THE PERMEATION KINETIC PARAMETERS OF FLUOROURACIL

Enhancer (%)	Azone (LDA)		*n*-decylmethyl sulphoxide (LDB)	
	Flux ($\bar{X} \pm$ S.D.) $mg \cdot cm^{-2} \cdot h^{-1} \times 10^{3}$ [a]	K_p ($\bar{X} \pm$ S.D.) $cm \cdot h^{-1} \times 10^{3}$ [b]	Flux ($\bar{X} \pm$ S.D.) $mg \cdot cm^{-2} \cdot h^{-1} \times 10^{3}$ [a]	K_p ($\bar{X} \pm$ S.D.) $cm \cdot h^{-1} \times 10^{3}$ [b]
0	2.95 ± 0.43	0.60 ± 0.08	2.95 ± 0.43	0.60 ± 0.08
1	129.70 ± 29.80	26.00 ± 5.98	12.50 ± 0.37	0.50 ± 0.07
5	148.00 ± 26.60	29.60 ± 5.33	2.50 ± 0.27	0.50 ± 0.05
10	140.00 ± 21.00	28.00 ± 4.20	–	–
15	156.00 ± 18.70	31.20 ± 9.74	5.80 ± 1.22	1.16 ± 0.24
40	167.00 ± 20.00	33.40 ± 4.01	213.00 ± 27.70	42.00 ± 4.62

[a] Calculated from the slopes of the individual cumulative plots divided by area = 0.78 cm^2.
[b] Calculated by means of Eqn. 2 (see text).

tion which may affect its interference in the skin permeation process, lower concentrations than 1% (0.1, 0.25 and 0.5%) were also tested (PG/aqueous solution). No increase in permeation was detected in this solvent system.

The data presented here indicates that although LDA and LDB are both surface-active molecules and have similar molecular weights, they appear to alter the skin permeation of fluorouracil by different mechanisms. This observation was further supported by the effect of LDA and LDB on the drug reservoir detected in

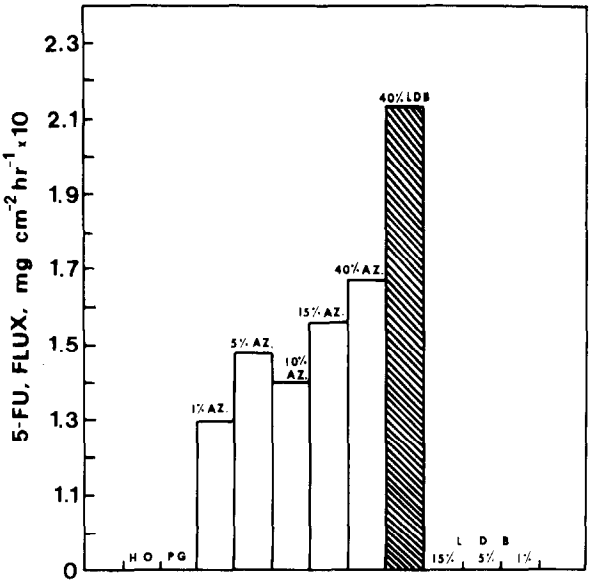


Fig. 7. Effect of various concentrations of enhancers on the permeation rate of 5-FU from solutions containing 5 $mg \cdot ml^{-1}$ drug.

TABLE 2

RESERVOIR EFFECT OF 5-FU IN HAIRLESS MOUSE SKIN FOUND DURING PERMEATION STUDIES * WITH VARIOUS SYSTEM COMPOSITIONS CONTAINING 5 mg·ml^{-1} 5-FU

K_p cm·h^{-1} × 10^3	5-FU concn. mg·(mg skin)$^{-1}$ × 10^4	System
0.1 ± 0.02	8.37 ± 0.93	Buffer pH 9
0.6 ± 0.08	2.79 ± 0.42	PG/Aq. soln. pH 9
26.0 ± 5.60	21.06 ± 3.10	PG/Aq. soln. pH 9 + 1% Azone
42.0 ± 4.60	15.73 ± 2.06	PG/Aq. soln. pH 9 + 40% LDB

* The results are presented as the mean ± S.D. of four determinations.

the skin. The effect of enhancers on the affinity of fluorouracil to the hairless mouse skin was evaluated by measuring the drug retention in the skin at the end of 30 h experiments. The results presented in Table 2 as mg fluorouracil·(mg skin)$^{-1}$ show that both LDA and LDB increased the concentration of drug retained in the skin. However, the quantity of drug retained by the skin from the LDB systems was found to be smaller than from LDA systems (15.7 versus 21.1, respectively); even the corresponding permeability coefficients appear to be in a reverse trend, e.g. 4.2×10^{-2} cm·h^{-1} for LDB and 1.6×10^{-2} cm·h^{-1} for LDA.

Examining the results in Table 2, it is also noteworthy that when the results

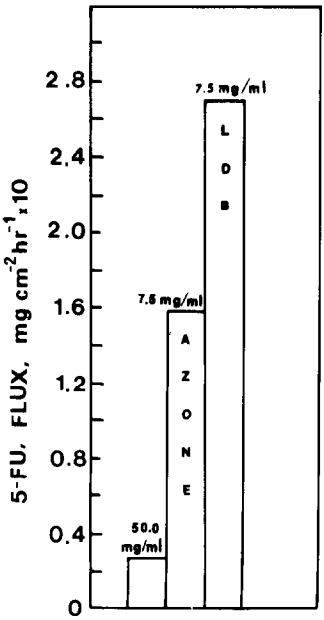


Fig. 8. Skin permeation flux of 5-FU from an aqueous solution of 50 mg·ml^{-1} as compared with solutions of 7.5 mg·ml^{-1} drug.

obtained with the two systems are compared, one containing the drug in buffer pH 9 and the other in PG/aqueous solution pH 9, the higher permeability and smaller reservoir effect was obtained with the PG system. This observation sustains the assumption made previously that PG may facilitate the transport of fluorouracil anion through the skin.

To assess a practical approach to some of the results obtained in these studies, the permeability coefficient of fluorouracil through the skin from a commercial solution of 50 $mg \cdot ml^{-1}$ drug was compared to the coefficient obtained with solutions containing only 7 $mg \cdot ml^{-1}$ drug in PG/aqueous solutions pH 9 with LDA 1% or LDB 40%. The results are schematically drawn in Fig. 8. The higher permeation from the preparations containing the enhancing agents and mainly from the 40% LDB system is evident. However, it will be important to judge if, and when, the system containing as much as 40% *n*-decylmethyl sulphoxide (LDB) is preferred to that containing only 1% Azone (LDA).

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