Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 298 (2005) 98–107
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Effects of ionization and penetration enhancers on the transdermal delivery of 5-fluorouracil through excised human stratum corneum

Brahma N. Singh *, Romi B. Singh [1], Jagdish Singh [2]

Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

Received 9 September 2004; received in revised form 28 January 2005; accepted 6 April 2005
Available online 23 May 2005

Abstract

The purpose of this study was to determine the effects of ionization and penetration enhancers on the transdermal delivery of 5-fluorouracil (5-FU) through excised human stratum corneum. The in vitro transport of 5-FU was determined at three physiologically relevant pH values of 5.0, 7.4 and 8.0, and in the presence of suitable penetration enhancers, namely Azone® (AZ), lauryl alcohol (LA), and isopropyl myristate (IPM). The results showed that passive permeation of 5-FU is dependent upon the pH of the donor solution, although did not fully conform to the pH-partition hypothesis. A further analysis of data suggested an inverse relationship (i.e., negative correlation) between steady-state flux and aqueous solubility of 5-FU at these pH values (correlation coefficient = −0.4205), although correlation was not statistically significant ($p = 0.7237$). In the absence of a penetration enhancer, the in vitro permeability of 5-FU was quite low ($0.82 \pm 0.06 \times 10^4$ cm/h). This delivery rate was enhanced by approximately by 3, 4 and 24-fold, respectively, when IPM, LA, and AZ were incorporated into the donor solution. All these enhancements were statistically significant ($p < 0.05$) compared to control, and occurred regardless of the polarity (solubility parameters) of these enhancers. Out of three examined enhancers, AZ appears to be a suitable enhancer for enhancing transport of 5-FU, which merits in vivo investigation in a suitable animal model. Possible mechanisms of enhancement by these penetration enhancers are also discussed.

Keywords: 5-FU; Azone®; Lauryl alcohol; Isopropyl myristate; Transdermal; Influence of pH

* Corresponding author at: Department of Pharmaceutics R&D, Emisphere Technologies, Inc., 765 Old Saw Mill River Road, Tarrytown, NY 10591, USA. Tel.: +1 914 785 4758; fax: +1 914 347 2498.
E-mail address: BSingh@Emisphere.com (B.N. Singh).
[1] Present address: Ranbaxy Research Laboratories, Gurgaon 122001, Haryana, India.
[2] Present address: Department of Pharmaceutical Sciences, College of Pharmacy, North Dakota State University, Fargo, ND 58105, USA.

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.04.004

1. Introduction

5-Fluorouracil (5-FU, Fig. 1) is an antimetabolite with promising antineoplastic activity against several premalignant and malignant conditions of the skin including Bowen's disease and superficial basal cell carcinomas (Bargman and Hochman, 2003; Epstein, 1985). Its topical application has also been proven to be a valuable and safe treatment for psoriasis and actinic keratosis (Pearlman et al., 1986; Robins and Gupta, 2002). Chemically, it is a diprotic acid with pK_a values of 8.0 and 13.0 (Rudy and Senkowski, 1973), and is highly polar in nature ($\log P_{\text{octanol/water}} = -0.89$; Williams and Barry, 1991). Because of its hydrophilic nature, the transdermal permeation of 5-FU through lipophilic stratum corneum is very low and marginal. This has prompted several investigators to explore alternate approaches to facilitate its transdermal delivery across skin. These approaches include iontophoresis (Merino et al., 1999), phonophoresis (Meidan et al., 1999), electroporation (Fang et al., 2004), laser treatment (Lee et al., 2002), prodrug approach (Beall and Sloan, 2002), and use of penetration enhancers (Gao and Singh, 1998; Goodman and Barry, 1988; Hirvonen et al., 1991).

Apart from clinical usefulness for topical treatment of skin related disorders, transdermal delivery of 5-FU may overcome certain limitations associated with oral and parenteral administration of 5-FU. After oral administration, 5-FU is poorly absorbed with significant variation in bioavailability ranging between 0 and 80% (Diasio and Harris, 1989). Following parenteral administration of 5-FU, there is a rapid elimination of the drug with an apparent terminal half-life of approximately 8–20 min (Diasio and Harris, 1989). These two problems make 5-FU a suitable candidate for transdermal delivery.

The objectives of this study were to examine the role of ionization and study the effects of penetration enhancers on the in vitro transport of 5-FU through excised human stratum corneum. From transdermal delivery point of view, a role of pH for percutaneous transport is obvious since contributions of ionization, solubility, lipophilicity, and pH are interrelated. Additionally, there is at least one recent evidence suggesting that cellular uptake of 5-FU by tumor cells exhibits pH-dependence (Ojugo et al., 1998). In this study, Azone®, lauryl alcohol (dodecyl alcohol) and isopropyl myristate were used as penetration enhancers. Azone® and isopropyl myristate were selected to quantitatively compare their effects since both enhancers seem to exert their effects by interacting with structured lipid bilayers of the stratum corneum. Likewise, lauryl alcohol was selected for comparing its penetration enhancing effect with Azone® because, like Azone®, it has a dodecyl side chain (Bhatt et al., 1991). The outcomes of various studies were assessed in terms of changes in rate of delivery (i.e., steady-state flux) and enhancement factor, whichever was applicable.

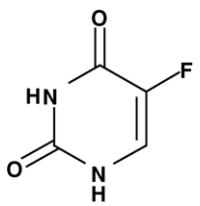

Fig. 1. Chemical structure of 5-fluorouracil.

2. Materials and methods

2.1. Materials

5-FU and Azone® (1-dodecylazacycloheptan-2-one) were supplied as free samples from Biochem Pharmaceutical Industries (Bombay, India) and Nelson Research and Development (Irvine, CA, USA), respectively. Lauryl alcohol (1-dodecanol) and isopropyl myristate were purchased from Sisco Research Laboratories Pvt. Ltd. (Bombay, India). All other chemicals were of analytical grade and were used without further purification.

2.2. Preparation of human skin membranes

Full thickness abdominal skin samples were obtained from cadavers at autopsy within 24 h of death from the department of forensic medicine. Skin samples were sealed in evacuated resealable plastic bags and stored frozen at −20 °C until used (≤24 h). All donors were males with an average age of 40 ± 9 (mean ± S.D.) years. The thickness of these specimens was approximately 1.5 cm, which based on average skin layer thickness includes the stratum corneum, the epidermis, dermis and some subcutaneous fat.

The subcutaneous fat was trimmed off with a scalpel and sheets of stratum corneum plus attached epidermis were prepared from the whole skin by a heat separation technique (Kligman and Christophers, 1963). Skin samples trimmed of fatty tissues were immersed in water at 60 °C for 2 min, after which the epidermal membranes were teased off the underlying dermis using ear buds (Johnson and Johnson Ltd., Bombay, India). The membranes were floated on water, cut into square pieces, and then placed dermal side down on filter papers. Then epidermal membranes were washed with distilled water, dried at room temperature (RT) and stored at −20 °C in a sealed petri dish. The epidermis was allowed to thaw at RT and rehydrated by immersing in water for 1 h before being used. Prior to use, each specimen was checked for its physical integrity and leakage.

2.3. Apparatus for permeation studies

In vitro passive permeation studies were carried out using two-chambered diffusion cells designed in our laboratory and reported earlier (Singh et al., 1992). The internal diameter and maximum capacity of each cell were 1.8 mm and 4.5 ml, respectively. Square pieces of isolated epidermis were mounted securely between the two halves of the diffusion cell with the dermal side exposed to the receptor compartment. The epidermis was supported in this position by a wire mesh. A thin film of silicone grease (Dow Corning Corporation, Michigan, USA) was applied on the lapped glass surfaces of the diffusion cell to provide a watertight glass to membrane seal. A spring-loaded clamp was used to hold the donor and receptor compartments together.

The assembled cells were immersed in a water bath maintained at 37 ± 0.5 °C. The solutions in both donor and receiver compartments were constantly stirred by a matched pair of starhead-shaped magnetic fleas (Thomas Scientific, NJ, USA) rotating at 40 rpm. The exposed surface area of the membrane available for drug permeation was 2.54 cm^2.

2.4. Passive diffusion studies

All diffusion studies were carried out at least in triplicates. The donor compartment was filled with 4.0 ml of freshly prepared solution of 5-FU in a buffer solution of desired pH. While investigating the effects of various permeation enhancers, solution formulations of 5-FU (1 mg/ml) were used as donor solutions. The receptor solution contained 4 ml of phosphate buffer (pH 7.4) throughout the studies. Samples of 0.5 ml each were withdrawn at regular intervals from the receptor compartment and immediately replaced with the same volume of fresh receptor solution. At the start and end of the experiment, 0.1 ml of the donor solution was also sampled for drug analysis to perform mass balance. The integrity of each specimen was examined at the end of each experimental run by quantifying the permeation of 0.5% benzyl alcohol for 1 h.

2.5. Effect of pH

The effect of change in the pH of the donor solution on the drug transport was studied at three different pHs: 5.0, 7.4, and 8.0 at 37 ± 0.5 °C. The buffer solution was prepared by mixing equal proportions of 30 mM each of trisodium citrate, sodium dihydrogen orthophosphate and glycine, and adjusting the final pH by adding 1 M NaOH or 1 M HCl. The same buffer of 90 mM was used to vary the pH of donor solution to minimize changes in transport efficiency. In addition, any changes in pH during drug transport were monitored and corrected for by the addition of micoliter amounts of 1 M HCl or 1 M NaOH solutions. By this method, the pH was kept within ± 0.2 units of the desired pH as determined at the end of the experiment.

2.6. Effects of penetration enhancers

The effects of the penetration enhancers on the skin permeation of 5-FU were studied using Azone®, lauryl alcohol, and isopropyl myristate. The concentrations of these enhancers in the donor solution were 3% (w/v), 5% (w/v), and 5% (w/w), respectively. In case of studies with Azone®, the drug solution was emulsified with the aid of 0.11% (w/v) of polysorbate 20, as suggested previously (Morimoto et al., 1986). All of these studies were performed at physiological pH, that is, the pH of the donor and receptor solutions were 7.4.

2.7. Analytical method

A standard curve of drug concentration versus absorbance was prepared in the expected range of drug

concentrations. Triplicates samples were used to check the reproducibility of the absorbance values. The repetition of the procedure gave concurrent absorbance values, which proved the reliability of the instrument in the analysis of the samples. The unknown samples were diluted as desired with phosphate buffer (pH 7.4) and the absorbances were measured at 266 nm on a Beckman Model-24 spectrophotometer. The absorbance at 266 nm showed no interference from water-soluble extracts of the skin.

2.8. Data analysis

The concentrations of 5-FU in unknown samples were determined by reference to a calibration plot that was linear. Appropriate corrections were made in calculating the cumulative amount of drug that permeated the skin using Eq. (1) (Touitou and Abed, 1985):

$$C_n = C_{n1} + \frac{0.5}{4} \sum_{S=1}^{S=n-1} C_p \quad (1)$$

where C_n is the corrected concentration of nth sampling, C_{n1} the measured concentration and C_p is the measured drug concentration of preceding samples.

The steady-state flux (J_{ss}) was determined from the slope of the linear portion of the plot of cumulative amount permeated per square cm of the skin versus time. The permeability coefficient (K_p) was calculated using Eq. (2) (Scheuplein, 1978):

$$K_p = \frac{J}{C} \quad (2)$$

where J is the flux and C is the concentration of 5-FU in the donor compartment. Finally, the enhancement ratio (ER) was calculated by Eq. (3) (Goodman and Barry, 1988):

$$\mathrm{ER} = \frac{K_p \text{ after application of penetration enhancer}}{K_p \text{ before application of penetration enhancer}} \quad (3)$$

2.9. Statistical analysis

Statistical comparisons were made using paired *t*-test (Microcal™ Origin™, Version 5.0). For the evaluation of any correlation, Pearson's correlation test was performed, and the correlation coefficients and associated probability values (two-tailed) were calculated using a statistical software (Graph PAD Instat®, CA, USA). The level of significance was considered at $p < 0.05$.

3. Results and discussion

3.1. Effect of ionization

The effect of ionization on the transdermal transport of 5-FU through human skin was studied at three different pH values, which were 5.0, 7.4 and 8.0. In aqueous medium, 5-FU behaves like a weak acid, with pK_a of 8.0 and 13.0. This implies that at pH values above 8, the molecule is predominantly negatively-charged (Rudy and Senkowski, 1973). It is also obvious that 5-FU would mainly exist in the unionized (free acid) form at the lowest pH studied (i.e., 5.0), and the proportion of unionized fraction would gradually decrease with an increase in pH, and at pH value equals to pK_a of 5-FU, an equal proportion of the ionized (monoanion) and unionized species would co-exist in the bulk of solution (Table 1). Values of pH lower than 5 or higher than 8 were not selected for this study since they are not physiologically relevant, and extreme acidic or alkaline conditions may cause alterations of membrane (Irwin et al., 1990).

The results from the diffusion studies determining the rate of delivery of 5-FU through human stratum corneum at different pHs are presented in Fig. 2, and the corresponding values are summarized in Table 1. As shown in Table 1, the steady-state flux increased by 1.6-fold when the pH of the donor solution changed from 5.0 to 7.4. On the other hand, the flux of 5-FU decreased

Table 1
Effect of pH on the fraction unionized[a] and the steady-state flux (mean ± S.D. of three determinations) of 5-FU transported through human stratum corneum

pH	Fraction unionized[b]	Steady-state flux (μg/cm²/h)		
		J_{ss} (total)	J_u	J_i
5.0	0.999	10.54 ± 0.04	10.53 ± 0.04	0.01 ± 0.00
7.4	0.799	16.4 ± 1.28	13.10 ± 1.02	3.3 ± 0.26
8.0	0.50	9.16 ± 1.48	4.58 ± 0.74	4.58 ± 0.74

[a] Fraction unionized (for acidic compounds) = 1/(1 + antilog(pH − pK_a)).
[b] Calculated by considering its pK_{a1} value of 8.0.

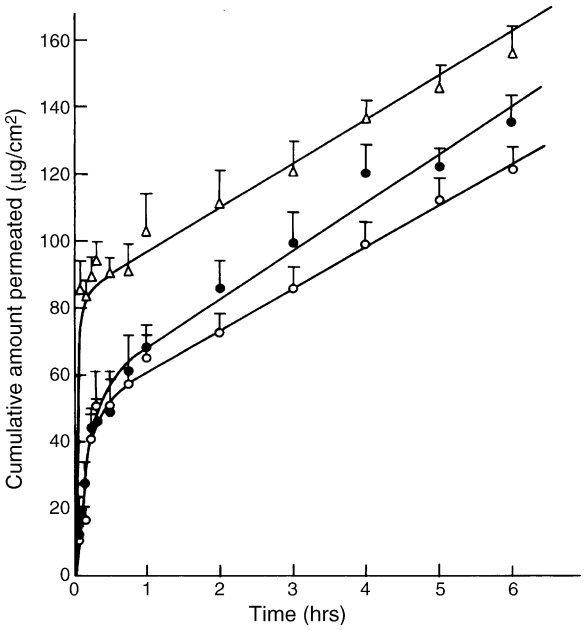

Fig. 2. In vitro permeation profiles of 5-FU through human cadaver skin at different pH values during passive diffusion. Key: (△) pH 8; (●) pH 7.4; (◯) pH 5. (Each point represents the mean ± S.D. of three experiments; only half bars have been shown for clarity.)

significantly with only a slight increase in pH from 7.4 to 8.0. The effect of pH on the passive permeation of 5-FU through porcine ear skin has been recently studied by Merino et al. (1999). Interestingly, these authors also reported an increase in passive flux of 5-FU by 1.6-fold when compared between flux values at pH 5.0 (41 ± 10 nmol/cm^2/h) and 7.4 (65 ± 27 nmol/cm^2/h), which is consistent with the findings of this study. However, in sharp contrast, they reported an unexpectedly significant increase in flux when flux at pH 7.4 is compared with that at pH 8.5 (101 ± 31 nmol/cm^2/h) for which they could not offer any rational explanation.

Theoretically, as a result of pH-partitioning mechanism, a decrease in passive flux is expected with an increase in pH from 5.0 to 7.4. However, this does not seem to be the case with 5-FU. In this case, the increase in flux occurred probably because both ionized and unionized species permeated through the skin and contributed to the total flux. This also seems conceivable in the light of fact that 5-FU being a hydrophilic molecule permeates through the stratum corneum via intercellular (hydrophilic) pathway (Moghimi et al., 1998).

Yet, there is another possible mechanism for permeation of hydrophilic molecules through the skin. The recent findings of Hadgraft and Valenta (2000) suggest that there might be a significant permeation of the ionized drugs through a lipophilic pathway, possibly as a result of ion pairing. The permeation of ionized (negatively-charged) species of 5-FU per se is not likely to occur through lipophilic pathway since their partitioning across lipid layer of membrane seems almost impossible in the absence of suitable counter ions. Thus, the increase in flux of 5-FU at physiological pH might have been contributed by transport of ionized species mainly through the intercellular pathway.

The relative contribution of ionized and unionized species to total observed flux (J_{ss}) was calculated using Eq. (4) (Irvin et al., 1990):

$$J_{ss} = \alpha J_i + (1 - \alpha) J_u \quad (4)$$

where J_i is the flux of the ionized fraction of 5-FU, J_u the flux of the unionized fraction, and α the degree of ionization. Furthermore, the fraction unionized was calculated by considering its first dissociation constant ($pK_{a1} = 8.0$). The second pK_a of 5-FU is considered too high ($pK_{a2} = 13.0$) to influence the ionization properties of aqueous solutions that have pH less than 9.0 (Monnot et al., 1990).

The calculated values of ionic flux (J_i) at various pHs are presented in Table 1. These values have been numerically deduced based on the proportion of ionized fraction; therefore, the data may not be solely explained just by considering the electrical properties of the skin. The isoelectric point (p*I*) of the human skin has been estimated to be about 4, therefore, under normal physiological conditions, with the surface of the skin also buffered at or near pH 7.4, the membrane bears a net negative charge (Burnette and Ongpipattanakul, 1987). Only lower pH values ($pH < pI_{Skin}$) are expected to alter the skin charge to net positive charge by providing hydronium ions, and the skin retains its net negative charge at pHs greater than pI of the skin (from 5.0 to 8.5) (Merino et al., 1999). If this holds true, then theoretically a relatively greater degree of electrostatic repulsion should occur between anions of 5-FU and negatively charged membrane, and as a result a decrease in ionic flux is expected at pH 8.0 (about 50% of 5-FU is ionized) compared to pH 7.4 (only 20% ionized). However, this in not consistent

Table 2
pH-solubility of 5-FU in 0.1 M phosphate buffer (data from Monnot et al., 1990)

pH	Solubility (mg/ml)
4.97	11.9
7.25	15.4
7.79	20.1
8.03	24.6
8.25	36.6

with the calculated values of ionic fluxes at pH 7.4 and 8.0. Thus, these values are unlikely to be suggestive of any effect of donor solution pH on the transport of ionic species, which is particularly mediated via altered membrane polarity or electrostatic repulsion.

In the second case, the total flux decreased significantly with a slight increase in pH from 7.4 to 8.0. This may be attributed to the decrease in unionized fraction of 5-FU, further suggesting that pH-partitioning was a predominant mechanism. However, a possible role of drug solubility in passive diffusion cannot be completely ruled out. The impact of drug solubility on percutaneous absorption has been studied mechanistically by considering the thermodynamic interrelationship between solubility, activity and activity coefficients where higher solubility is associated with lower activity coefficients and lower escaping tendencies of the solute from that solvent (Kurihara-Bergstrom et al., 1987). Since the aqueous solubility of 5-FU has been reported to be greater at higher pH compared to lower pH due to salt formation (Table 2), a relatively higher flux at pH 7.4 compared to pH 8.0 seems possible. However, this is in complete disagreement with the viewpoints of Hadgraft and Valenta (2000) that the maximum flux through the skin might occur at a pH where ionization is high since the aqueous solubility of the ionized material is significantly higher than the unionized, which would in turn compensate for the lower permeability of the ionized species.

The solubility of 5-FU in phosphate buffer (0.1 M) of various pHs is given in Table 2. Since these data were obtained in buffers of ionic strength similar to that used in present study (90 mM), it is worthwhile to correlate selected solubility values (at pH 4.97, 7.25 and 8.03) with corresponding flux values (at pH 5.0, 7.4, and 8.0). These two data sets are graphically displayed in Fig. 3. As shown in Fig. 3, the different fluxes reflect the dependence of the rate of permeation on ionization as well as on drug solubility in the pH range of 5–8. There was a negative correlation between aqueous solubility and steady-state flux values obtained at similar pHs ($p = 0.7237$, $r = -0.4205$). Collectively, these observations clearly suggest that pH-dependent partitioning, pore transport, and solubility-dependent mechanisms may play a concurrent role in the transport of hydrophilic drugs like 5-FU through the skin depending on the pH.

3.2. Effects of penetration enhancers

In absence of a suitable penetration enhancer or a formulation aid (e.g., vehicle, ion-pairing or

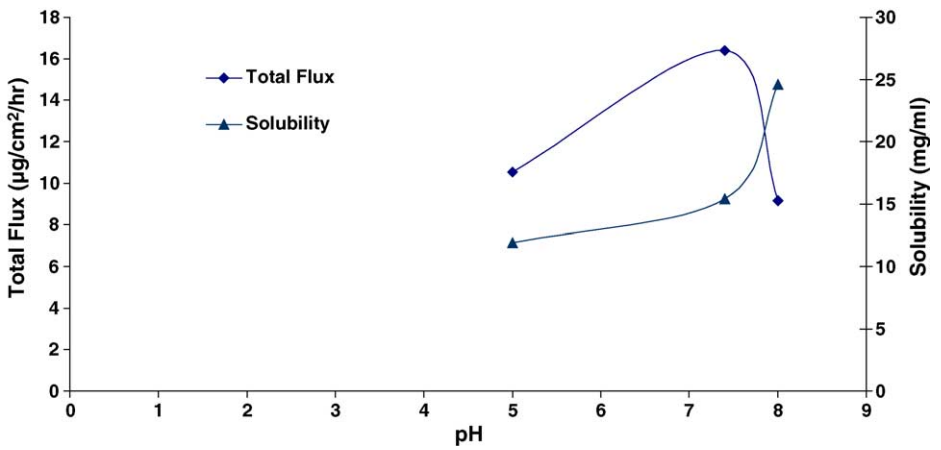

Fig. 3. Dependence of drug delivery rate on solubility and pH of donor solution.

complexing agent, etc.), the transport of 5-FU across skin is very limited. The experimentally determined diffusion coefficient of 5-FU through the human stratum corneum is about an order of 10^{-7} cm^2/h (Williams and Barry, 1991). The poor permeation of 5-FU through the stratum corneum is related to its hydrophilic nature, which is evident by the log P (stratum corneum/water) value of 0.46 (Williams and Barry, 1991). This has prompted several investigators to determine the permeability coefficients (K_p) of 5-FU across various skin types and a wide range of transdermal formulations and penetration enhancers. In this context, perhaps 5-FU is the most extensively studied hydrophilic model drug for transdermal research.

The effectiveness of various penetration enhancers on the transdermal transport of 5-FU is shown in Fig. 4. The corresponding results, in terms of their enhancement ratios, are tabulated in Table 3. In general, all studied enhancers promoted the in vitro transport of 5-FU across the stratum corneum, which occurred in the order IPM < LA < AZ. Three percent AZ increased the K_p by almost 24-fold, while 5% IPM and 5% LA increased by a factor of approximately 3 and 4, respectively. All these enhancements were statistically significant ($p < 0.05$) compared to control. Given the solubility parameter (δ) of 5-FU, which is equal to 14.99 (cal/cm^3)$^{1/2}$ (Beall and Sloan, 2002), and of these studied enhancers as reported in Table 3, it is apparent that enhancement in drug permeation occurred regardless of the polarity of these enhancers.

Goodman and Barry (1988) reported only eight-fold enhancement in permeation of 5-FU through human skin while using similar concentration of AZ (3% AZ with 0.1% Tween 20 in normal saline). This difference in results may be in part attributed to the ethnic differences in structure (e.g., number of hair follicles) and lipid composition of the stratum corneum in Caucasian versus Indian skin.

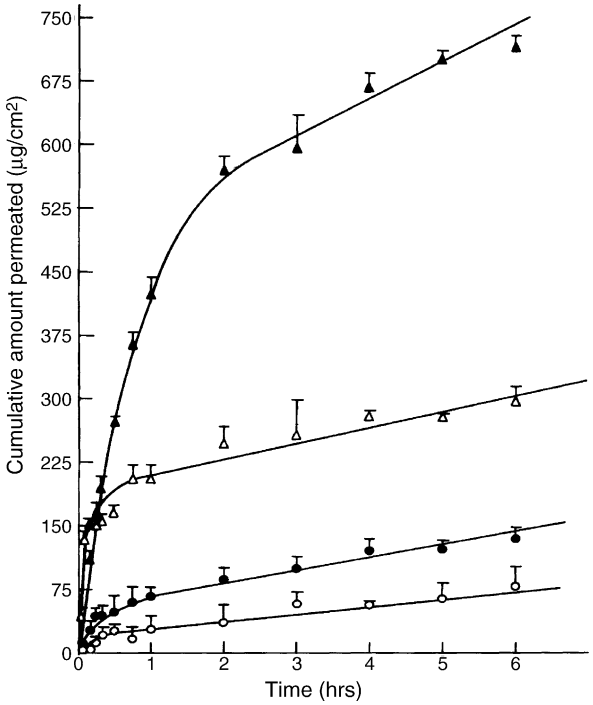

Fig. 4. Effect of various penetration enhancers on passive permeation of 5-FU through human cadaver skin. Key: (◯) 5% IPM; (●) control; (△) 5% LA; (▲) 3% AZ. (Each point represents the mean ± S.D. of three experiments; only half bars have been shown for clarity.)

The ability of AZ (also known as laurocapram) to greatly enhance the passive delivery of 5-FU across the hairless mice, rat, shed snake, and human skins has been demonstrated by several investigators

Table 3
Effect of various penetration enhancers on the steady-state flux (mean ± S.D. of three determinations) of 5-FU transported through human stratum corneum

Enhancers	Concentration	J_{ss} (μg/cm^2/h)	K_p (cm/h × 10^4)	Enhancement ratio
None (control)	0	16.4 ± 1.28	0.82 ± 0.06	1.0
Isopropyl myristate (δ = 8.02)[a]	5% (w/w)	44.26 ± 3.14	2.21 ± 0.16*	2.71 ± 0.29
Lauryl alcohol (δ = 9.51)[a]	5% (w/v)	61.45 ± 15.29	3.07 ± 0.76*	3.79 ± 1.12
Azone (δ = 9.06)[b]	3% (w/v)	397.19 ± 10.86	19.86 ± 0.54*	24.31 ± 1.94

δ = solubility parameter (cal/cm^3)$^{1/2}$.
[a] From Vaughan (1985).
[b] From Hadgraft et al. (1993).
* Significantly different from control ($p < 0.05$).

(Sugibayashi et al., 1985; Touitou and Abed, 1985; Morimoto et al., 1986; Díez-Sales et al., 1996; Itoh et al., 1992; Meidan et al., 1999). To date, there are three known mechanisms by which Azone® exerts its enhancer effect on drug permeation. First, it causes a fluidization of the structured lipids of the stratum corneum (Sugibayashi et al., 1992). This fluidization effect is mediated by the ability of Azone® molecules to insert themselves within the intercellular lipids (ceramide bilayers) of the stratum corneum (Meidan et al., 1999), and is related to its chemical structure, charge distribution and conformational state within the stratum corneum (Kim et al., 2001). As a result of this increased disorder, the diffusivity of 5-FU through the stratum corneum is significantly increased (Morimoto et al., 1986). Second mechanism, which is more relevant for penetration of hydrophilic compounds including 5-FU, is the fact that Azone® exerts a hydration effect on the stratum corneum (Sugibayashi et al., 1992). The hydrated stratum corneum, in turn, makes the penetration of hydrophilic compounds easier (Díez-Sales et al., 1996). Finally, the enhancing effect of AZ appears to involve a change in thermodynamic driving force across skin. The activation energy required for drug molecules to penetrate through the skin is significantly decreased in the presence of AZ compared to that in its absence (Ito et al., 1988). Out of these, first and third mechanisms might be responsible for enhancement in 5-FU permeation in the present study since our experiments utilized human stratum corneum that were fully hydrated in water for at least 1 h.

The effects of LA on the transdermal permeation of 5-FU have been investigated previously using shed snakeskin (Itoh et al., 1992). To authors' knowledge, this is the first investigation that examines the permeation enhancing effect of LA on transdermal delivery of 5-FU through human skin. Interestingly, the penetration enhancing effect of LA is much lower compared to AZ even though both enhancers contain a dodecyl side chain. Likewise, dodecyl *N*,*N*-dimethylamino acetate, which like AZ contains a C-12 alkyl chain, has been reported to enhance the K_p of 5-FU through human skin by 22-fold compared to only 11-fold with AZ (Hirvonen et al., 1991). Furthermore, the penetration enhancing effects of LA and AZ obtained in the present study exhibit a trend (LA < AZ) that is similar to that previously obtained for 5-FU across shed snakeskin (Itoh et al., 1992). This may be attributed to structural similarity between shed snakeskin and human stratum corneum (Itoh et al., 1990; Hirvonen et al., 1991). From mechanistic point of view, the penetration enhancing effect of LA is more likely to be mediated by a solubilization effect, which is common among alkanols. The drug solubility in the fatty matrix of the stratum corneum is considerably increased which in turn leads to improved partitioning of the drug into the skin (Bhatt et al., 1991).

IPM is an aliphatic ester, which has been widely used as a safe penetration enhancer in commercial dermatological formulations (e.g., Andro-Gel®). In addition, it has also been studied as a vehicle for transdermal delivery of 5-FU prodrugs to improve their stability (Sherertz et al., 1990). Its mechanism of action is not precisely understood, but it is thought that IPM exerts its effect by interacting with structured lipids of the stratum corneum. In a recent mechanistic study, it has been shown that IPM inserts (lateral) into the human stratum corneum, which results in densely packed bilayer lipids and a loss of order of the corneocyte-bonded lipids (Brinkmann and Müller-Goymann, 2003). This combined effect resulted in a decreased diffusion coefficient of hydrocortisone (a hydrophilic drug) in the stratum corneum and thus in a decreased permeation rate. Based on this mechanism, it is likely that diffusivity of the 5-FU would be decreased in the presence of IPM.

One intriguing aspect of this study is that the incorporation of 5% (w/w) IPM into donor solution increased the K_p by 2.7-fold, which suggests that the initial rate of delivery was faster. However, the overall permeation profile appeared to be lower compared to control. The increase in K_p may be probably attributed to an initial surge in "thermodynamic activity gradient" across the stratum corneum. The saturated solubility of 5-FU in IPM is 0.049 mM (Beall and Sloan, 2002), which is much lower than the aqueous solubility of 5-FU in phosphate buffer, pH 7.4 (57.82 mM; Gao and Singh, 1998). Thus, it is possible that by adding IPM to donor buffer, the solubility of 5-FU is decreased so that a relatively greater saturation of 5-FU is maintained during initial drug transport. As a result, 5-FU might exhibit a higher thermodynamic activity in presence of IPM. The decrease in overall drug transport may be related to decreased solubility of 5-FU within lipid matrix of stratum corneum due to presence of IPM, and increased packing of lipids, which is supported

by the findings of Brinkmann and Müller-Goymann (2003).

Sherertz et al. (1990) also observed higher flux rates of 5-FU through human and hairless mouse skins for suspension compared to solution formulations. They also attributed this effect to increased thermodynamic activity of 5-FU since suspended solid dissolved during the course of the experiment to maintain saturated solutions. Similar mechanism has been held accountable to explain the penetration enhancing effects of tetrahydrogeraniol on the percutaneous transport of 5-FU (Hanif et al., 1998). It should be, however, noted that an increased thermodynamic activity (or decreased activity coefficient) of drugs might underlie the penetration enhancing effect of an enhancer without exhibiting any apparent change in drug solubility. Such mechanism has been implicated for penetration enhancing effects of Azone® (Ito et al., 1988).

Overall, the enhancement effects obtained with various penetration enhancers used in the present study are well supported by their known mechanism(s) of action. Three percent Azone® exerted a much higher effect compared to 5% IPM because, unlike IPM, it fluidizes the lipid bilayers of stratum corneum and thus increases drug diffusivity. The inability of IPM to cause a fluidization effect is related to the specific manner in which IPM molecules get intercalated into lipophilic areas with an anchoring of the isopropyl group in the polar region of the stratum corneum microstructure (Brinkmann and Müller-Goymann, 2003). On the other hand, LA causes higher ER compared to IPM because of improved partitioning of 5-FU across the stratum corneum.

4. Conclusions

The results obtained in this investigation have clearly demonstrated that both pH-partitioning mechanism through lipidic pathway and ionic transport through the intercellular pathway may play a concurrent role, depending on the donor solution pH, and contribute to the overall transdermal transport of 5-FU through human skin. Secondly, with the application of suitable penetration enhancers, the transdermal delivery of 5-FU could be substantially enhanced compared to passive diffusion alone. A better understanding of the physicochemical and thermodynamic properties of 5-FU in presence of various formulation adjuvants, and safety and efficacy profiles of available penetration enhancers would be useful in developing commercially viable transdermal therapeutic systems for 5-FU.

Acknowledgements

This research work was performed for the partial fulfillment of the requirements for the degree of Master of Pharmacy (M. Pharm.). BNS gratefully acknowledges the financial assistance provided by the University Grants Commission (UGC), Government of India in the form of Junior Research Fellowship. We would also like to thank Dr. C.B. Tripathi (Head, Department of Forensic Medicine, Institute of Medical Sciences, BHU, Varanasi) for providing cadaver skin specimens. Finally, Biochem Pharmaceutical Industries (Bombay, India) and Nelson Research and Development (CA, USA) are acknowledged for providing the free samples of 5-fluorouracil and Azone®, respectively.

References

Bargman, H., Hochman, J., 2003. Topical treatment of Bowen's disease with 5-fluorouracil. J. Cutan. Med. Surg. 7, 101–105.

Beall, H.D., Sloan, K.B., 2002. Topical delivery of 5-fluorouracil (5-FU) by 1,3-bisalkylcarbonyl-5-FU prodrugs. Int. J. Pharm. 231, 43–49.

Bhatt, P.P., Rytting, J.H., Topp, E.M., 1991. Influence of Azone® and lauryl alcohol on the transport of acetaminophen and ibuprofen through shed snake skin. Int. J. Pharm. 72, 219–226.

Brinkmann, I., Müller-Goymann, C.C., 2003. Role of isopropyl myristate, isopropyl alcohol and a combination of both in hydrocortisone permeation across the human stratum corneum. Skin Pharmacol. Appl. Skin Pharmacol. 16, 393–404.

Burnette, R.R., Ongpipattanakul, B., 1987. Characterization of the permselective properties of excised human skin during iontophoresis. J. Pharm. Sci. 76, 765–773.

Diasio, R.B., Harris, B.E., 1989. Clinical pharmacology of 5-fluorouracil. Clin. Pharmacokinet. 16, 215–237.

Díez-Sales, O., Watkinson, A.C., Herráez-Domínguez, M., Javaloyes, C., Hadgraft, J., 1996. A mechanistic investigation of the in vitro human skin permeation enhancing effect of Azone®. Int. J. Pharm. 129, 33–40.

Epstein, E., 1985. Fluorouracil paste treatment of thin basal cell carcinomas. Arch. Dermatol. 121, 207–213.

Fang, J.Y., Hung, C.F., Fang, Y.P., Chan, T.F., 2004. Transdermal iontophoresis of 5-fluorouracil combined with electroporation and laser treatment. Int. J. Pharm. 270, 241–249.

Gao, S., Singh, J., 1998. Effect of oleic acid/ethanol and oleic acid/propylene glycol on the in vitro percutaneous absorption of 5-fluorouracil and tamoxifen and the macroscopic barrier property of porcine epidermis. Int. J. Pharm. 165, 45–55.

Goodman, M., Barry, B.W., 1988. Action of penetration enhancers on human skin as assessed by the permeation of model drugs 5-fluorouracil and estradiol I. Infinite dose technique. J. Invest. Dermatol. 91, 323–327.

Hadgraft, J., Valenta, C., 2000. pH, pK(a) and dermal delivery. Int. J. Pharm. 200, 243–247.

Hadgraft, J., Williams, D.G., Allan, G., 1993. Azone, mechanisms of action and clinical effect. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York, pp. 175–197.

Hanif, R.M., Qineng, P., Zhan, G., 1998. Penetration enhancing effect of tetrahydrogeraniol on the percutaneous absorption of 5-fluorouracil from gels in excised rat skin. J. Controlled Rel. 55, 297–302.

Hirvonen, J., Rytting, J.H., Paronen, P., Urtti, A., 1991. Dodecyl *N*, *N*-dimethylamino acetate and azone enhance drug penetration across human, snake, and rabbit skin. Pharm. Res. 8, 933–937.

Irwin, W.J., Sanderson, F.D., Li Wan Po, A., 1990. Percutaneous absorption of ibuprofen: vehicle effects on transport through rat skin. Int. J. Pharm. 66, 193–200.

Ito, Y., Ogiso, T., Iwaki, M., 1988. Thermodynamic study on enhancement of percutaneous penetration of drugs by Azone. J. Pharmacobiodyn. 11, 749–757.

Itoh, T., Xia, J., Magavi, R., Nishihata, T., Rytting, J.H., 1990. Use of shed snake skin as a model membrane for in vitro percutaneous penetration studies: comparison with human skin. Pharm. Res. 7, 1042–1047.

Itoh, T., Wasinger, L., Turunen, T.M, Rytting, J.H., 1992. Effects of transdermal penetration enhancers on the permeability of shed snakeskin. Pharm. Res. 9, 1168–1172.

Kim, N., El-Kattan, A.F., Asbill, C.S., Kennette, R.J., Sowell Sr., J.W., Latour, R., Michniak, B.B., 2001. Evaluation of derivatives of 3-(2-oxo-1-pyrrolidine) hexahydro-1*H*-azepine-2-one as dermal penetration enhancers: side chain length variation and molecular modeling. J. Controlled Rel. 73, 183–196.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88, 70–73.

Kurihara-Bergstrom, T., Flynn, G.L., Higuchi, W.I., 1987. Physicochemical study of percutaneous absorption enhancement by dimethylsulfoxide: dimethyl sulfoxide mediation of vidarabine (ara-A) permeation of hairless mouse skin. J. Invest. Dermatol. 89, 274–280.

Lee, W.R., Shen, S.C., Wang, K.H., Hu, C.H., Fang, J.Y., 2002. The effect of laser treatment on skin to enhance and control transdermal delivery of fluorouracil. J. Pharm. Sci. 91, 1613–1626.

Meidan, V.M., Walmsley, A.D., Docker, M.F., Irwin, W.J., 1999. Ultrasound-enhanced diffusion into coupling gel during phonophoresis of 5-fluorouracil. Int. J. Pharm. 185, 205–213.

Merino, V., López, A., Kalia, Y., Guy, R.H., 1999. Electrorepulsion versus elctroosmosis: effect of pH on the iontophoretic flux of 5-fluorouracil. Pharm. Res. 16, 758–761.

Moghimi, H.R., Williams, A.C., Barry, B.W., 1998. Enhancement by terpenes of 5-fluorouracil permeation through the stratum corneum: model solvent approach. J. Pharm. Pharmacol. 50, 955–964.

Monnot, E.A., Kindberg, C.G., Johnson, T.S., Riley, C.M., Stobaugh, J.F., Slavik, M., 1990. Stability of intravenous admixtures of 5-fluorouracil and spirogermanium, a novel combination of cytotoxic agents. Int. J. Pharm. 60, 41–52.

Morimoto, Y., Sugibayashi, K., Hosoya, K., Higuchi, W.I., 1986. Penetration enhancing effect of Azone on the transport of 5-fluorouracil across hairless rat skin. Int. J. Pharm. 32, 31–38.

Ojugo, A.S., McSheehy, P.M., Stubbs, M., Alder, G., Bashford, C.L., Maxwell, R.J., Leach, M.O., Judson, I.R., Griffiths, J.R., 1998. Influence of pH on the uptake of 5-fluorouracil into isolated tumor cells. Br. J. Cancer 77, 873–879.

Pearlman, D.L., Youngberg, B., Engelhard, C., 1986. Weekly pulse dosing schedule of fluorouracil: a new topical therapy for psoriasis. J. Am. Acad. Dermatol. 15, 1247–1252.

Robins, P., Gupta, A.K., 2002. The use of topical fluorouracil to treat actinic keratosis. Cutis 70, 4–7.

Rudy, B.C., Senkowski, B.Z., 1973. Fluorouracil. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances, vol. 2. Academic Press, New York, pp. 221–244.

Scheuplein, R.J., 1978. Skin as barrier. In: Jarret, A. (Ed.), The Physiology and Pathophysiology of Skin. Academic Press, New York, pp. 1693–1730.

Sherertz, E.F., Sloan, K.B., McTiernan, R.G., 1990. Transdermal delivery of 5-fluorouracil through skin of hairless mice and human skins in vitro: a comparison of the effect of formulations and a prodrug. Arch. Dermatol. Res. 282, 463–468.

Singh, S., Jayaswal, S.B., Upadhyay, S.N., Singh, J., 1992. Iontophoretic delivery of propranolol hydrochloride through human epidermis. J. Controlled Rel. 18, 165–170.

Sugibayashi, K., Hosoya, K.I., Morimoto, Y., Higuchi, W.I., 1985. Effect of the absorption enhancer, Azone, on the transport of 5-fluorouracil across the hairless rat skin. J. Pharm. Pharmacol. 37, 578–580.

Sugibayashi, K., Nakayama, S., Seki, T., Hosoya, K., Morimoto, Y., 1992. Mechanism of skin penetration-enhancing effect by laurocapram. J. Pharm. Sci. 81, 58–64.

Touitou, E., Abed, L., 1985. Effect of propylene glycol Azone and *n*-decylmethyl sulfoxide on skin permeation kinetics of 5-fluorouracil. Int. J. Pharm. 27, 89–98.

Vaughan, C.D., 1985. Using solubility parameters in cosmetics formulation. J. Soc. Cosmet. Chem. 36, 319–333.

Williams, A.C., Barry, B.W., 1991. Terpenes and the lipid-protein partitioning theory of the skin penetration enhancement. Pharm. Res. 8, 17–24.