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Effect of vehicles on topical delivery of 5-fluorouracil (5FU) by 1-acyl-5FU prodrugs

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Abstract

The solubilities of selected 1-alkylcarbonyl prodrugs of 5-FU and of 5-FU in isopropyl myristate (IPM), miglyol-812 (MG), tributyrin (TB) and triacetin (TA) were determined. The solubilities were correlated with the solubility parameters of the prodrugs, δ_i, and the vehicles, δ_v. The stabilities of the prodrugs in the vehicles in contact with hydrated skin were also determined. The rates of delivery of 5-FU through hairless mouse skin (transdermal delivery) by suspensions of 5FU and the prodrugs, and the accumulation of 5-FU in the skins (dermal delivery) were determined. In addition, the damage done to the skins by the application of 5-FU or its prodrugs in the vehicles was estimated from the rates of delivery (J_j) of a standard solute (theophylline) from a nondamaging vehicle (propylene glycol-PG) subsequent to the application of 5-FU or its prodrugs. 5-FU and its prodrugs were less soluble in the more lipophilic vehicles: $S_{IPM} < S_{MG} < S_{TB} < S_{TA}$. On the other hand, the hydrolytically unstable prodrugs were more stable in the more highly hydrophobic vehicle, IPM. The prodrugs delivered more 5-FU through (J_i) and accumulated more 5-FU in the skins from the more lipophilic vehicles in which they were less soluble. However, more damage was also done to the skins by the more lipophilic vehicles: IPM > MG > TB > TA. Thus, the greater delivery of 5-FU by the prodrugs from the more lipophilic vehicles was predominantly due to greater damage done to the skins by the more lipophilic vehicles. On the other hand, the ratios of delivery of 5-FU to damage (J_i/J_j) were greater for 1-acetyl-5FU (**1**) and 1-hexanoyl-5FU (**2**) from IPM than those from MG and TB, and **1** and **2** were less soluble in IPM than in MG and TB, so that an apparent inverse relationship between rates of delivery and solubility exists for those solute/vehicle combinations. © 1997 Elsevier Science B.V.

Keywords: Transdermal delivery; 5-Fluorouracil (5-FU) prodrugs; 1-Alkylcarbonyl-5-FU; Isopropyl myristate; Triglycerides; Skin damage

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1. Introduction

The antimetabolite/anticancer agent 5-fluorouracil (5-FU) is used in the treatment of proliferated skin diseases such as actinic keratoses (AK) (Dillaha et al., 1965), superficial basal cell carcinoma (BCC) (Epstein, 1985) and psoriasis (Tsuji and Sugai, 1972). 5-FU has a role in the treatment of these diseases because it effectively inhibits DNA synthesis and therefore cell replication (Goette, 1981; Pinedo and Peters, 1988). The problem with the use of 5-FU is not lack of efficacy at the site of action but rather its poor penetration of biological membranes and thus poor delivery to the site of action. After topical application for the treatment of AK, 5-FU is absorbed through permeable areas of the skin such as face and scalp, but the absorption of 5-FU is greatly decreased through less permeable areas of skin such as the arms and hands and it is not effective when applied to those areas (Robinson and Kligman, 1975). Similarly, treatment of moderate to severe psoriasis with 5-FU under occlusion leads to complete clearing of lesions (Tsuji and Sugai, 1972) but it is ineffective without occlusion (Van Scott and Reinertson, 1959). Thus, inadequate absorption of 5-FU limits its clinical usefulness in the topical treatment of AK and psoriasis.

The poor topical absorption of 5-FU can be attributed to its physicochemical properties. 5-FU contains two functional groups, an amide and an imide, which are involved in intermolecular hydrogen bond formation and which results in the high crystal lattice energy of 5-FU and its high melting point. This accounts for the low water and lipid solubility of 5-FU (Sloan and Beall, 1993). On the other hand, adequate lipid and water solubility are important criteria for predicting optimal topical delivery of drugs. The drugs must be able to diffuse through the lipid-aqueous bilayers in the intercellular spaces of the skin for effective topical absorption (Sloan, 1992).

In order to change the undesirable solubility properties of 5-FU, homologous series of 1-acyl 5-FU prodrugs have been synthesized. Examples of such series are the 1-alkylcarbonyl (Beall and Sloan, 1996), the 1-alkyloxycarbonyl (Beall et al., 1994), and the 1-alkylaminocarbonyl derivatives of 5-FU (Sloan et al., 1993; Sasaki et al., 1990). In each case, an acyl group was introduced to transiently mask the amide group and consequently change the undesirable physicochemical properties of 5-FU. Studies evaluating the ability of these prodrugs to deliver 5-FU through hairless mouse skins have shown that topical absorption of 5-FU can be increased up to 40 times that of 5-FU using this approach (Beall, 1991). Interestingly, for each series of more lipid soluble prodrugs, the most water soluble member was the most effective at delivering 5FU through the skin.

One vehicle, isopropyl myristate (IPM), was used in almost all of the studies reporting enhancement of topical absorption of 5-FU by 1-acyl prodrugs. Therefore, the effect of other aprotic solvents on the ability of three members of the 1-alkylcarbonyl-5-FU series of prodrugs of 5-FU to deliver 5-FU topically has been studied here. The 1-alkylcarbonyl series was chosen for this study since members of the series were among the most effective prodrugs reported for enhancing the topical delivery of 5-FU. Aprotic triacylglycerols, where the length of the alkyl chain was varied from C_7 to C_9 (Miglyol-812) to C_1 (triacetin) in addition to IPM, were used as the vehicles because (a) the 1-alkylcarbonyl prodrugs readily decomposed in protic vehicles; (b) the physicochemical properties of the triacylglycerols covered a sufficiently wide range to test the effect of the solubilities of the prodrugs in the vehicles on their ability to deliver 5-FU; and (c) the solubilizing functional groups (ester and alkyl chain) were the same. The solubilities and stabilities of the prodrugs in the vehicles, damage caused by the vehicles, and flux and skin accumulation values of 5-FU have been determined.

2. Experimental

5-FU and theophylline were obtained from Sigma. Miglyol-812 (MG) was obtained from Huls America. Acetyl chloride, triacetin (TA) and the bulk solvents were obtained from Fisher. In general, all other reagents were obtained from Aldrich. The female hairless mice (SKH-hr-1)

were obtained from Charles River. The diffusion cells were obtained from Crown Glass, Somerville, NJ with a 4.9 cm^2 surface area and a 20 ml receptor phase volume. The temperature of the diffusion cells was maintained at 32°C by a Fisher circulating water bath model 80. Melting points were determined with a Meltemp capillary melting point device. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Infrared (IR) spectra were obtained with a Perkin-Elmer 1420 spectrophotometer. TLC analyses were run on Brinkman Polygram Sil G/UV 254 plates. Elemental analysis was obtained for the new compound, **3**, through Atlantic Microlab, (Norcross, GA). The 1-acetyl-(**1**), 1-hexanoyl-(**2**) and 1-decanoyl-5-FU (**3**) prodrugs were synthesized according to a previously published procedure (Beall et al., 1996). The ^{1}H NMR, UV and IR spectra of **3** (mp 90–90.5°C) were consistent with the reported spectra of 1-alkylcarbonyl derivatives, its elemental analysis was $\pm$ 0.1% of calculated values and TLC analysis using ether as the eluent showed that **3** (as well as **1** and **2**) was one component.

2.1. Lipid solubility of prodrugs

The lipid solubility of each derivative was determined in miglyol-812 (MG), tributyrin (TB) and triacetin (TA). The solubility of 1-decanoyl-5-FU was also determined in isopropyl myristate (IPM). Three suspensions of each derivative in each vehicle were stirred at room temperature (22 ± 1°C) for 48 h (Beall and Sloan, 1996). The suspensions were allowed to settle at room temperature for 48 h. The suspensions were filtered through 0.45 μm nylon filters, and the solutions were diluted with acetonitrile. The solutions were analyzed by UV spectroscopy. Molar absorptivities for each prodrug were predetermined in acetonitrile at 261 nm.

2.2. Solubility of water in vehicles

The solubility of water in each vehicle was estimated by measuring the ratio of the $H_2O/2$ absorption to the C*H*-O_2C absorption of the vehicle by ^{1}H NMR spectroscopy in DMSO-d_6 for TB and TA or in DMSO-d_6:$CDCl_3$ (3:2) for MG and IPM. This ratio gave the mole fraction of water from which mmol amounts of water were calculated. Mixtures of 1 ml of vehicles and 0.5 ml of H_2O ($n = 3$) were stirred vigorously for 24 h then allowed to sit at room temperature for 24 h before a sample (approximately 65–100 μl) of the organic phase was transferred to an NMR spin tube containing the appropriate solvent. The amount of water in the sample was determined by subtracting the average of the integrations ($n = 4$) for H_2O in the NMR solvent before the water saturated vehicles were added from the average of the integrations ($n = 4$) for H_2O after the vehicles were added. The samples of IPM and MG were not completely soluble in pure DMSO-d_6 so a DMSO-d_6:$CDCl_3$ mixture was used.

2.3. Diffusion cell experiments

The procedure used for the cell diffusion experiments has been described previously (Beall and Sloan, 1996). The hairless mice were sacrificed by cervical dislocation and the skins were removed by blunt dissection. The dorsal portion of each skin was immediately mounted on diffusion cells and the dermal side of the skins were placed in contact with the receptor phase. The receptor phase consisted of a 0.05 M phosphate buffer (pH = 7.1, I = 0.12 M) containing 0.11% formaldehyde as preservative to insure the integrity of the skin during the course of the experiment (Sloan et al., 1991). The temperature was maintained at 32°C with the skins in contact with the buffer for 48 h prior to application of suspensions of 5-FU or its prodrugs. During this preapplication period, the receptor phase was changed three times to eliminate any UV absorbing material present in the skin and to condition the skins. Then, a suspension (0.3–1.0 M) of 5-FU or its prodrug in a vehicle was applied to each cell (0.5 ml). The suspensions were prepared 48 h prior to application by adding excess prodrug to the vehicles and stirring them for 48 h. After suspensions of 5-FU or its prodrugs were applied to the donor phase, 3 ml aliquots of the receptor phases were removed at approximately 4, 7, 11, 19, 22, 25, 28,

34, 43 and 48 h and subsequently analyzed by UV spectroscopy. Each time an aliquot was removed, the entire receptor phase was replaced with fresh buffer to maintain sink conditions. The donor phases were replaced with fresh 0.5 ml samples of suspensions of 5-FU or its prodrugs at 24 h. No methanol wash was used prior to reapplication of suspensions. The donor phases for each combination of prodrug/vehicle were combined and saved for analysis by ^{1}H NMR spectroscopy to assess the stability of the prodrugs during the first application period. At 48 h, the donor phases were again removed, combined and saved for analysis by ^{1}H NMR spectroscopy. The epidermal sides of the skins were washed twice with 5 ml of methanol to remove any residual 5-FU or its prodrugs. This was done as quickly as possible to avoid prolonged exposure of the skins to methanol. Then the skins were exposed to receptor phase for 24 h to allow any 5-FU retained in the skin to leach out. The receptor phase at the end of the 24 h leach period was subsequently analyzed by UV spectroscopy to determine the amount of 5-FU accumulated in the skin at the end of the first application period (C_s). The receptor phase was replaced with fresh buffer and a second application was made: in this case of a standard drug in a vehicle (0.5 ml of a suspension of 400 mg of theophylline in 6 ml of propylene glycol). Aliquots (3 ml) of the receptor phase were removed at 1, 2, 4 and 8 h and subsequently analyzed by UV spectroscopy. Each time an aliquot was removed, the entire receptor phase was replaced with the fresh buffer. Aliquots of the receptor phases from the first application period and from the 24 h leaching period were analyzed at 265 nm for 5-FU ($\epsilon = 7.13 \times 10^3$ l/mol). Aliquots of the receptor phases from the second application period were analyzed for theophylline at 270 nm ($\epsilon = 1.02 \times 10^4$ l/mol).

Vehicle control experiments were run using the same protocol except that only the vehicles were applied during the first application period.

The rates of deliveries of 5-FU (J_i) and theophylline (J_j) through skin were determined by plotting the cumulative amount of 5-FU or theophylline (μmol) in the receptor phase versus time and dividing the slopes of the steady-state or linear portion by the diffusion cell surface area (4.9 cm^2).

The solid and liquid portions of the combined donor phases that were removed at 24 and 48 h were separated by filtration. The stabilities of the prodrugs in both portions of the donor phases were determined from the integrations of the ^{1}H NMR C^6-H absorptions due to 5-FU at δ 7.7 and the 1-alkylcarbonyl prodrugs at δ 8.2 (DMSO-d$_6$) as previously described (Beall and Sloan, 1996). ^{1}H NMR spectra were run in DMSO-d$_6$ from freshly opened ampules immediately after preparing the samples to prevent the hydrolysis of the samples during acquisition of the spectra.

3. Results and discussion

3.1. Synthesis and structure determination

Two of the three prodrugs synthesized for this work, 1-acetyl-5-FU (**1**) and 1-hexanoyl-5-FU (**2**), had been synthesized before (Beall, 1991; Beall et al., 1996). The melting points and ^{1}H NMR spectra agreed with those reported in the literature. The melting points decreased as the chain length increased. However, the trend changed for 1-decanoyl-5-FU (**3**), which showed an increase in the melting point compared to 1-octanoyl-5-FU (Beall et al., 1996). The ^{1}H NMR spectrum for the new compound, **3**, was consistent with the spectra of other 1-alkylcarbonyl-5-FU prodrugs. The position of the C^6-H absorption for this series of prodrugs is about $\delta = 8.25$ in $CDCl_3$. This represents a downfield shift compared to the absorption of C^6-H in 3-alkylcarbonyl-5-FU derivatives and in 5-FU. The downfield shift of the C^6-H in the 1-alkylcarbonyl-5-FU derivatives is due to the deshielding brought about by the electronegative nature of the carbonyl group, which is coplanar with the 5-FU ring and oriented toward the C^6-H group (Ozaki et al., 1977; Beall et al., 1993).

The IR spectra of the synthesized prodrugs were consistent with the 1-alkylcarbonyl-5-FU structure (Beall et al., 1993). The broad, strongly intense IR absorption in the region of 1770–1640 cm^{-1} can be attributed to the carbonyl and C=C

Table 1
Solubilities of 5-FU and selected 1-alkylcarbonyl-5-FU prodrugs in selected ester vehicles[a]

Compound, Alkyl =	S_{IPM}(±S.D.) mM	S_{MG}(±S.D.) mM	S_{TB}(±S.D.) mM	S_{TA}(±S.D.) mM
5-FU	0.049 (0.018)[b]	0.23 (0.036)	1.6 (0.14)	8.1 (0.14)
1, CH_3	22 (0.70)[b]	52 (0.41)	220 (8.1)	667 (13)
2, C_5H_{11}	113 (2.6)[b]	194 (7.5)	554 (30)	746 (30)
3, C_9H_{19}	59 (0.14)	82 (0.28)	169 (49)	162 (15)

[a] Isopropyl myristate, IPM; miglyol-812 (a triglyceride of C_8–C_{10} fatty acids), MG; tributyrin, TB and triacetin, TA.
[b] From: Beall and Sloan, 1996.

stretching vibrations. These absorptions became more defined in this region (1745, 1710 and 1685 cm^{-1}) for 1-decanoyl-5-FU. The broad, moderately intense absorptions in the region of 3200–3000 cm^{-1} are characteristic of N–H stretching vibrations. As the number of methylene groups increased from 1-acetyl-5-FU to 1-decanoyl-5-FU, the absorptions due to aliphatic C-H vibrations became sharp and prominent at 2920 and 2850 cm^{-1}.

The UV spectra were also consistent with those in the literature (Beall et al., 1996). The molar absorptivities determined for **1** and **2** in acetonitrile at 261 nm are in agreement with those reported before, and that for **3** is essentially identical to those reported for **1** and **2**.

3.2. Solubility

The solubilities of each prodrug in IPM (S_{IPM}), miglyol-812 (S_{MG}), tributyrin (S_{TB}) and triacetin (S_{TA}) are presented in Table 1. The solubilities of 5-FU, 1-acetyl- (**1**) and 1-hexanoyl-5-FU (**2**) in IPM were reported in a previous paper and are included in Table 1 (Beall et al., 1996). For **1** and **2** the solubilities of the prodrugs increased as the polarity of the vehicles increased (from S_{IPM} to S_{TA}), as the melting point decreased (from 282 to 102°C), and as the length of the alkyl chain in the promoiety increased (from C_1 to C_5). Generally, as the difference between the values for the solubility parameters for the vehicle (δ_v) and for the solute prodrug (δ_j) decreases, the solubility of the prodrug increases until $\delta_v = \delta_i$ where a maximum in solubility is reached (Martin et al., 1985). In this case, the solubility parameters for IPM, MG, TB and TA increase from 8.02, 8.29, 9.97 to 10.77 $(cal/cm^3)^{1/2}$, respectively, as the polarity of the vehicles increased going from IPM to TA (Vaughan, 1988). Similarly, the calculated solubility parameters for 5-FU **1**, **2** and **3** decrease from 15.0, 14.1, 12.23 to 11.32 $(cal/cm^3)^{1/2}$, respectively, as the polarity of the prodrugs decrease going from 5-FU to **3** (Beall et al., 1996). Thus, although a maximum in solubility was not observed because $\delta_v \neq \delta_i$, the trend to converging δ values and increasing solubilities of 5-FU and the prodrugs **1** and **2** in IPM to TA was predicted by solubility theory.

However, the trend observed for **1** and **2** was not continued for 1-decanoyl-5-FU, **3**. The solubility of **3** in all vehicles was less than that of **2** and even less than that of **1** in the more polar vehicles, TB and TA. A portent of such a reversal in the trend was the solubility of 1-octanoyl-5-FU in IPM. 1-Octanoyl-5-FU exhibited a significantly lower melting point than 1-hexanoyl-5-FU (mp 83–84°C versus 101–102°C, respectively) yet its S_{IPM} value was somewhat less (111 versus 113 mM, respectively) (Beall and Sloan, 1996). The 1-decanoyl prodrug, with an even longer alkyl chain promoiety than the 1-octanoyl prodrug, extends the reversal of the solubility trend seen with 1-octanoyl-5-FU, and exhibits an even lower S_{IPM} value (59 mM). In addition, the melting point of **3** was higher than that of the 1-octanoyl prodrug (mp 90–90.5 versus 83–84°C, respectively) reversing the trend to lower melting points with increasing alkyl chain length.

This overall trend of decreasing melting point and concomitant increasing IPM solubility with increasing length of the alkyl chain in the promoiety of an homologous series of prodrugs, followed by an increase in melting point and decrease in

the IPM solubility with further increases in the length of the alkyl chain has been observed previously (Beall et al., 1996; Waranis and Sloan, 1987). The causes of this behavior have been discussed by Yalkowsky, 1977. Generally, the initial increases in IPM solubility can be attributed to masking a hydrogen bond donor (N^1-H) and to decreasing crystal packing efficiency by the short alkyl chain prodrugs. For the long alkyl chain prodrugs, van der Waals interactions between the alkyl chains become stronger and lead to increased crystal lattice energy and decreased IPM as well as MG, TB and TA solubility. Thus, although **3** would appear to be more lipophilic than the other members of the 1-alkylcarbonyl series because of the additional methylene groups, its general lipid solubility is less. In addition, the solubility of **3** in increasingly more polar vehicles seems to have reached a maximum in TB and TA. Thus, a maximum was reached well before the calculated value of δ_i was equal to that of δ_v. This result may be due to the fact that calculation of δ values from group contribution methods does not take into account increasing van der Waals interactions with increasing alkyl chain lengths which initiate a decrease in solubility regardless of the vehicle.

The aqueous solubilities (S_{H2O}) of 1-alkylcarbonyl-5-FU prodrugs **1** and **2** have been previously reported (Beall and Sloan, 1996). Only S_{H2O} values estimated from partition coefficients and solubility ratios were reported because of the rapid aqueous hydrolysis of the prodrugs (Beall et al., 1996). However, even an estimated value could not be determined here for **3** because of its highly hydrophobic nature and hydrolytic instability. It is reasonable to assume that its S_{H20} is much less than that of 1-octanoyl and the other 1-alkylcarbonyl-5-FU prodrugs.

The solubility of water in the vehicles was estimated because the vehicles were in contact with highly hydrated skin and exposed to atmospheric moisture. If the more polar vehicles such as TA were capable of solubilizing significant amounts of water, this could adversely affect the stability of the prodrugs in the vehicle and hence any attempt to compare the abilities of the intact prodrugs to deliver 5-FU through skin. The molar ratios of vehicle to water rounded off to the nearest whole integer obtained by ^{1}H NMR spectroscopy were as follows: IPM (34:1), MG (9:1), TB (5:1), TA (2:1). Thus, assuming that the applied vehicles became saturated with water and given that the densities of IPM, MG, TB and TA were 0.85, 0.94, 1.03 and 1.15 mg/ml, respectively, the amounts of water in 0.5 ml of each vehicle was 0.047, 0.116, 0.345 and 1.26 mmol, respectively.

3.3. Diffusion cell experiments

The results from the diffusion cell experiments are presented in Table 2. Because of the short half-lives of the 1-alkylcarbonyl derivatives ($t_{1/2}$ = 3–5 min) (Beall et al., 1996), only 5-FU was observed in the receptor phases. A typical plot of cumulative μmol of 5-FU in the receptor phase versus time for the delivery of 5-FU by **1**, **2** and **3** from MG is shown in Fig. 1. In general for 5-FU, **1**, **2** and **3**, the rates of delivery of 5-FU through hairless mouse skin (J_i) was greatest from IPM and least from TA for those compounds. When the solubilities were factored out of the J_i values to give log permeability coefficients (log P_i), the trend became even more pronounced (Table 3).

The rates of delivery of theophylline from propylene glycol (PG) in the second application studies (J_j) followed the same trend as the first application studies using 5-FU, 1, 2 and 3: the flux of theophylline from PG was greater after treatment with IPM and least after treatment with TA. Since the rates of skin penetration by theophylline from PG has been used as a measure of skin damage caused by an initial application of a prodrug or drug/vehicle combination (Sloan et al., 1986), the general rank order of apparent damage to the hairless mouse skin is IPM > MG > TB > TA. This trend also holds when J_j was determined after treatment with the vehicles alone: $J_j = 1.2 \pm 0.17$ (IPM), 0.23 ± 0.048 (MG), 0.14 ± 0.067 (TB) and 0.012 ± 0.0016 (TA) μmol/cm^2/h. However, in three cases either there was no significant difference between the J_j values for MG and TB or the order was reversed. Hence, MG and TB may not be much different in the damage they cause, but IPM always causes the

Table 2
Rates of delivery of 5-FU (J_i) by 5-FU and 1-alkylcarbonyl prodrugs from IPM, MG, TB and TA, rates of delivery of theophylline (J_j) from PG after treatment with 5-FU or its prodrugs in IPM, MG, TB and TA, and the ratios of J_i/J_j

Compound, Alkyl =	Vehicle (mmol)[a]	$J_i(\pm$ S.D.) (μmol/cm^2/h)	$J_j(\pm$ S.D.) (μmol/cm^2/h)	J_i/J_j
5-FU	IPM (0.15)[b]	0.23 (0.085)	1.2 (0.23)	0.19
	MG (0.15)	0.077 (0.012)	0.51 (0.19)	0.15
	TB (0.25)	0.052 (0.015)	0.12 (0.039)	0.43
	TA(0.15)	0.0024 (0.0011)	0.038 (0.026)	0.063
1, CH_3	IPM (0.50)[b,c]	9.3 (0.30)	1.7 (0.056)	5.5
	MG (0.33)	1.8 (0.41)	0.83 (0.17)	2.2
	TB (0.18)	0.68 (0.062)	0.26 (0.10)	2.6
	TA (0.45)	0.015 (0.0077)	0.015 (0.0033)	1.0
2, C_5H_{11}	IPM (0.30)[b,d]	1.1 (0.0001)	0.47 (0.020)	2.3
	MG (0.22)	0.45 (0.10)	0.28 (0.044)	1.6
	TB (0.40)	0.53 (0.029)	0.34 (0.078)	1.6
	TA (0.50)	0.020 (0.0062)	0.027 (0.0061)	0.74
3, C_9H_{19}	IPM (0.15)	0.13 (0.0046)	1.4 (0.028)	0.093
	MG (0.15)	0.085 (0.0077)	0.37 (0.067)	0.23
	TB (0.19)	0.11 (0.0077)	0.52 (0.039)	0.21
	TA(0.19)	0.012 (0.0054)	0.031 (0.019)	0.39

[a] Total mmol of solute in 0.5 ml of suspension used.
[b] From: Beall and Sloan, 1996.
[c] Suspension applied every 9 h.
[d] Suspension applied every 12 h.

most damage and TA the least to hairless mouse skin. In fact, the average J_j value for TA (0.025 ± 0.011 μmol/cm^2/h) is not significantly greater than the J_j value for a control experiment where the skins were not treated with anything except a methanol wash during the first 48 h application period ($J_j = 0.013 \pm 0.0022$ μmol/cm^2/h) (Koch and Sloan, 1987). Thus, the predominant factor in the increased rates of delivery of 5-FU by the prodrugs from the more lipophilic vehicles is the greater damage to hairless mouse skins they cause.

In order to attempt to factor out the contribution of damage caused by each vehicle to J_j, the ratios of J_i/J_j were calculated and are given in Table 2. Although they are at best crude criteria, the J_i/J_j ratios suggest that the delivery of 5-FU by 5-FU or by **3** from any vehicle and that the delivery of 5-FU by any of the solutes from TA cause more damage than the delivery of 5-FU by **1** and **2** from IPM, MG and TB relative to their rates of delivery. Thus, since the J_i/J_j ratios for the delivery of **1** and **2** from IPM were greater than those for the delivery of **1** and **2** from MG and TB and **1** and **2** are less soluble in IPM than in MG and TB, the rates of delivery of 5-FU by **1** and **2** through hairless mouse skin increased as their solubilities in the vehicles decreased. It is assumed that a more favorable partition coefficient is responsible for the increased rate.

The data for **3** does not fit the trend to higher J_i with lower solubilities in lipid vehicles such as IPM exhibited by **1** and **2**. **3** is less soluble than **2** in all the vehicles yet delivers up to 8 times less 5-FU than **2**. The reason for this is that in the initial members of this series, as the length of the alkyl chain increased, melting point decreased, S_{H20} decreased and S_{IPM} increased. However, the decrease in J_i as the alkyl chain increased was primarily due to a decrease in S_{H20} and not to an increase in S_{IPM}. For **3**, and for the 1-octanoyl derivative as well, both S_{IPM} and S_{H20} decreased due to increased intermolecular van der Waals forces caused by association of the long alkyl chains in crystals of **3**.

When the log P_i values were plotted against the calculated solubility parameters, δ_i, for the prodrugs (Beall and Sloan, 1996), a reasonable fit to the straight line plot for the log P_i values from IPM was obtained for **3** (slope = 0.88, $r = 0.99$): **3** behaves like the other members of the series in IPM (Fig. 2). However for the other vehicles, the plots of log P_i versus δ_i became curved as the solubility parameter values for the prodrugs, δ_i, approached those of the vehicles, δ_v. Curvature in such plots is to be expected because as the δ_i values approach those of δ_v, a maximum in solubility and hence a minimum in log P_i should be approached (Sloan et al., 1986). In fact, the solubility data (Table 1) suggest that a maximum in solubility in TA is reached by the longer alkyl chain prodrugs; and the plot of δ_i versus log P_i is in the shape of a parabola which suggests that $\delta_i = \delta_v$ at about 13–13.4 $(\text{cal/cm}^3)^{1/2}$.

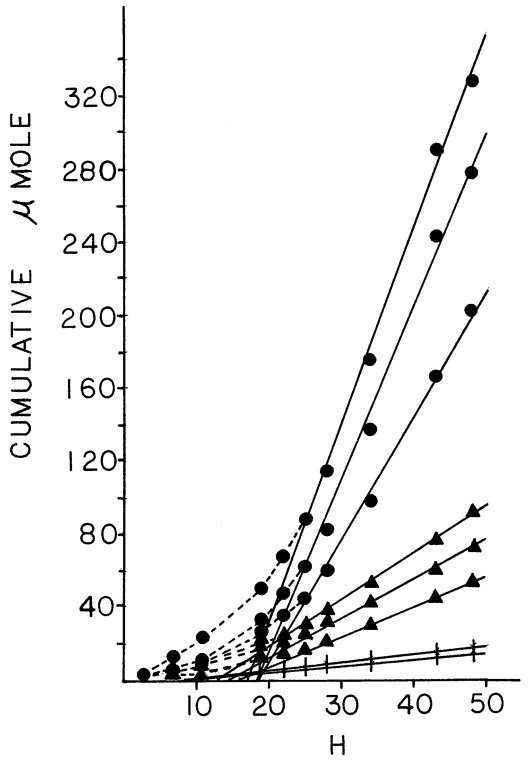

Fig. 1. Plots of cumulative μmol of 5-FU delivered by **1**(●), **2** (▲) and **3** (+) from MG versus time.

Table 3
Permeability coefficients for delivery of 5-FU by 1-alkylcarbonyl prodrugs (log P_i), and amounts of 5-FU retained in skin (C_s)

Compound, Alkyl =	Vehicle	log P_i (cm/h)	C_s(± S.D.) (μmol)
5-FU	IPM	0.69[a]	3.7 (0.85)[a]
	MG	−0.48	4.5 (0.15)
	TB	−1.48	4.5 (1.1)
	TA	−3.52	0.20 (0.14)
1, CH_3	IPM	−0.38[a]	68 (10)[a]
	MG	−1.46	24 (4.8)
	TB	−2.51	13 (2.2)
	TA	−4.65	0.85 (0.31)
2, C_5H_{11}	IPM	−2.01[a]	11 (3)[a]
	MG	−2.64	5.8(0.12)
	TB	−3.02	4.3 (0.31)
	TA	−4.57	2.0 (0.69)
3, C_9H_{19}	IPM	−2.66	2.2 (0.69)
	MG	−2.98	1.8 (0.15)
	TB	−3.19	1.8 (0.38)
	TA	−4.13	0.92 (0.15)

[a] From: Beall and Sloan, 1996.

The skin accumulation data (C_s) (Beall et al., 1994; Beall and Sloan, 1996) are given in Table 3. The C_s values follow the same trend that the J_i values followed: the greatest accumulation of 5-FU was generally from IPM and least from TA. Also, the largest C_s values were achieved by **1** from each vehicle (except for **2**/TA and **3**/TA) while **3** failed to achieve C_s values even as large as those by 5-FU itself (except for **3**/TA).

^{1}H NMR spectroscopic analyses of the donor phases that were removed every 24 h (every 9 or 12 h in previous experiments for **1**/IPM and **2**/IPM, respectively (Beall and Sloan, 1996)) showed only intact prodrug in the solutions. However, since the limit of detection of the C^6-*H* absorption due to 5-FU was only approximately 1% of the C*H*-O_2C absorption due to the solvent and the solubility of 5-FU in the vehicles was less than 0.15% mole ratio at best (TA), any 5-FU in the solution would not have been detected by ^{1}H NMR. In addition, for analyses of the solutions the ratios of the ^{1}H NMR absorptions of C^6-*H* in the prodrugs to C*H*-O_2C in the vehicles before the donor phases were applied was the same as after

the donor phases were removed from contact with skins: saturation was maintained during the experiment. However, ^{1}H NMR spectroscopic analyses of the solids in equilibrium with the solutions of the donor phase suspensions after contact with hydrated skin showed that in some cases the solid contained not only intact prodrug but also 5-FU. The 5-FU was apparently the result of hydrolysis of the prodrug during the experiment since no 5-FU was detected by TLC analyses of the prodrugs before they were used to make the suspensions. The solids from the donor phases prepared with **3** in all of the vehicles, with **1** and **2** in IPM and with **1** in MG contained only intact prodrug. The donor phases prepared with **2** in MG contained excess prodrug (1.6:1) while those prepared with **1** and **2** in TB and TA contained excess 5-FU (1.2 to 5.3:1). Due to the small number of solid portions of the donor phases available for analysis it was not possible to obtain more than a qualitative estimation of the ratios.

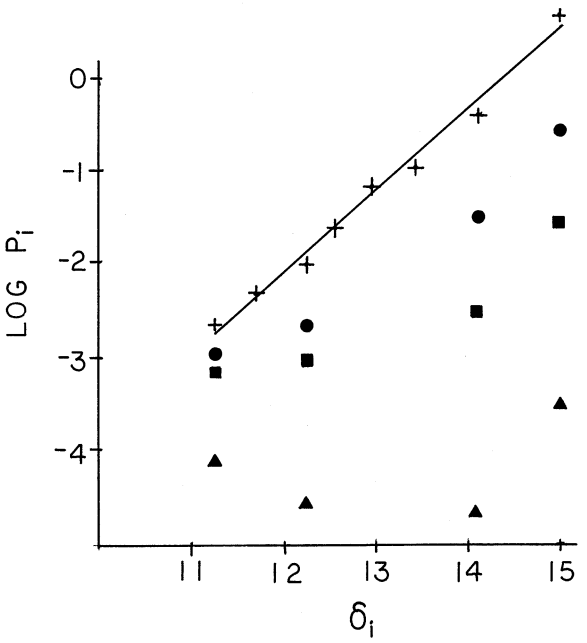

Fig. 2. Plots of experimental log permeability coefficients (P_i) versus solubility parameters for the 1-alkylcarbonyl-5-FU prodrugs (δ_i) for the delivery of 5-FU through hairless mouse skin from isopropyl myristate (+), Miglyol-812 (●), tributyrin (■) or triacetin (▲).

Regardless of the fact that more decomposition of the prodrugs was observed in suspensions of the vehicles which could solubilize more water, there was always some intact prodrug in equilibrium with the saturated solutions and the solutions contained only intact prodrug within the limits of detection of 5-FU (see above). For those combinations of prodrug/vehicle where 5-FU was observed in the donor phases (**1**/TB, **1**/TA, **2**/MG, **2**/TB and **2**/TA) and hence the delivery of 5-FU by 5-FU/vehicle could be a competing process, the J_i values for 5-FU/vehicle were only 7, 16, 17, 10 and 12%, respectively of the corresponding J_i values observed in prodrug/vehicle experiments. Thus, the competing process (5-FU/vehicle) would not change the overall trends observed. Although the mmol amounts of water that could have been absorbed by TB and TA in contact with the hydrated skin were greater than the mmol amounts of prodrug suspended in those vehicles (except for **2**/TB), the majority of the prodrug was in solution and it was intact in that solution. In addition, ^{1}H NMR spectroscopic analyses of the solutions did not show the presence of water. Thus, although the TB and TA solutions were apparently absorbing water that was causing some hydrolysis of the prodrugs to 5-FU, they apparently were not becoming saturated with water during their contact with hydrated mouse skin.

The pattern of stability observed with these combinations of prodrugs/vehicle suggests that for vehicles such as IPM, which can solubilize very little water, even hydrolytically unstable prodrugs will remain intact in contact with highly hydrated skin. For vehicles that can solubilize more water, such as TA, more hydrolysis of the prodrugs will take place. On the other hand, prodrugs with long alkyl chain promoieties that tend to self-associate, such as **3**, will tend to be stable even in TA.

4. Conclusions

There did not appear to be any advantage to using the prodrugs with the more polar triacylglycerol type vehicles. Although IPM caused more

apparent damage to hairless mouse skin than MG, TB or TA based on its high J_j values, for **1** and **2** IPM was even more efficient than MG, TB or TA at delivering 5-FU based on the fact that its J_i/J_j values were greater than those of the other vehicles. In addition, the hydrolytically unstable 1-alkylcarbonyl prodrugs were more stable in IPM than the more polar triglyceride vehicles. This result is probably due to the fact that the triglycerides are capable of absorbing more water than the highly hydrophobic IPM.

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