

Topical delivery of 5-fluorouracil (5-FU) by 3-alkylcarbonyl-5-FU prodrugs

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Abstract

The solubilities in isopropyl myristate (S_{IPM}) and pH 4.0 buffer (S_{AQ}) and the partition coefficients between IPM and pH 4.0 buffer ($K_{IPM:AQ}$) have been measured for a series of 3-alkylcarbonyl-5-fluorouracil prodrugs (3-AC-5-FU). The 3-AC-5-FU prodrugs were all 100 times more soluble in IPM and the first two members of the series were also more soluble in pH 4.0 buffer than 5-FU. The abilities of the 3-AC-5-FU prodrugs to deliver total 5-FU species through hairless mouse skin from IPM suspensions (J_i) were also measured. The 3-propionyl derivative **3**, which exhibited the highest S_{AQ} in the series, gave the highest J_i value. The S_{IPM} , S_{AQ} and molecular weights (mw) of the 3-AC-5-FU series correctly predicted the rank order and very closely (0.10 log units) predicted the absolute values for $\log J_i$ using the transformed Potts–Guy equation. Although the series of 3-AC-5-FU prodrugs was generally quite effective at increasing J_i (2–20 times), the best 3-AC-5-FU prodrug was not as effective as the best 1-alkylcarbonyl-5-FU prodrug (1-AC-5-FU) at increasing J_i and the ability of the 3-AC-5-FU prodrugs to increase the concentration of total 5-FU species in the skin was 2–5 times less than the 1-AC-5-FU prodrugs. Thus, the 1-AC-5-FU prodrugs remain as the best prodrugs with which to enhance the topical delivery of 5-FU. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Reports of attempts to transiently improve the physicochemical properties of 5-fluorouracil (5-FU, **1**) and hence improve its topical, oral or

rectal delivery are quite extensive in the prodrug literature because of the potential for increasing the breadth of its indicated uses (Sloan, 1992). For example, improvement in its topical delivery could allow it to be used for the treatment of psoriasis under ambient conditions, whereas present formulations require occlusion to be effective (Tsuji and Sugai, 1972). Generally, for highly polar molecules such as 5-FU, which exhibit low

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lipid solubility, increased topical delivery can be achieved by masking one of the polar functional groups and hence increasing its lipid solubility. However, it has also been shown for a homologous series of more lipophilic prodrugs, that those members that exhibit the higher aqueous solubilities in the series and not necessarily the higher lipid solubilities are the members that deliver the highest flux (Sloan, 1992). Thus, aqueous as well as lipid solubilities are important criteria to use in identifying the best prodrug for improving topical delivery.

The suggestion that a balance of lipid and aqueous solubility is necessary for compounds to exhibit relatively high permeation through skin was first made by Michaels et al. (1975) for non-homologous, unrelated compounds and later by Sloan et al. (1984) for prodrugs. Subsequently Roberts and Sloan (1999) developed a transformation of the Potts–Guy equation (Potts and Guy, 1992) which contained explicit parameters for lipid (solubility in isopropyl myristate, S_{IPM}) and aqueous (solubility in pH 4.0 buffer, S_{AQ}) solubility and was used to predict flux from lipid vehicles. Fit of the S_{IPM} , S_{AQ} and molecular weight (mw) data from seven series of more lipophilic prodrugs of polar parent drugs to the transformed Potts–Guy equation showed a strong dependence on S_{AQ} (see below) as well as S_{IPM} and mw. Although the transformed Potts–Guy equation was developed from in vitro data using IPM as a vehicle and hairless mouse skin, Roberts and Sloan (2001) have also shown that the mw, lipid and aqueous solubility data for non-steroidal anti-inflammatory drugs can also be fit to the transformed Potts–Guy equation to predict their flux from mineral oil through the skin of human volunteers (Wenkers and Lippold, 1999). There is a substantial dependence on aqueous as well as lipid solubility for permeation in vivo through human skin: 0.28 log S_{AQ} from in vivo human skin data versus 0.47 log S_{AQ} from in vitro hairless mouse skin data for the log S_{AQ} parameter.

Some time ago, Buur and Bundgaard (1984) identified several members of the 3-alkylcarbonyl series of derivatives of 5-FU (3-AC-5-FU) as exhibiting greater lipid and aqueous solubilities than 5-FU and suggested that these properties made

them good prodrug candidates. Subsequently, Buur and Bundgaard (1987) showed that 3-propionyl-5-FU improved the rectal delivery and bioavailability of 5-FU to 93%. Since then the unique structure of 3-AC-5-FU derivatives has been elucidated (Beall et al., 1993b) and their improved syntheses, thermal and hydrolytic stabilities have been reported (Beall et al., 1997). Recently, the abilities of 1-alkylcarbonyl-5-FU (1-AC-5-FU) prodrugs to enhance the topical delivery of 5-FU have also been reported (Beall and Sloan, 1996). 1-Acetyl-5-FU in IPM was found to increase the delivery of 5-FU 40-fold, which is the best performance by a 5-FU prodrug to date. In this paper the solubilities in lipid — isopropyl myristate — (S_{IPM}) and in pH 4.0 buffer (S_{AQ}) of a series of 3-AC-5-FU prodrugs and their abilities to delivery 5-FU through hairless mouse skin are reported and compared with the performance of the 1-AC-5-FU series.

2. Methods and materials

Melting points were determined with a Meltemp capillary melting point apparatus and are uncorrected. ^1H NMR spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Ultraviolet (UV) spectra were obtained on a Shimadzu UV-265 spectrophotometer. The diffusion cells were from Crown Glass, Somerville, NJ (surface area 4.9 cm², 20 ml receptor phase volume). The diffusion cells were maintained at 32°C with a Fisher circulating water bath model 25. TLC analyses were run on Brinkman Polygram Sil G/UV 254 plates. Isopropyl myristate (IPM) was obtained from Givaudan, Clifton, NJ. Theophylline (Th) and 5-fluorouracil (5-FU) were purchased from Sigma Chemical Co.; acid chlorides and all other reagent chemicals were from Aldrich Chemical Co.; all other solvents were from Fisher. The female hairless mice (25–30 g, 12–16 weeks old SKH-*hr-1*) were from Temple University, Skin and Cancer Hospital. The 3-alkylcarbonyl-5-FU prodrugs (**2–5**) were synthesized as previously described (Beall et al., 1997) and were identical with the literature prodrugs by ^1H NMR, TLC and mp.

2.1. Solubilities and partition coefficients

Lipid solubilities for **2–5** were determined in IPM (S_{IPM}) according to a previously described procedure (Beall et al., 1993a). Three suspensions of each prodrug were stirred at $22 \pm 1^\circ\text{C}$ for 48 h. The suspensions were filtered through 0.45 μm nylon filters. The saturated solutions were diluted with dry acetonitrile and quantitated by UV spectrophotometry using molar absorptivities determined in triplicate at 267 nm in acetonitrile (Beall et al., 1997).

Partition coefficients ($K_{IPM:AQ}$) were determined using the saturated IPM solutions ($n = 3$) from the lipid solubility determinations (Beall et al., 1993a). The saturated IPM solutions were partitioned against 0.05 M acetate buffer (pH 4.0). The two phases were shaken vigorously for 10 s then allowed to separate for 60 s. In previous experiments (Beall et al., 1993a) it had been shown that there was no significant effect on $K_{IPM:AQ}$ if the two phases were shaken for 10, 20 or 30 s. Aliquots from the IPM layers were diluted with dry acetonitrile and the UV absorbances were determined. The K values were calculated as follows:

$$K_{IPM:AQ} = [A_a/(A_b - A_a)]V_{AQ}/V_{IPM},$$

where A_b and A_a were the absorbances from the IPM layer before and after partitioning, respectively, and V_{AQ} and V_{IPM} were the volumes of the buffer and IPM phases, respectively. For compounds **2–5** volume ratios (IPM:AQ) were 1:1.

Estimated aqueous solubilities (S_{AQ}) were calculated from S_{IPM} and $K_{IPM:AQ}$ values: $S_{AQ} = S_{IPM}/K_{IPM:AQ}$. Direct S_{AQ} were determined by stirring suspensions of **2–5** in pH 4.0 acetate buffer (0.05 M) at $22 \pm 1^\circ\text{C}$ for 60 min ($n = 2–4$). The suspensions were filtered through 0.45 μm nylon filters. The saturated solutions were diluted with dry acetonitrile and quantitated by UV spectroscopy as above.

2.2. Diffusion cell experiments

The diffusion cell experiments were run according to previously described procedures (Sloan et al., 1986). Female hairless mice were sacrificed by

cervical dislocation. Their skins were removed by blunt dissection and placed epidermal side up in glass Franz diffusion cells with the dermal side in contact with pH 7.1 phosphate buffer (0.05 M, $I = 0.11$ M, 32°C) containing 0.11% formaldehyde (2.7 ml of 36% aqueous formaldehyde per liter) to prevent microbial growth and to insure the integrity of the mouse skins during the course of the experiment. [In control experiments to determine the effect of time on the integrity of the mouse skin, Th flux from propylene glycol (PG) applied 4 h after sacrifice was $6.1 \pm 0.6 \times 10^{-3}$ $\mu\text{mol cm}^{-2} \text{h}^{-1}$; 24 h after sacrifice was $8.3 \pm 1.9 \times 10^{-3}$ $\mu\text{mol cm}^{-2} \text{h}^{-1}$; 48 h after sacrifice was $9.4 \pm 1.2 \times 10^{-3}$ $\mu\text{mol cm}^{-2} \text{h}^{-1}$; 120 h after sacrifice was $10.0 \pm 1.2 \times 10^{-3}$ $\mu\text{mol cm}^{-2} \text{h}^{-1}$ (Sloan et al., 1991)]. The skins were kept in contact with the buffer for at least 48 h to condition the skins and to allow UV absorbing materials to leach from the skins; the receptor phases were changed at least three times during this time to facilitate the leaching process. The epidermal sides of the skins were exposed to ambient conditions and were untreated during the preapplication period.

After the preapplication period, 0.5 ml aliquots from suspensions of **2–5** in IPM were applied to the epidermal sides of the skins (the IPM suspensions were stirred at $22 \pm 1^\circ\text{C}$ for 48 h prior to application to ensure that saturation was attained). Total concentrations of the IPM suspensions ranged from 0.4–0.6 M which ensured that enough excess solid was present to maintain saturation for the duration of the application period (see below). Each prodrug–vehicle combination was run in triplicate. The donor phases were changed every 12 h and saved for analysis by ^1H NMR spectroscopy. Samples were taken from the receptor phases at 4, 8, 12, 21, 24, 27, 30, 33, 36, 45 and 48 h after donor phase application. The receptor phases were changed following removal of each sample so that sink conditions were maintained. Samples were analyzed by UV spectroscopy ($\varepsilon = 7.13 \times 10^3$ 1 mol $^{-1}$ at 266 nm) for total 5-FU species that had diffused after allowing at least 72 h for complete prodrug hydrolysis. Cumulative amounts of total 5-FU species in the receptor

phase (μmol) were plotted against time (h), and the slopes of the linear, steady-state regions (usually from 27 to 36 h) were calculated using linear regression. The slopes divided by 4.9 cm^2 (the area of the donor surface) gave the steady-state fluxes (J_i in $\mu\text{mol cm}^{-2} \text{ h}^{-1}$). Permeability coefficients (P_i) were determined by dividing the values of J_i by the solubility of the corresponding prodrugs in IPM (S_{IPM}).

Following removal of the donor phases after the 48 h application period, the epidermal sides of the skins were washed three times with 5 ml portions of methanol to remove all remnants of prodrug and vehicle from the skin surfaces. This was accomplished quickly (< 3 min) to minimize contact time between the skins and methanol. The receptor phases were changed again, and the dermal sides were again left exposed to the air for 24 h. After this leaching period, another sample was taken from each cell and quantitated by UV spectroscopy ($\epsilon = 7.13 \times 10^3 \text{ 1 mol}^{-1}$ at 266 nm) to measure the skin accumulation of total 5-FU species (C_S).

Second applications to the epidermal sides of the skins were made after the leaching period with a standard drug-vehicle suspension. Theophylline in PG (0.4 M) was applied to assess the damage to the skins from application of the initial prodrug–vehicle combinations. Samples were taken at 1, 2, 3, 5, 7 and 9 h after the second application. The samples were analyzed for theophylline by UV spectroscopy ($\epsilon = 1.02 \times 10^4 \text{ 1 mol}^{-1}$ at 271 nm) and second application fluxes (J_j) were determined as described above.

In a separate $n = 1$ diffusion cell experiment, run as above, except that fresh donor phase was applied every 24 h instead of every 12 h, HPLC was used to determine intact prodrug content in the receptor phase at each sampling time. Aliquots were removed from the receptor phase and chromatographed immediately. Total 5-FU species that had diffused was measured as above.

2.3. Analysis

The ^1H NMR spectra of the donor phases from the applications of **2–5** were run in $\text{DMSO}-d_6$. The $\text{C}^6\text{-H}$ for 5-FU appeared at $\delta 7.73$ and the

$\text{C}^6\text{-H}$ for the 3-AC prodrugs appeared at $\delta 7.90$ in $\text{DMSO}-d_6$. Since this area of each spectrum was free from interference by IPM absorbances, the two signals were easily quantified by integration.

The HPLC system used was previously described (Beall et al., 1993b). The mobile phase contained 18–50% methanol in 0.025 M acetate buffer (pH = 5.0). Retention times (% methanol) were as follows: **2** = 6.1 min (18%), **3** = 11.2 min (18%), **4** = 26.9 min (18%), **5** = 8.5 min (50%), 1-acetyloxymethyl-5-FU = 6.6 min (18%), 3-acetyloxymethyl-5-FU = 8.3 min (18%).

Since authentic samples of the proposed 1- and 3-alkylcarbonyloxymethyl-5-FU (1- and 3-ACOM-5-FU) products from the hydrolysis of the 3-alkylcarbonyl-5-FU (3-AC-5-FU) prodrugs in the receptor phase containing formaldehyde (Beall et al., 1993b, 1997) were not available (except 1- and 3-acetyloxymethyl-5-FU), each of the 3-AC-5-FU prodrugs ($1.12\text{--}1.28 \times 10^{-4} \text{ M}$) was allowed to hydrolyze completely in pH 7.1 phosphate receptor phase buffer containing 0.11% formaldehyde as control experiments. Values of molar absorptivities (ϵ) for these hydrolysate mixtures were obtained and compared to the value of ϵ for 5-FU under the same conditions.

2.4. Solubility parameters

The calculated solubility parameters (δ_i) were obtained using the method of Fedors (1974) as illustrated by Martin et al. (1985) and Sloan et al. (1986).

2.5. Statistical analysis

Statistical analyses were accomplished using Student's *t*-test. Unless otherwise indicated, statistical significance is for $P < 0.05$.

3. Results and discussion

3.1. Solubilities and partition coefficients

The lipid solubilities (S_{IPM}) of **2–5** and their solubilities in pH 4.0 acetate buffer (S_{AQ}) are given in Table 1. The lipid solubilities of the

3-AC-5-FU prodrugs are generally less than those of the corresponding 1-AC-5-FU prodrugs (Beall and Sloan, 1996). In particular, the 1-acetyl and 1-propionyl-5-FU derivatives are five and 2.5 times more soluble in IPM than **2** and **3**, respectively. Only **4** is slightly more lipid soluble than the corresponding 1-butyryl derivative. This is unusual because the apparent mp of the 3-AC-5-FU prodrugs are 10–30°C lower than those of the corresponding 1-AC-5-FU prodrugs. Although ΔH_f values were not obtained for these two series of prodrugs, the higher mp yet higher lipid solubilities of the 1-AC-5-FU series might suggest that their ΔH_f values must be lower. However, based on the DSC analyses of the 3-AC-5-FU prodrugs (Beall, et al., 1997), the observed mp are more likely the temperatures at which the 3-AC-5-FU prodrugs rearrange to the 1-AC-5-FU prodrugs and are not true mp.

Although the S_{IPM} values increase as the alkyl chain length increases up to **4**, the S_{IPM} value for **5** decreases dramatically. It is not unusual for the lipid solubility in a homologous series to begin to decrease as the chain length increases above a certain length (Sloan et al., 2000). However, it usually occurs later in the series and not as sharply.

It was possible to directly measure S_{AQ} values at pH 4.0. At pH 7.1 in the absence of formaldehyde the $t_{1/2}$ values for **2** and **3** were only 200 and 300 min, respectively (Beall et al., 1997). This is

5–6 times longer than the reported $t_{1/2}$ values at pH 7.4 for **2** and **3** of 43 and 50 min, respectively (Buur and Bundgaard, 1984), but still not long enough for **2–5** to be sufficiently stable that their S_{AQ} values could be measured directly at pH 7.1. The reason for the difference between the $t_{1/2}$ values reported by the two groups is that Buur and Bundgaard failed to recognize the existence of a terminal phase in the hydrolyses of the 3-AC-5-FU prodrugs (Beall et al., 1993b), which leads to the longer $t_{1/2}$ values reported by Beall et al. However, the shape of the pH versus rate profiles for the 3-AC-5-FU prodrugs reported by Buur and Bundgaard is accurate and shows a dramatic drop (1.2 log units) in the rate of hydrolyses at pH 4.0 compared to at pH 7.4. Thus, we can estimate that the rate of hydrolysis at pH 4.0 is about 2000 and 3000 min for **2** and **3**, respectively; and, during the time the solubilities were being measured (60 min), less than 5% of the prodrugs could have hydrolyzed to 5-FU.

The directly measured S_{AQ} value for **2** is much less than than reported by Buur and Bundgaard (1984) for **2**, while the value for **3** is only about 5% more — a reasonably good agreement. The poor agreement on the S_{AQ} for **2** may be due to the fact that Buur and Bundgaard (1984) used an ultrasonic water-bath for 30 min to solubilize the 3-AC-5-FU prodrugs before rotating their samples on a mechanical spindle for 1 h at 22°C and subsequent HPLC analyses. It has previously been

Table 1
Melting points (mp), solubilities in IPM (S_{IPM}) and direct, estimated and literature solubilities in pH 4.0 acetate buffer (S_{AQ})

3-R(C=O)-5-FU, R=	mp (°C) ^a	S_{IPM} ^{b,c}	S_{AQ} ^{b,d}	S_{AQ} ^{b,e}	S_{AQ} ^{b,f}
1 , 5-FU	280–282	0.049	96	—	85
2 , CH ₃	115–117	4.3	166	105	249
3 , C ₂ H ₅	102–103	14.0	198	135	190
4 , C ₃ H ₇	111–112	22.0	53	23	—
5 , C ₄ H ₉	110–111	9.2	5	5.5	—

^a From Beall et al., 1997.

^b Units of mM.

^c Standard deviations from the mean were within \pm 3% except for **4** which was \pm 10%.

^d Standard deviations from the mean for the direct S_{AQ} were within \pm 8%.

^e Standard deviations from the mean for the partition coefficients from which estimated S_{AQ} were obtained were 5, 5, 13 and 8%, respectively.

^f From Buur and Bundgaard, 1984.

Table 2

Log of partition coefficients between IPM and pH 4.0 acetate buffer ($K_{IPM:AQ}$), log solubility ratios ($SR_{IPM:AQ}$), associated methylene π values and solubility parameters (δ_i) for the prodrugs

Compound	Log $K_{IPM:AQ}$	π^a	log $SR_{IPM:AQ}$	π^b	δ_i^c
1, 5-FU	−3.24				15.0
2	−1.39	—	−1.59	—	14.09
3	−0.97	0.42	−1.14	0.45	13.45
4	−0.01	0.96	−0.38	0.76	12.96
5	0.22	0.23	0.27	0.65	12.56

^a $\pi = (\log K_{n+m} - \log K_n)/m$, where n is the number of methylene units in the promoiety of one prodrug and m is the number of additional methylene units in the promoiety of the prodrug with which it is compared.

^b Substitute SR for K in footnote a.

^c Units of $(\text{cal cm}^{-3})^{1/2}$ calculated using the method of Fedors (1974) as illustrated by Martin et al. (1985), Sloan et al. (1986).

noted that the use of ultrasonic water baths can lead to hot spots in solution and high solubility values (Kerr et al., 1998). We believe that this is the explanation here. However, the phenomenon does not occur uniformly since the efficiency of the ultrasonic water bath depends in part on where the sample is placed in the bath. Thus, not all values will be high.

The S_{AQ} values estimated from $S_{IPM}/K_{IPM:AQ}$ were generally much lower than the directly measured S_{AQ} values. This trend, although not as exaggerated, has been noted previously for other, more water soluble prodrugs which are sufficiently stable that their aqueous solubilities can be measured directly as well as estimated (Beall et al., 1993a). However, these estimated values are useful for comparing S_{AQ} values for series of prodrugs that exhibit different aqueous stabilities. In a comparison of the 3-AC-5-FU prodrugs, which are reasonably stable at pH 4.0, with the 1-AC-5-FU, which are not, estimated S_{AQ} must be compared. Here, except for 1-acetyl-5-FU, the 3-AC-5-FU prodrugs are all significantly more water soluble than the corresponding 1-AC-5-FU prodrugs.

The partition coefficients between IPM and pH 4.0 buffer, from which the estimated S_{AQ} values were determined, are given in Table 2. Usually the mean methylene π value calculated from the differences in $\log K_{IPM:AQ}$ for homologous series of prodrugs is about 0.60 ± 0.05 (Beall and Sloan, 1996). Here the mean methylene π value is 0.54 ± 0.38 . The mean methylene π value calculated from

the solubility ratios (Table 2) is only marginally better: 0.62 ± 0.16 . These results suggest that the solubilities of the series are not well-behaved. The reason for the poor behavior may be due to the fact that the 3-acyl group is perpendicular to the plane of the 5-FU ring (Beall et al., 1993b) only in this series of 5-FU prodrugs. This could cause non-uniform packing in the crystal lattices of adjacent members of the series.

3.2. Diffusion cell experiments

The results from the diffusion cell experiments ($n = 3$) are given in Tables 3 and 4 and the Fig. 1. IPM, a nonprotic solvent, was used as the vehicle for the delivery of 5-FU by the 3-AC-5-FU prodrugs so that the results from these diffusion cell experiments can be compared not only with those from the 1-AC-5-FU prodrugs in particular but also other series of prodrugs as well. The 1-AC-5-FU prodrugs had to be evaluated using a non-protic vehicle such as IPM because they are hydrolytically unstable in protic solvents (Beall and Sloan, 1996). Using IPM as the vehicle, only intact 3-AC-5-FU prodrugs were observed in the donor phases when the residues from the donor suspensions were analyzed by ^1H NMR spectroscopy.

Also all of the other series of prodrugs of 5-FU, and series of prodrugs of other parent drugs as well, have been evaluated in diffusion cell experiments which used 0.11% formaldehyde in the receptor phase to prevent deterioration of the

Table 3

Experimental rates of delivery of total 5-FU species by the prodrugs from IPM through hairless mouse skin (J_i), predicted J_i based on S_{IPM} , S_{AQ} and molecular weight, concentrations of total 5-FU species retained in the skin (C_S), dermal/transdermal (D/T) delivery ratios, and the rates of delivery of theophylline from PG (J_j) after removal of the prodrugs

Compound	J_i (\pm SD) ^a	Predicted J_i ^{a,b}	C_S (\pm SD) ^c	D/T ^d	J_j (\pm SD) ^a
1, 5-FU	0.24 (0.09)	0.34	3.7 (0.9)	0.131	1.2 (0.2)
2	4.4 (0.5)	2.75	16 (3)	0.031	1.6 (0.1)
3	5.2 (0.7)	5.24	15 (2)	0.024	1.8 (0.2)
4	2.2 (0.3)	2.57	7.1 (1.6)	0.027	1.1 (0.1)
5	0.55 (0.07)	0.74	3.3 (1.1)	0.051	1.1 (0.1)

^a Units of $\mu\text{mol cm}^{-2} \text{h}^{-1}$.

^b Predicted from Equation 3 in Roberts and Sloan, 1999, using estimated S_{AQ} values.

^c Units of μmol .

^d Calculated from $\{C_S/[(4.9 \text{ cm}^2)(24 \text{ h})]\}/J_i$ to give a dimensionless ratio.

mouse skins with time caused by microbial growth in the receptor phase (Sloan et al., 1991). Again, since it was essential to be able to compare results from these diffusion cell experiments where the 3-AC-5-FU series of prodrugs have been evaluated with those from experiments evaluating other series, 0.11% formaldehyde was used in the receptor phase in these experiments. However, during the evaluation of the 3-AC-5-FU prodrugs, an unexpected rearrangement and trapping of the intermediate by the formaldehyde in the receptor phase occurred which led to the formation of approximately equal amounts of 1- and 3-alkylcarbonyloxymethyl derivatives of 5-FU (1- and 3-ACOM-5-FU) in addition to 5-FU (Beall et al., 1993b, 1997). Only authentic samples of 1- and 3-acetyloxymethyl-5-FU were available to enable quantitation of their formation from the corresponding 3-acetyl-5-FU, **2**, in the receptor phase. Thus, it was not possible to quantitate each component in the receptor phase when samples were taken for analysis after the application of **3**, **4**, and **5** in IPM. Instead, each of the 3-AC-5-FU prodrugs were allowed to hydrolyze in pH 7.1 phosphate buffer containing 0.11% formaldehyde until HPLC analysis showed no 3-AC-5-FU remained in the solution: only 5-FU and the proposed 1- and 3-ACOM-5-FU. The measured molar absorptivities (ε) of these hydrolysates in control experiments (λ max at 266 nm) were 1.4, 0.6, 4.8 and 5.3% less than the molar absorptivity of 5-FU (λ max at 266 nm) measured under the

same conditions. Since the molar absorptivities (ε) of the hydrolysates were so close to that of 5-FU, and it was not possible to accurately measure how much intact **2–5** diffused into the receptor phase intact, rearranged and hydrolyzed or formed 1- and 3-ACOM-5-FU, the value of ε for 5-FU itself was used to calculate the concentrations of total 5-FU species in the receptor phase. Use of the ε value for 5-FU in the calculation leads to less than 5% underestimation of total 5-FU species in the receptor phase.

All of the 3-AC-5-FU prodrugs were more effective than 5-FU at delivering total 5-FU species through hairless mouse skin from IPM suspensions. As observed for similar previously studied series (Sloan, 1992), there was no direct relation-

Table 4

Log permeability coefficients ($\log P_i$) from $\log J_i - \log S_{IPM}$. Experimental rates of delivery of total 5-FU species (J_i) and intact prodrug (J_{Pro}), percent of flux as intact prodrug at steady-state and at 11 h and rates of delivery of theophylline from PG (J_j) after removal of the prodrug in IPM for the $n=1$ diffusion cell experiments

Compound	$\log P_i$ ^a	J_i ^b	J_{Pro} ^b	% J_{Pro} (11 h)	J_j ^b
1, 5-FU	0.69				
2	0.01	2.13	0.51	24 (19)	1.27
3	-0.43	3.78	1.87	49 (43)	1.28
4	-1.00	1.82	1.13	62 (52)	1.13
5	-1.22	0.34	—	—	0.81

^a Units of cm h^{-1} .

^b Units of $\mu\text{mol cm}^{-2} \text{h}^{-1}$.

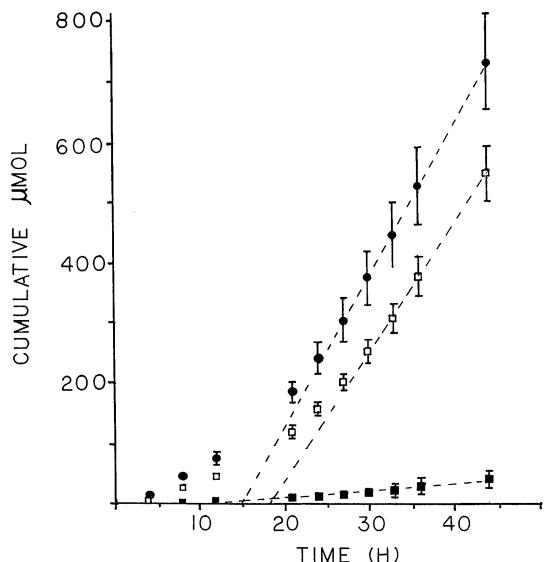


Fig. 1. A plot of the cumulative amount of total 5-FU species diffused versus time from 5-FU (■), 3-acetyl-5-FU (□) and 3-propionyl-5-FU (●).

ship between the lipid solubilities (S_{IPM}) of the prodrugs and their abilities to deliver total 5-FU species through skin (J_i). On the other hand, the rank order of the pH 4.0 aqueous buffer solubilities (S_{AQ}) for **2–5** is the same as the rank order of J_i for **2–5** which also had been observed for previously studied series. **3** was the most water soluble member of the series and it gave the highest flux, however it gave only half the flux of the best 1-AC-5-FU derivative: 1-acetyl-5-FU. As a series the 3-AC-5-FU derivatives were comparable to the 1-AC-5-FU series. J_i for **2** and **5** were less than J_i for 1-acetyl- and 1-valeryl-5-FU, while J_i for **3** and **4** were greater than J_i for 1-propionyl- and 1-butyryl-5-FU. The lower S_{IPM} values for the 3-AC-5-FU series are compensated by their higher S_{AQ} values (see below). The S_{AQ} data for 5-FU does not fit the rank order relationship found in the series because 5-FU contains a different mixture of functional groups and is 100 times less lipid soluble than the least lipid soluble member of the series.

This qualitative relationship between S_{AQ} , S_{IPM} and J_i has recently been put on a quantitative basis using similar data from seven other series of

prodrugs and a transformation of the Potts–Guy equation which accommodates J_i values obtained using lipid vehicles (Roberts and Sloan, 1999). In order to determine if the experimental S_{AQ} , S_{IPM} and molecular weights (mw) for this series of 3-AC-5-FU prodrugs and 5-FU accurately predicted experimental J_i data, values of predicted J_i were calculated from the transformed Potts–Guy equation 3 in Roberts and Sloan (1999): $\log J_M = -0.211 + 0.534 \log S_{IPM} + 0.466 \log S_{AQ} - 0.00364 \text{ mw}$. The predicted values for J_i given in Table 3 are reasonably accurate and correctly predict the rank order of the abilities of the members of the series to deliver total 5-FU species through hairless mouse skin. The average error in predicting $\log J_i$ (or average $\Delta \log J_M$) is given by (experimental $\log J_M$ – predicted $\log J_M$) = $\Delta \log J_M$. For the 3-AC-5-FU series the average $\Delta \log J_M$ was only 0.11 ± 0.08 log units which is less than the average $\Delta \log J_M$ (0.126 log units) for the members of the $n = 42$ data base of the seven series of prodrugs from which the parameter estimates of the transformed Potts–Guy equation were obtained. Thus, the data from the 3-AC-FU series of prodrugs is homogeneous with that from all of the other series of prodrugs for which similar data is available.

Although all of the 3-AC-5-FU prodrugs were more effective than 5-FU at causing accumulation of total 5-FU species in the skin based on the C_S values in Table 3, they were not nearly as effective as 5-FU itself based on the D/T delivery ratios also listed in Table 3. The C_S values reflect how much of total 5-FU species was in the skin when the donor phases were removed while the D/T delivery ratios normalize the C_S values for J_i . D/T delivery ratios less than that of 5-FU itself indicate that the prodrugs were not effective in preferentially increasing dermal delivery compared to 5-FU. Thus, the 3-AC-5-FU prodrugs increased C_S but not as much as J_i was increased. For instance **3** increased J_i by 20 times but only increased C_S by four times. By comparison, three members of the 1-AC-5-FU series of prodrugs gave D/T delivery ratios comparable to that of 5-FU whereas the best 3-AC-5-FU prodrug gave a D/T delivery ratio that was less than half of that given by 5-FU. The reason for this difference has

been discussed elsewhere in detail (Beall and Sloan, 1996). Essentially the 1-AC-5-FU prodrugs hydrolyze much faster than the 3-AC-5-FU prodrugs during their respective transits through the skin. Since both series of prodrugs have been designed to incorporate solubility properties that will allow them to partition into and through the skin better, the more stable series will deliver more intact prodrug through the skin and less 5-FU in the skin.

The differences in delivery of total 5-FU species by the members of the 3-AC-5-FU series of prodrugs does not appear to be due to significant differences in damage to the skin caused by their application in IPM. Application of a standard solute/vehicle (theophylline/PG), after removal of the prodrug/IPM donor phase and a 24 h leaching period, gave the J_j values listed in Table 3. In an identical experiment the value for J_j after the application of IPM alone was $1.2 \mu\text{mol cm}^{-2} \text{h}^{-1}$. Thus, using J_j values as one measure of damage, the damage caused by **2–5** was comparable to the damage caused by IPM itself or 5-FU/IPM. Normalization of J_i values by dividing by the respective J_j values (data not shown) did not lead to any changes in the rank order of performance by the 3-AC-5-FU prodrugs or their relative superiority over 5-FU/IPM (15-fold for **3**).

When the log permeability coefficients ($\log P_i$) from Table 4 were plotted against the respective calculated solubility parameters (δ_i) for the 3-AC-5-FU prodrugs from Table 2, a straight line (plot not shown) was obtained with a slope (0.80, $r = 0.99$) which is similar to that obtained for the 1-AC-5-FU prodrugs (0.89, $r = 0.99$). Thus, the 3-AC-5-FU series behaves in a manner that is similar to the 1-AC-5-FU series. As the members of the series become more lipophilic based on decreasing δ_i values, they become less efficient at delivering the parent drug from a lipoidal vehicle (decreasing P_i values).

In a separate experiment, the fluxes of intact prodrugs **2**, **3** and **4** were measured by HPLC as in Beall et al., 1994. The flux of **5** could not be determined because of the overlap of a contamination peak with that of **5**. The flux of total 5-FU species (J_i), flux of intact prodrug (J_{Pro}) and flux of theophylline from PG in a second application

(J_j) for this separate experiment are given in Table 4. The rank order of the values for J_i are the same as in Table 2, but their absolute values are 48, 73, 83 and 62% of those in Table 3 for **2**, **3**, **4** and **5**, respectively. The latter result may be due to the fact that fresh donor phases were applied every 24 h in this $n = 1$ experiment (Table 4), while they were applied over 12 h in the $n = 3$ experiments (Table 3). The absolute values of J_i in Table 4 are not less than those in Table 3 because of less damage since the values for J_j are generally higher in this separate experiment.

On one hand, the values for J_{Pro} are higher than they should be in intact animals because of the deterioration of the metabolic viability of the mouse skins in the diffusion cells during the course of the experiments. On the other hand, the values for J_{Pro} , which constitute a considerable portion of the fluxes of total 5-FU species, should be considered as the minimum amount of intact prodrug that had diffused under these conditions. Even if the receptor phase was analyzed immediately after a sample was taken, a portion of the intact prodrug that diffused into the receptor phase had 3 h to hydrolyze. The $t_{1/2}$ of the 3-AC-5-FU prodrugs under these conditions are sufficiently short (100 min for **2**) to allow significant amounts of the prodrugs in the receptor phase to hydrolyze in the 3 h between samples. In most cases, one cannot tell whether the 5-FU in the receptor phase comes from hydrolysis of the prodrug in the receptor phase or from hydrolysis of the prodrug in the skin and diffusion of the parent drug into the receptor. Because of the unusual reaction of the 3-AC-5-FU prodrugs with formaldehyde, the presence of significant amounts of proposed 1- and 3-ACOM-5-FU derivatives in the receptor phases from application of all of the 3-AC-5-FU prodrugs suggests that an undetermined additional amount of intact 3-AC-5-FU had diffused into the receptor phase intact, rearranged and reacted with formaldehyde or hydrolyzed. The extent of that additional amount cannot be estimated except for **2** because authentic samples of the corresponding 1- and 3-ACOM-5-FU were not available for **3**, **4** and **5**. However, for example in the 11 h receptor phase sample after the application of **2**, in addition to 19% **2**

there was a little over 3% of 3-acetyloxymethyl-5-FU and a little under 5% of 1-acetyloxymethyl-5-FU. Since the reaction of formaldehyde with the rearranged 3-acetyl-5-FU is at best only 50% effective (Beall et al., 1993b), we estimate that an additional $2 \times (3 + 5\%) = 16\%$ of intact 3-acetyl-5-FU may have diffused into the receptor phase intact during the 3 h period between samples. The trend in increasingly larger amounts of intact prodrug with increasing chain length of the 3-AC-5-FU prodrugs is consistent with the increasingly longer $t_{1/2}$ values in buffer and plasma with increasing chain length reported by Buur and Bundgaard (1984).

4. Conclusion

The 3-AC-5-FU prodrugs are all much more lipid soluble and the first two members of the series are also more water soluble than 5-FU. The most water soluble member of the series, 3, gives the greatest increase in flux: 20 times. However, none of the 3-AC-5-FU prodrugs are as effective as the best 1-AC-5-FU prodrug and their greater stability leads to lower D/T delivery ratios which is generally not desired when the final goal is to preferably treat local, as opposed to systemic disease states.

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