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Transdermal delivery of 5-fluorouracil (5-FU) through hairless mouse skin by 1-alkylaminocarbonyl-5-FU prodrugs: Physicochemical characterization of prodrugs and correlations with transdermal delivery

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Summary

The abilities of the members of a homologous series of 1-alkylaminocarbonyl-5-fluorouracil (5-FU) prodrugs to deliver 5-FU, **1**, through hairless mouse skin from isopropyl myristate (IPM) have been evaluated (alkyl = CH_3 to C_8H_{17}). The most effective member of the series was the propyl derivative which also exhibited the highest water (buffer) solubility of any of the prodrugs. Generally the members of the series were not very effective, with a maximum enhancement of only 3-times the rate of delivery of 5-FU by itself. All of the prodrugs were more lipid soluble than 5-FU and the prodrugs exhibited partition coefficient values that were comparable to other, more effective types of *N*-acyl prodrugs. The lack of effectiveness appears to be due to the lower buffer solubilities exhibited by these 1-alkylaminocarbonyl-5-FU prodrugs compared to the other types of *N*-acyl prodrugs. The lower buffer solubility, in turn, appears to be due to the fact that the hydrogen bonding donor ability of the *N*-alkylaminocarbonyl type of prodrug is not reduced compared to the other types of *N*-acyl prodrugs. Some differences in the literature for the physicochemical properties of the *N*-alkylaminocarbonyl series of prodrugs have been reconciled by an examination of their thermal properties.

Introduction

5-Fluorouracil (5-FU, **1**) is an important drug in the topical treatment of actinic keratoses and basal cell carcinomas of the face and other, more permeable areas of skin (Goette, 1981). However, present commercial formulations of 5-FU are ineffective in the treatment of actinic keratoses of the hands and arms where the skin is less permeable (Robinson and Kligman, 1975). 5-FU has also been reported to be useful in the treatment of vitiligo or psoriasis. However, effective treatment required either abrasion (Tsugi and Hamada, 1983) for the treatment of vitiligo, or

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occlusion (Tsugi and Sugai, 1972) or intradermal injection of the psoriatic plaques for the treatment of psoriasis (Lowe et al., 1990). In addition, the present commercial formulations are irritating. It is not clear whether the irritation is due to components of the formulation such as propylene glycol or to 5-FU itself, or a combination of the two factors. The irritant effect of 5-FU may be due to its physicochemical properties or the mechanism of its activity at the biochemical level. Since intradermal injection of 5-FU is effective in treating psoriasis but is reported not to cause the irritation observed from its topical application (Lowe et al., 1990), the latter possibility seems remote. Thus, it is generally conceded that a method of improving the topical delivery of 5-FU which is not irritating is desirable.

A prodrug approach to improving the topical delivery of 5-FU offers several advantages over formulation approaches. Since a prodrug transiently changes the physicochemical properties of the parent drug, the prodrug (1) may be designed to be non-irritating itself upon its delivery into the skin and (2) may be designed to be delivered from vehicles that in combination with the prodrug are less irritating to the skin than the combination of 5-FU and propylene glycol. However, before the irritancy problem can be addressed, the problem of identifying a particular prodrug approach to significantly improve the dermal delivery must be addressed. *N*-Acyl prodrugs are one general type of prodrug of 5-FU whose physicochemical properties have been extensively evaluated. Buur and Bundgaard (1984, 1985, 1986, 1989) have investigated the stability and solubility properties of selected members of the 1-alkylcarbonyl-, 3-alkylcarbonyl-, 1,3-bisalkylcarbonyl-, 1-alkyloxycarbonyl- and 1-alkylaminocarbonyl-5-FU series of *N*-acyl prodrugs. In particular, interest in the 1-alkylaminocarbonyl derivatives of 5-FU has led to the publication of several papers describing their synthesis (Ozaki et al., 1977) and physicochemical properties (Buur and Bundgaard, 1985), and it is the only series from which selected members have been evaluated for their ability to enhance the transdermal delivery of 5-FU (Sasaki et al., 1990). It was assumed that if transdermal delivery was improved, dermal delivery would be improved as well. However, only the longer alkyl chain, more lipophilic members of the series were evaluated for their abilities to enhance the transdermal delivery of 5-FU.

Based on previous experience with other series of different types of prodrugs of theophylline (Sloan and Bodor, 1982; Sloan et al., 1988), 6-mercaptopurine (Waranis and Sloan, 1987, 1988) and 5-FU (Sloan et al., 1988), it seemed likely that optimization of the transdermal delivery of 5-FU with the 1-alkylaminocarbonyl-5-FU derivatives had not been achieved. The previous literature suggested that the shorter alkyl chain, more water soluble members of the 1-alkylaminocarbonyl-5-FU series would be the more effective members of the series, and Sasaki et al. (1990) had not evaluated them. In this report, the evaluations of these shorter chain members of the 1-alkylaminocarbonyl-5-FU series of prodrugs and a comparison with the longer alkyl chain members of the series are presented. In addition, some of the thermal properties of the 1-alkylaminocarbonyl-5-FU prodrugs have also been examined.

Experimental

5-Fluorouracil (5-FU, **1**) was a gift from Hoffmann-La Roche, isopropyl myristate (IPM) was obtained from Givaudan, Clifton, NJ, the other reagents from Aldrich, and the bulk solvents from Fisher. TLC analyses were run on Brinkman Polygram Sil G/UV 254 plates. The female hairless mice (SKH-hr-1) were obtained from Temple University Skin and Cancer Hospital. The diffusion cells were from Crown Glass, Somerville, NJ (surface area 4.9 cm^2, 20 ml receptor phase volume). The diffusion cells were maintained at 32°C by a Fisher circulating water bath model 25. The ^{1}H-NMR spectra were recorded on a Varian EM-390, the IR spectra on a Perkin-Elmer 1420, and the UV spectra on a Cary 210 equipped with a constant temperature circulating water bath, or on a Shimadzu UV-265 spectrophotometer. DSC analyses in hermetically sealed pans were carried out using a Perkin-Elmer DSC-7 scanning

calorimeter and TGA analyses were performed with a Perkin Elmer TGA-7 thermogravimetric analyzer, both controlled by an IBM PS/2 Model 50Z microcomputer. Scan rates were 5°C/min in a nitrogen atmosphere for both DSC and TGA. Melting points were obtained on a Thomas-Hoover capillary apparatus. The HPLC system consisted of a Beckman Model 110A pump with a model 153 UV detector, a Rheodyne 7125 injector with a 20 μl loop, and a Hewlett-Packard 3392A integrator. The column was a 250 × 4.6 mm Lichrosorb RP-8 10 μm reverse-phase column. A mobile phase of 20% methanol and 80% 0.025 M acetate buffer pH 5.0 (Buur and Bundgaard, 1985) at a flow rate of 1.0 ml/min was used.

Synthesis

The 1-alkylaminocarbonyl-5-FU prodrugs were synthesized according to the general procedure of Ozaki et al. (1977). 5-FU, **1** (1.3 g, 0.01 mol), was suspended in 5 ml of dimethylacetamide (DMA) containing 0.10 g (0.001 mol) of triethylamine at room temperature. The appropriate alkyl isocyanate was added, and the suspension was heated with stirring at 90°C for 3 h in a flask equipped with a reflux condenser and drying tube. In all cases except for methyl isocyanate, a solution. was obtained upon heating. The solution was cooled to room temperature and poured into 10 ml of a well-stirred, dilute (9 ml of water + 1 ml of concentrated HCl) acid solution. The white precipitate that formed was filtered. The residue was washed with 4–10 ml of water and dried under atmospheric conditions. The white solid that resulted was triturated with ether overnight, filtered from the ether and dried. This crude product was crystallized from acetone-hexane to give a pure product which was characterized by ^{1}H-NMR, IR, UV, TLC, HPLC, DSC and melting point where possible.

1-Methylaminocarbonyl-5-FU (2) Crude yield, 58%; pure yield, 53%; ^{1}H-NMR (DMSO-d_6) δ 12.2 (broad m, 1, NH), 8.9 (broad m, 1, NH), 8.32 (d, 1, J = 7.5 Hz, CH=), 2.85 and 2.80 (2s, 3, CH_3-NH); IR (KBr) 3335 cm^{-1} (NH) 3300–2700 cm^{-1} (NH and CH stretch) and 1740 cm^{-1} (broad C=O); UV ($CH_3C{\equiv}N$) λ_{max} 256 ($\epsilon = 1.19 \times 10^4$ l/mol).

1-Ethylaminocarbonyl-5-FU (3) Crude yield, 85%; pure yield, 33%; ^{1}H-NMR (DMSO-d_6) δ 12.2 (broad m, 1 NH), 9.0 (broad m, 1, N-H, 8.32 (d, 1, J = 7.5 Hz, CH=), 3.28 (q, 2, J = 7.5 Hz, CH_3CH_2-N), 1.12 (t, 3, J = 7.5 Hz, CH_3CH_2-N); IR (KBr) 3350 cm^{-1} (NH), 3300–2700 cm^{-1} (NH and CH stretch) and 1740 cm^{-1} (broad C=O); UV ($CH_3C{\equiv}N$) λ_{max} 256 ($\epsilon = 1.16 \times 10^4$ l/mol).

1-Propylaminocarbonyl-5-FU (4) Crude yield, 47%; pure yield, 29%; ^{1}H-NMR (DMSO-d_6) δ 12.2 (broad m, 1, NH), 9.1 (broad m, 1, NH), 8.32 (d, 1, J = 7.5 Hz, CH=), 3.22 (q, 2, J = 6 Hz, CH_2CH_2N), 1.75–1.32 (m, 2, $CH_3CH_2CH_2N$), and (t, 3, J = 7.5 Hz, CH_3CH_2); IR (KBr) 3320 cm^{-1} (NH), 3300–2700 cm^{-1} (NH and CH stretch) and 1740 cm^{-1} (broad C=O); UV ($CH_3C{\equiv}N$) λ_{max} 256 ($\epsilon = 1.15 \times 10^4$ l/mol); TLC (ether), R = 0.46; m.p. = 138–140°C.

1-Butylaminocarbonyl-5-FU (5) Crude yield, 48%; pure yield, 28%; ^{1}H-NMR (DMSO-d_6) δ 12.2 (broad m, 1, NH), 9.05 (broad m, 1, NH), 8.32 (d, 1, J = 7.5 Hz, CH=), 3.4–3.13 (m, 2, CH_2CH_2N), 1.7–1.05 (m, 4, $CH_3CH_2CH_2CH_2N$) and 0.90 (t, 3, J = 6 Hz, CH_3CH_2); UV ($CH_3C{\equiv}N$) λ_{max} 256 ($\epsilon = 1.16 \times 10^4$ l/mol); TLC (ether), R_f = 0.50; m.p. = 133–135°C.

1-Octylaminocarbonyl-5-FU (6) Crude yield, 35%; pure yield, 30%; ^{1}H-NMR (DMSO-d_6) δ 12.2 (broad m, 1, NH), 9.05 (t, 1, J = 6 Hz, N-H, 8.32 (d, 1, J = 7.5 Hz, CH=), 3.4–3.1 (m, 2, CH_2CH_2N), 1.7–1.1 (m, 12, $(CH_2)_6$), and 1.0–0.7 (m, 3, CH_3CH_2); UV ($CH_3C{\equiv}N$) λ_{max} 256 (ϵ = 1.09 × 10^4 l/mol); TLC (ether), R_f = 0.50; m.p. = 91–94°C.

Solubilities and partition coefficients

The solubilities of the 1-alkylaminocarbonyl-5-FU prodrugs in IPM and pH 4.0 buffer were determined as previously described (Beall et al., 1993) by stirring an excess of the prodrug in IPM for 48 h and in pH 4.0 buffer for 1 h at room temperature, filtering the suspension through a 0.45 μm nylon filter and quantitating the diluted ($CH_3C{\equiv}N$) solution of prodrug by UV or by HPLC (λ_{anal} = 254 nm). In addition, the pH 4.0 buffer solubilities (S_{H_2O}) of the prodrugs were esti-

mated from their solubilities in IPM (S_{IPM}) and their partition coefficients between IPM and pH 4.0 buffer (K). The buffer solubilities were estimated from: $S_{H_2O} = S_{IPM}/K$. These solubility and partition coefficient values are reported in Table 2.

Diffusion cell experiments

The diffusion cell experiments were run in essentially the same way as previously described (Sloan et al., 1988; Saab et al., 1990). Briefly, the hairless mice were killed and the dorsal portion of each mouse skin was placed in contact with the receptor phase (pH 7.1 phosphate buffer, 0.05 M, $\mu = 0.11$ M, containing 2.7 ml of 36% formaldehyde/l of buffer) at 32°C for 48 h. The receptor phases were changed three times during this preapplication leaching-conditioning period. Then, 0.5 ml aliquots of 0.6 M suspensions of each prodrug in IPM were applied to the donor side of each of three diffusion cells. The suspension samples were prepared in the same way that the solubility samples were prepared: excess prodrug in IPM was stirred for 48 h at room temperature. After the suspensions were applied, 3 ml samples of the receptor phases were removed, generally at 4, 8, 12, 21, 24, 27, 30, 33, 36, 45 and 48 h. The amount of 5-FU in each sample was determined from the UV absorption at 265 nm ($\epsilon = 7.13 \times 10^3$ l/mol) after the samples had been allowed to sit at room temperature for 72 h. The samples were kept for 72 h to ensure that all the prodrug had hydrolyzed to 5-FU before analysis. Each time a sample was removed, the entire receptor phase was replaced with fresh receptor fluid. When the initial application period of 48 h was completed, the donor phase was carefully removed and the solid portion was analyzed for intact prodrug by ^{1}H-NMR (CH=absorption at δ 8.32). The donor surfaces were quickly washed with three 5 ml portions of methanol to remove any residual prodrug or 5-FU. After the methanol wash, the skins were kept in contact with fresh receptor fluid for 23–24 h to allow any 5-FU or 5-FU prodrug in the skins to leach out. Subsequently, these receptor phases were analyzed for 5-FU by UV spectrophotometry as above to give the amount of 5-FU in the skins (C_s). The receptor phases were replaced with fresh fluid and 0.5 ml aliquots of a standard drug/vehicle (theophylline/propylene glycol) were applied. This second application period was only for up to 12 h. Samples (3 ml) of receptor phases were generally taken at 1, 2, 3, 4, 6 and 12 h, and the amounts of theophylline in the receptor phases were quantitated from the UV absorptions at 270 nm ($\epsilon = 1.02 \times 10^4$ l/mol). Each time a sample was removed the entire receptor phase was replaced with fresh receptor fluid.

In all cases the rates of delivery of 5-FU (J_i) or theophylline (J_j) through skin were determined by plotting the cumulative amount (mg) of 5-FU or theophylline measured in the receptor phase against time, and dividing the slopes of the steady-state portions of those plots by the surface area of the diffusion cells. Permeability coefficients were determined by dividing the J values by the mg equivalent solubility of the corresponding prodrugs in IPM.

In a separate diffusion cell experiment, a suspension of each prodrug in IPM was applied to one diffusion cell. The same procedure as above was used except that the 24 h samples (which contained diffused 5-FU and 5-FU prodrug that had been collected for 3 h) were quantitated for 5-FU and intact 5-FU prodrug by HPLC immediately after the sample was taken for the methyl-, ethyl- and propylaminocarbonyl prodrugs.

Solubility parameters

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

Statistical analysis

Statistical analysis was accomplished using Student's t-test. Unless otherwise indicated, statistical significance is for $p < 0.05$.

Results and Discussion

Syntheses and characterizations

Although all of the 1-alkylaminocarbonyl-5-FU prodrugs that were synthesized for this report

were described before, there are significant differences between literature values for some of the physicochemical properties and those reported here. For instance, Ozaki et al. (1977) have reported that the prodrugs undergo thermal decomposition with loss of isocyanate to regenerate 5-FU, yet they have reported melting points from a Buchi capillary melting point apparatus for all five prodrugs (**2–6**, Table 1). Buur and Bundgaard (1985) synthesized **2**, **3** and **5** and reported melting points obtained using differential scanning calorimetry (Table 1). The melting points reported by Buur and Bundgaard are much higher than those reported by Ozaki for **2** and **3**. On the other hand, no melting points were observed for **2** and **3** in this work, and **4**, **5** and **6** each resolidified after having undergone an apparent melting process in a capillary tube at the reported values (Table 1). Prodrugs **4** and **5** resolidified immediately while **6** resolidified at about 120–130°C. Each sample subsequently remelted at about 280°C. This behavior is consistent with loss of isocyanate during melting to give 5-FU which then melted at 280°C.

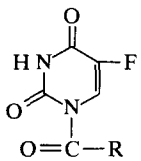
The thermal properties of **2–6** were also investigated using DSC and TGA in this work. The temperature of the peak of each endotherm is recorded in Table 1. The peak temperatures observed for **2** and **3** are much lower than the DSC melting points reported by Buur and Bundgaard (1985) but much higher than the capillary melting points reported by Ozaki et al. (1977). The DSC onset temperatures exhibited by **2** and **3** (187 and 165°C, respectively) were much lower than their endotherm peak temperatures. The DSC of **6** exhibited two endotherms at lower temperatures (76 and 91°C) and another at an intermediate temperature (127°C). In all cases the samples exhibited a large endotherm at about 276°C: 5-FU exhibited an endotherm at about 277°C.

The TGA results were consistent with thermal decomposition of the prodrugs in which the alkyl isocyanate was lost. For **3–5**, there were clearly two steps in mass loss: stoichiometric loss of isocyanate, then vaporization of 5-FU. For **2**, loss of 5-FU and isocyanate occurred simultaneously. For **6**, the boiling point of the isocyanate is so high that it did not completely volatilize until after some 5-FU also was lost.

For **2** and **3**, the temperature at which isocyanate loss began in the TGA was at or below the temperature for the endotherm peaks in the DSC attributed to melting of the prodrugs, so it is reasonable that no capillary melting point was

TABLE 1

Melting points, differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) data for 1-alkylaminocarbonyl-5-FU prodrugs



R	m.p. [a]	DSC [b]	m.p.	DSC [d]	TGA [e]
2, $NHCH_3$	170	225–228	– [c]	212	194
3, NHC_2H_5	165	190–196	– [c]	180	180
4, NHC_3H_7	145	–	138–140	139	144
5, NHC_4H_9	137	136	133–135	133	141
6, NHC_8H_{17}	98–100	–	91–94	76 and 91	119

[a] Ozaki et al. (1977).
[b] Buur and Bundgaard (1985).
[c] No melting point could be observed.
[d] Temperature of peak for endotherm.
[e] Temperature at which onset of mass loss occurred.

observed for them. For **4** and **5**, the temperature at which loss of isocyanate in the TGA began was only a few degrees higher than the temperature of the endotherm peak in the DSC so the observed melting and immediate resolidification in capillary tubes is consistent with the TGA and DSC. The TGA and DSC of **6** are also consistent with the observed capillary melting process where resolidification of the sample after melting at 94°C did not occur until 120°–130°C.

The ^{1}H-NMR spectra of the prodrugs given under Experimental are consistent with a 1-acyl structure (Beall, 1991; Kametani et al., 1980). The position of the C<u>H</u>=C absorptions is downfield at about δ 8.32 compared to the C<u>H</u>=C absorptions in 3-acyl derivatives (C<u>H</u>=C for 3-alkylcarbonyl-5-FU at about δ 7.25 in $CDCl_3$; Beall, 1991), but is analogous to other 1-acyl derivatives (C<u>H</u>=C for 1-alkyloxycarbonyl-5-FU at about δ 8.15 in DMSO-d_6 and for 1-alkylcarbonyl-5-FU at about δ 8.25 in $CDCl_3$; Beall, 1991). Although the 1-hexylaminocarbonyl derivative described in detail by Ozaki et al. (1977) was not investigated here, none of those that were investigated exhibited the appearance of two C<u>H</u>=C absorptions in going from $CDCl_3$ to DMSO-d_6 that Ozaki et al. observed. However, only freshly opened glass ampules of DMSO-d_6 were used in this study. Since it is known that DMSO picks up water very rapidly after a bottle or ampule is opened and 1-alkylaminocarbonyl derivatives hydrolyze fairly rapidly in the presence of water (Buur and Bundgaard, 1985), the ^{1}H-NMR spectra observed by Ozaki et al. (1977) may have been due to a mixture of intact prodrug and 5-FU in DMSO-d_6.

The UV and IR spectra of **2–6** were consistent with the assigned 1-acyl structure. The sharp, moderately intense IR absorption at about 3300 cm^{-1} may be attributed to a hydrogen bonded NH, possibly between the alkylaminocarbonyl chain and the 2-carbonyl oxygen of the 5-FU ring. Clear TLC could only be obtained for **4–6**; TLC of **2** and **3** exhibited tailing. HPLC of **2–4** showed that essentially one component was present, and that less than 2% of 5-FU was present in solutions of pH 4.0 acetate buffer that were analyzed immediately after being prepared. All of the prodrugs were stable in IPM. They were also stable in the donor phases after 48 h of contact with hydrated mouse skin; only intact prodrug was observed by ^{1}H-NMR analysis.

Solubilities and partition coefficients

The solubilities of the prodrugs in IPM (S_{IPM}), partition coefficient values between IPM and pH 4.0 buffer (K), direct and estimated pH 4.0 buffer solubilities (S_{H_2O}), and literature values for S_{IPM} and S_{H_2O} have been discussed in a previous paper (Beall et al., 1993). However, the values are reproduced here (Table 2) for convenience. The solubilities generated there agree well with those in the literature except for a few discrepancies which have already been discussed (Beall et al., 1993). The π values for the CH_2 group are consistent with π values for CH_2 from other series of *N*-acyl derivatives (Beall et al., 1993)

TABLE 2

Solubilities in IPM (S_{IPM}) and pH 4.0 buffer (S_{H_2O}) and partition coefficient values (K) between IPM and pH 4.0 buffer

Compound	S_{IPM} [a]	S_{IPM} [a,b]	K	S_{H_2O} [a,c]	Direct S_{H_2O} [a,d]		
1	0.0064	0.010	0.00058	–	–	11.7 [b]	11.1 [d]
2	0.056 (0.039)	–	0.081	0.69	0.65	–	0.62 [d]
3	0.56 (0.36)	–	0.36	1.56	1.55	–	1.50 [d]
4	2.67 (1.61)	–	1.39	1.93	2.03	–	–
5	5.63 (3.20)	8.72 (4.96)	4.79	1.17	–	1.10 [b]	0.82 [d]
6	13.36 (6.09)	13.80 (6.29)	84.8	0.16	–	0.0086 [b]	–

[a] Solubility in mg/ml (equivalent mg of 5-FU).
[b] Sasaki et al. (1990).
[c] S_{H_2O} calculated from S_{IPM}/K.
[d] Buur and Bundgaard (1985).

except for the value generated from the log K of **6**. However, if the literature value of K for **6** (Sasaki et al., 1990) is used, that π value for the CH_2 group is consistent with those from the other *N*-acyl series. Thus, the solubilities and K values are consistent with an homologous series of 1-alkylaminocarbonyl-5-FU prodrugs.

It is important to note that the K values for these 1-alkylaminocarbonyl-5-FU prodrugs are only a little less than (about one-half) those for the first four members of the 1-alkylcarbonyl-5-FU series (Beall, 1991; Beall et al., 1993) and are even a little greater than (two to five times) those for the comparable 1-alkyloxycarbonyl series (Beall, 1991; Beall et al., 1993). However, the water solubilities for the first two members of this series are 7–25-times less than the corresponding members of other series and their IPM solubilities are 5–12-times less than the corresponding members of the other series. This decrease in general solubility compared to the other 1-acyl derivatives is not reflected in the K values and illustrates the danger of using K values to estimate differences in lipophilicity or hydrophilicity, especially between two different types of homologous series. Apparently, the reason that the solubilities of the members of this series are generally low is because formation of this type of derivative does not result in a reduction in the total number of N-H groups. The alkylaminocarbonyl type of prodrug merely trades one N-H for another and does not reduce the overall hydrogen bonding potential, especially for the first few members of the series. Thus, the crystal lattice energy remains high and the observed endotherms in the DSC occur at higher than expected temperatures. However, as the alkyl chain gets longer in this series, and in the other series as well, the alkyl group, as opposed to the type of carbonyl group (N-(C=O)-C, N-(C=O)-O, or N-(C=O)-N), begins to dominate the solubility characteristics and the trends in the differences between this series and the other series become less distinct.

Diffusion cell experiments

The results from the diffusion cell experiments are given in Table 3, along with the literature results from Sasaki et al. (1990) for two of the same prodrugs, but using shaved rat skin instead of hairless mouse skin. Although there are differences in the relative performances of the two prodrugs that are common to this study and the previous literature, the trends in the solubilities of the members of the series versus their ability to deliver 5-FU through skin are essentially identical. The literature results show that in each comparison the more water soluble member and not the more lipid soluble one was the more efficient member at delivering 5-FU through the skin. The same result was observed in this study

TABLE 3

Rates of delivery of 5-FU by 1-alkylaminocarbonyl-5-FU prodrug (J_i) and rates of delivery of theophylline (J_j) through hairless mouse skin, log permeability coefficients (log P_i), and amount of 5-FU retained in skin (C_s)

Compound	δ [a]	J_i (±SD) [b]	J_i [b,c]	C_s (±SD) [d]	J_j (±SD) [b,e]	Log P_i [f]
1	15.0	0.031 (0.012)	0.0043	0.48 (0.11)	0.215 (0.042)	0.68 (−0.37)
2	14.45	0.027 (0.0094)	–	0.71 (0.17)	0.313 (0.045)	−0.16
3	13.82	0.078 (0.018)	–	0.45 (0.13)	0.196 (0.0065)	−0.66
4	13.31	0.097 (0.018)	–	0.65 (0.093)	0.330 (0.083)	−1.22
5	12.94	0.067 (0.014)	0.016	0.38 (0.13)	0.173 (0.017)	−1.68 (−2.49)
6	11.81	0.0078 (0.00037)	0.0057	0.32 (0.042)	0.171 (0.017)	−2.89 (−3.04)

[a] Units: $(cal/cm^3)^{1/2}$.
[b] Units: mg/cm^2 per h.
[c] Sasaki et al. (1990).
[d] Units: mg.
[e] The application of theophylline/PG to hairless mouse skins in diffusion cell experiments without pretreatment with 5-FU prodrug/IPM, but after brief washing with methanol, gave $J_j = 0.0024$ mg/cm^2 per h (Sloan et al., 1986).
[f] Units: cm/h from J_i/S_{IPM} (generated from literature values: Sasaki et al., 1990).

but the trend is more compelling here than in Sasaki et al. (1990) because of the larger number of prodrugs evaluated, regardless of the fact that there are no significant differences between the rates of delivery of 5-FU by **3**, **4** or **5**. The rate of delivering 5-FU through the skin increases in proceeding from **2** to **3** to **4** as the pH 4.0 buffer solubility increases. Prodrug **5** is more soluble in pH 4.0 buffer than **2** but less soluble than **3** and the value for the rate at which **5** delivers 5-FU through the skin falls between that for **2** and **3**. Prodrug **6** is the least soluble in buffer and it is the least efficient member of the series at delivering 5-FU through skin. In should also be noted that, although all of the prodrugs are more soluble in IPM than 5-FU (6–1000-times), there is absolutely no correlation between lipid solubility and relative ability to deliver 5-FU through skin. Prodrug **2**, which is the least buffer soluble of the shorter alkyl chain prodrugs and is the least soluble in IPM of all the prodrugs, still is significantly more efficient at delivering 5-FU than the most lipid soluble (S_{IPM}), longer alkyl chain prodrug, **6**.

In a separate experiment in which all five prodrugs from this study were evaluated at the same time, the same rank order of rate of delivery of 5-FU was observed: **4** > **3** > **5** > **2** > **6**. In that same experiment, the amount of intact prodrug that was delivered through the skin was measured by HPLC. Over a 3 h period ending at the 24 h sample, 10% of intact **2**, 10% of intact **3** and 6% of intact **4** were detected in the receptor phases. In comparable experiments, Sasaki et al., (1990) observed about 5% of intact **5** and 1% of intact **6** in the receptor phases of their diffusion cell experiments.

There do not appear to be any trends in the amounts of 5-FU leached from the skin in the 24 h after the donor phases had been removed from the skins (C_s), vs rates of delivery of 5-FU through the skins (J_i). Regardless, the amounts of 5-FU leached from the skins after their treatments with the prodrugs/IPM combinations are generally not significantly larger than after treatment with 5-FU/IPM. Taking C_s values as a relative measure of dermal delivery, the 1-alkylaminocarbonyl prodrugs offer no apparent advantage over 5-FU itself at delivering 5-FU into the skin. In similar experiments, Sasaki et al. (1990) found that all of the prodrugs were somewhat better than 5-FU at causing accumulation of 5-FU and intact prodrug in the skin. However, this accumulation was only significantly greater using **5** which was also the more efficient prodrug with which to deliver 5-FU through skin in their study. That result is consistent with comparisons between C_s and J_i observed for other *N*-acyl prodrugs of 5-FU (Beall, 1991): the greater the value for J_i the greater the value for C_s.

Similarly, there are no apparent trends in rates of delivery of theophylline from propylene glycol (J_j) vs J_i. The J_j values represent a measure of how much damage was done to the skins by the initial application of the prodrugs/IPM relative to an application of 5-FU/IPM. According to that criterion the damage done by **4**, which gave the highest value for J_i, was no greater than that done by **2**, which gave the next to lowest value for J_i. In addition, there were no differences among the damages caused by **1**, **3**, **5** and **6**; and the values of J_j for **2** and **4** are not significantly higher than that for **1**.

The solubility parameters calculated for the prodrugs (δ_i) are also given in Table 3 along with their respective log permeability coefficients calculated from $P_i = J_i/S_{IPM}$ (Waranis and Sloan 1987, 1988). Results for homologous series of prodrugs have shown that as the members of the series became more lipophilic (decreasing value for δ_i) they became less effective at delivering the parent drug. A plot of δ_i vs log P_i is given in Fig. 1 along with a plot of δ_i versus log P_i generated from the literature values (Sasaki et al., 1990). A value for the 1-hexylaminocarbonyl-5-FU derivative is also included in the plot using the literature data. The literature data was generated using shaved rat skin, so quantitatively it is quite different from the present data where hairless mouse skin was used. However, qualitatively the plots are very nearly parallel. Thus, the expectation is that, if Sasaki et al. (1990) had evaluated the shorter alkyl chain prodrugs, they would have obtained qualitatively the same results as obtained here. Also, since both plots show the same linear relationship between δ_i and P_i as had been previously observed (Waranis and Sloan

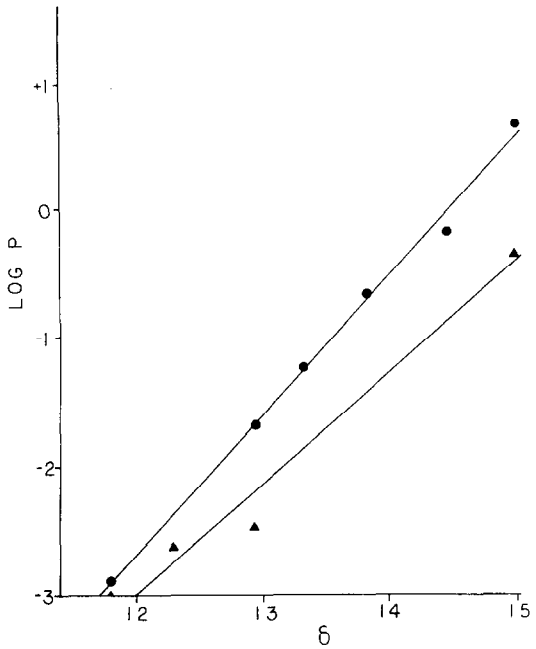


Fig. 1. Plots of solubility parameters for the 1-alkylaminocarbonyl-5-FU prodrugs (δ) vs their experimental log permeability coefficients (log P) for the delivery of total 5-FU through hairless mouse skin (●), and vs literature values for log P (▲).

1987, 1988; Saab et al., 1990), this suggests that (a) the alkylaminocarbonyl-5-FU prodrugs behave in the same way as all the other series of prodrugs that have been examined in diffusion cell experiments, and that (b) increased lipophilicity in an homologous series of prodrugs does not necessarily result in increased delivery.

Conclusion

These 1-alkylaminocarbonyl-5-FU derivatives represent an homologous series of *N*-acyl type prodrugs which exhibit significantly higher lipid solubility than their parent drug (6–1000-times), yet are incapable of enhancing the transdermal delivery of 5-FU by more than 3-times. Although the 1-alkylaminocarbonyl-5-FU derivatives are thermally and hydrolytically unstable, their poor performance is not due to their decomposition in the donor phases of the diffusion cell experiments. ^{1}H-NMR analyses of the donor phases immediately after those experiments were completed showed that the prodrugs were intact in the donor phases. Also, the partition coefficient values exhibited by the members of this series are comparable to the partition coefficient values exhibited by the members of other series of different types of *N*-acyl prodrugs of 5-FU which were much more efficient at enhancing the transdermal delivery of 5-FU (Beall, 1991). Instead, the poor performance of the 1-alkylaminocarbonyl-5-FU prodrugs appears to be due to the fact that at least the first few members of this series of *N*-acyl prodrugs exhibit significantly lower water (buffer) solubilities than do the corresponding members of those other series. In fact, the most buffer soluble member of this series, **4**, is only one-tenth as soluble as 5-FU, whereas several members of each of the other series are at least as soluble as 5-FU in buffer and some are 1.3–2-times more soluble (Beall, 1991).

This low water (buffer) solubility of the 1-alkylaminocarbonyl-5-FU prodrugs appears to be due to the fact that the number of hydrogen bonding donor groups is not reduced for this type of *N*-acyl derivative. One N-H group is exchanged for another, whereas for the other types of *N*-acyl derivatives an N-H group is masked by the *N*-acyl promoiety and the promoiety does not contain an amide-like N-H group. These results for the 1-alkylaminocarbonyl-5-FU prodrugs fit the previously observed requirement that the promoiety introduce enhanced biphasic solubility into the prodrug in order to significantly enhance the transdermal delivery of the parent drug by that type of promoiety (Sloan, 1989). Thus, the 1-alkylaminocarbonyl type derivatives are sufficiently soluble in lipids but not in water.

The relative abilities of members of this series of 1-alkylaminocarbonyl type prodrugs to enhance transdermal delivery also fits the previously observed trends (Sloan, 1989). The most water soluble member of an homologous series of more lipid soluble prodrugs will be the member which will be most efficient at enhancing transdermal delivery. In this series it is the third member of the series, **4**. The previous evaluation of the abilities of this type of *N*-acyl derivatives to enhance transdermal delivery of 5-FU (Sasaki

et al., 1990) did not even evaluate **4**, or for that matter **3**, which happens to be the next most water soluble and next most efficient prodrug of 5-FU in the series. This type of omission in the literature is not unusual (Mollgaard et al., 1982; Sloan et al., 1982). The basis for such omissions is the concept that increased lipophilicity or partition coefficient values is a sufficient criterion for identifying the members of a series of prodrugs to evaluate. The alkylaminocarbonyl-5-FU series then, represents another example of the failure of that concept to predict the optimum member of such series.

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