Pharmaceutical Research, Vol. 4, No. 4, 1987

Report

N-Mannich-Base Prodrugs of 5-Iodo-2′-deoxycytidine as Topical Delivery Enhancers

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Received October 22, 1986; accepted March 10, 1987

Two Mannich-base prodrugs of 5-iodo-2′-deoxycytidine (5-IDC) have been synthesized. The prodrugs exhibit increased lipid solubility compared to 5-IDC and rapidly revert to 5-IDC in buffer. One of the prodrugs delivered about twice as much 5-IDC from isopropyl myristate (IPM) through hairless mouse skin in diffusion-cell experiments as did 5-IDC from IPM. Subsequent applications of theophylline/propylene glycol onto the diffusion cells to determine the effect of prodrug/IPM, 5-IDC/IPM, or IPM on the resistance of the skins to subsequent applications showed that the prodrug/IPM had no more effect than IPM itself.

KEY WORDS: N-Mannich-base prodrugs; 5-iodo-2′-deoxycytidine; dermal delivery; diffusion cell.

INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is usually manifested in orofacial lesions (cold sores) (1). 5-Iodo-2′-deoxycytidine (5-IDC) has been found to be an effective inhibitor of HSV-1 replication *in vitro* (2). However, 5-IDC is not as effective against the virus *in vivo* (3). This decrease in efficacy *in vivo* could be due to an inability to cross through biological membranes such as the skin.

Many polar heterocyclic compounds such as 5-IDC are characterized by high melting points and poor solubility, which can lead to poor diffusion across biological membranes. One way to overcome this problem is to decrease the melting point of the compound (decreased hydrogen bonding) through the synthesis of prodrugs which tend to exhibit increased thermodynamic activity and solubilities compared to the parent compound (4).

As shown with other polar, heterocyclic compounds—theophylline, 5-fluorouracil, and 5-fluorocytosine—N-Mannich-base prodrugs can increase the lipid solubility of the parent drug and lead to an increase in the delivery of the parent drug through skin (5,6). The N-Mannich bases are formed through the reaction of a NH-acidic group (parent drug) with formaldehyde or paraformaldehyde and a secondary amine.

$$\text{Drug–NH} + \text{H}_2\text{C=O} + \text{HN–R}_2 \rightleftharpoons \text{Drug–N–CH}_2\text{–NR}_2 + \text{H}_2\text{O}$$

The resultant prodrug reverts to the parent compound through chemical hydrolysis (7). Therefore, these N-Mannich bases should be effective as topical prodrugs, where their sensitivity to water should not be a liability. This paper discusses the results of using N-Mannich-base prodrugs to increase the topical delivery of 5-IDC.

MATERIALS AND METHODS

The materials used were the same as those used for 5-fluorocytosine (5-FC) (6) except that high-performance liquid chromatography (HPLC) was used to quantitate 5-IDC and 5-iododeoxyuridine. The HPLC system used was a Beckman Model 110A pump with an Altex Model 210 injector (50-μl sample loop) and a Beckman fixed-wavelength UV detector fitted with a 280-nm filter. A Dupont Zorbax ODS (4.6 × 250-mm) reversed-phase column was used.

Syntheses

Reaction of 5-Iodo-2′-deoxycytidine with Formaldehyde and a Secondary Amine

To 0.33 g (0.004 mol) of 37% aqueous formaldehyde was added 0.004 mol of a secondary amine, and the solution was diluted to 10 ml with tetrahydrofuran. This solution was added to 0.35 g (0.001 mol) of 5-IDC. The solution was stirred for 1 to 2 days, and then it was triturated with ether two times. The oil that resulted was dried in a vacuum drying oven at 37°C overnight to give the desired products.

N⁴-(1″-Piperidinyl)methyl-5-iodo-2′-deoxycytidine Hydrate (Ia). Amorpous solid, 71% yield, IR (KBr) 3430 cm^{-1} (M) (OH), 1660 (S), 1630 (shoulder, M), and 1560 (M) (C=O and C=N); ^{1}H NMR (DMSO-d_6) δ 8.40 and 8.33 (2s, 1, 6-C*H*), 6.97 (t, 1, J = 6 Hz, N*H*, disappears in D_2O), 6.12 (t, 1, J = 6 Hz, anomeric *H*), 5.33–4.97 (m, 2, 3′- and 5′-O*H*, disappears in D_2O), 4.20 (m, 3, NH–CH_2–N and 3′-C*H*), 3.90–3.73 (m, 1, 4′-C*H*), 3.73–3.53 (m, 2, 5′-CH_2), 2.63–2.33 (s, 4, CH_2–N–CH_2), 2.27–2.08 (m, 2, 2′-CH_2),

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0724-8741/87/0800-0317$05.00/0

and 1.67–1.27 (s, 6, CH_2–CH_2); UV (CH_3CN) max 296 nm (ϵ = 5.57 × 10^3 l/mol) shoulder 254 nm (ϵ = 5.25 × 10^3 l/mol), UV (HCl) 313 nm (ϵ = 8.64 × 10^3 l/mol), and UV (NaOH) max 296 nm (ϵ = 5.34 × 10^3 l/mol) shoulder 254 nm (ϵ = 5.21 × 10^3 l/mol).

Anal. Calcd for $C_{15}H_{23}IN_4O_4 \cdot 0.5\ H_2O$: C, 39.23; H, 5.26. Found: C, 39.22; H, 5.11.

N^4-(4"-Morpholinyl)methyl-5-iodo-2'-deoxycytidine Hydrate (IIa). Amorphous, hygroscopic solid, 74% yield, ^{1}H NMR (DMSO-d_6) δ 8.34–8.30 (2s, 1, 6-C*H*), 7.10 (t, 1, *J* = 6 Hz, N*H*, disappears in D_2O), 6.10 (t, 1, *J* = 6 Hz, anomeric *H*), 5.30–4.93 (m, 2, 3'- and 5'-O*H*, disappears in D_2O), 4.40–4.00 (m, 3, 3'-C*H* and NH–CH_2–N, collapses to singlet in D_2O), 4.07–3.40 (m, 6, CH_2–N–CH_2 and 5'-CH_2), 2.63–2.33 (s, 4, CH_2–O–CH_2), and 2.23–1.93 (m, 2, 2'-CH_2); UV (CH_3CN) max 296 nm (ϵ = 5.38 × 10^3 l/mol) shoulder 255 nm (ϵ = 5.39 × 10^3 l/mol), UV (HCl) max 313 nm (ϵ = 7.21 × 10^3 l/mol), and UV (NaOH) max 296 nm (ϵ = 5.30 × 10^3 l/mol) shoulder 255 nm (ϵ = 5.32 × 10^3 l/mol).

Anal. Calcd for $C_{14}H_{21}IN_4O_5 \cdot H_2O$: C, 35.76; H, 4.93; N, 11.91. Found: C, 35.76; H, 5.12; N, 11.45.

Reaction of 5-Iodo-2'-deoxycytidine with Paraformaldehyde and a Secondary Amine

To 0.12 g (0.004 mol) of paraformaldehyde in 20 ml of methylene chloride was added 0.004 mol of a secondary amine and the suspension was stirred overnight. The solution that resulted was concentrated and the residue was diluted with 10 ml tetrahydrofuran. The solution was added to 0.35 g (0.001 mol) of 5-iodo-2'-deoxycytidine and the suspension was stirred overnight. The solution that resulted was concentrated, and the residue was triturated twice with ether and then a mixture of ether and petroleum ether (1:1). The solid was filtered and dried under vacuum with potassium hydroxide pellets as a desiccant to give the desired products.

N^4-(1"-Piperidinyl)methyl-5-iodo-2'-deoxycytidine Hydrate (Ib). Hygroscopic solid, 68% yield, spectral data same as **Ia**, R_f [ether:methanol (10:1)] 0.26.

Anal. Calcd for $C_{15}H_{23}IN_4O_4 \cdot 0.5\ H_2O$: C, 39.23; H, 5.26; N, 12.20. Found: C, 39.24; H, 5.27; N, 12.09.

N^4-(4"-Morpholinyl)methyl-5-iodo-2'-deoxycytidine Hydrate (IIb). Hygroscopic solid, 84% yield, spectral data same as **IIa**, R_f [ether:methanol (10:1)] 0.15.

Measurement of Half-Lives and Rates of Hydrolysis

The hydrolysis of the N-Mannich-base prodrugs in pH 7.1 buffer (0.05 *M*, μ = 0.15) was quantitated by UV spectroscopy at 294 nm, by measuring the appearance of 5-IDC. A plot of log ($A_t - A_\infty$) versus time was linear so first-order reaction kinetics were assumed. Each hydrolysis was performed in triplicate at a constant temperature of 24.0 ± 0.1°C and the half-lives and rates of hydrolysis were obtained using the Guggenheim method (8). Stock solutions of the N-Mannich bases were made in dry acetonitrile, and 0.1 ml of solution was added to 3 ml of buffer in a cuvette. The final concentration in the cuvette was between 10^{-4} and 10^{-5} *M*. The acetonitrile did not affect the pH of the buffer. The pH values of the solutions in cuvettes were checked after the hydrolyses were completed and were found to have remained constant.

Determination of Lipid Solubilities

The lipid solubilities of 5-IDC and its N-Mannich-base prodrugs were determined in trplicate in isopropyl myristate (IPM) at 23 ± 1°C as previously described for 5-FC (6). The concentration of compound in solution was quantitated by UV spectroscopy: 294 nm in methanol for 5-IDC and 296 nm in acetonitrile for the N-Mannich bases. The N-Mannich bases were stable in IPM based on inspection of the ^{1}H NMR spectra of the residues from the solubility determinations.

Diffusion-Cell Experiments

Diffusion-cell experiments were performed in triplicate for each compound tested as previously described for 5-FC (6). In this case, the donor phases were applied as suspensions (0.5 ml, 0.05 *M*) to the skin surface. Samples were taken over a 48-hr period and filtered (0.45-μm membrane filter), then analyzed using the HPLC system described above. A mobile phase of 13% acetonitrile in water at a flow rate of 1.0 ml/min was used to quantitate 5-IDC in the samples. The retention time of 5-IDC under these conditions was 4.6 min. Calibration curves (r = 0.9989, n = 7) of the peak area (mm^2) versus the concentration were used to calculate the concentration of 5-IDC in the samples. In order to detect the formation of 5-IDU, samples removed at 3, 9, and 48 hr were also analyzed by HPLC using a mobile phase of 12% tetrahydrofuran in water. The retention time of 5-IDU was 5.2 min under these conditions.

After the 48-hr application period, the remaining donor phase was removed by washing the skin surface with 20 ml of methanol. Each wash was diluted to 100 ml with methanol and analyzed by UV spectroscopy (294 nm, ϵ = 5.79 × 10^3 l/mol) to determine the amount of 5-IDC remaining in the donor phases. The Mannich bases decompose to 5-IDC in methanol. The receptor phases were then replaced with fresh buffer and the skins were allowed to remain in contact with the buffer for an additional 24 hr. The receptor phase was then sampled and analyzed by HPLC to determine the amount of 5-IDC left in the skin. These last two steps were used to determine the mass balance. The mass balances were approximately 91% of the applied dose (8.83 mg as 5-IDC).

After this last sampling, the receptor phase was again changed. The skin was left in contact with the receptor phase for 30 min, then a 3-ml sample was taken and analyzed by HPLC to ensure that all of the 5-IDC had been removed from the skins. A suspension of theophylline in propylene glycol (Th/PG; 0.5 ml, 0.4 *M*) was then applied to each diffusion cell as previously described for 5-FC (6), and the flux of theophylline was determined over a 48-hr period. Theophylline was quantitated by UV spectroscopy at 270 nm.

For each diffusion-cell experiment the flux of theophylline or 5-IDC was determined as previously described for 5-FC (6). Student's t test (one tailed) was used to test for significant differences between the fluxes (6).

RESULTS AND DISCUSSION

Syntheses and Structure Determination

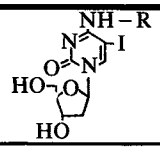
The N-Mannich-base prodrugs synthesized with 5-IDC are novel prodrugs (Table I). Although Mannich-base type adducts with purine and pyrimidine bases have recently been reported by Sloan and Siver (9) and Koch and Sloan (6), this is the first report of the synthesis and characterization of Mannich-base adducts with nucleosides. The prodrugs were made in the same two ways as previously reported for 5-FC (6). Although other derivatives could be synthesized using these procedures, only the piperidinyl and morpholinyl derivatives could be isolated in sufficient purity to give correct elemental analyses and ^{1}H NMR spectra. The N-Mannich bases obtained by either method were identical to each other by spectral and elemental analyses.

In 5-IDC there are two possible sites of N-alkylation—at the 3-position and on the exocyclic amino group (N^4). The reaction of formaldehyde with nucleosides and nucleic acids has been well documented (10–12). McGhee and von Hippel studied the reaction of formaldehyde with cytidine and isolated and characterized the N^4-hydroxymethyl derivative by ^{1}H NMR (10). The ^{1}H NMR spectra of these present derivatives showed only one peak that corresponded to a NH–CH_2–N group. The multiplicity of the CH_2 absorption was masked by the absorption due to the 3'-CH; however, its chemical shift was identical to that of NHCH_2N hydrogen in the ^{1}H NMR spectra of N-Mannich-base derivatives of 5-FC (6). Moreover, the NH absorption was a triplet, which established that the NH group was next to a CH_2 group. This result strongly supports a N^4-substitution site. Also, when the UV absorptions of the prodrugs were compared with those of various monosubstituted cytidines, the UV absorptions more closely resembled those of a N^4-substituted cytidine (13). Therefore, the N-Mannich-base derivatives of 5-IDC were assigned to the N^4-position.

Kinetic and Solubility Data

The hydrolyses of the piperidinyl (**Ib**) and morpholinyl (**IIb**) adducts of 5-IDC were monitored in pH 7.1 buffer at 24°C. The half-life ($t_{1/2}$) of **Ib** was 0.89 ± 0.06 min; the $t_{1/2}$ of **IIb** was 12.8 ± 0.6 min. These values are approximately the same as those previously reported for the respective N-Mannich bases of 5-fluorocytosine (6) and are of the same rank order as reported by Bundgaard and Johansen (7) for N-Mannich bases of amides.

Table I. Solubility of 5-Iodo-2'-deoxycytidine (5-IDC) and Its N-Mannich-Base Prodrugs in Isopropyl Myristate (IPM)



R	mg/ml ± SD (× 10²)	M (× 10⁵)
5-IDC:H	—[a]	
Ia:CH_2–N⬡	2.8 (0.3)*	6.2
Ib:CH_2–N⬡	1.6 (0.5)*	3.6
IIb:CH_2–N⬡O	1.3 (0.8)	2.9

[a] The solubility was so low it could not be detected by HPLC.
* Significantly different from each other ($P > 0.025$).

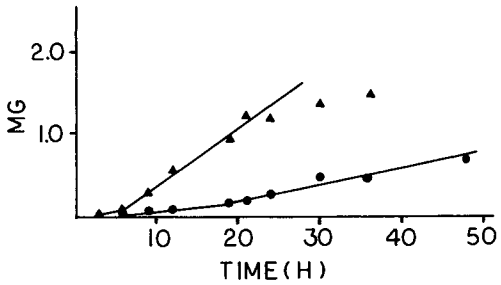


Fig. 1. A plot of the average cumulative milligrams of 5-IDC diffused through hairless mouse skin after treatment with 5-IDC in IPM (●) or **Ia** in IPM (▲).

The N-Mannich-base derivatives showed a significant increase in lipid solubility (i.e., in IPM) compared with that of 5-IDC (Table I). Since these prodrugs are unstable in protic solvents, IPM was also used as the vehicle in which to test these derivatives in the diffusion cells.

Diffusion-Cell Experiments

Hairless mouse skin has been found to be a good approximation for human skin for poorly soluble drugs (14) and was therefore used to examine the delivery of 5-IDC by the piperidinyl adduct (**Ia**) from IPM and of 5-IDC from IPM. **Ia** was chosen for this study since it was the more soluble prodrug. Suspensions of the two compounds (0.05 M, 0.5 ml) were applied in order to keep a constant driving force for

Table II. Data for the Rate of Delivery of 5-Iodo-2'-deoxycytidine (5-IDC) from Isopropyl Myristate (IPM) and by **Ia** from IPM, and the Flux of Theophylline from Propylene Glycol After a Subsequent Application of Theophylline in Propylene Glycol (Th/PG)

Compound/IPM[a]	Flux (mg/cm²/hr) of 5-IDC[b] (±SD × 10³)	Intercept (hr)
5-IDC	5.8 (1.3)	14.0
Ia	13.5 (4.1)	5.3
First application	**Flux (mg/cm²/hr) of Th/PG[c] (±SD × 10³)**	**Intercept (hr)**
IPM[d]	184 (23)	1.6
5-IDC/IPM	246 (17)	0.6
Ia/IPM	192 (38)[e]	1.0
Control[f]	2.4 (0.4)	4.7

[a] Applied as 0.05 M suspensions.
[b] Steady-state flux reported as milligrams of 5-IDC.
[c] Applied as 0.4 M suspension.
[d] See Ref. 15.
[e] Not significantly different from the flux after the first application of IPM.
[f] No pretreatment with a vehicle but with a methanol wash before the application of Th/PG (6).

diffusion (15). Since 5-IDC can be deaminated to 5-iodo-2′-deoxyuridine (5-IDU) (which is also an active antiviral agent) by mammalian deaminases, it was necessary to determine if this conversion had occurred in the diffusion-cell system (16). No 5-IDU was detected in the receptor phases of the diffusion cells by HPLC.

The steady-state delivery of 5-IDC by **Ia** from IPM was approximately twice that of 5-IDC from IPM (Fig. 1, Table II). The steady-state rate of delivery of 5-IDC had a faster onset for the prodrug as well. The falloff in the rate of delivery of 5-IDC after about 20 hr was due to decomposition of the prodrug to 5-IDC as determined by ^{1}H NMR spectroscopy.

The flux of theophylline from PG after application of the prodrug in IPM was not significantly different from that after application of IPM alone ($P > 0.10$). Therefore, the increased delivery of 5-IDC by the prodrug must be due to the improved physicochemical properties of the prodrug and not due to some effect of the prodrug or its decomposition product(s) on the resistance of the skin to permeation.

In conclusion, the N-Mannich-base prodrugs are potentially useful topical agents. When used dermally, the lability of these compounds in water should not be a problem. These prodrugs are chemically hydrolyzed and their degree of stability and solubility can be modified by varying the secondary amine used in the syntheses (6). Although these products liberate formaldehyde upon hydrolysis, the Cosmetic Ingredient Review (Cosmetic, Toiletry and Fragrance Association) has set the maximum allowable level of formaldehyde in dermatological formulations at 0.2% (17). Upon complete hydrolysis of the prodrug at a concentration of 0.05 *M*, only 0.15% formaldehyde would be released, which is well below the allowable limit.

REFERENCES

1. B. D. Davis, R. Dulbecco, H. N. Eisen, and H. S. Ginsberg. *Microbiology*, 3rd ed., Harper and Row, Hagerstown, Md., 1980, pp. 1061–1076.
2. I. Shildkraut, G. M. Cooper, and S. Greer. *Mol. Pharmacol.* **11**:153–158 (1975).
3. E. Declerq, J. Descamps, G. Verhust, R. T. Walker, A. S. Jones, P. F. Torrence, and D. Shugar. *J. Infect. Dis.* **141**:563–574 (1980).
4. A. J. Repta, B. J. Rawson, R. D. Shaffer, K. B. Sloan, N. Bodor, and T. Higuchi. *J. Pharm. Sci.* **64**:392–396 (1975).
5. K. B. Sloan, S. A. M. Koch, and K. G. Siver. *Int. J. Pharm.* **21**:251–264 (1984).
6. S. A. M. Koch and K. B. Sloan. *Int. J. Pharm.* **35**:243–252 (1987).
7. H. Bundgaard and M. Johansen. *J. Pharm. Sci.* **69**:44–46 (1980).
8. W. P. Jencks. *Catalysis in Chemistry and Enzymology*, McGraw–Hill, New York, 1969, pp. 562–564.
9. K. B. Sloan and K. G. Siver. *Tetrahedron* **40**:3997–4001 (1984).
10. J. D. McGhee and P. H. von Hippel. *Biochemistry* **14**:1281–1296 (1975).
11. J. D. McGhee and P. H. von Hippel. *Biochemistry* **14**:1297–1303 (1975).
12. P. K. Bridson, J. Jiricny, O. Kemal, and C. B. Reese. *J. Chem. Soc. Chem. Comm.* **1980**:208–209 (1980).
13. L. Sun and B. Singer. *Biochemistry* **13**:1905–1912 (1974).
14. R. B. Stoughton. In H. I. Maibach (ed.), *Animal Models in Dermatology*, Churchill Livingstone, New York, 1975, pp. 121–137.
15. K. B. Sloan, S. A. M. Koch, K. G. Siver, and F. P. Flowers. *J. Invest. Dermatol.* **87**:244–252 (1986).
16. M. A. Creasey. *J. Biol. Chem.* **238**:1772–1776 (1963).
17. R. L. Elder. *J. Am. Acad. Dermatol.* **11**:1168–1174 (1984).