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Research Article

Selection of a Derivative of the Antiviral Agent 9-[(1,3-Dihydroxy-2-propoxy)-methyl]Guanine (DHPG) with Improved Oral Absorption

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Various diesters of 9-[(1,3-dihydroxy-2-propoxy)-methyl]guanine (DHPG) were screened in order to identify a derivative with improved oral absorption. The solubilities and dissolution rates decreased with increasing chain length and branching of the ester group. However, the dipropionate ester showed an anomalously faster dissolution rate. The rates of hydrolysis to DHPG in the presence of intestinal homogenates were found to increase with increasing carbon number for the straight-chain alkyl esters and decreased with branching. The shorter-chain alkyl esters were relatively more stable in intestinal homogenates than in liver homogenates. Therefore they may have a better membrane permeability than DHPG due to their intact ester group. The hydrolysis rates in human blood increased with increasing carbon number for the straight-chain alkyl esters. The dipropionate ester appeared to be the most promising derivative because of its rapid dissolution rate, slower hydrolysis in the intestine, and rapid conversion to DHPG in liver and blood.

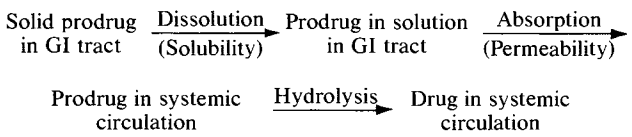
KEY WORDS: 9-[(1,3-dihydroxy-2-propoxy)-methyl]guanine (DHPG); oral absorption; prodrug; derivative.

INTRODUCTION

The acyclic nucleoside analogue 9-[1,3-dihydroxy-2-propoxy)-methyl]guanine (DHPG; I) is a selective antiviral agent which has shown activity against herpes simplex viruses I and II, cytomegalovirus, varicella zoster, and Epstein–Barr virus (1,2). DHPG is structurally similar to acyclovir (9-[(2-hydroxyethoxy)-methyl]guanine), which is currently available for parenteral, oral, and topical treatment of herpes simplex virus under the trade name of Zovirax (3,4). The absorption of acyclovir upon its oral administration has been reported (5) to be incomplete and species dependent (3.7–75% at a 25-mg/kg dose). The apparent bioavailability in man from a 200-mg dose was 20% (6). This limited absorption was believed to be due to the poor water and lipid solubility of acyclovir. Since DHPG has an additional hydroxy group, it is more polar than acyclovir and is expected to pass through the intestinal membrane less readily. Therefore, it was decided to identify potential derivatives of DHPG with improved oral absorption.

The rationale and the results of the *in vitro* screening studies are described in this paper. The studies described in the present work are by no means unique; however, they do illustrate the usefulness and importance of *in vitro* studies emphasized in the literature for the selection of a prodrug (7–9). Prodrug approaches to improve delivery characteristics of the antiviral agents acyclovir (10) and ara-A (11–12) have also been attempted.

The major steps involved in the absorption of a solid prodrug are shown in Scheme I.



Scheme I

The first step in the absorption process is the dissolution of the prodrug in the GI tract fluid. Since the rate of dissolution of the solid is a function of its solubility, the solubility becomes an important factor in this process. Once in solution the prodrug should be reasonably stable toward enzymatic and chemical hydrolysis, since the intact diester is expected to have superior permeability. After absorption the prodrug must hydrolyze in blood or during passage through liver to generate DHPG, which is the active species. Thus the *in vitro* screening studies consisted of evaluation of solubilities, dissolution rates in 0.1 *N* HCl, octanol/water partition coefficients, and stabilities in buffer, in plasma and in the presence of intestinal and liver homogenates.

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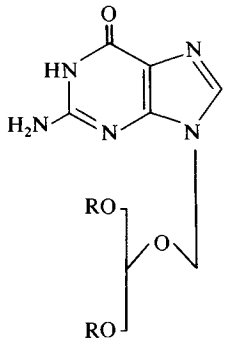
Diesters II–VIII, the synthesis and biological activities of which have been described by Verheyden and Martin (13), were studied. The short-chain alkyl esters were included because of their successful use as nucleoside derivatives (14,15) with improved oral absorption. Dihemisuccinate (VII) was selected as a compound with superior water solubility. The dimethyoxycarbonate derivative (VIII) contains a carbonate group.



I.	DHPG	R = H
II.	Diacetate	R = CH_3CO
III.	Dipropionate	R = CH_3CH_2CO
IV.	Dibutyrate	R = $CH_3(CH_2)_2CO$
V.	Dipivaloate	R = $(CH_3)_3CCO$
VI.	Dihexanoate	R = $CH_3(CH_2)_4CO$
VII.	Dihemisuccinate	R = $HOOCCH_2CH_2CO$
VIII.	Dimethoxycarbonyl	R = CH_3OCO

MATERIALS AND METHODS

Materials. DHPG and the diesters were obtained from the Institute of Bioorganic Chemistry (Syntex Research). All other materials used were commercially available and used without further purification.

Analytical Methods. All samples other than plasma were analyzed simultaneously for diester, monoester, and DHPG using a dual-column high-performance liquid chromatographic (HPLC) method described previously (16). A 10-μm cation-exchange column, 25 cm × 4.6 mm (Partisil 10 SCX, Whatman), was connected in series with a 10-μm reversed-phase column, 5 cm × 4.6 mm (Partisil 10 ODS-3, Alltech). The mobile phase was composed of methanol and 0.001–0.005 *M* ammonium phosphate, pH 2.5. Typical chromatograms are shown in Fig. 1. A reversed-phase HPLC method was utilized to assay plasma samples for DHPG. A 5-μm reversed-phase column, 25 cm × 4.6 mm (Spherisorb ODS, Regis), with a mobile phase of water containing 0.1% phosphoric acid was used.

Preparation of Tissue Homogenates. Intestine and liver were obtained from male Sprague–Dawley-derived rats and male rhesus monkeys. After excision the tissues were immediately washed by perfusion with ice-cold buffered saline (0.15 *M* NaCl with 0.05 *M* potassium phosphate, pH 7.4). The tissues were homogenized using a Potter–Elvehjen glass tube with a Teflon pestle in buffered saline at 4°C. The final homogenate concentration was 10% (w/v). The homogenates were then divided into individual vials and stored at −20°C until used.

In Vitro *Incubation with Homogenate.* Incubations were carried out in stoppered 50-ml Erlenmeyer flasks at 37°C with shaking under an atmosphere of oxygen/carbon dioxide (95/5). The incubation mixtures consisted of 2 ml of methanolic diester solution, 8 ml of buffered saline, and 2 ml of tissue homogenate. The final substrate and homogenate concentrations were 0.118 m*M* and 1.6% (w/v), respectively, unless indicated. At appropriate intervals, 1-ml aliquots of the incubation mixture were mixed with 4 ml of ice-cold acetonitrile to precipitate the protein. These solutions were then centrifuged at 2000 rpm for 10 min and the supernatants were injected directly onto a HPLC.

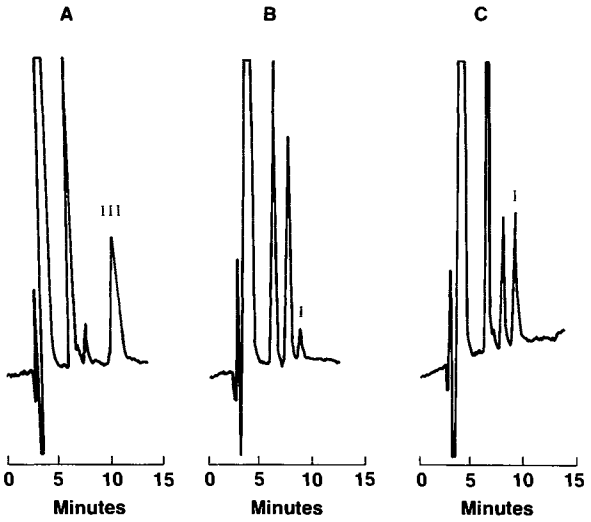


Fig. 1. Chromatograms of the incubation mixture of the dipropionate ester (III) of DHPG and rat liver homogenate at the initial time (A) and after 30 min (B) and 4 hr (C) of incubation at 37°C.

In Vitro *Incubations with Plasma.* Fifty microliters of a 30 μg/ml solution of the diester was pipetted into a tube. One milliliter of plasma was added to the tube. The tubes were vortexed for 5 sec and then placed in a water bath maintained at 37°C. After various incubation times, a trace amount of ^{3}H-DHPG was added to monitor recovery, and acetonitrile was added to the plasma in order to terminate the hydrolysis. A separate tube was used for each time point in the study. The samples were centrifuged and the supernatant was evaporated to dryness under a stream of nitrogen. The residue was dissolved in water and the solution applied to a reversed-phase C18 disposable extraction column (J. T. Baker). The column was washed sequentially with 1 bed vol of water and with 1 bed vol of 10% methanol in water. The analyte and the ^{3}H-DHPG were eluted with 10% methanol. A portion of the eluent containing the analyte and ^{3}H-DHPG were subjected to liquid scintillation counting to monitor the recovery. An equal portion was injected onto the HPLC for quantification of DHPG by an external standard method.

Solubility Measurements. An excess of the compound was suspended in 1–2 ml of the 0.04 *M* acetate buffer, pH 5.0. These suspensions were equilibrated for 2 days in a constant-temperature bath (25 ± 0.5°C) equipped with a sample holder that rotated at 30 rpm. Each sample was filtered through a 0.8-μm Metrical G.A. filter membrane, diluted with the mobile phase, and assayed by HPLC.

Intrinsic Dissolution Rate Determination. The intrinsic dissolution rates were determined using the rotating disk method of Wood *et al.* (17) described previously (18). The

dissolution medium was 300 ml of 0.1 *N* HCl and the rotation speed was 200 rpm. Absorbance changes at 254 nm were continuously recorded.

Partition Coefficient Measurements. Water and *n*-octanol presaturated with each other were used. The compounds were either dissolved in water (I–III, VII, VIII) or *n*-octanol (IV–VI). Typically 7 ml of a 1.6×10^{-3} *M* solution of the compound was shaken with 7 ml of the other phase for 24 hr at 25°C in a rotating bath. The samples were centrifuged at 2000 rpm for 30 min, and an aliquot from the aqueous layer was removed, diluted with the mobile phase, and injected onto a HPLC.

RESULTS AND DISCUSSION

Solubilities, Intrinsic Dissolution Rates, and Partition Coefficients. The solubilities and intrinsic dissolution rates (Table I) decreased with increasing number of carbon atoms for the alkyl esters except the dipropionate ester (III), which showed an anomalously higher solubility and intrinsic dissolution rate. This is consistent with its lower melting point and reflects a lowering of the crystal lattice energy as a result of the reduction in symmetry and compactness of the molecule (19). The possibility of polymorphism was ruled out, as crystals obtained from various solvents gave the same solubility and melting point. Similar behavior was reported for the 3-pentanoyloxymethyl prodrug of phenytoin (20) and the pentyloxycarbonyl prodrug of mitomycine (21). It is interesting to note that the dipentanoate ester of DHPG did not have an anomalous melting point. The dipropionate ester may have a unique side-chain length, resulting in anomalously low lattice energy, low melting point, and high aqueous solubility.

The dihemisuccinate ester (VII) had the highest solubility at physiological pH of all compounds studied. Based on solubility and intrinsic dissolution rate data, diesters III and VII appear promising. The partition coefficients are also included in Table I and tend to increase with increases in chain length. The log partition coefficients of diesters V and VI are about 2 or greater, which make them desirable for efficient oral absorption (22). However, they have the lowest aqueous solubilities, indicating dissolution limited absorption. The log partition coefficient of diester III is 12 times more positive than that of DHPG. This reflects a 10- to 12-fold greater partitioning of III in the lipophilic phase, and this property may improve intestinal permeability (22).

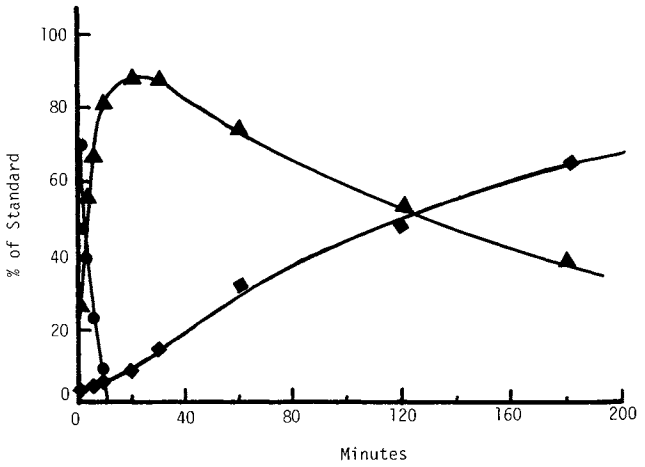


Fig. 2. Concentration versus time profiles for various species during the *in vitro* enzymatic hydrolysis of the dibutyrate ester (IV) in the presence of 1.6% (w/v) rat intestinal homogenate at 37°C. (●) Dibutyrate; (▲) monobutyrate; (◆) DHPG.

Stability in Intestinal Homogenates. DHPG was found to be stable in the rat intestinal homogenate. The excellent stability observed for DHPG indicates that it is the end product of diester hydrolysis in the intestinal homogenate. Similar observations were made in rat liver and monkey intestinal and liver homogenates. The concentrations of various species formed during the enzymatic hydrolysis of diester IV in the presence of rat intestinal homogenate are shown in Fig. 2. Conversion of dibutyrate to monobutyrate was rapid and essentially complete within 10 min. The concentration of monobutyrate reached a maximum at about 20 min, after which there was a gradual decrease due to the formation of DHPG. These reactions could be represented by the following equation:

$$\text{DHPG-(OR)}_2 \xrightarrow{k_1} \text{DHPG-OR} \xrightarrow{k_2} \text{DHPG} \qquad (1)$$

where k_1 and k_2 are the first-order rate constants for the hydrolysis of diester, DHPG-$(OR)_2$, and monoester, DHPG-

Table I. Solubilities, Intrinsic Dissolution Rates, and Partition Coefficients of DHPG (I) and Its Various Diesters

Compound	Melting point (°C)[a]	Solubility (mg/ml)	Intrinsic dissolution rate (mg/min/cm²)	Partition coefficient	Log partition coefficient
I	245–250	3.60	5.57	0.022	−1.66
II	237–239	0.58	0.84	0.113	−0.95
III	191–193	2.80	1.99	0.731	−0.14
IV	199–201	0.14	0.18	6.74	0.83
V	230–232	0.007	0.042	50.90	1.71
VI	179–181	—[b]	—	—[c]	—
VII	165–170	47.3	—	—[d]	—
VIII	178–179	0.143	—	0.133	−0.88

[a] From Ref. 13.
[b] Undetectable amount.
[c] Essentially all in the *n*-octanol phase.
[d] Essentially all the aqueous phase at pH 6.0.

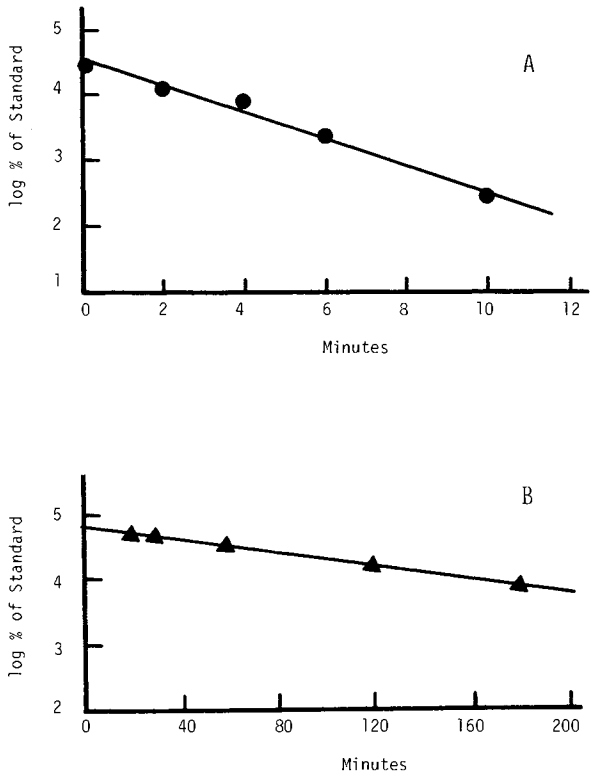


Fig. 3. First-order plots for the *in vitro* hydrolysis of the dibutyrate (A) and monobutyrate (B) esters in the presence of 1.6% (w/v) rat intestinal homogenate at 37°C.

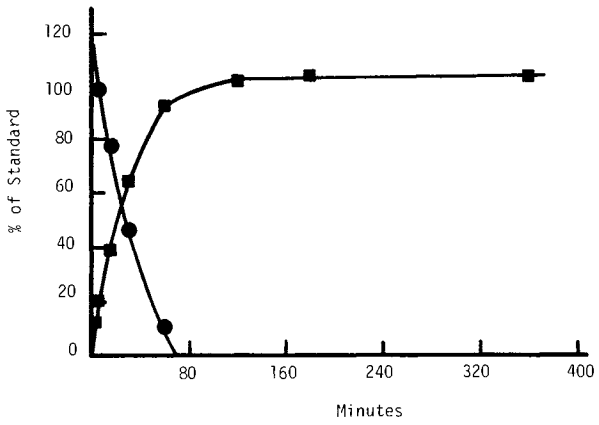


Fig. 4. Concentration versus time profile for various species during the *in vitro* enzymatic hydrolysis of dihexanoate ester (VI) in the presence of 1.6% (w/v) rat intestinal homogenate at 37°C. (●) Monohexanoate ester; (■) DHPG.

OR, respectively. The rate constant k_1 was obtained from the slope of the plot of ln % DHPG-$(OR)_2$ versus time and k_2 was obtained either by plotting ln (% DHPG at t_∞ − % DHPG at t) versus time or from the linear portion of the first-order plot for the disappearance of monoester. These plots for diester IV are shown in Fig. 3 and the rate constants and half-lives are listed in Table II.

The hydrolysis of the dihexanoate ester was very rapid and essentially complete conversion to monoester occurred within 1 min (Fig. 4). On the other hand, the diacetate and dihemisuccinate esters were found to be quite stable in the presence of intestinal homogenates. The hydrolysis of dipropionate and dimethoxycarbonyl was very slow. Figure 5 shows the time course for the hydrolysis of the dipivaloate ester. Although the rate of hydrolysis to monoester was reasonably fast, the monopivaloate ester appeared to convert to DHPG very slowly. Only 4.5% DHPG was formed after 6 hr of incubation. Each diester was also incubated in buffer without homogenate as a control and they all showed excellent stability at pH 7.4 and 37°C. Therefore these rate constants represent enzyme-mediated hydrolysis.

Similar trends were observed in the presence of monkey intestinal homogenates. Only the slow reaction, formation of DHPG, was followed. The rates of enzymatic hydrolysis in the presence of intestinal homogenates could be arranged in the order dihexanoate > dibutyrate > dipropionate > diacetate > dipivaloate, dihemisuccinate, dimethoxycarbonyl. Figure 6 shows the relationship between the alkyl chain length of the ester and the rate of enzymatic hydrolysis. As was found with other nucleoside esters (23,24), the rate increases with increases in chain length. The slow hydrolysis of dihemisuccinate ester is consistent with the resistance of anionic esters to esterase activity (25).

Stability in Liver Homogenates. The hydrolysis pattern of the diesters during incubation with rat and monkey liver

Table II. Half-Lives and Rate Constants for the Enzymatic Hydrolysis of Various DHPG Diesters at 37°C in Rat and Monkey Intestine Homogenates

	Rat homogenates (1.6%, w/v)				Monkey homogenates (1.6%, w/v), Monoester to DHPG	
	Diester to monoester		Monoester to DHPG			
Compound	k_1 (min^{-1})	$t_{1/2}$ (min)	k_2 (min^{-1})	$t_{1/2}$ (min)	k_2 (min^{-1})	$t_{1/2}$ (min)
II	—[a]		—[a]		0.00028	2487
III	0.0055	126	0.00055	1260	0.0015	463
IV	0.203	3	0.005	130	0.006	115
V	0.015	72	—[b]		—[a]	
VI	—[c]	<1	0.036	18	0.053	13
VII	—[a]		—[a]		—[a]	
VIII	0.0027	257	—[a]		—[a]	

[a] No significant hydrolysis up to 3 hr.
[b] Very slow reaction, only 4.5% DHPG formed after 6 hr of incubation.
[c] Very fast reaction, essentially complete within 1 min.

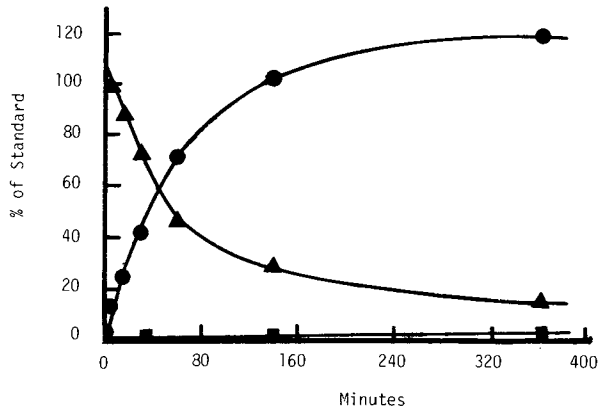


Fig. 5. Concentration versus time profile for various species during the *in vitro* enzymatic hydrolysis of the dipivaloate ester (V) in the presence of 1.6% (w/v) intestine homogenate at 37°C. (▲) Dipivaloate; (●) monopivaloate; (■) DHPG.

homogenates was the same as found previously [Eq. (1)]. The first-order rate constants and half-lives for the hydrolysis of the diesters and monoesters are presented in Table III. As in intestinal homogenates, DHPG was stable in liver homogenates. The hydrolysis of diester to monoester (k_1) was similar to that in intestinal homogenates. However, hydrolysis of the monoesters showed some interesting differences. Plots of ln k_2 versus chain length for the straight-chain alkyl esters are presented in Fig. 6. The shorter-chain esters, II and III, appear to be more rapidly hydrolyzed compared to their hydrolysis in the intestinal homogenates. This is inconsistent with the reported chain-length reactivity relationship of liver esterases (26) and indicates that the specificity of the liver homogenate for short-chain esters of DHPG is different from that seen for the intestinal homogenate. The hydrolysis of the dipivaloate ester was rapid, however, further hydrolysis to DHPG was very slow.

Stability in Human Plasma. Hydrolysis in human plasma was followed by quantitation of DHPG formed after 2 hr of incubation (Table IV). Hydrolysis of dibutyrate and dihexanoate ester was essentially complete in 2 hr. The diacetate and dipropionate esters were substantially hydrolyzed. No DHPG was formed from the dihemisuccinate, and the dipivaloate ester was only slightly hydrolyzed. The extent of enzymatic hydrolysis of the dipivaloate ester to DHPG was different in different species. The mouse plasma showed the most and the human plasma showed the least activity toward pivaloate esters. This finding suggests that

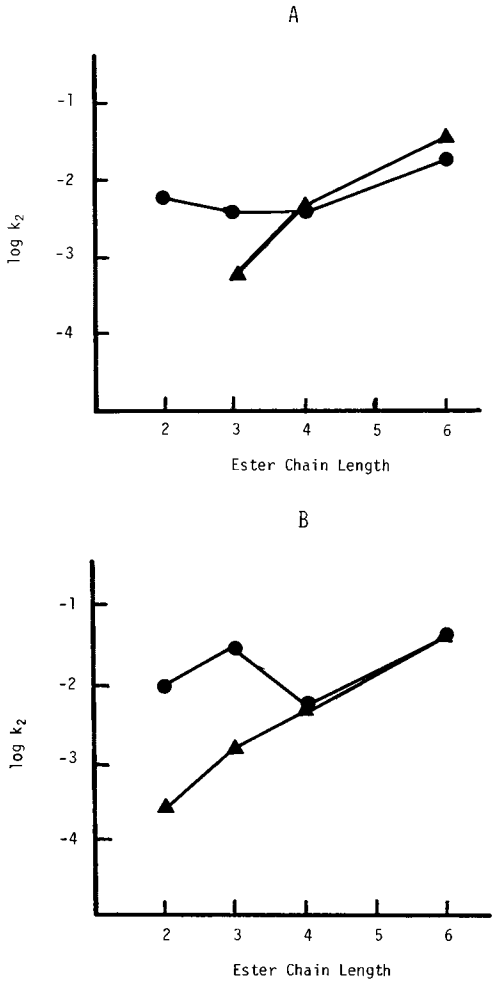


Fig. 6. Relationship between the alkyl chain length and the rates of enzymatic hydrolysis k_2 in the presence of intestinal (▲) and liver (●) homogenates from rat (A) and monkey (B).

Table III. Half-Lives and Rate Constants for the Enzymatic Hydrolysis of Various DHPG Diesters at 37°C in Rat and Monkey Liver Homogenates

	Rat homogenates (1.6%, w/v)				Monkey homogenates (0.83%, w/v), Monoester to DHPG	
	Diester to monoester		Monoester to DHPG			
Compound	k_1 (min^{-1})	$t_{1/2}$ (min)	k_2 (min^{-1})	$t_{1/2}$ (min)	k_2 (min^{-1})	$t_{1/2}$ (min)
II	0.020	35	0.0063	110	0.0096	73
III	0.183	4	0.0040	171	0.0351	20
IV	0.360	2	0.0040	171	0.0055	126
V	0.200	3	—[a]		—[a]	
VI	—[b]	<1	0.0212	33	0.0479	14
VII	0.019	37	0.0017	415	—[c]	
VIII	0.0047	149	—[a]		0.0023	297

[a] No significant hydrolysis up to 3 hr.
[b] Very fast reaction, complete within 1 hr.
[c] Incubation not run.

Table IV. Stabilities of Various Esters of DHPG in Human, Monkey, and Mouse Plasma at 37°C

Compound	% DHPG formed after 2 hr of incubation		
	Human	Monkey	Mouse
II	47		
III	71		
IV	102		
V	5	33	56
VI	102	95	101
VII	—[a]		

[a] No reaction.

the selection of derivatives based on only *in vivo* animal absorption data should be made with caution. The dipivaloate ester would be expected to yield good blood levels of DHPG upon administration to mouse, however, the blood levels upon oral administration to human are expected to be very low. These results suggest that dihemisuccinate and dipivaloate esters would not serve as potential prodrugs of DHPG.

CONCLUSIONS

The ideal prodrug (a) should have a good solubility in water, (b) should have a reasonable stability in the GI tract, and (c) once in the general circulation should hydrolyze back to DHPG in blood or during passage through the liver. The shorter-chain alkyl esters, II and III, are relatively more stable in intestinal homogenates and therefore may have a better membrane permeability because of the diester being intact. These two esters undergo considerable hydrolysis in blood and have selectively higher hydrolysis rates in liver homogenates. Therefore they will regenerate DHPG rapidly in blood and during the first pass through the liver. In addition, diesters II and III have better aqueous solubilities. The dipropionate ester appears to be the most promising derivative due to its anomalously high solubility and rapid dissolution rate, reasonable stability in the GI tract, and rapid conversion to DHPG in blood and liver. It is possible that such deviations from the usual structure–activity relationships exist for the solubility and esterase specificity of similar nucleosides. These possibilities should be exploited in designing new orally active nucleosides.

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