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Journal of Chromatography A, 707 (1995) 367–372

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Quantitative structure–retention relationships of acyclovir esters using immobilised albumin high-performance liquid chromatography and reversed-phase high-performance liquid chromatography

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First received 27 October 1994; revised manuscript received 7 March 1995; accepted 7 March 1995

Abstract

Acyclovir and 18 of its esters have been investigated by systematic measurement of their reversed-phase high-performance liquid chromatographic retention using differing mobile phase compositions. The methanol content of the mobile phase was varied between 5 and 95%. By linear least squares regression of the logarithmic retention factor (log k') against methanol concentration, the slope (S) and intercept (log k'_0) of the so obtained straight lines were calculated for each compound. The chromatographic hydrophobicity index ($\phi_{0,MeOH}$) calculated from the S and log k'_0 values ($\phi_{0,MeOH} = -\log k'_0/S$) showed significant correlation ($r > 0.96$) to the calculated octanol–water partition coefficients (cLog P). The albumin-binding properties of the compounds were characterised by the log k'_{HSA} values obtained by using an immobilised human serum albumin (HSA) HPLC column and 1% propan-2-ol 99% aqueous 10 mM phosphate buffer pH 7.0 as mobile phase. The measured albumin-binding parameters showed significant correlations to the cLog P, $\phi_{0,MeOH}$, S and log k'_0 values, establishing the importance of hydrophobic properties to the interaction of the acyclovir derivatives with HSA.

1. Introduction

Acyclovir, 9-(2-hydroxyethoxymethyl) guanine was the first non-toxic drug developed against herpes viral infections [1,2]. It has an acyclic side chain which has been shown to be phosphorylated by the herpes specified thymidine kinase and is converted to the triphosphate in herpes-infected cells to a much greater extent than in uninfected cells. The triphosphate of acyclovir is more inhibitory to the viral DNA polymerase than to the α-DNA polymerase of the cell [1]. Pharmacokinetic studies [3,4] have demonstrated that acyclovir has an advantageous kinetic profile and metabolic disposition and it can be administered parenterally as well as orally. However, its penetration through the skin was not ideal [5–7]. To increase skin-penetration efforts have been made to develop prodrugs [8].

Several papers have been published about the use of high-performance liquid chromatography (HPLC) for the analysis of acyclovir in various formulations [9–12] or biological samples [12–16]. Reversed-phase HPLC has been used with a few percent of organic modifier (3–5% methanol [9,15] or 3% acetonitrile [10]). Ion-pair chroma-

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SSDI 0021-9673(95)00339-8

tography has also been used with alkylsulphonates (heptansulphonic acid or octanesulphonic acid) with higher volume percent of organic modifier [11,13,16].

In this paper an investigation of 18 O-carboxylic esters and/or N-carboxamide derivatives of acyclovir, which are potential prodrugs, is presented. Their reversed-phase high-performance liquid chromatographic (RP-HPLC) retention behaviour was studied to reveal their hydrophobic properties. The HSA-binding properties of the compounds were measured by using immobilised HSA on a silica stationary phase in an HPLC column. It has been reported that chromatographic retention data correlate with ultrafiltration measurements of binding to HSA for a series of coumarin derivatives [17]. The applications of biochromatography to the determination of drug–protein interactions were discussed in detail by Aubry and McGann [18]. Excellent correlation was found for a structurally heterogeneous group of compounds between the protein-binding data obtained by HPLC and equilibrium dialysis [19] supporting the applicability of the method for the fast estimation of drug-binding. Kaliszan et al. [20] reported a quantitative structure–retention investigation of benzodiazepins on immobilised HSA, and found that two types of binding sites on HSA have a hydrophobic region with steric restrictions and a cationic region can also interact electrostatically with the compounds.

In this study the correlations between measured albumin-binding data, the chromatographic hydrophobicity data and the cLog P values (logarithm of calculated octanol–water partition coefficients) for the acyclovir derivatives were established.

2. Experimental

The compounds investigated are listed in Table 1. The compounds were synthesised at Burroughs Wellcome (NC, USA) or at the Wellcome Laboratories (Beckenham and Dartford, Kent, UK). Compounds 6–9 were synthesised by Professor Bundgaard (Royal Danish School of Pharmacy, Copenhagen, Denmark) with the aim of better skin absorption of the derivatives. All the compounds analysed were checked for purity chromatographically. Solutions of 1 mg/ml of the compounds in methanol–water (50:50, v/v) were used for the analysis. Slow hydrolysis of the esters has been observed during the measurements. Solutions were kept refrigerated (4°C) when not in use to minimise such degradation.

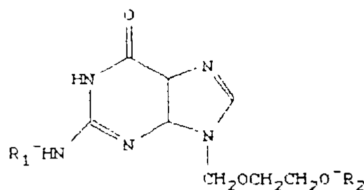
2.1. HPLC instrumentation

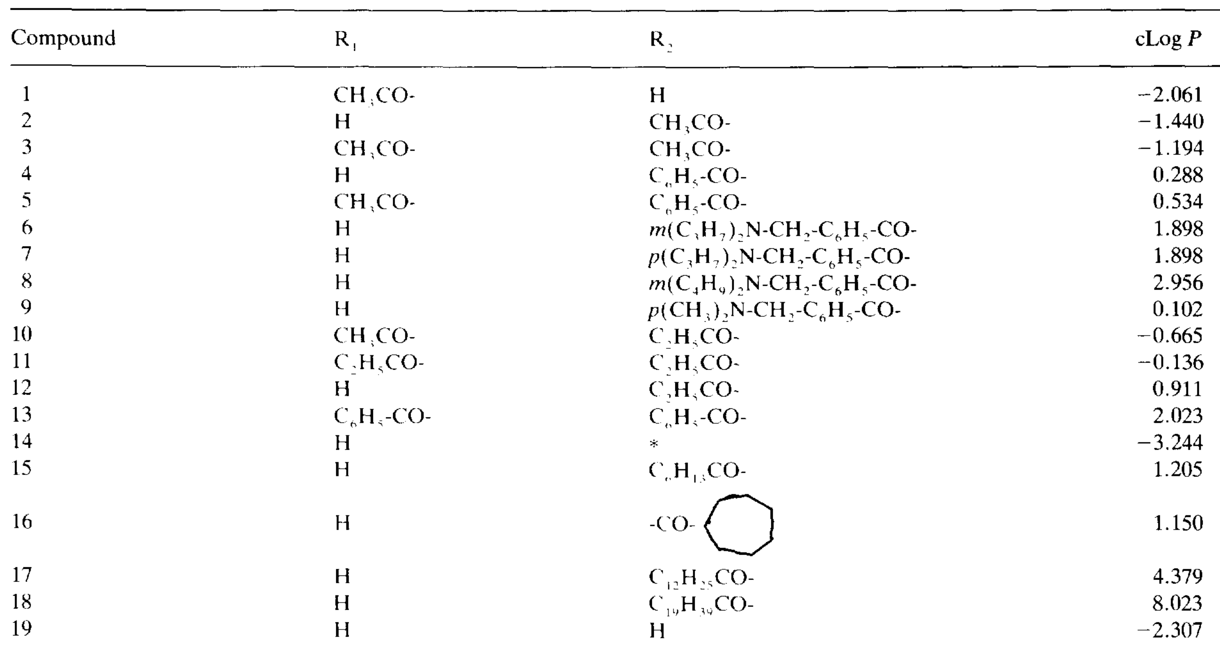
The HPLC equipment consisted of two Model 510 Waters pumps with automated gradient controller used together with a Waters 712 WISP autosampler and Waters 490E programmable multiwavelength detector (Milford, MA, USA). The column temperature was maintained using an oven unit obtained from Jones Chromatography (Hengoed, Mid Glamorgan, UK). Detection of the compounds was carried out at 254 nm UV with a sensitivity range of 0.05 AUFS. Quantitative evaluations of the chromatograms from the UV absorbance were made using a Multichrom data acquisition and analysis system (VG Data Systems, Altrincham, Cheshire, UK).

2.2. Reversed-phase measurements

A Zorbax C_8 (250 × 4.6 mm I.D.) (DuPont, Wilmington, DE, USA) reversed-phase column was used. The column was maintained at 30°C during the measurements. The mobile-phase flow-rate was 1.0 ml/min. The mobile phase was aqueous methanol with the methanol concentration ranging from 5 to 95%. The HPLC grade methanol was purchased from Rathburn (Loughborough, UK). Water was obtained from a Milli-Q system (Millipore, CA, USA). For compounds 6–9 the mobile phase contained also 10 mM phosphate buffer pH 7.0 (HPLC grade, HiPerSolv, BDH, Poole, UK). The dead time of the system was estimated from the solvent peak. From the retention time and the dead time values the chromatographic retention factor (log k') was calculated. The log k' values were then regressed against the methanol concentration and the slope (S) and the intercept (log k'_0)

Table 1
The chemical structure of the compounds and their calculated octanol/water partition coefficients (cLog P)





Compound	R_1	R_2	cLog P
1	CH_3CO-	H	−2.061
2	H	CH_3CO-	−1.440
3	CH_3CO-	CH_3CO-	−1.194
4	H	C_6H_5-CO-	0.288
5	CH_3CO-	C_6H_5-CO-	0.534
6	H	$m(C_3H_7)_2N$-CH_2-C_6H_5-CO-	1.898
7	H	$p(C_3H_7)_2N$-CH_2-C_6H_5-CO-	1.898
8	H	$m(C_4H_9)_2N$-CH_2-C_6H_5-CO-	2.956
9	H	$p(CH_3)_2N$-CH_2-C_6H_5-CO-	0.102
10	CH_3CO-	C_2H_5CO-	−0.665
11	C_2H_5CO-	C_2H_5CO-	−0.136
12	H	C_3H_7CO-	0.911
13	C_6H_5-CO-	C_6H_5-CO-	2.023
14	H	*	−3.244
15	H	$C_6H_{13}CO$-	1.205
16	H	-CO-(cycloheptyl ring)	1.150
17	H	$C_{12}H_{25}CO$-	4.379
18	H	$C_{19}H_{39}CO$-	8.023
19	H	H	−2.307

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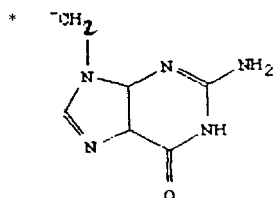

values of the least squares fitted straight lines were calculated. The chromatographic hydrophobicity index (ϕ_0) was calculated from the S and log k'_0 values ($-\log k'_0/S$) as previously described [19].

2.3. Albumin-binding measurements

An immobilised human serum albumin (HSA) column with the dimensions of 50 × 4.6 mm I.D. was obtained from Shandon HPLC (Life Science, Runcorn, UK) and was used for the measurement of the albumin-binding ability of the derivatives. The column temperature was maintained at 25°C. The mobile phase contained 1% propan-2-ol (HPLC grade, Fisons, Loughborough, UK) and 10 mM phosphate buffer pH 7.0. The flow-rate was 0.5 ml/min. For compounds 6–9 the pH was increased to 7.4 and the propan-2-ol concentration ranged from 4 to 8% in order to obtain shorter retention times and improved peak shapes. The binding properties of the compounds were expressed as the log k'_{HSA} values obtained from the retention time and

dead time data. The log k'_{HSA} values for compounds 6–9 were obtained by extrapolation from the data at higher propan-2-ol concentrations to 1% propan-2-ol.

The cLog P values (the calculated octanol–water partition coefficients) were obtained by using MedChem Ver. 3.54 (Pomona College, Claremont, CA, USA). The correlation study was carried out by using the DrugIdea program (Chemicro, Budapest, Hungary).

3. Results and discussion

The structure and the cLog P values of the compounds investigated are shown in Table 1. The reversed-phase retention parameters and the retention factor obtained by the drug-binding column are listed in Table 2.

Acyclovir (compound 19) showed only very weak binding (6%) to HSA which is in agreement with earlier results [9] obtained by ultrafiltration. No data has been reported on the drug-binding properties of the investigated esters. Compounds 6–9 showed extremely strong binding on the HSA column using 1% propan-2-ol and pH 7.0 buffer in the mobile phase. The propan-2-ol concentration had to be increased to 4% to obtain reasonable retention times. In order to be able to compare the binding parameters to the other compounds the measurements were repeated by using 7% and 8% propan-2-ol in the mobile phase. The log k'_{HSA} values were calculated and plotted as a function of propan-2-ol concentration. Straight lines were obtained which allowed the extrapolation of the log k'_{HSA} values to 1% propan-2-ol. Table 3 shows the slope and the intercept values obtained using these extrapolated parameter values (S_{HSA}, log $k'_{0,HSA}$).

Compounds 17 and 18 showed low solubility in methanol–water (50:50, v/v) and they had long retention times on a C_8 column even with a high percentage (99%) of methanol in the mobile phase. Compound 18 showed an extremely long retention time and gave a wide peak on the HSA

Table 2
Parameters from the measured reversed-phase retention data [the slope (S) and the intercept (log k'_0) from the log k' vs. methanol concentration regression and the correlation coefficient, r], the chromatographic hydrophobicity index (ϕ_0) and the albumin-binding parameter (log k'_{HSA})

Compound	S	log k'_0	r	ϕ_0	log k'_{HSA}
1	0.0448	1.098	0.998	24.5	−1.164
2	−0.0379	1.042	0.996	27.5	−0.725
3	0.0418	1.555	0.999	37.2	−0.752
4	0.0285	1.579	0.997	55.4	0.224
5	−0.0316	1.877	0.999	59.4	0.134
6	−0.0377	3.144	0.998	83.4	0.499
7	0.0396	3.326	0.999	84.0	0.581
8	0.0497	4.323	0.999	87.0	0.842
9	0.0184	1.161	0.998	63.1	0.302
10	−0.0347	1.639	0.999	47.2	−0.440
11	0.0240	1.368	0.999	57.0	−0.129
12	−0.0365	1.392	0.999	38.1	−0.461
13	0.0463	3.336	0.999	72.1	0.230
14	−0.0292	0.290	0.995	9.9	−1.164
15	0.0422	3.002	0.998	71.1	0.823
16	−0.0414	2.891	0.999	67.9	0.717
17	−0.0589	5.251	0.996	89.2	1.370
18	0.0819	8.069	0.999	94.8	2.2
19	−0.0351	0.412	0.997	11.7	−1.182

The reversed-phase retention data for compounds 6–9 were obtained by using phosphate buffer pH 7.0 in the mobile phase.

Table 3
The albumin-binding parameters of the four basic compounds obtained by varying the propan-2-ol concentration

Compound	S_{HSA}	$\log k'_{0,HSA}$	r
6	−0.0429	0.542	0.999
7	−0.0432	0.624	0.999
8	−0.0470	0.889	0.997
9	−0.0309	0.333	0.999

S and $\log k'_0$ are the slope and the intercept of the least squares fitted straight line, and r is the correlation coefficient; the number of data points was 6.

column using 8% propan-2-ol in the mobile phase. The cLog P value of compound 18 is also unrealistically high. The data of compound 18 was often an outlier in the correlation study and therefore its data were omitted from further calculations.

Eq. 1 describes the correlation between the cLog P values and the $\log k'_0$ values.

$$\text{cLog } P = 1.35(\pm 0.13) \log k'_0 - 2.54 \quad (1)$$

$n = 18$, $r = 0.935$, and $s = 0.71$

The correlation coefficients between the slope and the intercept values was small (−0.728) showing that the compounds differ from each other significantly with respect to their partition behaviour [23].

The correlation was further studied by using the S and $\log k'_0$ values as two independent variables in relation to the cLog P and the albumin-binding parameter ($\log k'_{HSA}$). A highly significant relationship ($p > 0.95$) were obtained and shown in Eq. 2.

$$\text{cLog } P = 72.79(\pm 12.07)S + 1.76(\pm 0.08) \times \log k'_0 - 0.80 \quad (2)$$

$n = 18$, $r = 0.994$, $s = 0.33$, and $F_{2,15} = 305.0$
where n is the number of compounds, r is the multiple correlation coefficient, s is the standard error of the estimate, F is the Fisher-test value. Fig. 1 shows the fit of the predicted cLog P vs. cLog P values from Eq. 2.

Eq. 3 describes the correlation between the $\log k'_{HSA}$ and $\log k'_0$ values.

$$\log k'_{HSA} = 0.50(\pm 0.07) \log k'_0 - 1.09 \quad (3)$$

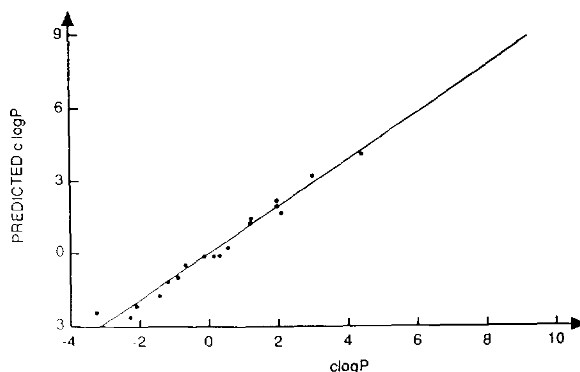

Fig. 1. The plot of the predicted cLog P vs. cLog P by Eq. 2.

$n = 18$, $r = 0.876$, and $s = 0.38$

Again much better correlation was obtained when the S values were also introduced as a second independent variable, showing the importance of the contact hydrophobic surface area in the drug–protein binding. Eq. 4 describes the relationship between the reversed-phase retention data and the albumin-binding data of the compounds. The relationships were again highly significant.

$$\log k'_{HSA} = 48.16(\pm 7.92)S + 0.75(\pm 0.06) \log k'_0 + 0.30 \quad (4)$$

$n = 18$, $r = 0.983$, $s = 0.21$, and $F_{2,15} = 104.2$

Fig. 2 shows the fit of the predicted $\log k'_{HSA}$ vs. $\log k'_{HSA}$ values from Eq. 4.

The regression of the albumin-binding data

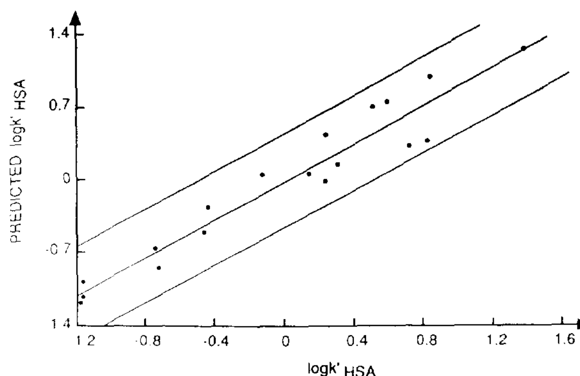

Fig. 2. The plot of the predicted $\log k'_{HSA}$ vs. $\log k'_{HSA}$ by Eq. 4 together with the 95% confidence interval.

against the cLog P values and the chromatographic hydrophobicity index ($\phi_{0,MeOH}$) values separately is described by Eqs. 5 and 6, respectively.

$$\log k'_{HSA} = 0.372(\pm 0.031)\text{cLog } P - 0.109 \quad (5)$$

$n = 18$, $r = 0.949$, $s = 0.252$, and $F_{1,16} = 144.4$

$$\log k'_{HSA} = 0.029(\pm 0.002)\phi_{0,MeOH} - 1.1613 \quad (6)$$

$n = 18$, $r = 0.953$, $s = 0.241$, and $F_{1,16} = 158.7$

Highly significant correlations were found in both cases.

In conclusion, the reversed-phase retention data obtained on the C_8 column by using methanol and pH 7.0 phosphate buffer in the mobile phase showed significant correlation to the calculated octanol–water partition coefficients. The albumin-binding parameters measured on an immobilised HSA column showed high correlation to the calculated octanol–water partition coefficients and the hydrophobicity indices measured by reversed-phase chromatography.

Acknowledgement

The authors would like to thank Alan Hill and Rachel Siertsema for the calculations of the cLog P values.

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