International Journal of Pharmaceutics, 57 (1989) 229–234
Elsevier

IJP 01947

Lipophilicity of guanine derivatives

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(Received 22 April 1989)
(Accepted 30 June 1989)

Key words: Acyclovir; Deoxyacyclovir; Physico-chemical properties; Partition coefficient; Partition method; Constant of ionization

Summary

Lipid–water partition coefficients for acyclovir and deoxyacyclovir were determined by partition experiment. The ionisation constants and the real partition coefficients were calculated by linear regression methods. The contradiction in lipophilicity and water solubility for acyclovir and deoxyacyclovir as determined previously (Kozjek et al., 1988) was explained by these data and with the aid of differential scanning calorimetry.

Introduction

The majority of the viruses induce the production of their own specific enzymes in the host cell. These processes can be inhibited without disturbing the function of the cell. In this manner the effective and selective agents against viruses can be designed.

Acyclovir (ACV) (9-(2-hydroxyethoxymethyl) guanine) is one of the first widely used agents against viruses acting on this principle. ACV is first phosphorylated by virus-induced thymidine kinase in the cell to ACV-monophosphate (Fletcher and Bean, 1985; Dorsky and Crumpacker, 1987). This ACV-monophosphate metabolite is furthermore transformed by host enzymes to the triphosphate derivative of ACV. ACV-triphosphate competes with viral deoxyguanosine triphosphate for binding to viral DNA polymerase. ACV is used mostly against herpes viruses type 1 and 2 and varicella zoster viruses (Fletcher and Bean, 1985; Dorsky and Crumpacker, 1987). It is a safe drug administered either intravenously, orally or topically.

ACV is poorly absorbed from gastrointestinal tract after oral administration; bioavailability of oral administered ACV is only 15–30% (Laskin, 1983; Fletcher and Bean, 1985; Dorsky and Crumpacker, 1987). This problem was approached by the introduction of new analogues of ACV. One of them is deoxyacyclovir (DCV), 2- amino-9-(2-hydroxyethoxymethyl)-9-H-purine. DCV as a prodrug has a higher bioavailability and at least 75% of the orally administered dose is absorbed from the gastrointestinal tract (Petty et al., 1987). DCV is transformed to ACV in the liver through oxidation with xanthine oxidase. DCV can there-

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fore be used in peroral therapies where ACV is applicable only by intravenous administration.

In the present work we wish to elucidate the contradictory results related to the water solubility and to the water–lipid distribution of ACV and DCV. DCV is 18 times (Krenitski et al., 1984) more soluble in water than ACV (the solubility for ACV in water is 2.7 mg/ml at 37 °C) and the partition coefficient (water/*n*-octanol) for DCV is much higher than that for ACV (Kozjek et al., 1988). In the previous work (Kozjek et al., 1988) we suggested the possibility of different ACV molecule associations. The mechanism and the degree of association could be different in both phases.

In order to prove or to reject the above mentioned hypothesis we have carried out the partition experiment for both substances, ACV and DCV. To achieve good statistical evaluation the experiments were repeated many times and with different volumes of organic phase to enable the transfer of greater amounts of samples in the organic phase after distribution. Additionally, differential scanning calorimetry of both substances was done to characterise their physical state.

Experimental

Reagents and solutions

Acyclovir (ACV) and deoxyacyclovir (DCV) were synthesized at Krka Pharmaceuticals (Novo mesto, Yugoslavia) (Kobe et al., US patent 4701526 and Kobe and Perhavc Yu-P-2188/84).

The buffers used for distribution studies were the following: a citrate (pH = 1.5–6.5), a phosphate (pH = 7.5) and a borate (pH = 8.5–10.5). The ion forces of all buffers were adjusted to 0.35 with NaCl. The solvent used for partition studies was water saturated *n*-octanol.

Partition experiment

Five ml solutions of ACV and DCV (concentration 500 mg/l) in corresponding buffers were shaken with 5 ml, 10 ml, 15 ml and 20 ml of *n*-octanol for 1 h at room temperature. After shaking, 1 ml of water phase was diluted 50 times with corresponding buffer and absorbances (*Apo*) and pH values of these solutions were measured. The nonshaken solutions of ACV and DCV were treated in the same manner and absorbances (*Apd*) and pH values were measured as well. Apparent partition coefficients (*Pn*) were calculated from obtained values by eq. (1):

$$Pn = \frac{Apd - Apo}{Apo} \tag{1}$$

where *Apd* indicates the measured absorbance of the nonshaken solutions and *Apo* after shaking.

When different volumes of water and lipid phase were used, the apparent partition coefficients (*Pn*) were calculated by eq. (2):

$$Pn = \frac{(Apd - Apo) \cdot Vv}{Apo \cdot Vo} \tag{2}$$

where *Vv* is the volume of water phase and *Vo* of lipid phase.

The absorbances were measured with a UV-VIS Perkin Elmer 554 spectrophotometer at $\lambda = 250$ for ACV and $\lambda = 303$ for DCV. pH values were obtained on the ISKRA-MA 5705 apparatus.

Using calculated values for apparent partition coefficients and corresponding pH values we have determined the ionization constants and real partition coefficients by least-squares linear regression method with eqs. (3,4) for ACV and eq. (3) for DCV.

$$1/Pn = 1/P + (1/P \cdot Ka) \cdot H^+ \tag{3}$$

$$1/Pn = 1/P + (Ka/P) \cdot 1/H^+ \tag{4}$$

Thermal analysis

The differential scanning calorimetry (DSC) diagrams for ACV and DCV were obtained on a Perkin Elmer 4 Differential Scanning Calorimeter. All thermal analysis measurements were performed with a heating rate of 10 °C per min in the dynamic nitrogen atmosphere (40 ml/min). The sample sizes were in the order of 1–5 mg. The sensitivity was 10 mcal/s.

Results and Discussion

Tables 1 and 2 represent the results obtained at different ratios water/lipid phase: (a) 1 : 1, (b) 1 : 2, (c) 1 : 3 and (d) 1 : 4. The use of least-squares linear regression method gave the following eqs. (5,6) for ACV:

$$1/Pn = 44.22 \pm 12.43 + (11040.29 \pm 324.17) \cdot H^+ \quad (5)$$

$$1/Pn = 38.42 \pm 28.45 + (2.24 \cdot E-8 \pm 1.39 \cdot E-9) \cdot 1/H^+ \quad (6)$$

TABLE 1

Absorbances of ACV solutions before and after shaking with n-octanol and the calculated values of apparent partition coefficients

pH	*Apd*	*Apo*	*Pn*
Ratio water/lipid phase 1 : 1			
1.48	0.412	0.411	0.0024
2.54	0.490	0.483	0.0145
3.55	0.499	0.490	0.0184
3.56	0.478	0.467	0.0235
5.54	0.467	0.461	0.0130
6.41	0.435	0.426	0.0211
6.50	0.455	0.445	0.0225
7.52	0.495	0.489	0.0123
Ratio water/lipid phase 1 : 2			
1.49	0.401	0.399	0.0025
3.56	0.478	0.457	0.0230
6.41	0.435	0.416	0.0228
9.55	0.382	0.374	0.0107
Ratio water/lipid phase 1 : 3			
3.56	0.478	0.452	0.0192
6.41	0.435	0.400	0.0292
7.53	0.502	0.478	0.0167
8.43	0.472	0.447	0.0186
Ratio water/lipid phase 1 : 4			
6.50	0.468	0.430	0.0221
7.53	0.502	0.462	0.0216
7.57	0.507	0.444	0.0355
8.43	0.472	0.429	0.0251
10.36	0.411	0.408	0.0018

TABLE 2

Absorbances of DCV solutions before and after shaking with n-octanol and the calculated values of apparent partition coefficients

pH	*Apd*	*Apo*	*Pn*
Ratio water/lipid phase 1 : 1			
5.55	0.324	0.297	0.0909
7.52	0.329	0.300	0.0967
9.46	0.256	0.232	0.1034
9.55	0.328	0.297	0.1044
Ratio water/lipid phase 1 : 2			
3.56	0.232	0.214	0.0421
6.42	0.343	0.283	0.1060
9.55	0.328	0.275	0.0964
Ratio water/lipid phase 1 : 3			
3.56	0.232	0.205	0.0439
6.42	0.343	0.264	0.0997
7.57	0.289	0.222	0.1006
8.44	0.334	0.260	0.0949
9.55	0.328	0.255	0.0954
10.36	0.359	0.279	0.0956
Ratio water/lipid phase 1 : 4			
5.58	0.326	0.240	0.0896
6.50	0.329	0.242	0.0899
7.53	0.316	0.226	0.0996
7.57	0.289	0.206	0.1007
8.44	0.334	0.244	0.0922
10.36	0.359	0.262	0.0926

and eq. (7) for DCV:

$$1/Pn = 10.40 \pm 1.08 + (44868.94 \pm 4123.90) \cdot H^+ \quad (7)$$

The constants of ionization and the partition coefficients obtained from these equations are: ACV: $pKa1 = 2.41 \pm 0.27$, $pKa2 = 9.06 \pm 0.88$, $\log P = -1.615 (P = 0.024)$; DCV: $pKa = 3.63 \pm 0.085$, $\log P = -1.02 (P = 0.096)$.

From eq. (8), which represents the multiple least-squares linear regression method, another set of results for the constants of ionization and the partition coefficient for ACV was calculated.

$$1/Pn = 11087.12 \cdot H^+ + 2.21 \cdot E-8 \cdot (1/H^+) + 45.46 \quad (8)$$

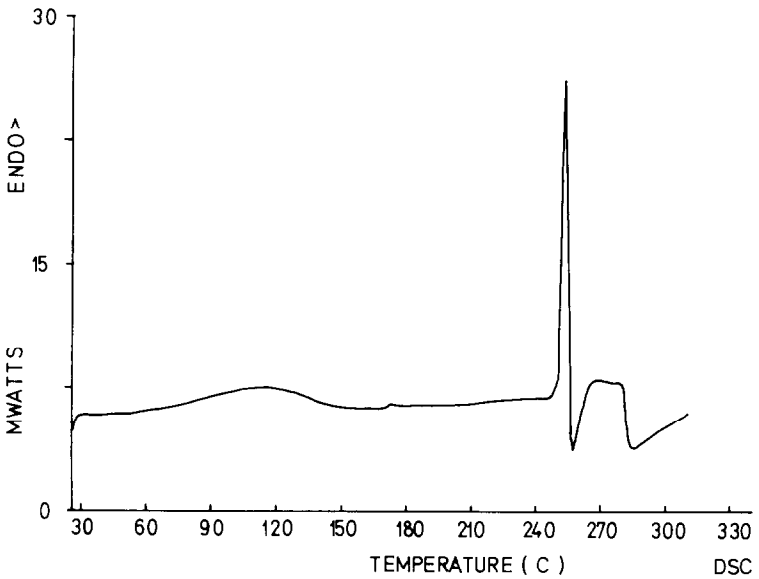

Fig. 1. DSC curve of ACV

where $pKa1 = 2.39$, $pKa2 = 9.31$, $\log P = 1.657$ ($P = 0.022$).

The differential scanning calorimetry (DSC) diagrams for ACV and DCV are illustrated in Figures 1 and 2, respectively.

There are some differences in the values for partition coefficients comparing them with previously determined (Kozjek et al., 1988), which exhibit the large mean standard deviation. Since these results represented rather unreliable values, a great number of partition experiments was additionally carried out in the present work and the ratio lipid/water phase was changing as well. Each partition experiment was repeated three times.

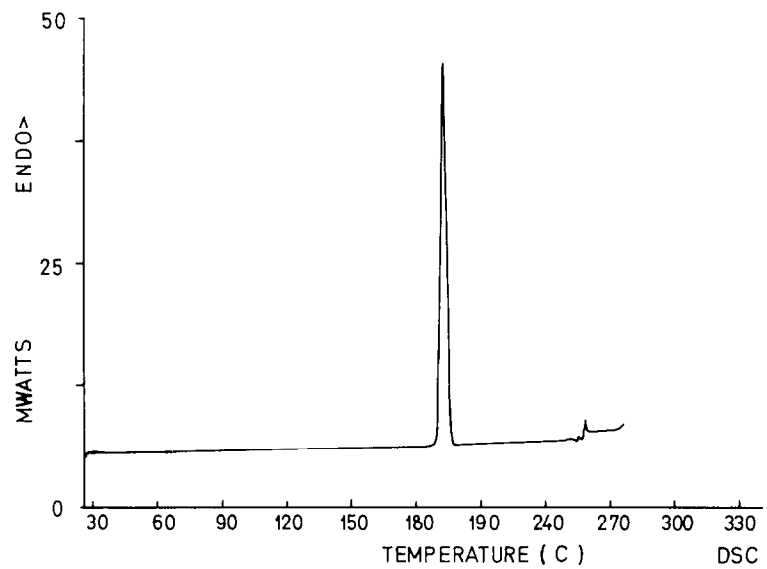

Fig. 2. DSC curve of DCV

Calculated values for constants of ionization and partition coefficients with linear regression methods in one way represent a simulation for experimentally determined values. The apparent partition coefficients at corresponding pH values were loaded in the linear regression equations. The values of the constants of ionization obtained by the least-squares linear regression method are in good agreement with experimental values (Kozjek et al., 1988). Therefore the real partition coefficients calculated by the same method are quite reliable. The multiple least-squares linear regression method, which has given another set of results for ACV, has only confirmed the above mentioned statement. We may conclude that the determined values are the reflection of the real state.

The obtained results still confirmed the established contradiction, that DCV was better distributed in the lipid phase and was better soluble in aqueous phase than ACV. Although the partition experiment gave reliable results, it could not explain the contradiction mentioned.

The association of the molecules of ACV was already suggested as a possible reason. The contradiction that the analog is more lipophilic and more hydrophilic than the drug itself was observed at some *N*-acyl derivates of 5-fluorouracil and allopurinol (Burer and Bundgaard, 1984; Bundgaard and Falch, 1985). This phenomenon was explained by the reduction of hydrogen bondings in the crystal structure. The contradictory data about solubility of ACV and DCV can be explained in the same way. The statement that intermolecular interactions (hydrogen bonds) between the molecules of ACV were involved could be supported by the higher melting point temperature for ACV (256.6–257 °C for ACV and 187–189 °C for DCV (Krenitski et al., 1984)). The connection between higher temperature of melting, intermolecular interactions, solubility and partition coefficient has often been discussed in the literature, for example Yalkovski (1981).

We wished to confirm the existence of the hydrogen bonds with differential scanning calorimetry. Our presumption was to detect the energetic changes in the melted substance at the temperatures higher than the melting point, which could be the result of intermolecular interactions. The thermograms did not confirm this assumption (Figs. 1 and 2). We discovered that the molecules of ACV are in solvated form and probably hydrated. Especially at temperatures higher than the melting point ACV was decomposed.

There are several data (Shefter and Higuchi, 1963; Fung and Nealon, 1974; Yalkovski, 1981) reporting about smaller solubility of the drugs in a solvated form. The solvates are defined as molecular complexes where the molecules of solvents are incorporated in their crystal structure. They are usually obtained after recrystallization from different solvents. The solvates usually possess smaller thermodynamic activity and are therefore in more stable state than nonsolvated forms (Yalkovski, 1981). The result is a smaller solubility.

We assume that the probable reason for the contradiction in lipophilicity and hydrophilicity of ACV and DCV is the solvated form of ACV molecules. The possibility that the molecules of ACV form associates in the water and in *n*-octanol phase can not be rejected yet.

Acknowledgement

The authors are thankful to Mag. F. Vrečer for DSC diagrams.

References

Bundgaard, H. and Falch, E., Allopurinol prodrugs 1. Synthesis, stability, and physicochemical properties of various N_1-acyl allopurinol derivatives, *Int. J. Pharm.*, 23 (1985) 223–237.

Burer, A. and Bundgaard H., Prodrugs of 5-fluorouracil 1. Hydrolysis kinetics and physicochemical properties of various *N*-acyl derivates of 5-fluorouracil, *Int. J. Pharm.*, 21 (1984) 349–364.

Dorsky, D.J. and Crumpacker, C.S., Drugs five years later: acyclovir, *Ann. Intern. Med.*, 107 (1987) 859–874.

Fletcher, C. and Bean, B., Evaluation of oral acyclovir therapy, *Drug Intell. Clin. Pharm.*, 19 (1985) 518–524.

Fung, H.-L. and Nealon, T., Solvent effects on comparative dissolution of pharmaceutical solvates, *Chem. Pharm. Bull.*, 22 (1974) 454–458.

Kobe, J., Gnidovec, J. and Zupet, P., US patent 4701526.

Kobe, J. and Perhavc, M., Yu-P-2188/84.

Kozjek, F., Mrhar, A., Bogataj, M. and Kobe, J., The effect of physicochemical properties of acyclovir and deoxyacyclovir on the in vitro diffusion, *Acta Pharmac. Jugosl.*, 38 (1988) 61–69.

Krenitski, T.A., Hall, W.W., Miranda, P., Beauchamp, L.M., Schaeffer, H.J. and Whiteman, P.D., 6-Deoxyacyclovir: a xanthine oxidase-activated prodrug of acyclovir, *Proc. Natl. Acad. Sci. USA*, 81 (1984) 3209–3213.

Laskin, O.L., Clinical pharmacokinetics of acyclovir, *Clin. Pharmacok.*, 8 (1983) 187–201.

Petty, G.B., Whitley, J.R., Liao, S., Krasny, H.C., Rocco, L.E., Davis, G.L. and Lietman, P.S., Pharmacokinetics and tolerance of descyclovir, a prodrug of acyclovir, in healthy human volunteers, *Antimicrob. Agents Chemother.*, 31 (1987) 1317–1322.

Shefter, E. and Higuchi, T., Dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals, *Pharm. Sci.*, 52 (1963) 781–791.

Yalkovski, H.S., Techniques of solubilization of drugs, Marcel Dekker Inc., New York, Basel, 1981, 160–195.