Negative Correlation of n-Octanol/Water Partition Coefficient and Transport of Some Guanine Derivatives Through Rat Jejunum *In Vitro*

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INTRODUCTION

Acyclovir (ACV) (9-[2-hydroxyethoxymethyl]-guanine) is a very potent selective antiviral agent. Because of its low bioavailability after oral application (only 15–30% (1)) many ACV prodrugs, analogs and similar compounds have been synthesized (2). One of these is deoxyacyclovir (DCV) 2-amino-9-(2-hydroxyethoxymethyl)-9-H-purine. DCV as a prodrug has higher bioavailability and at least 75% of the orally administered dose is absorbed from the gastrointestinal tract (3). At room temperature the solubility in water for DCV is almost 12 times higher than for ACV (7.15 mmol/l for ACV and 83.5 mmol/l for DCV) and the partition coefficient (logP n-octanol/water) increased from −1.57 for ACV to −1.08 for DCV (4).

The aim of present work was to compare the transport mechanisms of ACV and DCV and their acetyl congeners through rat small intestine and to find the origin of the improved bioavailability of DCV, compared to ACV. For this reason we performed transport experiments by applying two different side-by-side diffusion chambers, Ussing chambers (5) and Sweetana-Grass cells (6).

MATERIALS AND METHODS

Chemicals

Acyclovir (ACV) and deoxyacyclovir (DCV) with their acetyl congeners, given in Table I, were synthesized at the Institute of Chemistry, Ljubljana; the purity, determined by HPLC, is more than 99% (7). All other chemicals were of analytical grade.

In Vitro Intestinal Transport Studies

Transport of the guanine derivatives across living intestinal tissue was examined in Ussing chambers (5) and Sweetana-Grass diffusion cells (S.-G. cells) (6) in the manner reported previously (8). Both types of cells were custom built at the University of Utrecht. The cells are composed from two Lucite® chambers where the volume of donor and acceptor solution in Ussing chambers is 12.5 ml and exposed tissue surface is 0.79 cm^2 while these parameters for S.-G. cells are 2.5 ml and 1.22 cm^2, respectively. The exposed tissue area to chamber volume ratios for Ussing and S.-G. cells are 0.06 cm^{-1} and 0.49 cm^{-1}, respectively. Before the start and during an experiment in Ussing chambers, P_d (transepithelial potential) and I_{sc} (short-circuit current) were monitored on a custom built voltage clamp meter. Short-circuit conditions were maintained during the experiments with Ussing chambers, while no voltage clamp was applied on S.-G. cells.

Rat intestine was obtained from Wistar rats (200–300 g) which had access to a standard laboratory chow and tap water prior to experiments. After decapitation and laparotomy, the small intestine (jejunum, starting approximately 20 cm proximal to the ileocaecal junction) was quickly excised and placed into ice cold Ringer buffer containing (mM): Na^+, 140.6; Cl^-, 121.8; K^+, 5.0; Ca^{2+}, 1.2; Mg^{2+}, 1.2; $H_2PO_4^-$, 0.4; HPO_4^{2-}, 1.6; HCO_3^-, 25.0; pH = 7.4; if necessary corrected with 0.1 M HCl or NaOH. The intestinal segments were opened, flattened and placed between the two Lucite® chambers and bathed on both sides with the Ringer buffer containing 10 mM glucose at the serosal and 10 mM mannitol at the mucosal side. The solutions were gassed with 95% O_2-5% CO_2 and kept at 38°C (using thermostated waterjackets) to maintain the tissue viability.

During transport studies, tissue integrity was validated by measuring the permeability across the intestinal tissue of a fluorescent transport marker, fluorescein sodium.

After 30 minutes equilibration time, the tested compound was added to the mucosal or serosal side if studying m-to-s

Table I. The Values for logP (taken from ref. 4) and Papp (1/h·cm^2) with SEM (n = 6 for all substances or 18 for ACV and DCV) for Tested Guanine Derivatives and Fluorescein Obtained in Ussing Chambers in m-to-s Direction and S.-G. Cells in m-to-s and s-to-m Direction

		Ussing chambers	S.-G. cells	S.-G. cells
	logP	m-to-s	m-to-s	s-to-m
ACV	−1.57	5.02 ± 0.70	8.67 ± 0.61	7.90 ± 0.65
fluorescein		3.70 ± 0.81	7.00 ± 1.20	4.38 ± 0.59
NAcACV	−1.30	2.43 ± 0.44	6.61 ± 0.52	6.84 ± 0.54
fluorescein		1.66 ± 0.48	7.97 ± 0.85	7.37 ± 1.16
OAcACV	−1.07	1.80 ± 0.44	2.59 ± 0.24	2.55 ± 0.21
fluorescein		2.63 ± 0.34	5.78 ± 0.21	8.67 ± 1.80
diAcACV	−0.85	1.44 ± 0.29	2.78 ± 0.24	2.41 ± 0.14
fluorescein		2.32 ± 0.19	6.43 ± 0.35	5.78 ± 0.44
DCV	−1.08	2.39 ± 0.16	7.87 ± 0.48	8.30 ± 0.93
fluorescein		2.24 ± 0.30	6.34 ± 0.66	6.89 ± 0.78
NAcDCV	−1.33	2.85 ± 0.63	6.99 ± 0.21	7.42 ± 0.39
fluorescein		2.28 ± 0.62	7.12 ± 0.52	6.61 ± 0.62
OAcDCV	−0.61	0.89 ± 0.67	1.90 ± 0.46	1.77 ± 0.11
fluorescein		2.28 ± 0.62	7.80 ± 1.75	5.64 ± 0.87
diAcDCV	−1.05	1.15 ± 0.19	2.82 ± 0.45	2.03 ± 0.48
fluorescein		2.33 ± 0.22	7.80 ± 1.75	5.64 ± 0.87

Note: The values for Papp are given as Papp·10^5.

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(mucosal-to-serosal) or s-to-m (serosal-to-mucosal) transport, respectively and 250 μl samples were taken from the acceptor compartments at 20 min. intervals up to 240 min. After each sample, 250 μl Ringer solution containing 10 mM glucose or 10 mM mannitol was added to the acceptor phase. The concentration in the donor compartments was 1 mM for the guanine derivatives and 5 μM for fluorescein.

For ACV and DCV 18 and for the other substances tested 6 transport experiments were performed. Each 6 experiments were performed using the tissue obtained from one rat. The TEER (transepithelial electrical resistance) values were in a normal range between 30 and 80 $\Omega \cdot cm^2$ and stable throughout the experiment.

The average fluorescein fluxes in Ussing chambers experiments were in the range of 0.07–0.10 $nmol/h \cdot cm^2$ and in the case of S.-G. cells these values were from 0.15 to 0.30 $nmol/h \cdot cm^2$.

Analytical Procedures

The samples taken from the diffusion chambers were assayed for the guanine derivatives and fluorescein by reversed HPLC using a Nucleosil C-18 (5 μm, 120 × 4 mm I.D.) column and a mixture of methanol and 0.5% (V/V) acetic acid at various compositions as a mobile phase. The detection was UV absorption for the guanine derivatives and fluorescence for fluorescein. In the case of Ussing chambers, fluorescein concentration was determined using a fluorimetric detection without separation.

Analysis of Transport Data

The *in vitro* apparent permeability coefficient (Papp) was calculated (6) from the following equation:

$$P = \frac{dC}{dt} \cdot \frac{V}{C_o A}$$

where dC/dt is the change in concentration per unit time, V is the volume of acceptor phase, A is the exposed area of the membrane and C_o is the initial concentration of the diffusing drug in the donor compartment.

RESULTS AND DISCUSSION

The stability studies with all acetylated derivatives in intestinal homogenate (150 mg wet tissue homogenized in 50 ml Ringer at pH = 7.4) show at a concentration of 100 μM at 38°C a slow decomposition with a rate between 1 and 2 μmol/h (i.e. 1–2%/h), while ACV and DCV are stable. This indicates that during the experiment no important degradation of the substances tested at either side of the chambers can be observed; for this reason the driving force during each experiment was regarded as constant.

The calculated Papp values for all tested compounds are given in Table I, where also the values for the fluorescein permeabilities are given. The permeability values for fluorescein are in a normal range for this type of tissue, while also the TEER values as calculated from I_{sc} and P_d values measured during transport experiments were not changing after addition of the guanine derivatives tested, indicating no influence of the compounds on paracellular sieve function.

In the S-G cells the permeability was tested both in m-to-s and in s-to-m direction, but no polarization in transport can be observed. This is suggesting that ACV and congeners are transported passively and not by a carrier mediated process, as has been shown for ACV transport into erythrocytes (9). Our results are in agreement with a previous report showing ACV passive uptake in intestinal tissue, estimated with *in vitro* intestinal ring method (10). Comparison between the permeability data of the tested compounds is showing a good correlation between both chambers (Fig. 1), with an exception for ACV, exhibiting an inexplicable higher transport compared to the other compounds only in the Ussing set-up. However, the permeability of all compounds is much faster in the S-G cells, which can be attributed to the much higher area to volume ratio in these cells compared to the Ussing chambers (i.e. 0.49 vs 0.06 cm, respectively). The absence of a voltage clamp in the S-G cells can not be regarded as an influential factor, since these substances are hardly charged at this pH (11). The rank order of the compounds tested shows a significant correlation (Spearman rank order correlation coefficient is 0.833 at $p = 0.01$). It can be concluded that both cells give comparable results. The higher permeability of ACV compared to the other compounds in Ussing chambers goes with a small but significant increase in fluorescein permeability, but only in this diffusion cell (Table I); this might be attributed to some experimental scatter, and not regarded as an intrinsic influence of ACV on membrane level -TEER values did not change with these experiments.

Comparison of the Papp with the lipophilicity (logP n-octanol/water) of the tested compounds is showing a decrease of permeability with increasing logP values (Fig. 2). This is not in agreement with the regularly accepted rule that permeability of compounds are positively correlated with the lipophilicity, at least for compounds transported passively. This positive relation was e.g. shown for a homologous series of 6-fluoroquinolones with logP values ranging from -1.5 to 3.2 (12). In contrast, some others reported an inverse relation between transport and lipophilicity (13), which was explained by interaction of the compounds with the mucus barrier.

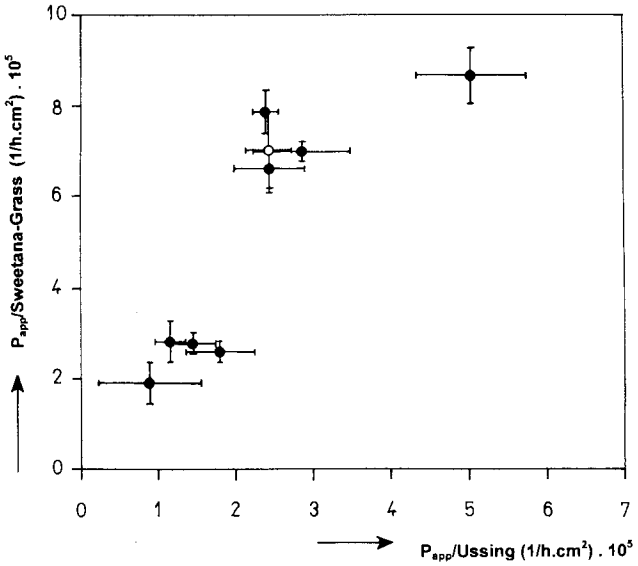

Fig. 1. Correlation of Papp values (mean ± SEM) obtained in Ussing and in S.-G. cells. The average of the permeabilities of fluorescein from all experiments is also indicated (opened symbol).

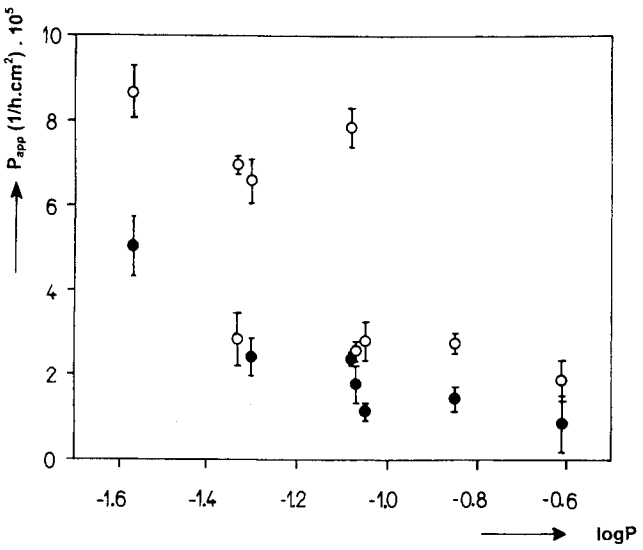

Fig. 2. The relationship between logP and Papp is given. The points represent the experimental data for Papp (mean ± SEM) for m-to-s flux determined in Ussing chambers (closed symbol) and in S.-G. cells (opened symbol).

In this respect an interesting result can be derived from the permeability data and the graph of the permeabilities in Fig. 1. In this graph also the average of the permeabilities of fluorescein from all experiments has been indicated (Fig. 1: open symbol). While the parent compounds and the N-acetylamides are permeating at a rate very similar to fluorescein (apart from the above mentioned faster permeation of ACV in the Ussing set-up), in contrast the ester-derivatives are permeating at a slower rate, and even more pronounced in the S-G cells. No explanation can be given for this phenomenon so far.

It is generally accepted that transport markers like fluorescein and mannitol permeate through the intestinal tissue predominantly, if not completely, via the paracellular pathway (8,14). It was hypothesized for mannitol that despite the paracellular permeation, solvent drag through this route is accounting for a large fraction absorbed *in vivo* (14). Solvent drag could thus result in rather high bioavailability of the substances transported passively. This might support the contradiction between the extreme low permeation of ACV and DCV through tissue *in vitro,* while the absorption *in vivo,* especially for DCV, is in the higher range. The very low permeability of all compounds tested, similar as or even slower than fluorescein, is suggesting that these compounds are permeating *in vitro* through the paracellular route only.

The primary aim of this investigation was to find an explanation on the intestinal absorption level for the increased bioavailability of DCV compared to ACV using an *in vitro* permeability testing approach. Based on the results presented here we can however not derive any explanatory conclusion for that phenomenon. DCV is permeating at a slower rate through intestinal tissue compared to ACV.

REFERENCES

1. O. L. Laskin. Clinical pharmacokinetics of acyclovir. *Clin. Pharmacok.* **8**:187–201 (1983).
2. G. Darly. Acyclovir and beyond. *J. Int. Med. Res.* **22**(Suppl 1):33A–42A (1994).
3. P. J. Rees, P. Selby, H. G. Prentice, P. D. Whiteman, and D. M. Grant. A 515 U: a prodrug of acyclovir with increased oral bioavailability. *J. Antimicrob. Chemother.* **18**(Suppl. B):215–222 (1986).
4. A. Kristl and G. Vesnaver. Thermodynamic investigation of the effect of octanol-water mutual miscibility on the partitioning and solubility of some guanine derivatives. *J. Chem. Soc. Faraday Trans.* **91**:995–998 (1995).
5. H. H. Ussing and K. Zehran. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta. Physiol. Scand.* **23**:110–127 (1951).
6. G. M. Grass and S. A. Sweetana. *In vitro* measurement of gastrointestinal tissue permeability using a new diffusion cell. *Pharm. Res.* **5**:372–376 (1988).
7. A. Štimac and J. Kobe. A new synthesis of acyclovir prodrugs. N^2-acetylacyclovir and 6-deoxyacyclovir. *Synthesis* 461–464 (1990).
8. P. W. Swaan and J. J. Tukker. Carrier-mediated transport mechanism of foscarnet (trisodium phosphonoformate hexahydrate) in rat intestinal tissue. *J. Pharmacol. Exp. Ther.* **272**:242–247 (1995).
9. W. B. Mahony, B. A. Domin, R. T. McConnell, and T. P. Zimmerman. Acyclovir transport into human erythrocytes. *J. Biol. Chem.* **263**:9285–9291 (1988).
10. K. C. Meadows and J. B. Dressman. Mechanism of acyclovir uptake in rat jejunum. *Pharm. Res.* **7**:299–303 (1990).
11. A. Kristl, A. Mrhar, and F. Kozjek. The ionization properties of acyclovir and deoxyacyclovir. *Int. J. Pharm.* **99**:79–82 (1993).
12. V. Merino, J. Freixas, M. del Val Bermejo, T. M. Garrignes, J. Moreno, and J. M. Pla-Delfina. Biophysical models as an approach to study passive absorption in drug development: 6-fluoroquinolones. *J. Pharm. Sci.* **84**:777–782 (1995).
13. I. Matthes, F. Nimmerfall, J. Vonderscher, und H. Sucker. Mucusmodelle zur Untersuchung von intestinalen Absorptionsmechanismen, 4. Mitteilung: Vergleich des Mucusmodells mit Absorptionsmodellen *in vivo* und *in situ* zur Vorhersage intestinaler Wirkstoffabsorption. *Pharmazie* **47**:787–791 (1992).
14. P. Krugliak, D. Hollander, C. C. Schlaepfer, H. Nguyen, and T. Y. Ma. Mechanisms and sites of mannitol permeability of small and large intestine in the rat. *Dig. Dis. Sci.* **39**:796– 801 (1994).