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RESEARCH PAPER

Comparative Permeability of Some Acyclovir Derivatives Through Native Mucus and Crude Mucin Dispersions

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ABSTRACT

The permeability of some guanine derivatives (acyclovir [ACV], deoxyacyclovir [DCV], and their N*-acetyl congeners) through native porcine mucus and crude porcine mucin dispersions (30% and 50% w/v) was investigated in two-compartment dialysis cells. High correlation between apparent permeability coefficients* P_{app} *of tested substances determined in these two models was observed, although the examined compounds permeated faster through the native mucus. It was also established that* P_{app} *values decrease with increasing hydrophilicity and molecular mass of the tested substances. Furthermore, the influence of some substances that affect mucus structure (cysteine,* N*-acetylcysteine [NCY], sodium taurocholate [ST], and sodium chloride) on the permeation rate of the examined compounds through mucus and mucin dispersions was examined. It was shown that the* P_{app} *values of guanine derivatives were generally lower after the addition of these substances to the native mucus and mucin dispersions, although the lowering effect was more pronounced in the case of native mucus.*

Key Words: *Acyclovir; Apparent permeability coefficient; Mucin; Mucolysis; Porcine gastric mucus*

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INTRODUCTION

Mucus forms a continuous viscoelastic and water-insoluble gel layer on the gastrointestinal cell surface, where its primary function is acting as a lubricant and a protective barrier against harmful agents, such as hydrogen ions and pepsin (1). Second, the gastrointestinal tract (GIT) mucus layer acts as a barrier to the diffusion and/or absorption of various drug molecules, although there are some disagreements about which components of mucus are responsible for the barrier properties (2–7). Recent studies (5,7) indicate that the most important is the distribution of drugs to the hydrophobic areas within the mucus since it was established that high lipophilicity correlates with low diffusion of drugs through pig intestinal mucus. Moreover, it was found that the purified (delipidized) pig gastric mucin gives limited information about drug-mucus interaction (7).

The primary aim of this study was to compare the permeability of some guanine derivatives (acyclovir [ACV], deoxyacyclovir [DCV], and their *N*-acetyl derivatives NAcACV and NAcDCV) through native pig gastric mucus and through the aqueous dispersions of crude pig gastric mucin (CPGM), which was evaluated as a potential model for native mucus. In addition, the effect of some agents that affect mucus structure (i.e., cysteine, *N*-acetylcysteine [NCY], sodium chloride, and sodium taurocholate [ST]) (3,8) on the permeability of tested guanine derivatives through native porcine mucus and CPGM dispersions also was investigated. Finally, an attempt was made to clarify the discrepancy between in vitro and in vivo data for ACV and DCV. It was reported (9) that DCV permeates slower than ACV through rat jejunum in side-by-side diffusion cells, although DCV exhibits much higher oral bioavailability in humans ($>75\%$) compared to ACV (15%–20%) (10).

EXPERIMENTAL

Materials

Acyclovir, deoxyacyclovir, and their *N*-acetyl congeners were synthesized at the National Institute of Chemistry, Ljubljana, Slovenia (11); the purity determined by high-performance liquid chromatography (HPLC) was more than 99%. The fluorescent marker, disodium fluorescein, was purchased from Fluka. Crude pig gastric mucin (type II), L-cysteine (CY), NCY, and ST were obtained from Sigma (St. Louis, MO).

Permeability Experiments

Porcine gastric mucus was obtained from an abattoir using healthy animals immediately after slaughter. The stomachs were split open; the luminal surface was washed with water; and the gel was collected by gently scraping the mucosal surface with a metal scoop. Pooled mucus scrapings (ca. 100 g) from 4–6 stomachs were stored at 4°C until the permeation studies (not longer than 24 h). The 30% and 50% (w/v) dispersions of CPGM were prepared in the same manner as described previously (12).

Permeability experiments carried out at room temperature ($23^{\circ}C \pm 2^{\circ}C$) were performed in equilibrium dialysis cells (Scienceware, USA) consisting of two half chambers (volume of each half chamber was 1 cm^3) separated by a dialysis cellulose membrane (exposed area was 2.01 cm^2) with a cutoff of 6000–8000 (Spectra/Por, molecular porous membrane tubing, Thomas Scientific, USA). The donor compartment was filled with an investigated dispersion (native mucus, 30% or 50% w/v dispersions of CPGM), while the acceptor compartment was filled with phosphate buffer (pH 6.7). In the case of reference experiments, both compartments were filled with phosphate buffer (pH 6.7) only. The initial concentrations of tested guanine derivative and the fluorescent transport marker (fluorescein) in the donor compartment were 0.5 mM and 5 μM, respectively. When necessary, the substance that affects mucus structure was added to the donor compartment in the concentration of 0.74 M [similar high concentrations were used previously in the rheological investigations of mucus and mucin dispersions (8) and showed a very pronounced effect on viscosity]. At 20-min intervals up to 80 min, 100-μl samples were taken from the acceptor compartment and replaced by the same volume of phosphate buffer (pH 6.7).

It was also established by monitoring the concentration of examined substances in the solution bathing the dialysis cells and cellulose membrane without native mucus or CPGM dispersions that there was no binding of examined substances to the cellulose membrane or to the dialysis cells. No change of concentration was observed for at least 2 h.

Because of the membrane high cutoff, the osmotic pressure cannot influence the diffusion of tested compounds; as well, the Donnan equilibrium, which might be established because of huge mucin molecules, could not affect the permeability of guanine derivatives since these compounds are not ionized at experimental pH.

Analytical Procedures

The samples taken from the acceptor compartment were assayed by HPLC analysis using a Nucleosil C-8 (5 μm, 250 × 4 mm, id) and a mobile phase composed of phosphate buffer (pH 7.9, $\mu = 0.05$), methanol, and acetonitrile at different proportions, depending on the substance analyzed. The detection was by ultraviolet absorption for the guanine derivatives and fluorescence for fluorescein.

Analysis of Transport Data and Statistic

The in vitro apparent permeability coefficient P_{app} for transport of the substance from donor to acceptor compartment was calculated according to the following equation:

$$P_{app} = \frac{dC}{dt} \cdot \frac{V}{C_0 \cdot A}$$

where dC/dt is the change in concentration per unit time in the acceptor compartment, V is the volume of acceptor compartment, A is the exposed surface area, and C_0 is the initial concentration of the diffusing molecule.

Means plus or minus standard deviation are provided throughout, and the differences (t test) were considered significant at the $P < .05$ level.

RESULTS AND DISCUSSION

The CPGM is commercially available and was tested as a possible alternative to the native mucus for permeation studies. The experiments were performed in the equilibrium dialysis cells similar to those used by Matthes et al. (4) for studies on drug-mucus interaction. CPGM cannot reconstitute the gel structure at the same concentration as native mucus (5% w/v) (12). Therefore, 30% and 50% (w/v) dispersions of CPGM were prepared.

It was shown previously that 30% CPGM dispersion has similar rigidity properties to the native porcine gastric mucus, although at this concentration, no gel is formed. The 50% CPGM dispersion is much more rigid than native porcine gastric mucus, and it forms a gel similar to that of native mucus, although not as strong (12). The results in Table 1 show that all P_{app} values obtained for the permeation of the tested substances through CPGM dispersions are lower than those obtained for native mucus, indicating that the interactions of these molecules with CPGM are much more pronounced than those with native mucus. In addition, the higher complex viscosity of 30% and 50% CPGM dispersions compared to the native mucus (8) goes well with the lower permeability of tested compounds in the case of CPGM dispersions. However, one can observe good correlation between the P_{app} values obtained in native mucus and the CPGM model (Pearson coefficient $r = 0.99$) (Fig. 1).

The P_{app} values obtained under control conditions (buffer solution in both compartments) and with native mucus differ significantly only for ACV ($P < .05$) and even more in the case of the marker fluorescein ($P < .0001$), while for DCV, NAcACV, and NAcDCV, we did not find any statistical differences (Table 1). This suggests that mucus represents an absorption barrier only for ACV and fluorescein in the case of the tested compounds. The marker fluorescein, which permeated through the native mucus at the lowest rate, is added to the donor compartment in the form of disodium salt, and ACV is the most hydrophilic substance (it has the lowest $\log P$ value, -1.57) among the tested guanine derivatives. Mucus/mucin interactions with polar/ionized groups of tested molecules are also supported with positive correlation between $\log P$ (octanol/water) and P_{app} values in the case of native mucus ($r = 0.91$), as well as in the case of CPGM ($r = 0.89$ and $r = 0.90$ for 30% and 50% w/v dispersions, respectively) (Table 1). On the other hand, P_{app} values for the permeation of tested substances through native mucus and CPGM dispersions decreased with the molecular weight of the substances.

The influence of some substances (which affect mucus structure) on the permeation of tested guanine derivatives through native mucus and CPGM dispersions was also studied. Cysteine and *N*-acetylcysteine act as mucolytic substances, and they degrade the disulfide bridges between the glycoprotein subunits. This leads to the reduction of viscoelastic properties of mucus (3,8,14). One can see that almost all P_{app} values of tested substances

Table 1

Average Values of P_{app} ($\cdot 10^{-5}$cm/s) *with Standard Deviations for the Permeability of Tested Guanine Derivatives and Fluorescein Through Native Mucus, 30% and 50% (w/v) (CPGM) Dispersions without (−) and with Addition of the Substances That Affect Mucus Structure to the Donor Side*

				Native Mucus					30% CPGM					50% CPGM				
Substance	M_w	log *P*	Buffer	—	CY	NCY	ST	NaCl	—	CY	NCY	ST	NaCl	—	CY	NCY	ST	NaCl
ACV	225	−1.57	1.87 ± 0.12	1.65 ± .05	1.45 ± 0.07[a]	1.10 ± 0.13[a]	0.64 ± 0.06[a]	1.57 ± 0.06[a]	0.92 ± 0.06	0.75 ± 0.04[a]	0.84 ± 0.04	0.65 ± 0.03[a]	0.76 ± 0.04[a]	0.65 ± 0.01	0.49 ± 0.05[a]	0.52 ± 0.03[a]	0.43 ± 0.02[a]	0.50 ± 0.01[a]
NAcDCV	251	−1.33	1.65 ± 0.09	1.67 ± 0.05	1.62 ± 0.06	1.38 ± 0.05[a]	0.73 ± 0.02[a]	1.55 ± 0.07[a]	0.93 ± 0.02	0.91 ± 0.02	n.d.	n.d.	0.96 ± 0.04	0.72 ± 0.04	0.84 ± 0.12	n.d.	n.d.	0.74 ± 0.10
NAcACV	267	−1.30	1.74 ± 0.04	1.73 ± 0.10	1.63 ± 0.06	1.13 ± 0.11[a]	0.70 ± 0.04[a]	1.72 ± 0.05	1.04 ± 0.02	1.00 ± 0.02	0.67 ± 0.04[a]	0.39 ± 0.01[a]	1.08 ± 0.04	0.83 ± 0.05	0.83 ± 0.02	0.54 ± 0.07[a]	0.38 ± 0.05[a]	0.80 ± 0.01
DCV	209	−1.08	1.99 ± 0.08	1.87 ± 0.06	1.58 ± 0.08[a]	1.63 ± 0.11[a]	0.86 ± 0.04[a]	1.93 ± 0.04	1.18 ± 0.05	1.13 ± 0.01	1.10 ± 0.09	0.79 ± 0.01[a]	1.24 ± 0.04	0.86 ± 0.01	0.89 ± 0.05	0.83 ± 0.04	0.71 ± 0.02[a]	0.83 ± 0.01
Fluorescein	376	—	1.33 ± 0.08	0.60 ± 0.06	0.43 ± 0.06[a]	0.04 ± 0.01[a]	0.18 ± 0.03[a]	0.48 ± 0.08[a]	0.25 ± 0.06	0.23 ± 0.01	0.06 ± 0.04[a]	0.09 ± 0.02[a]	0.20 ± 0.02[a]	0.14 ± 0.04	0.16 ± 0.04	0.04 ± 0.01[a]	0.09 ± 0.03[a]	0.14 ± 0.05

CY, cysteine; NCY, *N*-acetylcysteine; ST, sodium taurocholate and NaCl; n.d., experiment not done.

P_{app} values for the reference experiments (buffer), log *P* (octanol/water) values, and molecular weights (M_w, g/mol) are also given (Ref. 13).

Number of experiments: 3–4 for tested guanine derivatives and 12–16 for fluorescein.

[a]P_{app} value after the addition of CY, NCY, ST, or NaCl to the native mucus or CPGM dispersion was significantly lower ($P < .05$) than the corresponding P_{app} value without the addition.

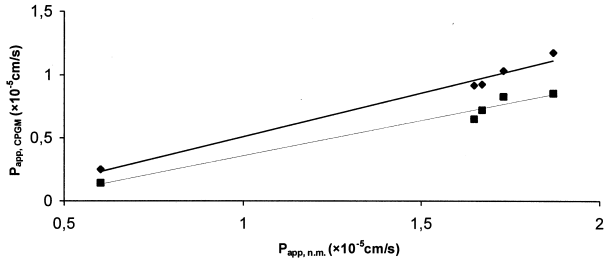

Figure 1. Correlation of P_{app} for CPGM 30% (◆, $r = 0.9914$) and CPGM 50% (■, $r = 0.9858$) with P_{app} for native mucus (values obtained from Table 1).

decreased significantly after the addition of cysteine or *N*-acetylcysteine to the native mucus, while for the CPGM dispersions, only a few values decreased (Table 1). The effect of these two mucolytic compounds is most probably based on the degradation of mucin macromolecules into smaller entities, and thus more interactions can take place between the permeated molecule and mucus. The degradation products of polymeric glycoproteins caused by cysteine or *N*-acetylcysteine in the donor compartment could be noticed also as additional peaks in HPLC chromatograms during analysis of the acceptor phase. Some degradation products are probably small enough and thus permeate through the dialysis membrane to the acceptor phase.

Sodium chloride and sodium taurocholate affect mucus structure by disrupting the ionic and secondary bonds between or within the carbohydrate chains (3). The action of sodium chloride results only in small changes in gel structure (15). Consequently, the smallest effects on P_{app} values were observed when this electrolyte was added to the native mucus and CPGM dispersions (Table 1). The most pronounced reduction of the permeability through native mucus and CPGM dispersions was observed after the addition of sodium taurocholate (Table 1). The explanation for this phenomenon could be that taurocholate and glycoproteins may compete with each other with respect to the binding of the diffusing compounds, resulting in a reduced permeation rate (16).

On the basis of the results obtained for the permeation of examined guanine derivatives through native mucus and CPGM dispersions, one cannot explain the above-mentioned discrepancy between in vitro and in vivo data for ACV and DCV permeability and bioavailability. However, the permeability experiments through mucin dispersions were designed in completely different ways than the permeability experiments through rat jejunum in vitro in a previous study (9), and therefore the results (P_{app} values) of both studies cannot be compared directly. On the other hand, the differences in P_{app} values for ACV and DCV determined in mucin dispersions are relatively small, indicating that the permeability of these substances through rat jejunum (i.e., mucosa) is the rate-limiting step rather than permeability through mucus.

CONCLUSION

Native porcine mucus and CPGM dispersions could offer relatively similar experimental conditions for the studied substances, but the values of P_{app} and the reduction of P_{app} values after the addition of substances that affect mucus structure in CPGM dispersions were in general lower than those for native mucus. Therefore, it might be questionable to replace native mucus with CPGM in the experiments when absolute values for P_{app} are needed. Because of stronger mucus/mucin interactions with more polar ACV and fluorescein, we suggest that native mucus represents a more rigorous absorption barrier for polar/ionized compounds than for nonpolar ones.

REFERENCES

1. Strous, G.J. Crit. Rev. Biochem. Mol. Biol. **1992**, *27*, 57.
2. Desai, M.A.; Mutlu, M.; Vadgama, P. Experientia **1992**, *48*, 22.
3. Marriot, C. Symp. Soc. Exp. Biol. **1989**, *43*, 163.
4. Matthes, I.; Nimmerfall, F.; Sucker, H. Pharmazie **1992**, *47*, 505.
5. Matthes, I.; Nimmerfall, F.; Sucker, H. Pharmazie **1992**, *47*, 609.
6. Matthes, I.; Nimmerfall, F.; Vonderscher, J.; Sucker, H. Pharmazie **1992**, *47*, 787.
7. Wikman Larhed, A.; Artursson, P.; Björk, E. Pharm. Res. **1998**, *15*, 66.
8. Kocevar-Nared, J. Master's thesis; University of Ljubljana: Slovenia, 1995.
9. Kristl, A.; Tukker, J.J. Pharm. Res. **1998**, *15*, 499.
10. Petty, B.G.; Whitely, R.J.; Liao, S.; Krasny, H.C.;

Rocco, L.E.; Davis, G.L.; Lietman, P.S. Antimicrob. Agents Chemother. **1987**, *31*, 1317.
11. Štimac, A.; Kobe, J. Synthesis **1990**, 461.
12. Kocevar-Nared, J.; Kristl, J.; Šmid-Korbar, J. Biomaterials **1997**, *18*, 677.
13. Kristl, A.; Vesnaver, G. J. Chem. Soc. Faraday Trans. **1995**, *91*, 995.
14. Livingstone, C.R.; Andrews, M.A.; Jenkins, S.M.; Marriot, C. J. Pharm. Pharmacol. **1990**, *42*, 78.
15. Bell, A.E.; Sellers, L.A.; Allen, A.; Cunliffe, W.J.; Morris, E.R.; Ross-Murphy, S.B. Gastroenterology **1985**, *88*, 269.
16. Poelma, F.G.J.; Tukker, J.J.; Crommelin, D.J.A. Acta Pharm. Technol. **1990**, *36*, 43.