Brief/Technical Note

Evaluation of Nanosuspensions for Absorption Enhancement of Poorly Soluble Drugs: *In Vitro* Transport Studies Across Intestinal Epithelial Monolayers

T. Lenhardt,¹ G. Vergnault,² P. Grenier,² D. Scherer,³ and P. Langguth^{1,4}

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INTRODUCTION

The absorption of drugs from the intestine is mainly dependent on their solubility in the intestinal fluids and their intestinal membrane permeability. Insufficient absorption of poorly soluble drugs is mainly due to slow dissolution and the generation of a small concentration gradient across the intestinal mucosa. To overcome these problems different formulations of nanosized drugs have been developed. Nanosuspensions are colloidal dispersions of solid drug particles in a liquid phase with average particle sizes below 1 μm stabilized by the use of surfactants (1).

Drug particle size reduction leads to an increase in surface area and consequently in the rate of dissolution as described by the Nernst–Brunner and Levich modification of the Noyes–Whitney equation (2–4). In addition, an increase in saturation solubility is postulated by particle size reduction due to an increased dissolution pressure explained by the Ostwald–Freundlich equation (5). Depending on the production technique applied changes in crystalline structure of drug particles may also occur (6). An increasing amount of amorphous drug fraction could induce higher saturation solubility. Furthermore, a general adhesiveness to tissues has been described for nanoparticles (7).

A well established model to study intestinal drug absorption is the Caco-2 cell monolayer system (8,9). Cell culture methods allow direct measurements of drug fluxes across an epithelium. In the present study the *in vitro* permeabilities of test substances formulated as nanosuspensions type DissoCubes® were tested on Caco-2 cell monolayers and were compared to saturated solutions prepared from micronized powders in the presence of the corresponding surfactant concentrations. The practically insoluble budesonide and spironolactone were selected for this investigation.

The aims of the present study were to evaluate whether providing the drug in the form of a nanosuspension may improve its epithelial transport. It was hypothesized that nanosuspensions will enhance drug flux resulting from higher transmembrane concentration gradients.

MATERIALS AND METHODS

Nanosuspensions

Budesonide and spironolactone nanosuspensions type DissoCubes® were manufactured by high-pressure piston gap homogenization (Emulsiflex®-C50, Avestin Inc, Ottawa, Canada). Budesonide nanosuspension consisted of 10% budesonide, 5% Octosol 449 (potassium oleate) and 85% water. Spironolactone nanosuspension type DissoCubes® consisted of 10% spironolactone, 0.5% Octowet70 PG (sodium dioctyl sulfosuccinate) and 89.5% water. The mean particle diameters in the nanosuspensions were 581 nm for budesonide and 515 nm for spironolactone, respectively (PCS, Zetasizer 3000 HS Malvern Instruments Ltd, UK).

Test Preparations

Test preparations for *in vitro* permeability studies were obtained by diluting nanosuspensions at ratios of 1:10, 1:30 and 1:100 with buffer and shaking until equilibrium was reached. The buffer consisted of Hanks' Balanced Salt Solution (HBSS, Biochrom KG, Berlin, Germany) supplemented with 25 mM 2 (*N*-morpholino)ethanesulfonic acid (MES, Merck, Darmstadt, Germany) adjusted to pH 6.5. For the test solutions an excess amount of micronized powder of each drug was shaken in HBSS/MES in the presence of the corresponding surfactant concentration until saturation concentration was reached. Separation of the solution from the sediment was performed by centrifugation at 4,500 $\times$ g for 15 min. As reference a saturated solution of drug without surfactant was used. Dilutions of budesonide nanosuspension resulted in surfactant concentrations of (a) 0.5%, (b) 0.17% and (c) 0.05% Octosol 449. Dilution of spironolactone nanosuspension resulted in surfactant concentrations of (a) 0.05%, (b) 0.017% and (c)

¹ Department of Biopharmaceutics and Pharmaceutical Technology, Johannes Gutenberg-University Mainz, 55099 Mainz, Germany.

² SkyePharma AG, Muttens, Switzerland.

³ ApisPharma AG, Laufen, Switzerland.

⁴ To whom correspondence should be addressed. (e-mail: langguth@mail.uni-mainz.de)

Table 1. P_{app} Values of Mannitol and Metoprolol as Integrity Markers During Permeation Experiments at Different Surfactant Concentrations of Budesonide Nanosuspension and Corresponding Saturated Solutions Derived from Micronized Material. (means $\pm$ SD; $n=3$)

	Octosol 449 conc. [%]	P_{app} mannitol [$\times 10^6$ cm/s]	P_{app} metoprolol [$\times 10^6$ cm/s]
Saturated solution (reference)	–	0.58 $\pm$ 0.14	15.0 $\pm$ 1.16
Saturated solution (a)	0.5	1.06 $\pm$ 0.25*	27.4 $\pm$ 0.47*
Saturated solution (b)	0.17	1.25 $\pm$ 0.27*	22.3 $\pm$ 0.49*
Saturated solution (c)	0.05	1.15 $\pm$ 0.32*	22.7 $\pm$ 0.3*
Nanosuspension 1:10 (a)	0.5	1.42 $\pm$ 0.17*	17.2 $\pm$ 0.1*
Nanosuspension 1:30 (b)	0.17	1.27 $\pm$ 0.16*	23.7 $\pm$ 0.51*
Nanosuspension 1:100 (c)	0.05	0.624 $\pm$ 0.02	14.0 $\pm$ 0.78

* $p < 0.05$ test versus reference

0.005% Octowet 70 PG. The same surfactant concentrations were adjusted for the saturated test solutions.

Drug Absorption Studies

Caco-2 cells (passage 33–41) were cultured for 21 to 25 days on 24 mm polycarbonate filter membranes 0.4 μ m pore size (TranswellTM, Corning Costar, Bodenheim, Germany). Samples from the receiver chamber were collected at 0, 30, 60, 90, 120 min and were analysed for the radiolabelled marker molecules by liquid scintillation counting (Beckmann Coulter, Unterschleissheim, Germany) and for budesonide or spironolactone by HPLC in accordance with published methods (10,11). TEER (transepithelial electrical resistance) measurements (Millicell ERS, Millipore, Bedford, USA) at the start and at the end of each experiment were conducted. As paracellular integrity marker mannitol (0.1 mM unlabeled and 1 μ Ci/ml ¹⁴C-mannitol) was used. In addition, metoprolol

(0.1 mM unlabeled and 1 μ Ci/ml ³H-metoprolol) was added as transcellular marker. The apparent permeability coefficients (P_{app}) were calculated according to Eq. (1),

$$P_{app} = \frac{F \times V}{A \times C_0} \left[\frac{\text{cm}}{\text{s}} \right] \quad (1)$$

where F is the flux rate of substance from the donor to the acceptor compartment given in concentration/time, V is the acceptor volume, A is the surface area of the filter membrane and C_0 the donor concentration of the substrate.

The fluxes of each drug were calculated from the slope of cumulative concentrations transported (dQ) versus time according to Eq. (2).

$$\text{flux} = \frac{dQ}{dt} \left[\frac{\mu\text{g}}{\text{ml}} \cdot \text{s}^{-1} \right] \quad (2)$$

Fig. 1. Fluxes [μ g/s] of budesonide nanosuspensions (NS) and saturated solutions (SS) with corresponding surfactant concentrations across Caco-2 cell monolayers, (means $\pm$ SD; $n=3$). Significant difference ($p < 0.05$) was observed between the fluxes from saturated solution vs. nanosuspension at all concentrations of surfactant

Table II. P_{app} Values of Mannitol and Metoprolol as Integrity Markers During Permeation Experiments at Different Surfactant Concentrations of Spironolactone Nanosuspension and Corresponding Saturated Solutions Derived from Micronized Material, (means $\pm$ SD; $n=3$)

	Octowet 70 PG conc. [%]	P_{app} mannitol [$\times 10^6$ cm/s]	P_{app} metoprolol [$\times 10^6$ cm/s]
Saturated solution (reference)	–	0.26 $\pm$ 0.006	19.7 $\pm$ 0.8
Saturated solution (a)	0.05	35.3 $\pm$ 0.96*	33.4 $\pm$ 2.0*
Saturated solution (b)	0.017	25.7 $\pm$ 1.76*	29.7 $\pm$ 2.6*
Saturated solution (c)	0.005	0.71 $\pm$ 0.08*	22.9 $\pm$ 0.06*
Nanosuspension 1:10 (a)	0.05	33.8 $\pm$ 0.93*	32.2 $\pm$ 1.5*
Nanosuspension 1:30 (b)	0.017	16.0 $\pm$ 0.03*	29.3 $\pm$ 0.8*
Nanosuspension 1:100 (c)	0.005	0.59 $\pm$ 0.08*	23.5 $\pm$ 0.9*

* $p < 0.05$ test versus reference

Data Analysis

Statistical significance was tested by two-tailed Student's t -test for differences between two groups and by one-way ANOVA for testing differences between means for more than two groups. Statistical significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

In the budesonide flux studies, the effect of nanosuspensions and saturated solutions on the integrity of cell monolayers was monitored by TEER measurement, mannitol and metoprolol permeability. TEER values decreased significantly during flux experiments, but were at least above $370 \Omega \text{ cm}^2$. As shown in Table I, P_{app} of mannitol as a paracellular marker, increased from $0.58 \times 10^{-6} \text{ cm/s}$ for the reference to $1.42 \times 10^{-6} \text{ cm/s}$ for the nanosuspension with the highest

surfactant concentration. Metoprolol permeability increased at maximum 1.8-fold and was still comparable to those values reported in the literature (12–14). These data suggest that the monolayers maintained their integrity during the flux studies.

The results of the budesonide fluxes across Caco-2 monolayers are summarized in Fig. 1. No significant differences in flux were found for the saturated solutions of budesonide in the presence of different surfactant concentrations. The flux of budesonide nanosuspension increased 2.2-fold from dilution 1:100 to 1:10. Based on comparison of the fluxes generated by the saturated solution and the nanosuspension, dilutions 1:100, 1:30 and 1:10, with Octosol 449 concentrations of 0.05%, 0.17% and 0.5%, respectively, caused significantly higher fluxes for the nanosuspension (1.3, 2.5 and 2.7-fold, respectively).

In the spironolactone permeation experiments, TEER values decreased strongly for both nanosuspension and saturated solution at concentrations of 0.05% and 0.017%

Fig. 2. Fluxes [µg/s] of spironolactone nanosuspensions and saturated solutions with corresponding surfactant concentration across Caco-2 cell monolayers, (means $\pm$ SD; $n=3$). Significant difference ($p < 0.05$) was observed between the fluxes from saturated solution vs. nanosuspension at all concentrations of surfactant

Octowet 70PG (TEER decrease by 88–93%). Mannitol permeability (Table II) increased from 0.26×10^{-6} cm/s for the reference solution without surfactant to 35.3×10^{-6} cm/s for the solution with 0.05% Octowet 70PG and 33.8×10^{-6} cm/s for nanosuspension with the same Octowet 70PG concentration, indicating an influence of high surfactant concentrations on paracellular integrity. The P_{app} values for the transcellular marker metoprolol, however, increased only by 1.7-fold at maximum. Thus it may be concluded that high surfactant concentrations resulted in an increase of mannitol permeability through opening of intercellular tight junctions, but had only a small effect on metoprolol permeability.

Spironolactone and budesonide are both BCS class II drugs with low water solubility and high permeability and will pass membranes by the transcellular pathway. Therefore, integrity for this pathway was postulated. Figure 2 shows the flux results of the spironolactone preparations. Only small differences in flux were found for the saturated solution of spironolactone in the presence of different surfactant concentrations. Significantly higher fluxes were observed for the nanosuspension. Comparing both formulations at each surfactant concentration 1.9, 2.4 and 4.9-fold higher fluxes were determined.

The higher fluxes for the budesonide and spironolactone nanosuspensions could arise from the higher saturation solubility for the nanosized drugs and/or by the larger contact area between drug and epithelial cell layer thus creating a steeper concentration gradient across the biological membrane. The exact mechanism(s) responsible for the present observations will need to be examined in future studies.

CONCLUSION

Higher fluxes were found for the nanosuspension formulations in comparison to saturated solutions of micronized material in the presence of corresponding surfactant concentrations. This factor may contribute to the higher bioavailability of nanosuspension formulations for poorly soluble compounds (15).

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