In vitro intestinal transport of a poorly aqueous soluble drug and efflux using biorelevant medium simulating the intestinal fluids

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INTRODUCTION

To enhance the physiological relevance of the Caco-2 cell culture model, biorelevant media (BM) simulating the intestinal fluids have been tested for compatibility with the model (1). Use of BM in the Caco-2 cell model has previously been shown to affect transport of drugs (2). Poorly aqueous soluble drugs in the intestinal fluids will participate between the aqueous phase and micelles (Fig. 1). However, the present knowledge on rate and mechanism of drug transport from the bulk into enterocytes is sparse. Further, components in the intestinal fluids may interfere with efflux transporteres in the cell membrane and thereby affect transport.

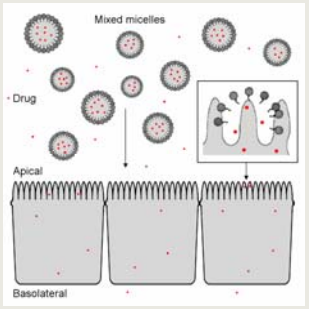

Fig. 1: Transport of drug (•) from aqueous phase and micelles into enterocytes.

AIM

The aim of this study was to assess the impact of BM simulating the intestinal fluids on the flux of a poorly water soluble model drug, estradiol, and on a substrate of efflux mechanisms, etoposide.

METHODS

BM contained sodium taurocholate (NaTC), lyso-phosphatidylcholine (lyso-PC), oleic acid (OA) and mono-olein (MO) (Table 1), in order to simulate the conditions in the upper small intestine. The colourless and CO_2-independent nutritional medium Leibovitz's L-15 (L-15) was chosen in order to increase cellular viability (1).

Table 1: Overview of media used in the transport studies.

Medium	Composition: Additives to L-15[1]	Application	Saturation solubility of estradiol[2] (µM) (mean ± SD)(n)	Hydrodynamic diameter[3] (nm) (mean ± SD) (n)
L-15 pH 6.0	20 mM MES	Apical	10.36 ± 0.86 (9)	-
BM- pH 6.0	5 mM NaTC, 1.25 mM lyso-PC, 20 mM MES	Apical	22.17 ± 1.70 (4)	4.85 ± 0.21 (15)
BM+ pH 6.0	5 mM NaTC, 1.25 mM lyso-PC, 0.5 mM OA, 0.25 mM MO, 20 mM MES	Apical	22.14 ± 1.19 (8)	5.38 ± 0.26 (16)
L-15 pH 7.4 + BSA	20 mM HEPES, 1 % (w/v) BSA	Basolateral	79.44 ± 1.28 (5)	-

[1]Leibovitz´s L-15 supplemented with penicillin (90 U/ml), streptomycin (90 µg/ml), glucose ad 4.5 g/L, and non-essential amino acids (L-15)
[2]Excess amount of estradiol was added to the media, rotated for 24 hours at 37°C. Samples were analysed by UV-HPLC.
[3]Particle size distribution (volume distribution) measured by dynamic light scattering (Zetasizer Nano, Malvern) at 37°C.

Transport of estradiol and etoposide

Passages 36-48 of Caco-2 cells were grown on Transwell inserts (pore size 0.4 µm) for 25-27 days. The viability of the cells was assessed by measurements of TEER. 0.5 ml BM or L-15 pH 6.0 was applied to the apical compartment and 1.0 ml L-15, pH 7.4 basolaterally.
Flux of estradiol was determined from A-B for 1 hour, using 4, 8 and 20 µM estradiol spiked with 0.1 µCi/ml ^{3}H-estradiol (45 or 150 rpm, 37°C). Flux (pmol/s x cm^2) was calculated at steady state appearance rate of estradiol in the receiver chamber (max. 20% of initial concentration).
Transport of etoposide was determined from A-B and B-A for 2½ hours, using 100 µM etoposide spiked with 1.0 µCi/ml ^{3}H-etoposide (60 rpm, 37°C). 0.5 µCi/ml ^{14}C-mannitol was added to control of monolayer integrity.

RESULTS

Estradiol

BM- and BM+ increased the solubility of estradiol by a factor 2 (Table 1). Fig. 2 shows that by decreasing the size of the unstirred water layer (agitation was increased from 45 to 150 rpm) the flux was increased and SD were decreased, too. Flux of estradiol decreased in the presence of the micelles and use of BM- or BM+ showed no differences (Fig. 3). Increasing the initial donor concentration of estradiol in BM by a factor of 2 or 5 resulted in approx. 2 and 5 times higher flux (Fig. 3), indicating that micelle solubilisation of estradiol improved solubility without further enhancement of transport. Apparently, the applied estradiol levels exerted no change in size distribution of micelles (Table 1), implying that the surface area of micelles remained constant.
In the present study, estradiol was quantified by scintillation counting, however the apical and basolateral media should be examined with respect to metabolites by use of other methods, e.g. LC-MS.

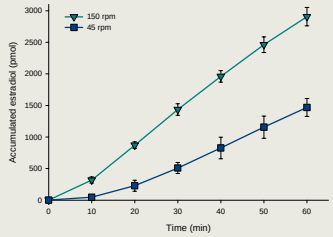

Fig. 2: Transport of estradiol across Caco-2 cells using 45 rpm or 150 rpm (mean ± SD, n=6). Initial donor concentration was 20 µM estradiol in BM+ (37°C).

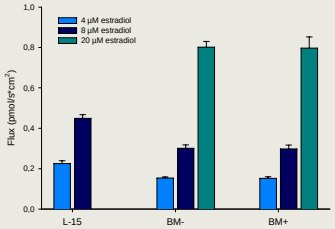

Fig. 3: Flux of estradiol using L-15, BM- or BM+ (mean ± SD, n=6-16). Initial donor concentration was 4, 8 or 20 µM estradiol (150 rpm, 37°C).

Etoposide

Permeability of etoposide was much lower from A to B that B to A (Fig. 4A). However, when BM- or 1:1 dilution of BM- were applied apically, a concentration dependent increase in etoposide transport was observed (Fig. 4A). The increase was not caused by decreased monolayer integrity since the permeability of mannitol was only slightly increased (Fig. 4B). Addition of OA and MO to BM+ did not further affect permeability of etoposide. These results suggest an inhibitory effect of compounds present in BM on efflux mechanisms.

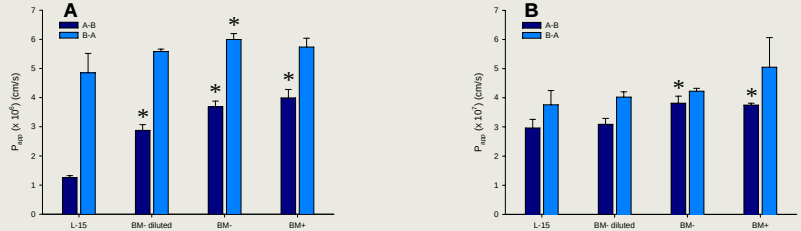

Fig. 4: Permeability of **A**: etoposide and **B**: mannitol from apical to basolateral or in opposite direction after application of different media in apical compartment (mean±SD, n=3) (60 rpm, 37°C).

CONCLUSION

The use of BM resulted in higher solubility and lower flux of a poorly soluble drug, estradiol, compared to L-15. Increasing the initial donor concentration of estradiol in BM from 4 to 20 µM, hence assuming an increased amount of drug in micelles, showed no impact on flux. Further, compounds present in BM inhibited the efflux mechanisms of etoposide.

References

(1) Lind, M.L. et al., unpublished data.
(2) Ingels, F. et al. 2002. Int. J. Pharm. 232, 183-192.

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