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Note

The prevention of cyclosporin A adsorption to Transwell® surfaces by human plasma

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Abstract

Prevention of cyclosporin A (CsA) adsorption onto the inner surface of Transwell® during transport experiments, by the addition of human plasma to the receiver compartment (basolateral side), was investigated. The addition of plasma to a level of 50% (v/v) of the transport medium led to a reduction in the adsorption of CsA (0.1 μM) down to a level of 5%. As a result, the apical to basolateral flux of CsA across the Caco-2 cell monolayer in the presence of 50% (v/v) plasma was estimated to be 2.7-fold higher than that obtained in the absence of plasma. Thus, the adsorption problem can be overcome simply by the addition of an appropriate volume of human plasma to the transport medium. This method appears to be applicable to the routine estimation of CsA flux across epithelial cell monolayers using Transwell®. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cyclosporin A; Adsorption; Transwell®; Human plasma; Caco-2 cell

The flux of xenobiotics including cyclosporin A (CsA), a powerful immunosuppressive agent (Kahan, 1989), across filter-grown monolayers of epithelial cells, such as Caco-2, is frequently measured using commercially available in vitro transport screening systems which involve the use of Transwell® (Coring Costar Corp., Cambridge, MA, USA) (Augustijns et al., 1993). In such experiments, however, the adsorption of xenobiotics onto the inner polystyrene surface of Transwell® often leads to a misinterpretation of the obtained data (Augustijns et al., 1993).

In order to examine how much CsA is adsorbed onto the inner surface of Transwell®, 1.5 ml of transport medium (TM, HEPES-buffered HBSS, pH 7.4) (Kahan, 1989) containing radiolabeled CsA (final CsA concentration of 0.1 and 0.5 μM, 0.02 μCi/ml each) was placed in the receiver compartment of Transwell® (with 1 cm^2, permeable polycarbonate inserts in 12-well plates, 0.4 μm pore size; Coring Costar Corp., Cambridge, MA,

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USA) followed by shaking at 37°C for 30 (for CsA 0.1 μM) or 90 min (for CsA 0.5 μM). 0.1 ml aliquots were withdrawn from the receiver side at predetermined time points, and the radioactivity remaining in the samples was measured by liquid scintillation counting (LSC). The loss of radioactivity compared with the initial radioactivity was regarded as the amount of CsA adsorbed to the wall of Transwell®. In fact, more than 95% of the radioactivity which was lost in 30 min after the addition of 0.1 μM CsA to the receiver side, for example, was recovered by rinsing the receiver compartment three times with 1.5 ml of 1 mM unlabeled CsA solution in ethyl alcohol.

The radioactivity in the receiver compartment decreased rapidly as a function of time yielding $78 \pm 3\%$ (mean $\pm$ S.D., $n = 6$) adsorption for 0.1 μM CsA in 30 min (Fig. 1). The adsorption for 0.5 μM CsA was somewhat smaller but still significant ,(i.e., approximately, 20 and 50% in 30 and 90 min, respectively, data not shown). These data indicate that adsorption is indeed a serious problem in transport experiments with Transwell® for micromolar levels of CsA.

To examine whether typically employed adsorption prevention techniques are applicable for CsA,

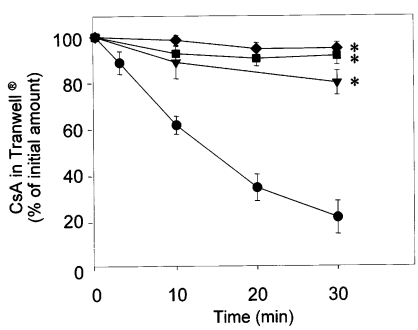

Fig. 1. Effect of human plasma (0, 5, 10, 50% v/v) on the adsorption of CsA to the inner surface of the receiver compartment of Transwell® from transport medium (HEPES-buffered HBSS, pH 7.4, 37°C) containing 0.1 μM CsA (0.02 μCi). CsA adsorption is expressed as the percent of the initial dose remaining (mean $\pm$ S.D. of three to six experiments) in the receiver compartment. (●), control (without plasma); (▼), in the presence of 5% (v/v) plasma; (■), in the presence of 10% (v/v) plasma; (◆), in the presence of 50% (v/v) plasma. (*), $P < 0.05$ from the control at 30 min, by ANOVA and Dunnet's multiple comparison.

the adsorption of the drug under the earlier reported prevention methods was assessed. Among these, the addition of PEGs, (i.e. PEG 400, 2000 or 6000) to the receiver side to levels of 0.5% (w/v) in TM (Horne, 1985) failed to significantly prevent the adsorption of 0.5 μM CsA (data not shown). Higher concentrations of PEGs have been reported to affect the permeability of poorly water-soluble drugs across epithelial cell monolayers (Saha and Kou, 2000), and thus, were not tested in the present study. Neither the addition of bovine serum albumin (BSA) to TM to levels of 0.175%, another reported method, significantly prevented the adsorption for the case of 0.5 μM CsA (less than 10%), which might be related to a weak binding of CsA with BSA (Bakhtiar and Stearns, 1995).

In the present study, we examined human plasma in terms of its potential to prevent the adsorption of CsA. This strategy appeared feasible because CsA is likely to bind with lipoproteins in the serum (Lemaire and Tillement, 1982). Human plasma was obtained from healthy Korean volunteers, and added to TM in the receiver compartment followed by an estimation of the adsorption of CsA. Contrary to the cases of PEGs and BSA, the adsorption of 0.1 μM CsA was dramatically reduced down to 15% of the initial dose in 30 min by the presence of 10% (v/v) human plasma in TM (Fig. 1). The prevention of adsorption became more significant as the concentration of human plasma in the TM was increased. For example, the adsorption was reduced to 5% when the plasma concentrations in the TM was increased to 50% (v/v). In other words, almost 95% of the CsA could be recovered from the TM in the presence of human plasma (50%). The recovery of CsA (0.1 μM) in the presence of 50% plasma was even higher than that achieved by rinsing the receiver compartment once with 1.5 ml of 1 mM unlabeled CsA in ethyl alcohol after incubation for 1 h, which achieved approximately an 87% recovery of the initial CsA ($n = 6$). In addition, the present method has an advantage over the rinsing method in that additional incubation for re-dissolution of the adsorbed CsA is not necessary.

The transport of CsA across a Caco-2 cell monolayer was then estimated in the presence and absence of adsorption prevention using the standard method (Li et al., 2001). Briefly, Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD), grown in 75 cm^2 tissue culture flasks at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity using Eagle's minimal essential medium supplemented with 10% fetal bovine serum (FBS) and 1% nonessential amino acids, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Before the experiments, the culture medium was removed, and the filter-grown Caco-2 cells were washed three times with warm TM [2]. After each wash, the plates were returned to the incubator for 30 min. Then, 0.5 ml of TM containing CsA (0.1 μM, 0.4 μCi) was added onto the apical side of the Transwell®, and the appearance of CsA in the receiver compartment (basolateral side), which contained 1.5 ml of TM in the presence or absence of plasma (50%), was determined at predetermined time points, (i.e. 30, 60, 90 and 120 min). In each experiment, three inserts were used for each treatment. The apparent permeability coefficient (P_{app}) of CsA was estimated from the apical-to-basolateral flux.

The P_{app} of CsA, as estimated in the presence of 50% (v/v) plasma in the receiver compartment (2.15×10^{-6} cm/s) was 2.9-fold larger ($n = 3$, $P < 0.05$ by Dunnet's multiple comparison) than that estimated in the absence of plasma (7.44×10^{-7} cm/s). This indicates that the adsorption of CsA onto the surface of Transwell® leads to substantial underestimation of the permeability of the drug. A significantly smaller P_{app} value (1.25×10^{-6} cm/s, $P < 0.05$, Dunnet's multiple comparison) compared with the present method (plasma addition) was obtained when the receiver compartment was rinsed once with 1.5 ml of ethyl alcohol containing 1mM cold CsA. The incomplete recovery of the drug especially from the lower surface of the insert in Transwell® might be responsible for the smaller P_{app} value obtained in the rinsing method. Thus, P_{app} of CsA should be estimated in the presence of plasma (50%) in the receiver compartment. In addition, the P_{app}, estimated in this experimental system, may be more physiologically relevant, since CsA, when transported across the intestinal epithelial layer, may rapidly bind to component(s), (e.g. lipoprotein) of the serum in vivo. In this study, we did not attempt to correct for the adsorption of CsA in the donor compartment, (i.e. the apical side), because the surface area which is in contact with the drug in the donor compartment was much smaller compared with that in the receiver compartment.

In conclusion, we suggest that addition of fresh human plasma to the receiver compartment represents an efficient procedure for overcoming adsorption problems, specifically the adsorption onto the inner surface of the receiver compartment, in estimating apical to basolateral transport of hydrophobic drugs, such as CsA in Transwell®. Extrapolation to other drugs and to plasmas from other species might be possible, but must be confirmed by experimental verification.

Acknowledgements

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