Bovine Serum Albumin in Caco-2 Permeability Testing and Effect on P_app Values Determined with Highly Protein Bound Compounds
Elke S. Perloff, Sudarshan Kapadnis, Andrew K. Mason, and David M. Stresser
Corning Incorporated, Life Sciences, Woburn, MA USA

Introduction

The apparent permeability (P_app) in Caco-2 cell monolayers is a routine aspect of many drug discovery programs. For many compounds, however, non-specific binding to the cells and/or filter plate assembly, possibly combined with limited solubility, results in substantially reduced compound recovery from the test system (<50%), and/or the lack of quantifiable concentrations in the receiver sample. To mitigate non-specific binding and improve solubility, the assay buffer is frequently supplemented with protein (e.g., bovine or human serum albumin, fetal bovine serum, calf serum, etc.), however, detailed protocols vary (protein added to both compartments, to the receiver compartment only, or to the basolateral compartment only) and limited systematic data is available comparing the impact of those varying approaches on P_app.

The present study determined the impact of adding 2% BSA to the assay buffer on P_app values for a set of compounds covering a wide range of plasma protein binding.

Methods

Bidirectional permeability in Caco-2 cell monolayers was determined for a set of 16 compounds covering a range of reported plasma protein binding and human intestinal absorption. Cell monolayers were set up in Falcon® 24-Multiwell 1 µm filter inserts and grown to confluence for 21 to 25 days. Membrane integrity was confirmed by TEER and Lucifer yellow A-B flux testing.

The assays were performed using four conditions: (1) protein-free assay buffer (HBSS with 10 mM HEPES, pH 7.4), (2) buffer supplemented with 2% bovine serum albumin in both compartments, (3) buffer with 2% BSA in the basolateral compartment only. Compounds were assayed at 10 µM and incubated for 90 minutes at 37ºC. Donor (0 and 90 min) and receiver samples (90 minutes) were quantified by LCMS/MS or liquid scintillation counting, and P_app values and efflux ratios were calculated and compared between conditions.

In addition, compound binding to human plasma and to 2% BSA in assay buffer was determined using the RED Device (Thermo Fisher Scientific) at 500 rpm for three hours at 37ºC.

Results

The degree of protein binding in assay buffer with 2% BSA was somewhat lower than that in human plasma (Figure 3).

The impact of 2% BSA in the Caco-2 assay buffer on A-to-B and B-to-A P_app varied widely for different compounds and conditions tested (Table 1).

P_app values for certain highly protein bound (>95% plasma protein binding) compounds (e.g., naproxen, warfarin) known to exhibit good apparent permeability in Caco-2 cells decreased substantially (>10-fold) in presence of BSA in both donor and receiver compartments, whereas effects on P_app values for compounds with moderate (verapamil, propranolol) or low (metoprolol, labetalol) protein binding were less pronounced (2-fold or less) (Table 1, Figure 1). As a result of the drastic change in P_app values, highly permeable compounds with high protein binding may be misclassified as low permeability compounds leading to underestimated intestinal absorption (Figure 1).

Altered P_app values in turn resulted in changes in efflux ratios which were most pronounced for highly protein bound efflux substrates (saline, furansulfone, sulfasalazine) (Table 2). In particular, presence of BSA in the basolateral compartment led to a decrease in efflux ratios, while presence of BSA in the receiver compartment only did not appear to affect the identification of efflux substrates.

Conclusions

Addition of protein to the assay buffer is a useful and frequently employed approach to mitigate non-specific binding, improve solubility, and/or better mimic physiological conditions in Caco-2 permeability screening assays. However, caution should be exercised when interpreting the resulting data as P_app values may be substantially altered depending on compound properties and the particular assay conditions used.

In particular when protein is added to the donor compartment (a useful approach to improve compound recovery), compounds exhibiting high plasma protein binding (95% or greater) may show substantially decreased (>10-fold) P_app values resulting in misclassification of high permeability compounds as low or moderate permeability.

Efflux ratios for highly protein bound efflux substrates were also altered. In particular, the presence of BSA in the basolateral compartment led to a decrease in efflux ratios.

Table 1. Protein Binding, Absorption, and Effect of 2% BSA on the Apparent Permeability of the Test Compounds

Table 2. Effect of 2% BSA on the Efflux Ratios of the Test Compounds

Figure 1. Effect of 2% BSA on the Relationship between Apparent Permeability and Human Absorption

Figure 2. Effect of 2% BSA on A-to-B P_app for Moderate/High P_app Compounds

Figure 3. Protein Binding in Human Plasma and Assay Buffer with 2% BSA

References


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