MDR1-MDCKII Permeability Assay

Background: Solubility, intestinal and CNS permeability are the most important factors determining oral absorption of drugs. MDR1-MDCKII is one of common in vitro models for a permeability assay and for identification of P-gp substrates and inhibitors. MDR1-MDCKII is a Madin-Darby canine kidney cell line transfected by human MDR1 gene, which encodes the efflux protein P-gp. The advantages of using this cell line are faster proliferation and formation of monolayer with tight junction which reduces the time required to conduct the in vitro transport studies. MDR1-MDCKII cells derive from MDCKII line (MDCKII is a subclone derived from the heterogeneous parent line MDCK) transfected with the human ABCB1 gene encoding the well characterized transmembrane drug efflux pump, P-glycoprotein (P-gp). Designed this way, MDR1-MDCKII became of a particular value to identify compounds with intestinal or blood barrier permeability, or select for P-gp substrates or inhibitors. For MDR1-MDCKII permeability assay, cells are grown on semi-permeable supports inside inserts in multi-well plates. The system is composed in the way that a semi-permeable support separates apical and basolateral compartments, as differentiated MDR1-MDCKII monolayer is asymmetrical. Therefore, this system enables measurements of drug transport in both directions (apical to basolateral or basolateral to apical/A-B and B-A), across the cell monolayer. After the selected incubation time point, the solutions of tested compounds and samples from appropriate compartments are collected and analyzed using LC-MS/ MS. Papp, the permeability coefficient which reflects the ability of a compound to penetrate cell monolayer, is calculated based on the compound concentrations measured. Transport assessment across the monolayer in both directions (A-B and B-A) enables to determine an efflux ratio which indicates whether a compound undergoes active efflux. A P-gp inhibitor, verapamil or cyclosporine A, can also be included into the assay settings to identify whether active transport is mediated by P-gp efflux pump. Both the MDR1-MDCKII and Caco-2 models produce comparable results with non P-gp substrates and compounds with low and medium permeability. However, MDR1-MDCKII model is generally considered to be a better tool to predict and classify compounds that are likely to pass through the blood–brain barrier, while Caco-2 model is the standard for predicting intestinal absorption.

Service Details: MDR1-MDCKII cell line (Sigma-Aldrich, cat.#MTOX1303) is cultured according to the supplier's recommendations. The MDR1-MDCKII assay is carried out in 24-well insert plates (Millipore). Prior to use, the integrity of MDR1- MDCKII monolayer is verified by transepithelial electrical resistance (TEER) measurements. The assay is performed by spiking a compound (in duplicates, at 10 μM) into the apical and/or basolateral compartments of the trans-well insert, and monitoring the appearance of this compound on the basolateral and/or apical side at a predetermined time point. Incubation time for tested compounds is 1.5 h; buffer pH is 7.4 (or 6.5 optionally) in the donor and 7.4 in the acceptor compartments. High and low permeability controls are run with every experimental batch to verify assay validity. Similar permeability assay using Caco-2 (human colorectal adenocarcinoma line) cell line is also available upon request.

Deliverable: Based on compound concentrations measured by LC-MS/MS, apparent permeability coefficient (P_{app}), reflecting the ability of a compound to penetrate cell monolayer, is calculated. Efflux ratios and P-gp substrate liability are determined if applicable. Full study report is provided.

Sample Submission: A minimal accurately weighable quantity of dry compound (2 μM) or 50 μL of 20 mM stock DMSO solution is required for this assay. For multiple assays, lesser amount of compound per assay may be sufficient. Brutto formulas are required for all studies involving MS detection.