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Original article

Comparison of MDCK-MDR1 and Caco-2 cell based permeability assays for anti-malarial drug screening and drug investigations

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ABSTRACT

Introduction: Malaria is a major health concern and affects over 300 million people a year. Accordingly, there is an urgent need for new efficacious anti-malarial drugs. A major challenge in developing new anti-malarial drugs is to design active molecules that have preferable drug-like characteristics. These "drug-like" characteristics include physiochemical properties that affect drug absorption, distribution, metabolism, and excretion (ADME). Compounds with poor ADME profiles will likely fail *in vivo* due to poor pharmacokinetics and/or other drug delivery related issues. There have been numerous assays developed in order to pre-screen compounds that would likely fail in further development due to poor absorption properties including PAMPA, Caco-2, and MDCK permeability assays. **Methods:** The use of cell-based permeability assays such as Caco-2 and MDCK serve as surrogate indicators of drug absorption and transport, with the two approaches often used interchangeably. We sought to evaluate both approaches in support of anti-malarial drug development. Accordingly, a comparison of both assays was conducted utilizing apparent permeability coefficient (P_{app}) values determined from liquid chromatography/tandem mass spectrometry (LC-MS) analyses. **Results:** Both Caco-2 and MDCK permeability assays produced similar P_{app} results for potential anti-malarial compounds with low and medium permeability. Differences were observed for compounds with high permeability and compounds that were P-gp substrates. Additionally, the utility of MDCK-MDR1 permeability measurements was demonstrated in probing the role of P-glycoprotein transport in Primaquine–Chloroquine drug–drug interactions in comparison with *in vivo* pharmacokinetic changes. **Discussion:** This study provides an in-depth comparison of the Caco-2 and MDCK-MDR1 cell based permeability assays and illustrates the utility of cell-based permeability assays in anti-malarial drug screening/development in regard to understanding transporter mediated changes in drug absorption/distribution.

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1. Introduction

Each year more than 300–500 million new cases of malaria are reported resulting in the death of over 2 million people annually (Na-Bangchang and Congpuong, 2007; Pimentel et al., 1998). Malaria's prevalence and worldwide health effects make it one of the most important parasitic infections to combat. The need for new anti-malarial drugs, which are effective against the various life cycle stages of the different *Plasmodium* species and against resistant strains, is growing. Anti-malarial activity is not the only parameter required for developing new efficacious drugs. Problems with physiochemical properties such as solubility, metabolic stability, and cell permeability can result in costly drug developmental failures if not screened out early in the development process. Fig. 1 illustrates a generalized testing strategy for anti-malarial drug discovery where physiochemical properties such as compound solubility, metabolic stability, and permeability are assayed early in the evaluation process to aid in the down selection of compounds that will likely fail in further development. Evaluation of permeability using cell-based assays can glean insight into potential issues with intestinal transport as well as the brain-uptake (Misfeldt, Hamamoto, & Pitelka, 1976). Both colorectal adenocarcinoma-2 (Caco-2) and MDCK (Madin–Darby canine kidney MDCK) assays have been successfully utilized in the study of drug transport; however there are differences between the two cell lines that are illustrated below.

Caco-2, a human adenocarcinoma cell line, has been widely used as intestinal drug absorption *in vitro* model (Artursson & Karlsson, 1991).

Abbreviations: MDCK, Madin–Darby canine kidney; MDR1, multi-drug resistance gene 1; Caco-2, colorectal adenocarcinoma; P-gp, P-glycoprotein; P_{app}, permeability coefficient; PAPMA, parallel artificial membrane permeability assay; LC-MS, liquid chromatography–mass spectrometry; CsA, cyclosporine A; ACN, acetonitrile; ADME, absorption–distribution–metabolism–excretion; PQ, primaquine; CQ, chloroquine carboxy; PQ, carboxy primaquine; PK, pharmacokinetic.

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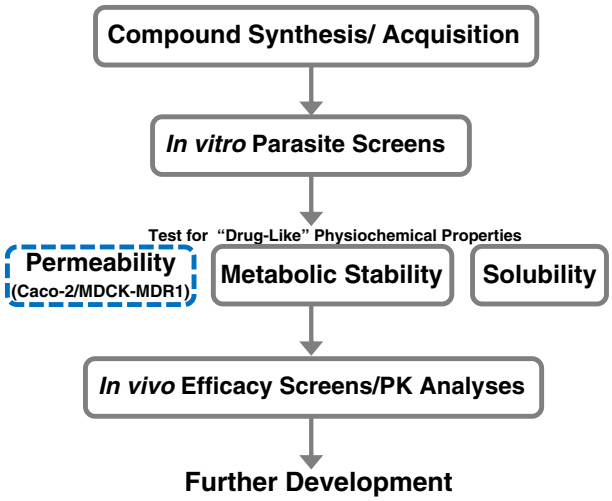

Fig. 1. Implementation of cell based permeability studies in anti-malarial drug development/screening. Permeability studies (highlighted with blue box) can be utilized early in the developmental life cycle of anti-malarial compounds to down select molecules that might have unfavorable intestinal transport/brain-uptake. Permeability studies can also be utilized to study effects of drug–transporter interactions.

Caco-2 monolayer membranes are routinely used in screening new drug candidates as drug transport across Caco-2 cell monolayers was shown to correlate with *in vivo* bioavailability and absorption (Artursson & Karlsson, 1991). Lennernas et al. also observed that drugs with rapid and complete passive transport in the Caco-2 cell model had comparable permeability with transport *in vivo* human jejunum (Lennernas et al., 1991). Caco-2, however, is not an ideal cell model for high throughput screening due to its slow growth and variable expression of transporters (Lennernas et al., 1991).

The other cell-based permeability assay utilizes MDCK cells. MDCK cells grow and differentiate quickly which significantly reduces the time required to complete *in vitro* transport studies. MDCK permeability based assays provide an attractive alternative to Caco-2 cells in terms of the increasing throughput, and reducing the time required to conduct transport studies of new anti-malarial compounds (Putnam, Pan, Tsutsui, Takahashi, & Benet, 2002). In addition to screening for and predicting passive permeability (Irvine et al., 1999), MDCK cells can be used to study drug efflux and active transport. MDCK-MDR1 transfected cells have been used to study efflux by P-glycoprotein (P-gp) (Horio et al., 1989), and it was suggested that the apical peptide transporter in MDCK behaved like intestinal PEPT1 transporter (Brandsch, Ganapathy, & Leibach, 1995). A study by Wang et al. also demonstrated that MDCK monolayers can be used to predict and classify compounds that were likely to pass through the blood–brain barrier based on the apparent permeability coefficients (A → B).

In the present study, the permeability of 28 anti-malarial compounds (from the 8-Aminoquinoline, 4-Aminoquinoline, and Guanidylimidazoline classes) was assayed using Caco-2 and MDCK-MDR1 cell-based permeability assays. Apparent permeability coefficient (P_{app}) values were determined for each compound based on results obtained from liquid chromatography/mass spectrometry (LC–MS) analyses. Additionally, the utility of MDCK-MDR1 permeability measurements is demonstrated in probing the role of P-glycoprotein transport in Primaquine pharmacokinetics and Primaquine–Chloroquine drug–drug interactions. This study provides a comparison of the Caco-2 and MDCK-MDR1 cell-based permeability assays for anti-malarial compounds and illustrates the usefulness of cell-based permeability assays in anti-malarial drug screening/development in regard to understanding transporter mediated changes in drug absorption/distribution.

2. Materials and methods

2.1. Materials and reagents

All tested compounds were obtained from the Walter Reed Army Institute of Research chemical repository (WRAIR) (Silver Spring, MD). The selection of reference compounds was based on availability of information with respect to the compounds' gut penetration. Caco-2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). MDCK (Madin–Darby Canine Kidney)-MDR1 (multi-drug resistance gene) cells were obtained from NIH (Bethesda, MD). DMEM (Dulbecco's modified eagle medium) with Glutamax, HBSS (Hank's Balanced Salt Solution), HEPPS (-[4-(2-Hydroxyethyl)-1-piperazinyl] propanesulfonic acid), 1 mM Trypsin-EDTA, and fetal bovine serum were purchased from GIBCO (Grand Island, NY). Penicillin–streptomycin solution stabilized 10,000 units penicillin & 10 mg/mL streptomycin, cyclosporine A (CsA), and Lucifer yellow CH di-lithium salt were purchased from Sigma (St Louis, MO).

2.2. Cell culture

Caco-2 (1×10^6 cells/mL) or MDCK-MDR1 cells (1×10^6 cells/mL) were cultured in DMEM with Glutamax supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. Caco-2 or MDCK cells were seeded onto cell culture inserts of 24-well plates (BD Biosciences, San Jose, California) at an approximate density of 12,000 cells/well for Caco-2 cells and 25,000 cells/well for MDCK-MDR1 cells. Plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2. For both Caco-2 and MDCK-MDR1 cells, the plate medium was replaced with fresh medium every other day and 24 h before use. Caco-2 cells were used for permeability studies 14 days post seeding, while MDCK cells were used 3–4 days post seeding.

2.3. Bidirectional permeability measurements

Bidirectional permeability assays were conducted according to the assay described in Wang et al. (2005). Briefly, after seeding of Caco-2 or MDCK-MDR1 membranes, the integrity of cell monolayers was assessed by measuring the transepithelial electrical resistance (TEER) using EVOM & EVOMX Epithelial Voltohmmeter (World Precision Instruments, Sarasota, FL). Medium from all apical and basolateral wells was replaced with 5 mM HEPES + HBSS solution and incubated for 10 min at 37 °C. Compound permeability was assayed by adding 10 μmol of compound in HEPES + HBSS solution to an apical insert for the apical to basolateral (A to B) transport or to a basolateral well for the basolateral to apical (B to A) transport. The treated cells were then returned to the incubator for 1 h at 37 °C. The assay was stopped after 1 h by separating apical insert from basolateral well. Samples were collected from both apical inserts and basolateral wells, and then analyzed using LC–MS. Membrane integrity was assayed using Lucifer yellow. To ensure the integrity of each membrane, Lucifer yellow solution (10 μM) was prepared in 5 mM HEPS + HBSS solution and added to apical inserts, while the basolateral wells were treated with plain 5 mM HEPS + HBSS solution. The cells were incubated for 30 min at 37 °C and then the transport was stopped by separating the apical inserts from the basolateral wells. The plate was placed into a fluorescent reader, and the fluorescence absorbance determined by using 485 nm excitation and 535 nm emission. Analyses were conducted in quadruplicate.

2.4. Bidirectional permeability measurements of anti-malarial compounds with or without P-gp inhibition

Permeability plates were prepared as described above. In order to study the effects of P-gp inhibition, the P-gp inhibitor cyclosporine A