The Effects of Media on Pharmaceutically Relevant Transporters in the Human HT-29 Adenocarcinoma Cell Line: Does Culture Media Need to be Controlled?

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ABSTRACT: The HT-29 cell line forms a confluent monolayer with tight junctions, but displays different phenotypes when cultured for 21 days in galactose-supplemented media (differentiated) versus glucose-supplemented media (dedifferentiated). This study is aimed at elucidating how media differences might affect selected drug transporter expression and peptide-based substrate transport toward reducing this variability. A vial of HT-29 cells was amplified and cultured over several passages in four different mediums (American Type Culture Collection recommended McCoy's 5A versus Dulbecco's modified Eagle's media containing glucose, galactose, or neither carbohydrate) with normal supplementation. Transporter mRNA expression was characterized at days 5 and 21 postseeding utilizing SABiosciences quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) drug transporter arrays. Transport studies using [H]histidine, [^{3}H]glycylsarcosine, [^{3}H]valacyclovir, and [^{3}H]carnosine were performed to assess the functional effects of oligopeptide transporter expression changes in HT-29 cells grown in each media. qRT-PCR arrays illustrated variable, media-dependent transporter expression between both the initial and differentiated time points. Permeability studies illustrated considerable media-dependent differences in both paracellular and transcellular substrate fluxes. The results demonstrate that these cells exhibit differing monolayer characteristics and genotypic/phenotypic profile properties when cultured under different media. The results suggest a need for standardization of culture methodologies for reducing inter- and intralaboratory variability. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:1616–1630, 2012

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INTRODUCTION

Optimizing predictive drug screening tools to improve lead candidate selection and optimization has the potential to result in a decrease in clinical compound attrition and is of considerable global interest in research laboratories.[1–4] Of particular interest to our laboratory has been the utilization of cell culture systems to delineate *in vitro* drug permeability across a physiologically relevant cell monolayer, in an attempt to correlate *in vitro* permeability with the drug's *in vivo* absorption, distribution, metabolism, and excretion (ADME) characteristics.[5,6] In response to growing concerns associated with cell variability, several studies have been conducted with the aim to improve and validate a variety of cell-based assay systems.[3,4,7–11] These studies were aimed at

Abbreviations used: ABC, ATP-binding cassette; ATCC, American Type Culture Collection; BLQ, below the limit of quantitation; ΔC_t, difference between the C_t levels of each gene in the treated versus control media; DMEM, Dulbecco's modified Eagle's media; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3 phosphate dehydrogenase; GI, gastrointestinal; HBSS, Hank's balanced salt solution; hPepT, human oligopeptide transporter; hPHT, human peptide/histidine transporter; MRP, mulidrug resistance-like protein; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; P-gp, P-glycoprotein (MDR1); POT, proton-dependent oligopeptide transporter; RP L13a, ribosomal protein L13a; SLC, solute carrier transporter.

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identifying the most important, often overlooked factors to be controlled to enhance method rigor, allowing for not only increased utilization of these high-throughput cell-based screening methods, but also more impactful and meaningful data. This is particularly true for assessing the role of pharmaceutically relevant drug transporters, for example, P-glycoprotein (P-gp, MDR1) or proton-dependent oligopeptide transporter (POT) 1 (PepT1), during lead candidate permeability selection and optimization.[7–10]

The primary goal of cell-based drug permeability screening is to develop predictive correlations between the *in vitro* results and human *in vivo* absorption.[2] Therefore, developing a better understanding of the critical characteristics that cellular models possess in relation to the human physiological barriers they mimic should ultimately enhance their predictive capability and efficiency for translation. There are a number of potential confounding factors that can alter the results from cell-based assays across laboratories.[3,11,12] In response to managing the variability, we have hypothesized that there should be a greater emphasis placed on identifying critical assay parameters that will minimize variability upon inter- and even intralaboratory comparisons. Moreover, better control of critical assay parameters will give researchers more mechanistic information about specific drug transport and metabolic pathways. This standardization will also potentially allow more meaningful interlaboratory comparisons.[13]

In predicting oral absorption, discovery scientists are interested in utilizing a cell line that grows and stably mimics the human gastrointestinal (GI) physiology upon differentiation. There are a number of critical factors, where slight changes in the culturing conditions may add confounding variability and these should be highlighted briefly.[3,11] For instance, Caco-2 cells are typically chosen because they exhibit morphological and biochemical characteristics resembling the absorptive enterocytes lining the small intestine of the GI tract after an extended period of culture.[3,14] However, the Caco-2 cell model also has several characteristics that limit their efficient predictability for human absorption. For example, a widespread issue when using cell culture models, for example, Caco-2 cells, is the interlaboratory variability with respect to the expression of transporters and their ability to accurately mimic human permeability for certain compounds.[3,15,16] The variability could be the result of many factors including differences in the serum and media used for culture, origin of cell acquisition, passage number,[17] cell seeding density, monolayer age (days cultured),[17] growth surface, and the addition of certain extracellular matrix and adhesive proteins that contribute to the growth and morphological variability.[14,18] This may lead to cellular phenotypic profile changes upon differentiation that are often overlooked without proper characterization of the system as a whole.[19,20] It is our laboratory's belief that standardized cell culture protocols may be critical, and that may allow better comparisons of data between laboratories and provide insights into where comparative approaches fail.

HT-29 cells represent an alternative cell line derived from a separate human adenocarcinoma, as initially described by Fogh and coworkers.[21] The HT-29 cell line has also been widely utilized to study nutrient and drug permeation, multidrug resistance, metabolism, and nutrient-induced enterocytic differentiation amongst other pharmaceutically relevant studies.[22] Other studies in the HT-29 cell line include investigating integrin expression, the role of insulin-like growth factors, and mechanisms of lipid metabolism.[22] One of the major differences between this cell line and the more widely utilized Caco-2 cell line is that the HT-29 cells can produce mucin at a relatively high level, which can act as a rate-determining barrier for drug absorption.[23–25] This is due in part because the parental HT-29 cells are considered to contain a more heterogeneous mixture of cells than the Caco-2 cell model that do not spontaneously differentiate under certain culture conditions. For example, it has been reported that the parental HT-29 cell line exhibits polarization and differentiation over a 21-day time course in glucose-free, galactose-supplemented media, whereas they undergo dedifferentiation in glucose-containing media.[26]

The studies described here focus on elucidating the differences in drug transporter expression and altered function that can occur when growing cells under the different recommended/suggested culture conditions. Specifically, we investigated the changes in passive paracellular transport, transport of small peptides, peptide-based drugs, and histidine, and selected solute carrier transporter (SLC) and ATP-binding cassette (ABC) transporter expression that occur when HT-29 cells are cultured in media [McCoy's 5A or Dulbecco's modified Eagle's media (DMEM)] supplemented with either galactose or glucose. The American Type Culture Collection (ATCC) recommends using McCoy's 5A media (3.0 g/L glucose) as opposed to the much more widely utilized DMEM media (4.5 g/L glucose), which may also contribute to variability across laboratories. In these studies, we have utilized the SABiosciences (Frederick, Maryland) quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) drug transporter array to determine differences in the mRNA expression of 84 pharmaceutically relevant transporters in HT-29 cells cultured utilizing the four different media conditions. We also held all other culturing conditions constant, including fetal bovine serum (FBS), endothelial growth factor concentrations, and lot numbers, throughout