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Variability in mRNA expression of ABC- and SLC-transporters in human intestinal cells: Comparison between human segments and Caco-2 cells

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ABSTRACT

The Caco-2 cell monolayer model is widely used as a tool for evaluating human intestinal permeability and interaction with transporters. Therefore, we determined mRNA levels for 15 of the most frequently studied uptake and efflux transporters (MDR1, MRP2–3, BCRP, OCTN2, PepT1, OATP-B, OATP8, OCT1–3, OAT1–3, MCT1) using real-time PCR in Caco-2 cells and in human jejunum and colon. The expression levels in the Caco-2 cells did not significantly vary between different passages (p29–43) and batches for any of the genes measured. However, levels increased with culture time (1–5 weeks) for PepT1, MDR1, MRP2, OATP-B and BCRP. The general rank order of the gene expression levels in Caco-2 cells was established as follows: MRP2 > OATP-B > PepT1 ≫ MDR1 > MCT1 ≈ MRP3 ≈ BCRP ≈ OCTN2 ≫ OCT3 > OCT1 > OAT2. Four genes were absent: OATP8, OCT2, OAT1, and OAT3. Ranking of 11 expressed genes showed a significant correlation between human jejunum and 2–5-week-old Caco-2 cells. The expression profile in colon was, however, very different compared to both Caco-2 cells and jejunum. We conclude that the Caco-2 cells in our hands express similar transporters as the human jejunum, but are different from colon, indicating their usefulness for obtaining small intestinal transport data. In addition, we also suggest that cells with a well-defined range of culture ages should be used to minimize variability in data from experiments and even erroneous conclusions.

1. Introduction

The intestinal epithelium is a semi-permeable membrane that allows molecules to pass via both passive diffusion and carrier mediated transport, facilitated by trans-membrane transporting proteins. Uptake transporters, e.g. PepT1, enhance the absorption of drugs into cells which can result in increased plasma level of the drug (Liang et al., 1995). Efflux transporters, e.g. MDR1, restrict the fraction absorbed of a drug by pumping the compound out of the intestinal cells into the lumen (Chan et al., 2004). Thus, transporter proteins may play an important role in the pharmacokinetic profile of drugs by controlling drug disposition.

Nowadays, the Caco-2 cell model plays an important role for intestinal permeability screening during discovery (Ungell and Karlsson, 2003). However, remarkable differences

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in the transepithelial electrical resistance, permeability coefficients and expression of enzymes and transporters have been reported between different laboratories as well as between different Caco-2 cell subclones (Artursson and Karlsson, 1991; Behrens et al., 2004; Kerns et al., 2004; Nakamura et al., 2002; Walter and Kissel, 1995). In addition, small changes, either in culture conditions, culture time, initial seeding density, or medium supplements may lead to significant differences in transporter expression (Anderle et al., 1998; Behrens and Kissel, 2003; Li et al., 2003). Therefore, cell cultures need to be carefully controlled to obtain reliable data.

The transporter properties can be identified by its functionality to transport substances and/or being inhibitable by specific inhibitors. Transporters can also be defined by use of Western blot analysis, i.e. their protein expression levels. These two techniques involve several experiments and utilisation of specific substrates, inhibitors or antibodies, which is time-consuming and often result in data, which in many cases is complex to interpret. A more sensitive and faster method is the quantification of the specific transporter mRNA expression. Presence or absence of mRNA expression levels of transporters in the screening model compared to the corresponding human organs is important basic knowledge, guiding which protein or functionality would be expected to contribute to drug transport.

Today, there are a broad variety of methods available to quantify mRNA expression levels, such as Northern blotting, in situ hybridisation, RNase protection assays, cDNA arrays, and real-time PCR (Giulietti et al., 2001). Among these methods, real-time PCR (TaqMan® analysis) has been reported to be the most sensitive and accurate of these quantification methods (Radonic et al., 2004; Wang and Brown, 1999). The method allows a comparison between expression levels of genes in different cell or tissue samples when an appropriate endogenous control gene is used.

Recently, many research groups have become engaged in the mapping of the gene expression in human intestinal cells (Caco-2 cells, duodenal enterocytes, colorectal tissue) and non-intestinal tissues (e.g. liver, kidney, and heart) (Anderle et al., 2004; Langmann et al., 2003; Nakamura et al., 2002; Pfrunder et al., 2003; Stephens et al., 2001; Sun et al., 2002; Taipalensuu et al., 2001). It is, however, difficult to compare expression values between laboratories unless the same endogenous control and technique are used or absolute quantitative levels of mRNA are presented (e.g. recombinantly produced). To our knowledge there is no complete report available comparing Caco-2 cells with both human jejunum and colon and also defining variability in gene expression by passage and age of Caco-2 cells.

The aim of the present study was to investigate the mRNA expression variability in Caco-2 cells of 15 of the most well known efflux and uptake transporters, and in two human intestinal segments, jejunum and colon. These data can support the suitability of Caco-2 cells as a human intestinal model expression system and allow quality assurance by the ability to define range of age and passage for best use of Caco-2 cells in transport experiments.

2. Materials and methods

2.1. Cell culture and tissue samples

Caco-2 cells were purchased from ATCC (Rockville, MD, USA) at passage 18. The cells were routinely sub-cultivated by trypsinization using trypsin (0.05%)–EDTA (0.02%) solution and seeded at a density of 2.0×10^6 cells per 175 cm^2 flask. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat inactivated foetal calf bovine serum, 1% non-essential amino acids and 1.5% L-glutamine, in an atmosphere of 95% air and 5% CO_2 at 37 °C. All tissue culture media were obtained from GIBCO, Life Technologies (Paisley, Scotland). From passage numbers 29, 32 (batch 2), 41, 43 (batch 1), monolayer cultures were grown on polycarbonate culture inserts. The cells were seeded at an initial density of 2.5×10^5 cells per 1.13 cm^2 filter with a pore size of 0.4 μm (Transwell®, Cat. No. 3401, Corning Costar Corporation, Cambridge, MA, USA). The medium was changed every second day. The medium for the plates contained antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). Total RNA was isolated from Caco-2 cells (passages 29, 32, 43) after 9, 16, 23, 30 and 38 days in culture on filters. Passage 41 was generally used 1 day earlier.

The human tissues were obtained upon written informed consent in agreement with local ethics committee. Jejunum tissue was obtained from Sahlgren's Hospital (Gothenburg, Sweden), from four healthy subjects of both genders (aged 49–79) undergoing gastric bypass surgery. Colon samples were from one male and one female subject, 57 and 69 years of age, after cancer resections at East Hospital (Gothenburg, Sweden). The mucosa was immediately scraped off the underlying tissue with a microscope glass, weighed and snap-frozen in liquid nitrogen before isolation of mucosal mRNA.

2.2. Preparation of RNA and cDNA synthesis

RNA was isolated using RNA STAT-60™ (Tel-Test Inc., Friendswood, TX, USA) and generally performed according to the product manual. For each Caco-2 monolayer sample, six filters were cut out from the inserts and mixed with 400 μl RNA STAT-60™. The scraped human intestinal tissues were homogenized in RNA STAT-60™ using a Polytron PT 1200 homogenizer (1 ml RNA STAT-60™ per 50–100 mg tissue).

The RNA preparations were treated with DNase (DNA-free™; Ambion Ltd., Huntingdon, Cambridgeshire, UK) and the quantity and purity of the RNA were determined spectrophotometrically using a GenQuant pro RNA/DNA calculator (Biochrom, Cambridge, UK). The RNA integrity was checked by the assessment of the sharpness of ribosomal RNA bands on a 1% agarose gel using a 1× TBE buffer (0.09 M Tris–borate, 0.002 M EDTA, pH 7.8).

cDNA was prepared from total RNA by using Superscript™ First-Strand Synthesis System for real-time PCR (Invitrogen Ltd., Paisly, UK) according to the manufacturer's protocol. The two-step reaction mixture contained 2 μg RNA, 100 ng random hexamers, 0.5 mM dNTP mix (dATP, dCTP, dGTP, dTTP), 10 mM Tris–HCl (pH 8.4), 25 mM KCl, 5 mM $MgCl_2$, 10 mM DTT, 40 units RNaseOUT™ recombinant ribonuclease inhibitor. The incu-