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International Journal of Pharmaceutics 297 (2005) 235–241
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Note

Validation of the 96 well Caco-2 cell culture model for high throughput permeability assessment of discovery compounds

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Received 10 December 2004; received in revised form 25 February 2005; accepted 8 March 2005
Available online 11 April 2005

Abstract

The use of Caco-2 cells for permeability screening of discovery compounds is quite well established and serves as the "method-of-choice" across the pharmaceutical industries worldwide. The typical permeability-screening model involves growing cells on a 12 well or 24 well transwell format. In this manuscript, we report the use of Caco-2 cells grown on 96 well transwell plates for screening of discovery compounds to assess their permeability characteristics. A set of standard compounds (marketed compounds) belonging to the various class of Biopharmaceutics Classification System (BCS) were used to assess the utility of the 96 well Caco-2 cells. Extensive validations were also performed with ∼160 Bristol-Myers Squibb (BMS) discovery compounds by comparing the permeability values in the 96 well Caco-2 cells with the in-house 24 well Caco-2 cells. Functional Caco-2 cells with intact monolayers could be established in the 96 well format using optimized seeding and culturing conditions. The permeability of BCS compounds in the 96 well format was found to be comparable to the permeability in 24 well format. Similarly, there was very good correlation ($R^2 = 0.93$) between the two formats for the extensive validation performed with in-house discovery compounds. The validated 96 well Caco-2 cell system presents a very attractive permeability screening tool that can perform much more efficiently than the conventional 12 well or 24 well systems while providing the same high quality permeability screening data.

Keywords: Permeability; Caco-2 cells; High throughput screening; Absorption; Transport; Drug discovery

Abbreviations: ADME, absorption, distribution, metabolism, elimination; BCS, biopharmaceutics classification system; BMS, Bristol-Myers Squibb Company; HBSS, Hank's balanced salt solution; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; IAM, immobilized artificial membrane; LSC, liquid scintillation counter; NCE, new chemical entity; PAMPA, parallel artificial membrane permeability assay; P_c, permeability coefficient; P-gp, P-glycoprotein; TEER, trans epithelial electrical resistance

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doi:10.1016/j.ijpharm.2005.03.008

In spite of many innovations in drug delivery methods, the oral route still remains the most desirable route of administration for new chemical entities (NCE). For a NCE to become a successful drug, there are a multitude of desirable characteristics it should possess: potency to a biological target, selectivity, good stability and physico-chemical properties, minimal toxicity and adequate ADME profile. Good permeability through intestinal membranes can lead to adequate systemic absorption that is a critically desirable property for NCE's. Different models are used in early discovery to screen the permeability properties of compounds (Artursson and Borchardt, 1997; Avdeef, 2001; Balimane et al., 2000; Hidalgo, 2001; Hillgren et al., 1995; Kerns, 2001). Commonly used models include cell based assays such as Caco-2, MDCK, LLC-PK1 cells; tissue based models such as ussing chamber, single pass perfusion; whole animal models such as in vivo screens; and even non-empirical in silico models (Artursson, 1991; Cho et al., 1989; Irvine et al., 1999; Tukker, 2000; Wessel et al., 1998). Some key characteristics desirable in a permeability screen in early drug discovery stage are: high efficiency, high accuracy, time, cost and space effectiveness and capability for high throughput.

Combinatorial chemistry and other advances in synthetic chemistry have led to a tremendous inflow of discovery compounds being fed into the screens for permeability assessment. Permeability-screening models have constantly been modified and improved to handle the deluge of compounds from chemistry laboratory. However, Caco-2 cells grown on 12 well or 24 well transwell plates have been the staple of the Pharmaceutical Industry for HT permeability screening of discovery compounds. Increasingly, a lot of companies have incorporated sophisticated levels of automation into these assays to make it amenable to higher throughput (Lenz et al., 1999; Russell et al., 1999). However, there is enough rationale for further miniaturization (i.e., 96 well Caco-2) that could help in streamlining these permeability assays (Alsenz and Haenel, 2003; Balimane et al., 2004). Apart from the obvious increase in throughput, miniaturization would lead to a tremendous cost reduction (decreased cost of media, plates, buffer, etc.). One additional key advantage that 96 well cells have over 12 well or 24 well set-up is the requirement of much less discovery compound to perform the same assay. This can be a significant advantage keeping in mind the hectic pace at which diverse chemotypes are synthesized in early stages of discovery. The use of 96 well Caco-2 cell system that can perform as well as the 12 well or 24 well Caco-2 cells can significantly increase the productivity of the cell based permeability assays. With adequate automation, the 96 well Caco-2 cell system can become an attractive and a valuable tool in early discovery. The article presents the validation data for the 96 well Caco-2 cells using marketed as well as discovery program compounds from active research projects within Bristol-Myers Squibb (BMS).

For this paper, Caco-2 cells were seeded onto filter membranes at a density of ~80,000 cells/cm^2 for both 24 well and 96 well Caco-2 cell plates. The cells were grown in culture medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 1% L-glutamine, 100 U/mL penicillin-G and 100 μg/mL streptomycin. The culture medium was replaced every 2 days and the cells were maintained at 37 °C, 95% relative humidity and 5% CO_2. Permeability studies were conducted with the monolayers cultured for approximately 21 days with the cell passage numbers between 22 and 40. Physiologically and morphologically well developed Caco-2 cell monolayers with TEER values greater than 400 Ω cm^2 were used for the studies reported in this manuscript. The formation of confluent Caco-2 cells with functional tight junctions in the 96 well transwells was confirmed by microscopy, TEER value measurements and permeability studies performed with mannitol (paracellular probe with low permeability). Direct visual inspection of Caco-2 cells under the microscope demonstrated uniform cell growth with no structural damage. Measurement of TEER values presented a quick confirmation of tight junction formation. Mannitol flux was monitored in the 96 well Caco-2 cells that had been cultured for 7, 14, 21, 28 and 38 days, respectively, post-seeding. Results from the growth study (Fig. 1) provided evidence of formation of intact tight junction as early as 14 days post-seeding with cell monolayers remaining intact for up to 28 days post-seeding. Low permeability values of mannitol ($P_c < 30$ nm/s) demonstrated that 96 well Caco-2 cells were optimum from 21 days to at least up to 28 days post-seeding.

For investigating the utility of 96 well Caco-2 cells, permeability studies were performed in parallel in both 96 well and the more conventional 24 well Caco-2