

3-Day Caco-2 Culture for In Vitro Intestinal Permeability Screening

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Results

- We have successfully developed a proprietary procedure for differentiated 3-day Caco-2 culture. The procedures are validated in our laboratory and in external laboratories.
- We have also developed procedures for the shipment of the cells (plated on Transwells for immediate use) worldwide without losing the desired differentiated properties.

Introduction

- Oral administration is the preferred route of drug delivery.
- Screening for intestinal permeability is therefore important for drug development.

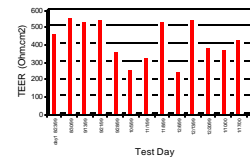
Caco-2 cells

- Derived from a human colon carcinoma.
- Known to differentiate into intestinal mucosal epithelium when cultured as confluent for an extended period (21 days).
- The 21-day culture is routinely used for screening of intestinal drug permeability.
- The 21-day culture is difficult to use due to the difficulty in scheduling for experiments and the increased probability of contamination during this prolonged culture period.
- We therefore embarked upon the development of a culturing procedure to allow Caco-2 cells to express intestinal mucosal epithelium properties in 3 days.

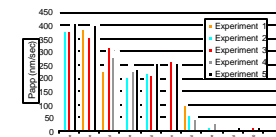
Probes for Transport Pathways

- Transcellular - Propranolol, Testosterone
- Paracellular - Mannitol, Inulin
- Carrier mediated - Glucose
- P-Glycoprotein mediated - Vinblastine inhibited by Verapamil

Caco-2 Assay Positive Controls Day to Day Variation



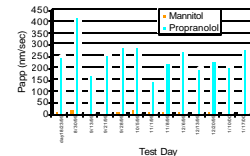
Caco-2 Assay Positive Controls FDA List (3H or 14C Labeled Compounds)



Caco-2 Electrical Resistance

Culture Age	Resistance Ohm/cm ²	Mannitol flux mm/sec
3 days	385	14
4 days	439	14
5 days	544	15
6 days	411	17
7 days	450	12

Caco-2 Assay Positive Controls Day to Day Variation

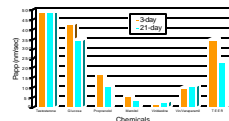


In Vivo - In Vitro Correlation

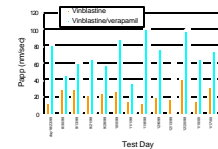
Drugs	Oral Absorption in Humans (%)	3-day Caco-2 Kp
Caffeine	100	227
Ibuprofen	100	261
Desipramine	95	261
Acetaminophen	95	218
Propranolol	90	265
Hydrazine	90	155
Ketoconazole	76	120
Terbutaline	73	56
Atenolol	50	30
Acetabutool	40	29
Nadolol	35	22
Losartan	33	42
Mannitol	16	40
Inulin	5	12

Chong, Dando & Morrison; Pharm. Res. 1997

Caco-2 Assay 3-day vs. 21-day Cultures



Caco-2 Assay PGP Controls Day to Day Variation



Conclusions

3-day Caco-2 Culture

- 3-Day Caco-2 intestinal permeability model developed.
- Little difference between the 3-day and the 21-day cultures, including Pgp expression.
- Robust assay with acceptable day-to-day variations demonstrated.
- Good correlation between in vivo absorption and 3-day Caco-2 results shown

3-Day Caco-2 assay can be used routinely for the screening of intestinal permeability