EVALUATION OF A CELL CULTURE SYSTEM TO STUDY MUCOSAL ABSORPTION OF DRUGS

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Cell culture systems reduce the need for animal experimentation, offer a reproducible, homogeneous population of cells for study, and can produce significant results with a small number of cells. The use of cultured epithelia to study drug transport has been reviewed by Handler (1983). The MDCK cell line has been proposed as a model cellular transport barrier (Cho et al 1989) and primary hamster buccal cells have been studied by Tavakoli-Saberi and Audus (1989a,b). The main difficulty is assessing the suitability and viability of the preparation.

The culture and characterisation of an oral mucosal cell line intended for the study of buccal drug absorption is here described. The cell line was derived from rat palatal epithelium as outlined by Jepsen (1974), and supplied by Dr A Jepsen (Royal Danish Dental School, Aarhus). The cells were grown at 32°C in an atmosphere of 5% CO_2 in air, in Minimum Essential Media with Earles salts, 5% foetal calf serum, 2mM 1-glutamine, 125IU/ml penicillin, 0.125µg/ml streptomycin and non-essential amino acids. Cells were cultured on Millicell HA and Transwell filters. Integrity of the cell layer was assessed using microscopy, electrical resistance (Epithelial Volt/Ohm meter), and a fluid phase permeability marker, poly ethylene glycol(PEG) m.w. 4000.

Fixed and stained filters were confluent after around 10 days, the cells appearing tightly packed under the light microscope. Examination of transverse sections by transmission electron microscopy (TEM) and the cell surface by scanning electron microscopy (SEM) was also carried out. TEMs at day 7 showed the cells to be mitotically active and beginning to form multilayers. At day 14 the filters were multilayered with other regions showing signs of cell vacuolation and cell death. The extracellular space was large, containing numerous spiny projections. SEMs of the cell surface at day 10 showed that the cells did not form a tight confluent monolayer. The filter could be seen between the cells at high power magnification. The spiny projections noted in the TEMs were clearly visible. Cell layer resistance increased, plateauing around day 10 at 115.8 +/- 16.7 ohm.cm^2, until day 14. PEG permeability decreased until day 8, reaching a minimum (around 10% transported by 240 minutes), between days 8-14.

Electrical resistance is a useful technique to follow the confluency of a cell layer, such that it remains intact for subsequent use in transport studies. Resistance indicates the bulk integrity of the cell layer and can be confirmed by fluid phase marker and microscopy studies. The cell line in this study demonstrated a peak resistance, however it did not form "tight multilayers". The extracellular space was wide, lacking membrane coating granules or lipid lamellae, which are believed to be the main permeability barrier in buccal mucosa (Siegel 1984). Thus the rat palatal cell line is not a suitable cell line for the study of buccal drug absorption.

Cho, M. et al (1989) Pharm. Res. 6: (1) 71-77
Handler, J.S. (1983) J. Exp. Biol. 106: 55-69
Jepsen, A. (1974) Scand. J. Dent. Res. 82: 144-146
Siegel, I. (1984) Permeability of oral mucosa. In: The structure and function of oral mucosa. Eds. Meyer, J. et al, Pergamon New York
Tavakoli-Saberi, M.R., Audus, K.L. (1989a) Int. J. Pharm. 56: 135-142
Tavakoli-Saberi, M.R., Audus, K.L. (1989b) Pharm. Res. 6: (2) 160-166