

Lecture: alternatives & education

Validation of an *ex vivo* human cervical tissue model for the local delivery of nucleic acid drugs

Udo Bock, Annette Amann, Eleonore Haltner

Across Barriers GmbH (Saarbruecken) (DE)

e-mail: u.bock@acrossbarriers.de

Subject: The human cervix is the point of origin of cervical cancer, the second most common female tumor entity worldwide. Every year about 230,000 women die from cervical cancer. The necessary cause of cervical cancer is the infection of the cervix with high risk types of human papilloma virus (HPV) (Walboomers et al., 1999). To address a local therapy with nucleic acid drugs it is important to understand the barrier properties of the target tissue to which the drug will be applied. The aim of this study was to establish and validate an *ex vivo* human cervical tissue model for delivery and permeation studies.

Material and Methods: Permeability studies were conducted using the static Franz cell system (Hiller et al., 2007). The cervical biopsies of an area of 0.125 cm² were inserted with the mucosal side oriented upwards. The transport experiments were performed at 37°C applying a pH gradient (donor pH 6.0/acceptor pH 7.4). 40 µM [¹⁴C]Mannitol and [³H]Propranolol were used. [¹⁴C]Dextran 10,000 was used at a concentration of 1.5 µg/ml without the addition of unlabelled Dextran 10,000 and 40 mg/ml FITC-Dextran were used. The diffusion studies were carried out over 24 hours. After a preincubation time of 15 minutes, the acceptor medium was sampled after 0.5, 1, 2, 4, 6, 18 and 24 h.

Results and Discussion: To determine the influence of the menopausal status of the investigated 34 patients (21 pre- and 13 postmenopausal) the Papp values were statistically evaluated for three subgroups of small molecules, hydrophilic molecules and larger molecules. These subgroups show nearly an equal distribution of patients of both menopausal status. The apparent permeability coefficients (Papp) of the various markers demonstrated that with increasing molecular weight the marker permeability decreases, an upper permeability limit between 10,000 to 20,000 Da, no significant intraindividual variability, because the Papp values were comparable among biopsies of the same patient a significant variation of the permeability among different patient samples. A continuous difference of one log value between the Papp of mannitol and dextran 4,000 make them suitable as an internal marker control pair for each biopsy. The Papp values of both markers across fresh and frozen tissue are comparable.

Conclusion: According to the presented data we concluded that the human cervical tissue model has been well characterized and is therefore suitable for delivery and permeation studies with a focus on nucleic acid drugs. To our knowledge the present studies are the first time investigating in a wide range a human *ex vivo* cervical model for permeability studies. The comprehensive aim was to use this *ex vivo* model to support preclinical lead optimization of locally acting medicaments at the cervical tissue. The validation of the *ex vivo* cervical model did not cover all ECVAM recommendations (Hartung et al., 2004) because our principle goal was not to establish a tissue model for the replacement of animal studies.

References

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