Chapter 16

In Vitro Nasal Models

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1. INTRODUCTION

Pharmaceutical companies are investing time and money in the development of alternatives to injectable formulations for the systemic delivery of therapeutics. Alternative delivery systems offer enormous market potential and increased patient compliance. The nasal route has proved effective and acceptable for several therapeutics. In fact, several biotechnology nasal products are currently in the U.S. market, including DDAVP (desmopressin acetate, Rhone-Poulenc Rorer), Synarel (narelin acetate, Syntex), Diapid (lypressin, Sandoz), and Syntocinon (oxytocin, Sandoz). Intranasal administration of therapeutics offers many advantages over other routes of administration. Therapeutics administered nasally avoid gastrointestinal degradation and first-pass metabolism associated with oral administration. In addition, the high vascularity of the nasal mucosa allows rapid absorption of some compounds. However, bioavailability of nasally administered therapeutics is normally low, making this route acceptable for only a few potent therapeutics.

The low bioavailability of nasally administered therapeutics is believed to result from both epithelial and protease barriers. An understanding of transport and metabolic properties of these barriers will provide information necessary to facilitate the development of successful nasal formulations for new therapeutic entities as well as for existing therapeutics currently delivered by alternative routes of administration. Several in vitro nasal models are currently being employed to study the transport and metabolic properties of the nasal mucosa.

The aim of this chapter is to provide methods to establish in vitro nasal models essential for investigating transport and metabolic properties of the nasal mucosa.
Models discussed include the excised nasal tissue model, isolated airway epithelial membranes, and nasal homogenates. Each model has its own benefits in studying transport and metabolism of potential therapeutics directed at the nasal mucosa as outlined in this chapter.

2. METHODS

2.1. Materials

Common reagents used for in vitro nasal model systems are as follows.

Bicarbonate-buffered Ringer solution (BBRS): 112 mM NaCl, 5.0 mM KCl, 1.2 mM CaCl$_2$·$\text{H}_2\text{O}$, 1.2 mM MgCl$_2$·$6\text{H}_2\text{O}$, 1.6 mM NaHPO$_4$, and 25 mM NaHCO$_4$. The BBRS is often oxygenated with 95% O$_2$/5% CO$_2$ and incubated at 37°C to maintain a pH of 7.4 (Wheatley et al., 1988).

Isotonic physiological buffer: A number of buffers are appropriate, such as phosphate-buffered saline (PBS), 0.1 M phosphate buffer, 0.9% NaCl solution, or KCl solution.

Mucosal bathing solution: BBRS containing 10 mM mannitol.

Serosal bathing solution: BBRS containing 10 mM glucose.

Membrane incubation buffer: 250 mM sucrose, 5 mM HEPES-Tris, 2 mM EDTA, and 1 mM dithiothreitol, pH 7.8.

Membrane homogenization buffer: 50 mM mannitol, 5 mM HEPES-Tris, 0.25 mM MgCl$_2$, and 1 mM dithiothreitol, pH 7.4.

Membrane isolation medium: 10 mM MgCl$_2$, 100 mM mannitol, and 5 mM HEPES-Tris, pH 7.4.

Alkaline phosphatase assay substrate (Sigma 104 phosphate substrate, Sigma Chemical Co., St. Louis, MO).

Alkaline phosphatase assay medium: 0.1 M Tris (pH 9.0), 2.5 mM MgCl$_2$, and 1% Triton X-100.

Glycine buffer: 133 mM glycine, 83 mM sodium carbonate, and 67 mM sodium chloride.

2.2. Excised Nasal Tissue Model

The utilization of excised nasal tissue mounted in Ussing chambers or modified Ussing chambers provides a rapid method to evaluate the permeability and the mechanisms of permeation of therapeutics across the nasal mucosa. In addition, the model provides a method to quantitate peptidase activity at the mucosal surface as well as to investigate the degradation of therapeutics directed at the nasal mucosa.