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The relationship between *in vivo* nasal drug clearance and *in vitro* nasal mucociliary clearance: Application to the prediction of nasal drug absorption

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ABSTRACT

Drug absorption after nasal application is dependent on drug clearance from the nasal cavity, which is determined by nasal mucociliary clearance (MC). We previously developed an *in vitro* method to evaluate MC *via* the translocation velocity of fluorescent microspheres (V_{FMS}) applied to excised rat nasal mucosa. In the present study, the relationship between *in vivo* nasal MC and *in vitro* V_{FMS} was examined to optimize our PK model for the prediction of nasal drug absorption. Appropriate inhibitors (propranolol and atropine) and enhancers (terbutaline and acetylcholine chloride) of MC were utilized to modify MC. *In vivo* clearance of drug from the nasal cavity was determined from the disappearance of fluorescent microspheres (FMS) from the nasal cavity following nasal application to rats. The first order elimination rate constant, k_{mc}, was determined from the disappearance profiles of FMS. k_{mc} was decreased to 35.8% by propranolol and 52.6% by atropine, but increased to 117% by terbutaline and 168% by acetylcholine chloride. A significant linear correlation was observed between k_{mc} and V_{FMS} ($r^2 = 0.9745$, $p < 0.001$). These results indicate that *in vivo* k_{mc} can be estimated from the *in vitro* parameter, V_{FMS}. By introducing linear correlation into our PK model, nasal drug absorption may be precisely estimated, even with changes in MC.

1. Introduction

Intranasal drug application has attracted significant attention as an alternative delivery route for poorly absorbed drugs or large molecules such as peptides, with several attempts at direct nose-to-brain drug delivery having been reported in recent decades (Kumar et al., 2016). Despite several studies reporting the advantages of nasal drug delivery, relatively few nasal drug preparations are available on the market. The absence of a method that allows the accurate estimation of nasal drug absorption may be one of the reasons why the development of nasal drug formulations has not progressed. The study using an accurate estimation system allows us to evaluate the nasal drug absorption quantitatively, which leads to the development of the nasal preparation. Furthermore, inter-individual variability is frequently observed, presenting a challenge to the accurate assessment of nasal drug absorption.

Drug absorption after intranasal administration depends on the nasal residence of the drug, which is determined by nasal mucociliary clearance (MC). The primary function of MC is protection against exogenous substances such as bacteria and viruses deposited by inhalation and thus the avoidance of infectious diseases (Merkus et al., 1998; Schipper et al., 1991). Drugs applied to the nasal cavity are cleared toward the pharynx by MC and transported to the stomach by swallowing. Given the large difference observed between nasal and intestinal absorption, MC is clearly an important determinant of the rate and extent of drug absorption after nasal application. In our previous study, we illustrated the importance of MC in nasal drug absorption, and established the first pharmacokinetic (PK) model to evaluate nasal drug absorption (Furubayashi et al., 2007a, 2007b). Since MC is fixed as a constant parameter in this first PK model, nasal bioavailability during changes in MC cannot be estimated. Some studies

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have reported that the *in vivo–in vitro* correlation of MC is not constant (Saldiva et al., 1995) and that the MC parameter can change significantly (Corbo et al., 1989).

Many investigations on MC have been reported where the transport rate of dyes or particles on nasal and/or tracheal tissue was measured. Nasal or tracheal tissues from various animal species have been utilized, such as human nasal (Zhang et al., 2008; Mason et al., 1995; Liote et al., 1989; Hardy et al., 1985), rat nasal (Zaki et al., 2007; Zhou and Donovan, 1996; Donovan and Zhou, 1995; Saldiva et al., 1995; Macchione et al., 1995), rat tracheal (Gatto, 1993), frog palate (Saldiva et al., 1995; Liote et al., 1989; Rubin et al., 1990; Braga et al., 1990, 1992) and bovine tracheal tissues (Shah and Donovan, 2007a, 2007b). In other *in vivo* studies, the clearance time of markers (dyes or particles) from the nasal cavity to the pharynx was evaluated by the collection of the markers from the pharynx region (Merkus et al., 1998). Recently, the *in vitro* and *in situ* measurements of MC have been reported, where the transport of particles on excised mucosa was observed directly and the movement velocity of particles was regarded as an index of MC (Shah and Donovan, 2007a, 2007b; Mason et al., 1995; Saldiva et al., 1995). In our previous study, an *in vitro* MC evaluation method was developed using excised rat nasal mucosa by measuring the velocity of microspheres on the nasal mucosa as an index of MC (Inoue et al., 2012, 2013), allowing us to investigate nasal MC and to estimate the effects of drugs, pharmaceutical additives, and formulations on MC, thus increasing understanding of nasal MC function. Using the information derived from the *in vitro* system, the estimation system by Furubayashi et al. (2007a, 2007b) can be optimized to further the development of nasal formulation.

The aim of this study was to clarify the effect of changes in MC on *in vivo* nasal clearance, and to evaluate the relationship between *in vitro* MC and *in vivo* nasal clearance. In order to improve our *in vitro* evaluation method, this relationship was subsequently applied to our PK model for the prediction of nasal drug absorption.

2. Material and methods

2.1. Materials

Fluorescent microspheres (FMS; Fluoresbrite® YG microspheres, particle diameter 6.00 μm) were purchased from Polysciences, Inc. (Warrington, PA, USA). Ethyl carbamate (urethane) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Phosphate-buffered saline (PBS, pH 7.4) was purchased from Nacalai Tesque (Kyoto, Japan). Benzalkonium chloride, (±)-propranolol hydrochloride, acetylcholine chloride, and atropine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Terbutaline sulfate was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. Animal study

Male Wistar rats weighing 250–300 g were used in all animal experiments. All animal studies were conducted under guidelines approved by the local Animal Care committee of Shujitsu University in accordance with the Principles of Laboratory Animal Care (NIH publication #85-23).

2.3. Pretreatment of rat nasal epithelium with MC modulators

Five drugs with different modes of pharmacological action were used as MC modulators as described previously (Inoue et al., 2012, 2013). Propranolol (PPL, 1 μM and 100 μM) and atropine (ATRP, 100 μM) are MC inhibitors, while terbutaline (TBL, 1 mM) and acetylcholine chloride (ACH, 100 μM) are MC enhancers. It has been reported that PPL, β-adrenergic antagonists (Bateman et al., 1980; Pavia et al., 1986), and ATRP, cholinergic antagonists (Groth et al., 1991), inhibit mucociliary function, while TBL, β-adrenergic agonists (Salathe, 2002, 2007; Hayashi et al., 2008), and ACH, cholinergic agonist (Gatto, 1993; Hayashi et al., 2008; Zagoory et al., 2002), promote mucociliary function. According to previous reports (Hayashi et al., 2008; Houtmeyers et al., 1999; Iravani and Melville, 1975), drug concentrations used in this study are sufficient to exhibit the pharmacological action. Benzalkonium chloride (BZC, 0.1%) is an irreversible MC inhibitor (Mallants et al., 2007; Hofmann et al., 2004) that completely blocks mucociliary transport by cytotoxicity (Inoue et al., 2013). The MC modulators were dissolved in PBS and 40 μL of each solution was placed in the rat nasal cavity 15 min prior to the nasal residence study.

2.4. In vivo study of drug nasal residence

In order to estimate *in vivo* drug nasal residence, the clearance of FMS, as a non-absorbable marker, from the nasal cavity was determined. A stock suspension of FMS (2.5% as solids-latex in water) was diluted by 10,000 times to prepare a dosing suspension. Under urethane anesthesia (1.0 g/kg, i.p.), rats were fixed in a supine position and 5 μL of FMS suspension was instilled at a depth of 10 mm from the nostril. Immediately after application, rats were changed to a prone position and residual FMS in the nasal cavity was collected at time intervals of 0, 5, 10, 15, 30, and 60 min. The surgery by Hirai et al. (1981) was performed on rats immediately prior to the collection of FMS. PBS (5 mL) and 50 mM DTT solution (5 mL) were passed through the nasal cavity from the esophagus, once and twice respectively, to collect FMS in the nasal cavity. The amount of FMS in the recovery solution was determined fluorometrically (excitation/emission = 444 nm/500 nm). Data were expressed as % recovery (%).

2.5. Estimation of the clearance rate constant from the nasal cavity

The disappearance rate constant of FMS was determined from the clearance profiles of FMS. The profile showed biphasic elimination when plotted on a semi-logarithmic scale. Given our assumption that the initial rapid clearance (α-phase) was attributable to MC, the disappearance rate constant of FMS (k_{mc}) was calculated from the slope of the linear elimination of α-phase plotted on the semi-logarithmic scale.

2.6. PK modeling of drug absorption after intranasal administration with respect to nasal MC

We have previously established a PK model with drug nasal clearance as a PK parameter (Furubayashi et al., 2007a, 2007b). The estimation of nasal drug absorption by this system is limited under normal MC conditions (Fig. 1). In the present study, in order to improve the flexibility and application of the model, change in MC was introduced as a PK parameter.

According to this model, total bioavailability (BA) after nasal administration (F_n^{Total}) is the sum of the nasal BA (F_n) and the intestinal BA (F_{gi}), as shown in Eq. 1.

$$F_n^{Total} = Fn + Fgi \tag{1}$$

Assuming first-order kinetics for drug absorption from the nasal cavity and drug clearance by MC, F_n can be calculated according to Eq. 2 from the first-order rate constants of absorption (k_n) and MC (k_{mc}).

$$Fn = \frac{kn}{kn + kmc} \tag{2}$$

F_{gi} can be calculated as the product of oral bioavailability (F_{po}) and the fractional clearance to the GI tract (Eq. 3), assuming negligible degradation of the drug in the nasal cavity.

$$Fgi = Fpo\cdot(1 - Fn) \tag{3}$$

The total bioavailability (F_n^{Total}) can be expressed as Eq. 5 by substituting Eq. 1 and Eq. 2 into Eq. 3.