Kinetic Model to Predict the Absorption of Nasally Applied Drugs from

in Vitro Transcellular Permeability of Drugs

Tomoyuki FURUYASHI,*a Akiko KAMAGUCHI,a Kazushi KAWAHARA,b Yoshie MASAOKA,b Makoto KATAOKA,b Shinji YAMASHITA,b Yutaka HIGASHI,a and Toshiyasu SAKANEa

aSchool of Pharmacy, Shujitsu University; 1-6-1 Nishigawara, Okayama 703–8516, Japan; and bFaculty of Pharmaceutical Sciences, Setsunan University; 45–1 Nagatoge-cho, Hirakata, Osaka 573–0101, Japan.

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The purpose of this study is to propose a kinetic model to predict the absorption of nasally applied drugs from their permeability to the Caco-2 monolayer (Pcaco2). Since a drug applied to the nose in an in vivo physiologic condition is translocated to the gastrointestinal (GI) tract by coordinated beats of cilia (mucociliary clearance, MC), the drug undergoes absorption both from the nasal cavity and from the GI tract. The detailed MC of the rat was examined, using inulin as a marker of the applied solution. Inulin disappeared monoeXponentially from the nasal cavity, indicating that the MC can be assumed to follow first-order kinetics. From the disappearance of inulin, the first order rate constant for MC (ka) was calculated as 0.0145 min−1. In the proposed kinetic model, the fractional absorption of the drug following nasal application is predicted as the sum of Fnc (fractional absorption from the nasal cavity) and Fgi (fractional absorption from the GI tract), both of which are estimated indirectly from Fcaco2. Fnc is calculated according to the equation, k/a/(k/a+kMC), where k/a is the absorption rate constant. Nasal drug absorption is assumed to follow first order kinetics. The k/a of four drugs was initially calculated from kMC and their Fnc; thereafter, the linear relationship between k/a and Pcaco2 from which k/a is predicted, was determined. Fnc is calculated as Fnc(1-Fgi), where Fnc is fractional absorption after oral administration. Fnc was predicted from the previously determined sigmoid curve between Fp.o, and Pcaco2. The proposed kinetic model is the first estimation system for nasal drug absorption based on drug disposition after nasal application and is useful for the development of nasal dosage forms.

Key words nasal absorption; prediction; Caco-2 monolayer; mucociliary clearance

In the last few decades, nasal administration has received a great deal of attention as a rationale for the systemic delivery of many drugs.1) The range of compounds investigated for possible nasal application varies greatly from very lipophilic drugs to polar, hydrophilic molecules including peptides and proteins.2) The relatively high permeability of the nasal epithelium, its high vascularization and the avoidance of hepatic first-pass metabolism makes nasal application a promising alternative, especially for drugs exhibiting high metabolism in the intestine and/or liver.

Oral drug absorption can be estimated from in vitro transepithelial transport across the Caco-2 monolayer with various systems.3–5) These systems are responsible for screening of huge number of new drug candidates which are synthesized through combinatorial chemistry and pharmacologically screened with an in vitro high throughput system. Although nasal administration has been considered important as an alternative to oral application, as mentioned above, no prediction system has been developed so far. A prediction system would greatly help the development of nasal medications.

Some respiratory epithelial cells possess cilia on their surface. The cilia beat in a coordinated fashion to transport the mucus layer, which covers the surface of the epithelium, to the nasopharynx, where it is swallowed.6–8) The combined action of the mucus layer and cilia is called mucociliary clearance (MC). It is an important nonspecific defense mechanism of the respiratory tract to protect the body against noxious inhaled materials. Due to MC, drugs applied to the nasal cavity are translocated to the nasopharynx and, thereafter, to the gastrointestinal (GI) tract, together with the mucus layer. Some fraction of the nasally-administered drug undergoes absorption from the GI tract. In order to develop a predictive system for fractional drug absorption after nasal application, the kinetic characteristics of mucociliary clearance must be clarified and correctly combined in the kinetic model.

In the previous manuscript,9) five non-degradable drugs were selected as model drugs and their fractional absorption following nasal and oral application, and their permeability to the Caco-2 monolayer (Pcaco2) were examined. The methods for the calculation of fractional absorption from the nasal cavity and from the GI tract after nasal application were also described. The relationship between fractional absorption and Pcaco2 was discussed, and the feasibility to predict drug absorption following nasal administration from Pcaco2 was indicated. The first aim of this research is to clarify the details of MC. For this purpose, the surgical operation reported by Hirai et al.10) was not done on the esophagus and trachea, and the animal was kept conscious for as long as possible during the animal study. Based on the information on MC, the second aim is to propose a kinetic model to predict drug absorption following nasal application to rats from Pcaco2. Various fractional absorptions reported previously were utilized in this study. From these values, the correlation between the kinetic parameter and Pcaco2 was determined and applied for the prediction of total drug absorption after nasal administration.

Theory and Kinetic Model Drugs applied nasally undergo absorption both from the nasal cavity and from the GI tract. Total fractional absorption following nasal drug application (Fp.o) is considered the sum of two fractional absorptions, Fnc and Fgi. Fnc is the fraction of the drug absorbed from the nasal cavity and Fgi is that from the GI tract following nasal drug application.
\[ F_x = F_{NC} + F_{GI} \]  

To simplify the prediction model, no degradation and metabolism of the drug in the nasal cavity was taken into consideration in this study. The model drugs had been selected in the previous report as not metabolizing and degrading in the nasal cavity.

**Prediction of Fractional Absorption from the Nasal Cavity**

The drug is eliminated from the nasal cavity both by absorption into systemic circulation and by mucociliary translocation. The assumptions listed below were made for the prediction of \( F_{NC} \).

**Assumption 1:** The same value of \( k_{MC} \), which is a first order rate constant for MC, can be applied to all drugs. Since the drug is translocated by MC, together with the dosing solution, the mucociliary translocation of the drug is independent of the physicochemical properties of the drug.

**Assumption 2:** Drug absorption from the nasal cavity is assumed to follow first order kinetics with a rate constant, \( k_a \).

**Assumption 3:** There exists a proportional relationship between \( k_a \) and \( P_{Caco-2} \).

The data from the study on the disappearance of inulin from the nasal cavity showed that MC follows first order kinetics with the rate constant, \( k_{MC} \). Based on Assumption 2, \( F_{NC} \) can be calculated according to the following equation.

\[ F_{NC} = \frac{k_a}{k_a + k_{MC}} \]  

To predict \( F_{NC} \) of the drug, \( k_a \) is initially estimated from the proportional relationship between \( k_a \) and \( P_{Caco-2} \) (Assumption 3) and then \( F_{NC} \) is calculated according to Eq. 2. A correlation equation between \( k_a \) and \( P_{Caco-2} \) was determined in this report using data from four drugs.

**Prediction of Fractional Absorption from GI Tract**

The prediction of \( F_{GI} \) is very simple. A non-degradable drug is translocated to the GI tract at the fraction of \( 1 - F_{NC} \) and is absorbed from the GI tract at the fraction of \( F_{p.o.} \). Consequently, \( F_{GI} \) can be calculated as follows.

\[ F_{GI} = F_{p.o.}(1 - F_{NC}) \]  

Substitution of Eqs. 2 and 3 into Eq. 1 results in the following equation.

\[ F_x = \frac{k_a + F_{p.o.} \cdot k_{MC}}{k_a + k_{MC}} \]  

Equation 4 indicates that the total fractional absorption of the drug after nasal application can be calculated from three parameters, \( k_a \) and \( F_{p.o.} \) are predicted from \( P_{Caco-2} \), while \( k_{MC} \) is a constant irrespective of the difference in the physicochemical properties of drugs.

**RESULTS AND DISCUSSION**

**Disappearance of Inulin from the Rat Nasal Cavity**

Figure 1 shows the elimination of inulin from the nasal cavity as a function of time. The disappearance of inulin from the nasal cavity was rapid. Inulin was eliminated 180 min after instillation. The semilogarithmic plot of the curve was almost linear. Akaike’s information criteria (AIC) calculated with a computer program of nonlinear regression analysis, MULTI, clarified that a monoequational equation is better for the elimination of inulin than a biexponential equation. The rate constant (disappearance rate constant, \( k_{dis} \)) was calculated with MULTI. The dashed line shown in Fig. 1 is the result and \( k_{dis} \) is obtained as 0.0152 min\(^{-1}\). Consequently, the disappearance half-life of inulin from the nasal cavity is 37.1 min.

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**Hardy et al.** reported the MC of humans. Radioactive albumin instilled or sprayed nasally into human subjects was detected by \( \gamma \)-camera. Examination of the disappearance time courses of albumin clarified that albumin in the human nasal cavity was eliminated monoequationally. Since the molecular size of albumin is so large, the contribution of absorption to the disappearance of albumin from the nasal cavity is negligible. This indicates that there may exist no species difference in MC irrespective of the large difference in the structure and anatomy of the nasal cavity.

**Determination of the First Order Rate Constant for MC**

If drug absorption from the nasal cavity is assumed to follow first order kinetics, mucociliary translocation of the drug also follows first order kinetics. Although inulin was poorly absorbable and used as a marker of the dosing solution, \( F_{NC} \) of inulin was 0.046. Consequently, both the translo-
cation of the drug and absorption contributed to the disappearance of inulin from the nasal cavity. The calculation shown in appendix 1 indicates that $k_{MC}$ is $0.0145 \text{ min}^{-1}$. The contribution of absorption and translocation to the disappearance of inulin from the nasal cavity is 4.6% and 95.4%, respectively.

Relationship of $F_{p.o.}$ and $F_n$ to $P_{Caco-2}$

Figure 2 shows the sigmoid curve between $F_{p.o.}$ and $P_{Caco-2}$. Since $F_{p.o.}$ of the model drugs was scattered from the sigmoid curve, the curves were obtained assuming a similar shape and location to those reported by Artursson and Karlsson. $3^3$ More data are necessary to identify the precise relationship between $F_{p.o.}$ and $P_{Caco-2}$. The relation between $F_n$ and $P_{Caco-2}$ is also indicated in Fig. 2. In contrast with $F_{p.o.}$, the shape of the curve is sigmoid.

Caco-2 is very popular and widely used for in vitro experiments.$^{13}$ The origin of Caco-2 is the human colon carcinoma$^{13}$; therefore, it is reasonable to use this cell line for the prediction of oral bioavailability. Recently, Calu-3 cells have been used for the in vitro system of pulmonary and nasal drug absorption.$^{14,15}$ Calu-3 was derived from human lung carcinoma. Judging from the origin of the cell line, Calu-3 cells was used in the present study. Many studies have been done on the characterization of Caco-2 and much information is available from the literature. Researchers have used Caco-2 for many years. The kinetic model utilizing Caco-2 has many advantages over that using Calu-3, which is why Caco-2 was used in this study. The permeability of drugs to Calu-3 was determined and compared. The results showed no significant differences in drug permeabilities to Caco-2 and Calu-3.

Determination of the Correlation between $k_a$ and $P_{Caco-2}$

Table 1 lists $P_{Caco-2}$, $F_n$, $F_{p.o.}$, $F_{NC}$ and $F_{GI}$ of the model drugs which were previously reported.$^9$ From $k_{MC}$ ($0.0145 \text{ min}^{-1}$) and $F_{NC}$ listed in Table 1, the $k_a$ of the drugs, except for methotrexate, were calculated according to Eq. 8. The result is also indicated in Table 1. Figure 3 shows the correlation of $k_a$ to $P_{Caco-2}$. The line in Fig. 3 is expressed as

$$k_a (\text{min}^{-1}) = 9975.8 \times P_{Caco-2} (\text{cm/s}) \quad (5)$$

with the correlation coefficient of 0.947 ($p<0.01$ by Student’s $t$-test). Data of methotrexate were excluded from Fig. 3 since the calculation of $k_a$ gives an erroneous result in the case of the drug with almost complete absorption (see appendix 2).

The reliability and precision of the estimation system is dependent on the correlation of $k_a$ with $P_{Caco-2}$. Equation 5 was determined using the data of four drugs, but these data are likely not sufficient. Since it is not feasible to determine $k_a$ of highly permeable drugs such as methotrexate, the model drugs for this purpose are restricted ($5 \times 10^{-7} < P_{Caco-2} (\text{cm/s}) < 10^{-5}$). A study to improve the reliability of the system is now under investigation.

From Eq. 4, $k_a$, $F_{p.o.}$ and $k_{MC}$ are required for the estimation of fractional absorption following nasal drug application. The relationship between $P_{Caco-2}$ and $F_{p.o.}$ in human is avail-
In conclusion, the proposed kinetic model is the first estimation system for nasal drug absorption based on drug disposition after nasal application and is useful for the development of a system with feasible application to humans.

Appendix 1: Calculation of $k_{MC}$ $k_{ds}$ of inulin is 0.0152 min$^{-1}$, equal to the sum of $k_a$ and $k_{MC}$.

$$k_{ds}=k_a+k_{MC}=0.0152$$

(6)

Since $F_{NC}$ of inulin is 0.046, Eq. 2 for inulin is as follows:

$$F_{NC} = \frac{k_a}{k_a+k_{MC}} = 0.046$$

(7)

From Eqs. 6 and 7, $k_{MC}$ was calculated as 0.0145 min$^{-1}$.

Appendix 2: Calculation of $k_a$ of Model Drugs $k_a$ of the drug was calculated from $k_{MC}$ and $F_{NC}$ according to the following equation which is obtained by rearrangement of Eq. 2.

$$k_a = \frac{F_{NC} \cdot k_{MC}}{1-F_{NC}}$$

(8)

In Eq. 8, $k_{MC}$ is 0.0145 min$^{-1}$ and the $F_{NC}$ of each drug listed in Table 1 is used. Since the denominator of Eq. 8 is $1-F_{NC}$, the calculation of $k_a$ of the drug with $F_{NC}$ almost equal to 1 is influenced very much by experimental error to give an erroneous result. Consequently, $k_a$ of methotrexate with an $F_{NC}$ of 0.963, was excluded from Fig. 3.

REFERENCES