Journal of
Pharmaceutical and BioTech Industry

MDPI

Communication

Influence of Nasal Mucociliary Activity on Levofloxacin Absorption Following Intranasal Administration in Rats

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Abstract

Background: Intranasal administration avoids the hepatic first-pass effect and allows relatively high-molecular-weight drugs to permeate the nasal mucosa, making it an advantageous alternative route for poorly absorbable drugs. Mucociliary clearance (MC) is a physiological factor that determines drug absorption after intranasal administration. However, the quantitative relationship between changes in MC activity and intranasal absorption remains unclear. Methods: Pharmacokinetic analysis of drug absorption of levofloxacin (LFX) after intranasal administration was conducted in rats with pharmacologically altered MC activity. LFX was administered intranasally to rats pretreated with propranolol as an MC inhibitor and terbutaline as an MC enhancer. Results: Terbutaline decreased LFX absorption, whereas the effect of propranolol was not significant, although a slight trend towards improved absorption was observed. These results indicated that LFX absorption after intranasal administration varied with MC modulator treatment. This study revealed that treatment with MC modulators could alter LFX absorption after intranasal administration, suggesting that the degree of variation in drug absorption may be due to the physicochemical properties of the administered drug. Conclusions: Considering that MC activity is essential for designing formulations that allow for the precise control of drug absorption in the development of nasal formulations, a quantitative analysis of MC activity is crucial. These findings provide fundamental insights for optimizing and accelerating the development of nasal formulations.

Keywords: transnasal absorption; mucociliary clearance; intranasal absorption; nasal formulation development

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Academic Editor: Fernando Albericio

Received: 22 October 2026
Revised: 24 November 2026
Accepted: 25 November 2026
Published: 26 November 2026

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1. Introduction

Intranasal (IN) administration is an attractive route for drug delivery because it is noninvasive, can provide rapid drug absorption, and avoids hepatic first-pass metabolism. The nasal cavity is highly vascularized, and its relatively permeable epithelium enables the absorption of a wide range of therapeutic agents, including small-molecule drugs and macromolecules [1–4]. Nevertheless, the extent and rate of drug absorption following IN administration are affected by several physiological and biopharmaceutical factors, including nasal mucosal permeability, drug physicochemical properties, formulation characteristics, and mucociliary clearance (MC) [2–6]. Understanding the contribution of these factors is important for predicting systemic drug absorption and optimizing the performance of nasal formulations.

MC is an essential defense mechanism of the respiratory tract that removes inhaled particles, microorganisms, and other foreign substances trapped in the mucus layer. This process is mediated by coordinated ciliary beating, which transports mucus toward the nasopharynx and facilitates its removal from the nasal cavity [1,6–8]. Although MC protects the respiratory tract from exogenous materials, it can also limit the absorption of intranasally administered drugs by shortening their residence time in the nasal mucosa. Following administration, a drug must remain in contact with the nasal epithelium for sufficient transnasal drug permeation. Consequently, the rate of disappearance by MC can influence both the rate and extent of systemic drug absorption after IN administration [1,2,5,6]. The relationship between nasal residence time and drug absorption is also affected by the physicochemical properties of the drug, dosage forms, and site of deposition within the nasal cavity [3–5,9]. Thus, MC should be considered an important physiological determinant of transnasal drug absorption.

Nasal ciliary activity can be affected by pharmacological agents and components of nasal formulations. Previous studies have demonstrated that certain drugs and excipients can alter ciliary beat frequency and mucociliary function, highlighting the importance of evaluating their effects on nasal physiology [10,11]. Propranolol has been reported to inhibit ciliary movement, whereas terbutaline has been used experimentally to enhance MC [12]. Therefore, these pharmacological agents provide a means to investigate the contribution of altered nasal ciliary function to drug absorption. However, changes in ciliary activity may also influence other physiological processes in the nasal cavity. Accordingly, pharmacokinetic alterations following pharmacological modulation of MC should be interpreted in the context of the potential effects of MC-modulating agents beyond ciliary movement itself.

In our previous study, we quantitatively investigated the influence of nasal MC on the systemic absorption of norfloxacin following intranasal administration in rats [13]. Using modulators of ciliary activity with propranolol and terbutaline, we demonstrated that the inhibition and enhancement of MC altered the bioavailability of norfloxacin. Furthermore, we developed a pharmacokinetic approach to quantitatively characterize the relationship between nasal clearance and drug absorption [13]. In a subsequent study, we investigated the relationship between in vivo nasal clearance and an in vitro measure of mucociliary transport, providing further support for the quantitative assessment of nasal clearance and its potential application to the prediction of drug absorption [14]. Together, these studies established a pharmacokinetic model to evaluate the contribution of nasal MC to systemic drug absorption. However, whether the magnitude of pharmacokinetic fluctuation in response to altered ciliary activity is comparable among different drugs remains insufficiently characterized.

Levofloxacin (LFX) is a fluoroquinolone antibacterial agent with well-established pharmacokinetic properties following oral administration. Intranasal delivery of LFX has also been investigated in rats as a potential approach for treating localized nasal infections. In a previous study, intranasal administration of a thermo-reversible in situ gel for LFX resulted in high drug concentrations in the anterior nasal mucosa, whereas systemic absorption was limited under the conditions examined [15]. These findings demonstrated that the pharmacokinetic behavior of intranasally administered LFX can depend on the intended delivery strategy and the distribution of the drug within the nasal cavity. However, the influence of altered nasal ciliary activity on the systemic pharmacokinetics and bioavailability of intranasally administered LFX has not been adequately considered.

In this study, we investigated the effects of pharmacological modulation of nasal ciliary activity using propranolol and terbutaline on the pharmacokinetics and bioavailability of LFX following IN administration in rats. This study aimed to extend our understanding of the relationship between nasal physiological function and systemic drug absorption

following IN administration by quantitatively evaluating the effects of MC inhibition and enhancement on LFX pharmacokinetics.

2. Materials and Methods

2.1. Materials

Levofloxacin (LFX) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), (±)-propranolol hydrochloride (PPL) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and phosphate-buffered saline (pH 7.4) (PBS) was purchased from Nacalai Tesque (Kyoto, Japan). Terbutaline sulfate (TBL), isoflurane, methanol, acetonitrile, and formic acid were purchased from FUJIFILM Wako Pure Chemical Co. (Tokyo, Japan).

2.2. Animals

Male Wistar rats (220–280 g) were used in all animal experiments. All animal studies were conducted in accordance with the relevant guidelines after approval from the local committee of Animal Care of Shujitsu University (011-001-2018), following the Principles of Laboratory Animal Care (NIH publication #85-23). The animals were kept under conditions of food and water intake ad libitum, at a room temperature of 24 ± 1 ∘C, humidity of 55 ± 5%, and a light/dark cycle of 7:00 to 19:00.

2.3. Plasma Pharmacokinetics of LFX After Intravenous Administration

The LFX solution for intravenous (IV) administration was prepared by dissolving LFX in PBS at a concentration of 1.0 mg/mL. Under isoflurane anesthesia (introduced concentration of 4%, maintained concentration of 2%), cannulation was performed with polyethylene tubing (SP-31, Natsume Co., Ltd., Tokyo, Japan) of the left femoral vein for drug administration and the right femoral artery for blood sampling. The rats were restrained in a cage (KN326 Type III, Natsume Co., Ltd.), and after recovery from anesthesia, the LFX solution was injected into the venous cannula at a concentration of 1.0 mg/kg. After dosing, 200 µL of blood was collected via an arterial cannula at the following time intervals: 0, 1, 2, 5, 10, 15, 30, 45, 60, and 120 min.

2.4. Pharmacokinetic Study of LFX After Intranasal Administration

For intranasal (IN) administration, LFX was dissolved in PBS at a concentration of 1.0 mg/mL, and 5.0 µL of the LFX solution was administered into the nasal cavity of the rats using a microsyringe. Rats that underwent cannulation for blood sampling from the right femoral artery were restrained in a cage, and the LFX solution was instilled into the nasal cavity. Blood samples were collected via an arterial cannula at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after dose administration.

2.5. Estimation of Pharmacokinetic Parameters

In this study, pharmacokinetic parameters were estimated from fitting calculations using the nonlinear least-squares regression analysis program, MULTI. The plasma concentration–time profiles of individual animals were fitted separately by nonlinear least-squares regression. An unweighted fitting procedure (weighting factor = 1) was used, and the pharmacokinetic model was selected based on Akaike's information criterion (AIC). The plasma concentration–time profiles of LFX were analyzed based on a two-compartment model for IV administration using the following Equation (1):

$$C_p(t)_{IV} = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (1)$$

where $C_p(t)_{IV}$ represents the plasma concentration of LFX after IV administration, A and B are the intercepts, and α and β are the rate constants for the distribution and elimination phases, respectively.

For intranasal administration, the profiles were estimated following a one-compartment model with a first-order absorption process, following Equation (2).

$$C_p(t)_{IN} = X/(ka - ke)\cdot\{(e^{-ket}) - (e^{-kat})\} \quad (2)$$

$$X = F\cdot D\cdot ka/V_d \quad (3)$$

where $C_p(t)_{IN}$ represents the plasma concentration of LFX after IN administration. X is a composite coefficient defined in Equation (3), F is the absolute bioavailability, D is the administered dose, *ka* is the absorption rate constant, *ke* is the elimination rate constant, and V_d is the apparent volume of distribution.

The area under the concentration–time curve (*AUC*) was calculated using the trapezoidal rule up to sampling time points of 120 min for IV administration and 6 h for intranasal administration. To estimate the *AUC* up to infinity time ($AUC_{0-\infty}$), the value of elimination clearance obtained from the fitting curve by MULTI was used. From the profile after IV administration, the elimination clearance rate was estimated to be 0.0136 ± 0.0014 min^{-1}, and the ratio of the extrapolated AUC from 120 min to infinity to the $AUC_{0-\infty}$ value was 18.62 ± 1.60%. In IN administration, the ratios were 6.82 ± 1.89%, 3.24 ± 0.39%, and 8.62 ± 0.94% for IN + PBS, IN + PPL, and IN + TBL, respectively.

2.6. Drug Treatment for MC Alteration

According to our previous study [13], propranolol (PPL, 0.1 mM) and terbutaline (TBL, 1.0 mM) were used as an MC inhibitor and enhancer to alter the nasal MC activity in rats. A 40 µL solution of MC modulators dissolved in PBS was pretreated intranasally and applied in rats 15 min before LFX administration. As a control group (IN + PBS), a condition was used in which the rats were pretreated with PBS, and a solvent of MC modulators was dissolved.

2.7. Sample Treatment for LFX Determination

Plasma samples were obtained by centrifuging the collected blood for 5 min at 16,100× *g* and stored at −80 °C until analysis. Plasma samples (100 µL) were deproteinized with 1.2 mL methanol, and after centrifugation for 10 min at 16,100 *g*, the supernatant (1 mL) was evaporated. The residue was reconstituted with 100 µL of the mobile phase for LC-MS/MS or HPLC. For the samples obtained from the intranasal administration experiments, the LFX concentration was determined using an LC-MS/MS system (6460 Triple Quad LC/MS, Agilent Technologies) equipped with an ODS column (CAPCELL PAK, MG-III, 2.0 mm I.D., 75 mm, Osaka Soda Co., Ltd., Osaka, Japan). The mobile phase of 0.2% formic acid (60%) and acetonitrile (40%) was run isocratically at a flow rate of 0.2 mL/min, and 5 µL of the sample was injected into the system. The MS conditions are summarized in Table 1. For the samples obtained from the IV administration experiments, the LFX concentration was determined by HPLC (LC-10AD, Shimadzu, Kyoto, Japan). The ODS column (CAPSELL PAK, MG-II, 4.6 mm I.D., 150 mm, Osaka Soda Co., Ltd., Osaka, Japan) was equipped in the HPLC system, and the mobile phase, 0.01 M potassium dihydrogen buffer, pH 3.4 (86%) and acetonitrile (14%), was flowed at a rate of 1.0 mL/min. A sample of 20 µL was injected and analyzed for 9 min under isocratic conditions. LFX was detected by UV detection at a wavelength of 294 nm.

Table 1. Analytical conditions of LC/MS for determining the plasma concentration of LFX.

Condition	Value
Ionization	Positive
Precursor ion (m/z)	362.2
Product ion (m/z)	318.8
Fragmentor (V)	120
Collision energy (V)	17
Cell accelerator voltage (V)	2
Capillary voltage (V)	4000 (+)

For plasma samples, decreased quantitative sensitivity was observed for LFX because the blood components were mixed as a matrix. However, the matrix effect was not considered to have a significant impact. To quantify the drug concentration, the external standard method was used to calculate the drug concentration in all samples. Calibration curves were generated from standard solutions in which known concentrations of LFX were spiked into blank plasma obtained from rats, and the LFX concentrations in the samples were determined quantitatively from the standard curves. The concentration range of the spiked solutions was optimized for each analytical system. The parameters for the accuracy and precision of the analysis and the analytical limitations are listed in Tables 2–4. In this study, the lowest plasma concentrations were 0.172 ng/mL in the sample obtained after intranasal administration and 166.2 ng/mL in the sample obtained after IV administration. As all samples were within the limits of quantification in both analytical systems, it was confirmed that the determination of LFX concentration in plasma has quantitative validity. Plasma samples were stored at −80 °C for approximately one week before analysis. Therefore, long-term stability was evaluated under the same storage conditions for a period equal to the actual sample storage period of one week. After thawing, the plasma samples were kept at room temperature for approximately 10 min before protein precipitation, and the processed samples were analyzed immediately.

Table 2. Analytical parameters of LC-MS/MS for determination of LFX concentrations in plasma after IN administration.

	Within-Run (3 Batches with 4 Times Each)			**Between-Run (3 Batches with 4 Times Each)**			**Matrix Effect**		**Stability**	
Spiked Conc. (ng/mL)	**Measured Conc. (ng/mL)**	**Precision (% RSD)**	**Accuracy * (%)**	**Measured Conc. (ng/mL)**	**Precision (% RSD)**	**Accuracy * (%)**	**Precision (% RSD)**	**Accuracy * (%)**	**1 Weeks at −80 °C (%)**	**Extraction Recovery (%)**
0.1	0.0984	0.61	98.43	0.104	1.75	103.76	6.87	91.46	94.65	88.35
0.5	0.498	0.24	99.70	0.529	2.22	105.79	5.98	93.46	96.78	92.45
1	0.996	0.54	99.58	0.974	3.05	97.42	4.21	95.13	97.23	90.58
5	4.948	0.56	98.97	5.021	1.36	100.42	3.87	94.88	98.66	91.23
10	9.962	0.97	99.62	10.320	1.13	103.20	4.45	94.56	98.12	93.65
50	49.673	0.12	99.35	50.108	0.63	100.22	4.12	93.88	97.31	92.76

* Each accuracy value was calculated as the ratio of the measured concentration to the nominal concentration.

Table 3. Analytical parameters of HPLC for determination of LFX concentrations in plasma after IV administration.

	Within-Run (3 Batches with 4 Times Each)			Between-Run (3 Batches with 4 Times Each)			Stability	
Spiked Conc. (ng/mL)	Measured Conc. (ng/mL)	Precision (% RSD)	Accuracy * (%)	Measured Conc. (ng/mL)	Precision (% RSD)	Accuracy * (%)	1 Weeks at −80 °C (%)	Extraction Recovery (%)
10	11.65	2.84	116.51	11.24	2.81	112.39	94.32	91.12
50	48.62	1.21	97.23	50.77	1.31	101.54	95.43	89.35
100	100.52	0.78	100.52	102.41	1.07	102.41	92.45	92.43
500	497.26	0.41	99.45	488.63	0.78	97.73	96.22	91.46
1000	1009.39	0.51	100.94	1031.06	1.11	103.11	92.46	94.32

* Each accuracy value was calculated as the ratio of the measured concentration to the nominal concentration.

Table 4. Analytical limitations of LC/MS/MS and HPLC for plasma concentrations of LFX.

System	LOD (ng/mL)	LOQ (ng/mL)	Linearity
LC-MS/MS	0.041	0.123	$r^2 = 0.999965$
HPLC	8.26	24.79	$r^2 = 0.999895$

2.8. Statistical Analysis

The required experimental data were analyzed statistically using IBM SPSS Statistics software (Ver. 29.0.0.0). One-way ANOVA was performed with a significance level of 95%, followed by Tukey's post hoc test to determine the level of significance. Results were considered significant at $p < 0.05$.

3. Results

3.1. Pharmacokinetics of LFX After IV Administration

The plasma concentration–time profile of LFX after IV administration is shown in Figure 1. The elimination of LFX in plasma after IV administration showed a biphasic pattern, and the estimated *AUC* from 0 to infinity was 26.98 (% of dose min/mL).

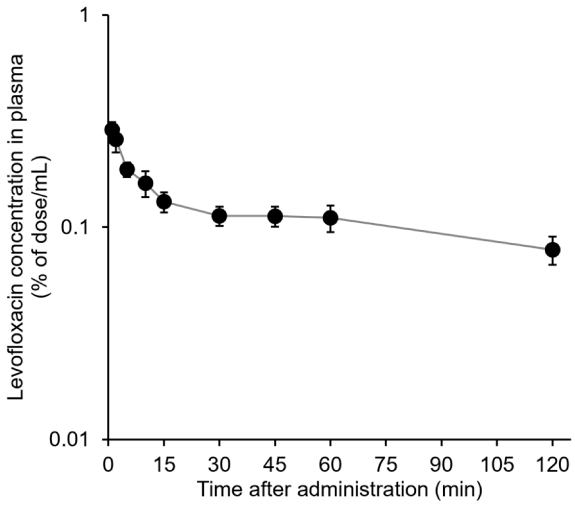

Figure 1. Plasma concentration–time profile following IV administration of LFX to rats. Data are expressed as the mean ± S.E.M. of independent experiments (n = 4).

3.2. Pharmacokinetic Alteration of LFX After IN Administration by Pretreating MC Modulators

The profiles of LFX concentrations in the plasma after IN administration are shown in Figure 2. The LFX concentration with PPL pretreatment (IN + PPL) was slightly higher than that in the group without MC modulators (IN + PBS), although the effect was not significant. In contrast, the LFX concentration with TBL pretreatment (IN + TBL) was lower than that of the other groups. These results indicate that pretreatment with MC modulators alters the absorption of LFX after IN administration.

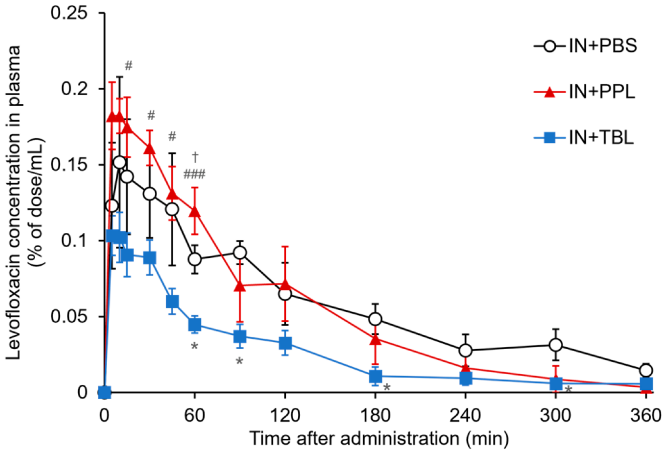

Figure 2. Effects of MC modulators on plasma concentration–time profiles following IN administration of LFX to rats. Rats were pretreated with MC modulators, PPL (0.1 mM) as an inhibitor and TBL (1.0 mM) as an enhancer, for 15 min before the application of LFX (5 μL as 1.0 mg/mL solution) into the nasal cavity. ○, IN + PBS; ▲, IN + PPL; ■, IN + TBL. Data are expressed as the mean ± S.E.M. of independent experiments (n = 4). * $p < 0.05$, compared between IN + PBS and IN + TBL; ### $p < 0.001$ and # $p < 0.05$, between IN + PPL and IN + TBL, and † $p < 0.05$, between IN + PBS and IN + PPL.

To clarify the effect of MC modulator treatment on LFX absorption after IN administration, a pharmacokinetic analysis was performed. The pharmacokinetic parameters of drug absorption estimated using MULTI are listed in Table 5. For MULTI estimation, we used AIC to evaluate model validity. We adopted pharmacokinetic parameters using a model estimation that minimizes the AIC value. The level of the maximum LFX concentration in plasma (C_{max}) was 1.24 times higher with PPL treatment compared to the control, indicating a trend of increase, although the effect was not significant. The C_{max} levels decreased by 0.73 times with TBL treatment compared to the untreated group. This suggests that LFX absorption can fluctuate in conjunction with changes in MC activity. The times to reach C_{max} (T_{max}) were not altered by either PPL or TBL treatment; they remained between 5–10 min in all groups, revealing the rapid appearance of LFX in plasma after IN administration. The absorption of LFX can vary depending on MC activity, suggesting that the total amount absorbed after IN administration changed accordingly. The estimated *AUC* value of LFX showed a 0.51-fold decrease with TBL treatment, whereas the increase with PPL treatment was not statistically significant (1.08-fold increase). As a result, it was revealed that bioavailability decreased by 0.51 times with TBL treatment and slightly changed by 1.08 times with PPL treatment compared to the control (Figure 3).

Table 5. Pharmacokinetic parameters following IN and IV administration of LFX.

Treated	C_{max} (% of Dose/mL)	T_{max} (min)	$AUC_{0-\infty}$ (% of Dose min/mL)	Bioavailability (%)
IN + PBS	0.158 ± 0.037	5	15.71 ± 2.43	58.24 ± 8.99
IN + PPL	0.196 ± 0.007	5–10	17.04 ± 2.01 #	63.16 ± 7.46 #
IN + TBL	0.116 ± 0.011	5	8.08 ± 0.92 *	29.94 ± 3.40 *
IV	-	-	26.98 ± 3.04	-

# $p < 0.05$ compared between IN + PPL and IN + TBL, * $p < 0.05$ between IN + PBS and IN + TBL.

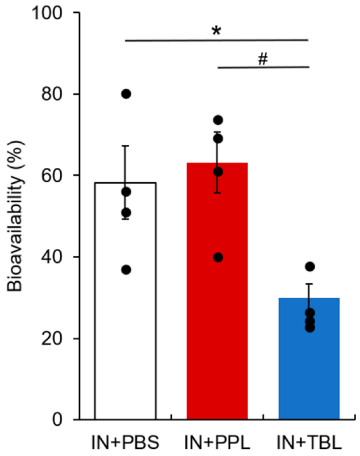

Figure 3. Effect of MC modulators on bioavailability of LFX following IN administration to rats. Data are expressed as the mean ± S.E.M. of independent experiments (n = 4). * $p < 0.05$ compared between IN + PBS and IN + TBL, # $p < 0.05$ between IN + PPL and IN + TBL.

4. Discussion

In this study, rats were used as the animal model for evaluating the effect of MC on drug absorption after IN administration. Typically, the nasal cavity volume of a rat is approximately 250–500 µL, and when evaluating intranasal absorption of a drug as the solution in rats, a volume of 50–100 µL is commonly used [16]. In our series of studies on evaluation of mucociliary movement, we have set the volume at 40 µL to minimize the influence of the solvent volume on the properties of the mucus layer, while ensuring that the MC-modulating agents spread throughout the nasal epithelium and exert sufficient pharmacological effects upon application. As detailed in our previous papers, we have already reported that when MC-altering agents are treated using this method, the activity of ciliary beating, in vitro MC transportability on the nasal mucosa, in vivo nasal residence time of the drug, and in vivo transnasal absorption after intranasal administration are significantly positively correlated [13,14,17,18]. We have demonstrated that transnasal absorption after altering intranasal MC can be quantitatively evaluated by pretreatment with MC-altering agents in this manner. Therefore, we consider that treatment with MC-altering agents in this study allows for the quantitative evaluation of total LXF absorption after intranasal administration of altering intranasal MC in rats.

To clarify the extent to which altering nasal MC activity affects drug absorption after IN administration in vivo, a pharmacokinetic analysis was performed on the fluctuations in LFX absorption in rats with altered MC activity. The absorption process after intranasal administration is thought to involve two processes: transnasal absorption, where the drug permeates through the nasal mucosa, and absorption via the gastrointestinal tract after drug translocation by mucociliary clearance. In this study and the series of previous studies evaluating the effect of MC on drug absorption after intranasal administration, we have attempted to quantitatively evaluate the process by which intranasally administered drugs

are actually absorbed under physiological conditions. Therefore, this study does not aim to strictly distinguish between transnasal absorption by membrane permeation through the nasal mucosa and estimate membrane permeability; rather, it focuses on evaluating the actual total absorption of LFX after intranasal administration, including intranasal mucosal permeation and absorption via gastrointestinal absorption.

PPL (0.1 mM) and TBL (1.0 mM) were used as MC modulators to alter the activity of rat nasal MC. According to previous reports [19,20], the drug concentrations used were sufficient to exert pharmacological action. In our previous study [17,18], we confirmed that the in vivo nasal residence of substances in the nasal cavity of rats was altered by pretreatment with these MC modulators. Our previous findings demonstrated that pharmacological modulation of nasal MC activity altered the nasal residence of substances in rats. Although the nasal residence time of LFX was not directly quantified in the present study, the observed changes in systemic absorption were consistent with the hypothesis that MC-mediated nasal clearance contributes to the extent of nasal drug absorption. Although PPL and TBL have been used to modulate nasal MC activity, their pharmacological effects may not be limited to changes in mucociliary movement. The potential effects on other nasal physiological processes, such as mucosal blood flow and mucus secretion, could also contribute to the observed changes in drug exposure. Therefore, the contribution of MC activity to LFX absorption should be interpreted considering these potential confounding effects.

The absorption of LFX after IN administration was evaluated in rats in which nasal MC activity was pharmacologically altered, and fluctuations in LFX absorption were observed depending on changes in MC activity (Figures 2 and 3). Regarding the degree of absorption, PPL treatment slightly changed LFX absorption by approximately 1.0–1.1 times, whereas TBL treatment reduced it by approximately 0.5–0.7 times (Table 5). Previous studies [13] have shown that the fluctuations in bioavailability with changes in MC activity were 1.9 times with PPL treatment and 0.79 times with TBL treatment for norfloxacin, suggesting that LFX and norfloxacin have different degrees of effect on drug absorption in response to changes in MC. Notably, the magnitude of the changes in LFX absorption was asymmetric between the PPL and TBL treatments. PPL treatment had less of an impact on absorption, whereas TBL treatment resulted in a more pronounced reduction. This finding suggests that the effects of altered nasal MC activity on drug absorption may not be linearly proportional to changes in nasal residence time. Further studies that directly correlate MC activity, nasal residence time, and systemic drug exposure are required to clarify this relationship.

The difference in the magnitude of the absorption changes between LFX and norfloxacin may be partly associated with the physicochemical and biopharmaceutical properties of the drugs. LFX is classified as a biopharmaceutical classification system (BCS) class 1 drug, whereas norfloxacin is classified as a BCS class 4 drug. The relatively high solubility and permeability of LFX may facilitate its absorption across the nasal mucosa within a short residence time. However, the BCS classification primarily reflects gastrointestinal drug absorption and does not directly predict nasal absorption. Therefore, the contributions of solubility, membrane permeability, and nasal residence time to the observed differences remain to be elucidated.

These findings indicate that the contribution of nasal MC activity to systemic drug absorption after IN administration may fluctuate depending on the physicochemical and biopharmaceutical properties of the drug. Quantitative evaluation of this relationship may help identify drugs for which prolonging the nasal residence time is particularly beneficial and guide the selection of formulation strategies, such as the use of mucoadhesive excipients. This approach may support the rational design of nasal formulations with controllable drug absorption profiles.

Author Contributions: Conceptualization, D.I.; methodology, D.I.; software, D.I.; validation, D.I. and H.T.; formal analysis, D.I.; investigation, D.I.; resources, D.I.; data curation, D.I.; writing—original draft preparation, D.I.; writing—review and editing, D.I. and H.T.; visualization, D.I.; supervision, D.I. and H.T.; project administration, D.I.; funding acquisition, D.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board of Shujitsu University (protocol code 011-001, 2018), approval date 1 April 2018.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed toward the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MC	Mucociliary clearance
IN	Intranasal administration
IV	Intravenous administration
LFX	Levofloxacin
PPL	Propranolol
TBL	Terbutaline
C_{max}	Maximum concentration of drug in plasma
T_{max}	Time to reach maximum concentration of drug in plasma
AUC	Area under the concentration–time curve
BCS	Biopharmaceutical classification system
LC/MS	Liquid chromatograph/Mass spectrometry

References

1. Schipper, N.G.M.; Verhoef, J.C.; Merkus, F.W.H.M. The Nasal Mucociliary Clearance: Relevance to Nasal Drug Delivery. *Pharm. Res.* **1991**, *8*, 807–814. [CrossRef] [PubMed]
2. Marttin, E.; Schipper, N.G.M.; Verhoef, J.C.; Merkus, F.W.H.M. Nasal Mucociliary Clearance as a Factor in Nasal Drug Delivery. *Adv. Drug Deliv. Rev.* **1998**, *29*, 13–38. [CrossRef] [PubMed]
3. Ugwoke, M.I.; Verbeke, N.; Kinget, R. The Biopharmaceutical Aspects of Nasal Mucoadhesive Drug Delivery. *J. Pharm. Pharmacol.* **2001**, *53*, 3–21. [CrossRef] [PubMed]
4. Illum, L. Nasal Drug Delivery—Possibilities, Problems and Solutions. *J. Control. Release* **2003**, *87*, 187–198. [CrossRef] [PubMed]
5. Davis, S.S.; Illum, L. Absorption Enhancers for Nasal Drug Delivery. *Clin. Pharmacokinet.* **2003**, *42*, 1107–1128. [CrossRef] [PubMed]
6. Kublik, H.; Vidgren, M.T. Nasal Delivery Systems and Their Effect on Deposition and Absorption. *Adv. Drug Deliv. Rev.* **1998**, *29*, 157–177. [CrossRef] [PubMed]
7. Gizurarson, S. The Effect of Cilia and the Mucociliary Clearance on Successful Drug Delivery. *Biol. Pharm. Bull.* **2015**, *38*, 497–506. [CrossRef] [PubMed]
8. Agu, R.U. Challenges in Nasal Drug Absorption: How Far Have We Come? *Ther. Deliv.* **2016**, *7*, 495–510. [CrossRef] [PubMed]
9. Djupesland, P.G. Nasal Drug Delivery Devices: Characteristics and Performance in a Clinical Perspective—A Review. *Drug Deliv. Transl. Res.* **2013**, *3*, 42–62. [CrossRef] [PubMed]
10. Jiao, J.; Zhang, L. Influence of Intranasal Drugs on Human Nasal Mucociliary Clearance and Ciliary Beat Frequency. *Allergy Asthma Immunol. Res.* **2019**, *11*, 306. [CrossRef] [PubMed]
11. van de Donk, H.J.M.; Merkus, F.W.H.M. Decreases in Ciliary Beat Frequency Due to Intranasal Administration of Propranolol. *J. Pharm. Sci.* **1982**, *71*, 595–596. [CrossRef] [PubMed]
12. Djupesland, P.G.; Messina, J.C.; Mahmoud, R.A. The Nasal Approach to Delivering Treatment for Brain Diseases: An Anatomic, Physiologic, and Delivery Technology Overview. *Ther. Deliv.* **2014**, *5*, 709–733. [CrossRef] [PubMed]

13. Inoue, D.; Kimura, S.; Kiriyama, A.; Katsumi, H.; Yamamoto, A.; Ogawara, K.; Higaki, K.; Tanaka, A.; Yutani, R.; Sakane, T.; et al. Quantitative Estimation of the Effect of Nasal Mucociliary Function on in Vivo Absorption of Norfloxacin after Intranasal Administration to Rats. *Mol. Pharm.* **2018**, *15*, 4462–4469. [CrossRef] [PubMed]
14. Inoue, D.; Tanaka, A.; Kimura, S.; Kiriyama, A.; Katsumi, H.; Yamamoto, A.; Ogawara, K.; Kimura, T.; Higaki, K.; Yutani, R.; et al. The Relationship between in Vivo Nasal Drug Clearance and in Vitro Nasal Mucociliary Clearance: Application to the Prediction of Nasal Drug Absorption. *Eur. J. Pharm. Sci.* **2018**, *117*, 21–26. [CrossRef] [PubMed]
15. Sousa, J.; Alves, G.; Fortuna, A.; Falcão, A. Intranasal Delivery of Topically-Acting Levofloxacin to Rats: A Proof-of-Concept Pharmacokinetic Study. *Pharm. Res.* **2017**, *34*, 2260–2269. [CrossRef] [PubMed]
16. Wong, C.Y.J.; Baldelli, A.; Tietz, O.; van der Hoven, J.; Suman, J.; Ong, H.X.; Traini, D. An Overview of in Vitro and in Vivo Techniques for Characterization of Intranasal Protein and Peptide Formulations for Brain Targeting. *Int. J. Pharm.* **2024**, *654*. [CrossRef] [PubMed]
17. Inoue, D.; Furubayashi, T.; Ogawara, K.; Kimura, T.; Higaki, K.; Katsumi, H.; Sakane, T.; Yamamoto, A.; Higashi, Y. In Vitro Evaluation of Nasal Mucociliary Clearance Using Excised Rat Nasal Septum. *Biol. Pharm. Bull.* **2012**, *35*, 889–894. [CrossRef] [PubMed]
18. Inoue, D.; Furubayashi, T.; Ogawara, K.; Kimura, T.; Higaki, K.; Shingaki, T.; Kimura, S.; Tanaka, A.; Katsumi, H.; Sakane, T.; et al. In Vitro Evaluation of the Ciliary Beat Frequency of the Rat Nasal Epithelium Using a High-Speed Digital Imaging System. *Biol. Pharm. Bull.* **2013**, *36*, 966–973. [CrossRef] [PubMed]
19. Mallants, R.; Jorissen, M.; Augustijns, P. Effect of Preservatives on Ciliary Beat Frequency in Human Nasal Epithelial Cell Culture: Single versus Multiple Exposure. *Int. J. Pharm.* **2007**, *338*, 64–69. [CrossRef] [PubMed]
20. Houtmeyers, E.; Gosselink, R.; Gayan-Ramirez, G.; Decramer, M. Effects of Drugs on Mucus Clearance. *Eur. Respir. J.* **1999**, *14*, 452. [CrossRef] [PubMed]

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