The Permeation of Nalmefene Hydrochloride across Different Regions of Ovine Nasal Mucosa

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The permeability of nalmefene hydrochloride (NH) across different regions of ovine nasal mucosa was investigated *in vitro*. Five different regions of ovine nasal mucosa (superior turbinate mucosa, middle turbinate mucosa, inferior turbinate mucosa, posterior septum mucosa, and anterior septum mucosa) were studied. The results showed that the permeability coefficients of NH through different regions of nasal mucosa were different, and the suitable regions for the absorption of NH were the middle turbinate mucosa, the posterior septum mucosa and the superior turbinate. At the same time, the middle turbinate mucosa was the largest region among the five regions, thus it was the main absorption region for NH. The high uniformity of the middle turbinate mucosa also made it the most suitable model for the permeation of NH *in vitro*.

Key words nasal drug delivery; permeation; different region; nalmefene hydrochloride

Nasal drug delivery has been generating widespread interest in the drug delivery field because it could not only be used in local treatment but also in systemic administration. Various properties of nasal drug delivery made it an alternative route for intravenous and oral administration, such as bypassing of enzymatic or acidic degradation and exempting the first-pass hepatic metabolism; the relatively large epithelial surface, porous endothelial membranes and highly vascularized epithelium also maintained the rapid absorption of drugs.

Various factors that might affect the permeability of drugs through the nasal mucosa could be broadly classified into three categories as follow: biological factors, formulation aspects and device-related factors.[1,2] The nasal structure feature was one of the main biological factors. The nasal cavity could be anatomically segregated into five different regions: nasal vestibule, atrium, respiratory area, olfactory region and nasopharnyx.[2] The structure features of different regions were quite different which resulted in different blood supply, nasal secretion and mucociliary clearance among them,[3] and thus may influence the permeability of drug and presystemic degradation. Moreover, different devices and dosage forms may result in differences of the deposition place and residence time of drugs in the nasal cavity. Therefore, the research on the permeability of drug through mucosas of different regions of nasal cavity *in vitro* may help us to find out the main absorption region of drugs and understand the mechanism, rate and extent of drug absorption across the nasal mucosa. Furthermore, it may also be helpful for choosing dosage forms to deposit drugs on the main absorption region and prolong the residence time.

However, there were few studies about the permeation of drugs through mucosas of different regions of the nasal cavity. In present study, the Valia-Chien chamber technique[4] and a hydrophilic model drug nalmefene hydrochloride [17-*N*-cyclopropylmethyl-3,14-b-dihydroxy-4,5-a-epoxy-6-methylene morphinan hydrochloride][5] which was chosen for its possibility to be developed as a product for emergency medicine,[6] was used to study the permeability of the five different regions. The permeability coefficient and accumulative drug permeation was investigated. The permeation of NH through different pieces obtained from the same middle turbinate mucosa and the same septum mucosa was also investigated to evaluate the uniformity of such mucosa.

The excised tissue of animals such as sheep,[7—9] pig,[10,11] rabbit,[4,12,13] and ox[14,15] had been employed by researchers to study the drug delivery through nasal mucosa. Among these animals, the morphology of the ovine mucosa is more comparable to that of humans because of the presence of ciliated and non ciliated cells, basal cells, goblet cells and serous glands.[16] In the present study, ovine nasal mucosa was employed. During the experimental process, it was easier to handle the tissue and keep the mucosa intact and unstrained. There was also an ethical advantage that the material was obtained from a slaughterhouse and the sacrifice of animals for only a small piece of tissue was thus avoided.

Experimental

Materials NH was synthesized in the laboratory. The Ringer's solution used throughout the studies consisted of NaCl (125 mM), KCl (5 mM), $CaCl_2$ (1.4 mM), NaH_2PO_4 (1.2 mM), NaH_2PO_4 (1.2 mM), $NaHCO_3$ (10 mM) and D-glucose (11 mM) was filtered through a 0.45 μm filter before use, and then stored in refrigerator. All substances were of pharmaceutical quality or analytical grade.

Preparation of NH Solution The drug was dissolved by pH 4.0 phosphate[17] buffer using ultrasound to get a solution contains 2 mg/ml of NH.

Tissue Preparation Ovine nasal mucosa of male sheep, 1—2 years old, weighing 35—40 kg, was used in this study .The time from slaughter to removal of the tissue was approximately 1 or 2 min. After sacrifice of the animal, the nose cavity of sheep was fully exposed by a longitudinal incision through the lateral wall of the nose but avoid the damage of the septum, as it was shown in Fig. 1. The nasal septum, the middle turbinate and the superior turbinate with their underlying bone were carefully isolated with a scissors; however the mucosa on the inferior turbinate should be carefully excised by the round-edged tweezers. Then all the tissues were immersed in the ice-cold gassed (O_2/CO_2=95%/5%) Ringer's solution. The mucosae were carefully stripped from the cartilage and the conchae before using and then immediately mounted on the two halves of Valia-Chien diffusion chamber which had an exposed tissue surface area of 0.785 cm^2. The thickness of the mucosa on different regions was estimated before the experiment using a micrometer.

Permeation Study Before the experiment, the excised mucosa was immediately mounted on the two halves of the Valia-Chien diffusion chamber, and both of the acceptor (serosal) side and the donor (mucosal) side were filled with 3 ml of Ringer's solution. The temperature was kept at 37±1 °C.

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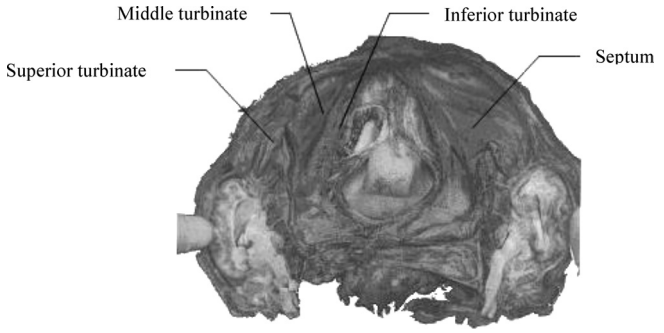

Fig. 1. The Photograph of a Sagittal Section of Ovine Nasal Cavity

Then the Ringer's solution on the donor side was replaced by the NH solution. During the experiment, 0.2 ml of sample was removed from the acceptor side at 20, 40, 60, 90 and 120 min, replaced by Ringers solution of the same volume and temperature. In this study, the experiment was carried out within 3 to 3.5 h since the obtaining of tissues, so the viability of the mucosa was maintained.[7,11)]

HPLC Method A HPLC system consisting of a pump (SPD-10A, SHIMADZU, Japan) and ultroviolet detector (LC-10AT, SHIMADZU, Japan) was employed, the mobile phase consisted of methanol : 0.02 M KH_2PO_4 : triethylamine (40 : 60 : 0.1, v/v/v) running at a flow rate of 1.0 ml/min. The detection wave length was 230 nm. The column was phenomenex Prodigy 5u ODS3 100A (U.S.A.) and the samples were centrifuged under 10000 rpm (LDZ5-2 high speed centrifuge, Beijing medical high speed centrifuge factory, China) for 10 min before analysis.

Calculation of Permeability Coefficient The flux was plotted as the accumulation amount of NH from the donor chamber to the acceptor side through mucosa *versus* time. The following equation was used to calculate the permeability coefficient (P):

$$P = \frac{dQ/dt}{C_0 A} \quad 4)$$

where dQ/dt represents the permeability rate, C_0 is the initial concentration in donor compartment and A is the permeation area of mucosa.

The steady state flux (J_{ss}) was calculated according to the equation as follows:

$$J_{ss} = \frac{V}{A} \cdot \frac{dC_n}{dt} \quad 18)$$

where dC_n/dt is the increase of NH concentration in the diffusion solution in the acceptor chamber, V represents the volume of the acceptor chamber and A is the permeation area of mucosa.

Student's t-test and ANOVA were used to determine statistical significance. Differences were considered to be significant for values of $p<0.05$.

Results and Discussion

The Permeability Difference of NH through Diverse Regions of Ovine Nasal Mucosa The permeability differences of NH across anterior septum, posterior septum, middle turbinate, inferior turbinate and superior turbinate *in vitro* were investigated. All the comparative mucosae were obtained from five sheep. From Fig. 3 and Table 1 we could see that the permeability coefficients of the NH across the posterior septum mucosa was 1.45-foldhigher than that of anterior septum mucosa, however, the permeability coefficients of the NH across the middle turbinate, the posterior septum and the superior turbinate were similar. Such phenomena might because that the nasal mucosa on the anterior septum was stratified squamous epithelium while the mucosa of middle turbinate, the posterior septum and the superior turbinate consisted of pseudostratified columnar epithelium that covered the respiratory epithelium. In conclusion, the permeability coefficients of NH through different regions of nasal mucosa were in the following order, middle turbinate≈posterior septum≈superior turbinate>anterior septum>inferior turbinate, which showed that the middle turbinate, the posterior septum and the superior turbinate were suitable for the permeation of NH, since the middle turbinate occupies the largest area of the nasal cavity, thus the middle turbinate was the main absorption region of NH.

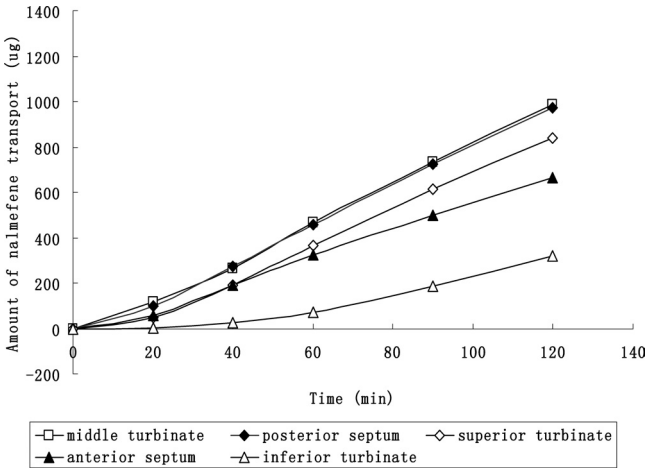

Fig. 2. The Permeation of NH through Diverse Sheep Nasal Mucosa *in Vitro*

Table 1. The Permeability Coefficients and the Steady State Fluxes of NH across the Different Mucosae of the Sheep *in Vitro* (Mean±S.D., $n=5$)

Mucosas	dQ/dt ($\times10^{-3}$)	P ($\times10^{-6}$) (cm s^{-1})	J_{ss} ($\times10^{-3}$) (μg·cm^{-2}·s^{-1})
Superior turbinate	138.38±34.76	88.11±22.15[a)]	98.56±25.08
Middle turbinate	147.18±12.76	93.76±8.14[a)]	105.6±9.90
Inferior turbinate	54.56±8.36	34.85±5.61[c)]	38.72±6.38
Anterior septum	101.64±18.71	64.66±11.99[b,c)]	73.26±13.64
Posterior septum	145.64±26.62	92.71±16.94[a,b)]	105.6±19.8

a) ANOVA was used to analyze the significance of posterior septum, middle turbinate and superior turbinate ($p>0.05$, no significant difference). *b*) The Student's t-test was used to analyze the significance between anterior septum and posterior septum ($p<0.05$, significant difference). *c*) The Student's t-test was used to analyze the significance between inferior turbinate and anterior septum ($p<0.05$, significant difference).

From Fig. 3, we could also see that a lag time appeared on each profile of the cumulative flux of NH through different mucosa, particularly in the case of the inferior turbinate mucosa. It might be related to the thickness of the mucosa which made it take some time for the drug to permeate across it. The inferior turbinate mucosa was the thickest among the mucosa whose thickness was about 1500—2000 μm, while the thickness of middle turbinate mucosa, superior turbinate mucosa, posterior septum mucosa, anterior septum mucosa were 450—600 μm, 900—1300 μm, 500—600 μm and 1200—1700 μm, respectively.

The Investigation of the Uniformity of Different Mucosa The middle turbinate mucosa and the septum mucosa were employed here because the area of these two mucosae was large enough for this experiment. The middle turbinate mucosa had the largest area among the five mucosae, most of which had an area of 3.5 cm×5.5 cm approximately. During the experiment, five pieces of middle turbinate mucosae and septum mucosa were used; each of them was cut into 4

Table 2. The Permeability Coefficients of NH across Different Pieces from the Same Middle Turbinate Mucosa and the Same Septum Mucosa *in Vitro* (Mean±S.D., $n=4$)

Different mucosa	P ($\times 10^{-6}$) (cm s^{-1})				
	1	2	3	4	5
Middle turbinate mucosa	92.86±3.15	102.10±2.11	95.33±4.51	90.57±1.66	101.66±3.61
Septum mucosa	76.75±23.80	55.25±19.62	68.50±17.52	86.21±24.67	80.25±12.61

pieces, and the drug permeation across each piece was tested.

As shown in Table 2, the permeability coefficient among the pieces from the same middle turbinate mucosa was comparable (average RSD=3.01%), while the permeability coefficient among the pieces from the same septum mucosa was quite different (average RSD=19.64%). The variation of permeation coefficient among different pieces of septum turbinate might because of the change of thickness and epithelium cell type from the anterior septum to the posterior septum while the thickness and epithelium cell type of the middle turbinate mucosa was kept unchanged.

Conclusion

The result of this study indicated that the permeability coefficients of NH on different sites of mucosa in the nasal cavity was quite different and followed such order: middle turbinate mucosa≈posterior septum mucosa≈superior turbinate mucosa>anterior septum mucosa>inferior turbinate mucosa. The main absorption region of NH was the middle turbinate because of its high permeability coefficient and large area. The middle turbinate was also chosen as the suitable model for *in vitro* study because of its many advantages, such as large surface area, highest drug permeability coefficient and easy to be conducted. The high reproducibility of permeation study across the middle turbinate mucosa was also helpful to reduce the deviation and the error emerged in the experiments.

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