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Rapid-onset intranasal delivery of metoclopramide hydrochloride Part I. Influence of formulation variables on drug absorption in anesthetized rats

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Abstract

Intranasal (IN) administration is a promising approach for rapid-onset delivery of medications and to circumvent their first-pass elimination when taken orally. Metoclopramide (MCP) is a potent antiemetic, effective even for preventing emesis induced by cancer chemotherapy. The feasibility of developing an efficacious intranasal formulation of metoclopramide has been undertaken in this study. The nasal absorption of MCP was studied in anesthetized rats over 60 min using the in vivo in situ technique. The influence of several formulation variables, vis., pH and the addition of preservative, viscosity and absorption enhancing agents on the nasal MCP absorption was examined. The data obtained showed that MCP was well absorbed nasally where almost 90% of the drug was absorbed after 60 min from the rat nasal cavity. The MCP absorption was pH-dependant such that the apparent first-order rate constant of absorption (K_{app}) was almost tripled when the pH of the solution was increased from 5 to 8. However, deviation from the classical pH-partition theory was observed pointing to the role of aqueous pore pathway in MCP nasal absorption. The K_{app} was significantly increased ($P < 0.05$) by incorporation of 0.01% of the preservative benzalkonium chloride. Conversely, increasing the solution viscosity by the use of hydroxylpropyl methylcellulose adversely affected the rate of absorption. The use of enhancers namely sodium deoxycholate, sodium cholate, chitosan low and high molecular weight, protamine sulphate and poly-L-arginine resulted in significant increase in MCP absorption. The highest promoting effect was observed with the bile salt sodium deoxycholate where about 92% of the drug was absorbed in 25 min from the rat nasal cavity and the K_{app} showed more than two-fold increase as compared to control (from 0.0452 to 0.1017 min^{-1}).

Keywords: Nasal absorption; Metoclopramide; Rapid-onset; Absorption enhancers

1. Introduction

The past decade has been marked by a steadily growing interest using the nasal route for systemic drug delivery. Compounds ranging in size from simple, traditional drug molecules of approximately 200–300 Da to complex proteins with molecular weights exceeding 100,000 have been investigated. The nasal delivery appears to be a desirable alternative to the parenteral medication because of the existence of a rich vasculature and a highly permeable structure within the nasal membranes (Behl et al., 1998a). The well perfused nasal mucosa provides an excellent site for rapid absorption of centrally acting drugs such as midazolam for the control of epileptic seizures, morphine and ketamine for the treatment of pain and dihydroergotamine for the relief of migraine (Illum, 2003). In addition, nasal application circumvents first-pass elimination and/or degradation of protein drugs prone to enzymatic degradation in the GI tract, and may be employed routinely without any pain (Hussain, 1998). Furthermore, studies demonstrated the existence of direct transport from the nasal cavity to the cerebrospinal fluid and proceeding to the brain (Illum, 2003).

The fast rate of absorption with rapid onset of action could be especially important in the management of crisis situations such as severe nausea and vomiting. Oral antiemetics often get vomited out before systemic absorption compelling parenteral administration which results in low patient compliance. Metoclopramide hydrochloride is a potent antiemetic, effective in the treatment of nausea and vomiting associated with migraine,

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cancer therapy, pregnancy, etc. It is well absorbed orally and peak plasma concentrations occur about 1–2 h after an oral dose. However, it undergoes hepatic first-pass metabolism and both the absolute bioavailability and the plasma concentrations are subject to wide inter-individual variation showing values between 32 and 98% (Harrington et al., 1983; Dollery, 1999).

Different factors affecting nasal absorption include: drug characteristics such as molecular weight, lipophilicity and pK_a of drug molecule, formulation factors such as pH, viscosity, particle size and various pharmaceutical excipients as well as physiological factors for example blood flow to the nasal mucosa and rate of mucociliary clearance (Behl et al., 1998a). Nasal bioavailability of drugs may be improved with the aid of absorption promoters which include classical anionic enhancers such as bile salts as well as the new cationic enhancers such as chitosan, protamine and poly-L-arginine (Davis and Illum, 2003).

In this study, nasal formulations of metoclopramide HCl were developed to increase the extent of absorption through by-pass of hepatic first-pass metabolism and also as a strong alternative antiemetic therapy to achieve rapid onset of therapeutic drug action. The nasal in vivo in situ method (Faraj et al., 1990) was adopted to study drug absorption from the nasal cavity. Different formulation variables such as pH, viscosity and the use of preservatives and absorption enhancers were investigated with the aim of optimizing nasal metoclopramide HCl delivery.

2. Materials and methods

2.1. Materials

Metoclopramide hydrochloride (Lepetit, Italy), hydroxypropyl methylcellulose (Methocel-E4M, molecular weight 86,000, 4000 centipoise (cps), urethane, sodium cholate, sodium deoxycholate, protamine sulphate, poly-L-arginine (molecular weight. 70–150 kDa) (Sigma, USA), chitosan low molecular weight (150 kDa), chitosan high molecular weight (600 kDa) both were 75–85% deacetylated (Fluka, Germany), disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, benzalkonium chloride analytical grade (ADWIC Co., Egypt).

2.2. Methods

2.2.1. Preparation of metoclopramide hydrochloride nasal solutions

- *Control solution:* Aqueous solution containing 9 mg/ml metoclopramide hydrochloride (MCP HCl) (Paget and Barnes, 1964) was prepared by dissolving the drug in distilled water using a magnetic stirrer (Thermolyne Corporation, USA) and adding the appropriate amount of sodium chloride to adjust the solution tonicity (Stoklosa and Ansel, 1996). The pH of the solution was determined to be 6.7 ± 0.2 using a pH meter (Jenway, UK).
- *Test solutions*: The control solution to which different additives were added to give the following solutions:
  (1) Solutions of different pHs: 5, 6, 7 and 8 using 1/15 M Sorensen's phosphate buffer as a vehicle.
  (2) Solutions containing 0.01 or 0.02% (w/v) of benzalkonium chloride (BAC).
  (3) Solutions of varying viscosities containing 0.2, 0.4 and 0.8% hydroxypropyl methylcellulose (HPMC).
  (4) Solutions containing 0.5% of different absorption enhancers, namely: sodium cholate (SC), sodium deoxycholate (SDC), chitosan low molecular weight (CS L), chitosan high molecular weight (CS H), protamine sulphate (Prot) and poly-L-arginine (Poly-L-arg).

Solutions tonicity was adjusted with sodium chloride.

2.3. In vivo in situ nasal MCP HCl absorption study

2.3.1.1. Surgical technique

The method described by Faraj et al. (1990) was adopted. Albino male rats, weighing 250–300 g each, were used. At the time of the experiment, the rats were anaesthetized where a 1.3 g/10 ml urethane solution was injected intraperitoneally in a dose of 1 ml/100 g rat body weight. Fig. 1 shows the experimental arrangement for the in vivo in situ nasal experiments. While the rat is kept in the supine position, an incision was made in the neck then the trachea was cannulated with a polyethylene tube to maintain respiration. The oesophagus was ligated with a thread to keep the solution in the nasal cavity and eliminate oral absorption. The nasopalatine was closed with an adhesive agent to prevent the drainage of the drug solution from the nasal cavity to the mouth. Prior to drug administration, the nasal cavity was washed carefully with 10 ml normal saline solution. The experimental procedures conform to the ethical principles of the Experiments and Advanced Pharmaceutical Research Unit (EAPRU), Faculty of Pharmacy, Ain Shams University, Cairo, Egypt on the use of the animals.

2.3.1.2. Deposition of drug solution

In each rat, 20 μl aliquots of drug solutions equivalent to 0.18 mg MCP HCl were deposited in one nostril by means of a micropipette. After specified times of deposition: 3, 5, 10, 15, 20, 25, 30, 40 or 60 min, the residual drug in the nasal cavity was recovered by washing thoroughly with 6 ml of normal saline at a flow rate 2 ml/min (Aikawa et al., 1998). The lavage fluid was filtered using Millipore filteration unit (Millipore, Milford, USA) and was used directly for analysis of drug content spectrophotometerically at λ_{max} 273 nm (Schimadzu UV 240 double beam spectrophotometer, Koyoto, Japan) after appropriate dilution. The concentration of drug remaining in the nasal cavity after each specified time interval was determined. The experiment was conducted at least in triplicate and the results are expressed as the mean ± S.D. of three measurements.

2.3.2. Data analysis and calculations

Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by the least significant difference method (LSD) for multiple comparison at $P < 0.05$.

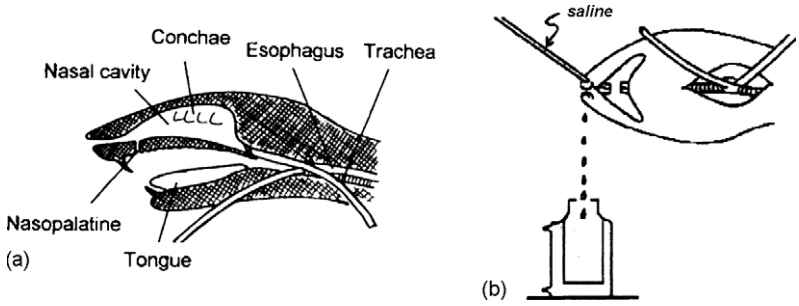

Fig. 1. Diagram of the surgical procedures for drug administration in rats (a) and experimental setup of the in vivo in situ technique used for studying nasal drug absorption (b).

The apparent absorption rate constant, K_{app}, was calculated using the first-order kinetic rate equation:

$$C_t = C_o e^{-K_{app} \cdot t} \quad (1)$$

where C_t is the concentration remaining unabsorbed at time t; C_o the initial concentration of the drug and K_{app} is the apparent absorption rate constant and obtained from the slope of the semilog plot of C_t versus t.

The percent unionized of MCP HCl was calculated using the following equation (Martin, 1993):

$$\%\text{unionized} = \frac{100}{[1 + \text{antilog}(pK_a - pH)]} \quad (2)$$

where pK_a of MCP HCl equals 9.71 (Pitrè and Stradi, 1987); pH is the pH of the deposited solution.

3. Results and discussion

In this study the more physiologically relevant in vivo in situ method (Faraj et al., 1990) was adopted as a modification of the in situ perfusion technique, first described by Hirai et al. (1981a) that was frequently used to determine nasal drug absorption kinetics. Although both methods employ the same surgical procedure whereby the nasal cavity of an anaesthetized rat is isolated to restrict drug absorption to the nasal mucosa, yet in the in vivo in situ method a small volume of the drug solution (20 μl) is nasally deposited simulating the use of nasal drops in humans contrary to a volume of 5 ml used in the in situ perfusion technique. Moreover, the solution is not circulated through the nasal cavity and in such way factors such as recirculation speed, volume of circulating solution and total surface area of the nasal cavity covered by the solution that influence the determination of the drug absorption rate constant are eliminated (Gizurarson, 1993). However, the limitations associated with the original in situ perfusion technique like the gradual erosion of the mucus covering the epithelium, the prolonged nasal drug residence time and the lack of nasal mucociliary clearance still exist. To surmount these limitations, we performed an in vivo nasal absorption study in rabbits as a complementary work to this study (Part II).

3.1. Effect of pH

A 'dual-pathway', one lipoidal and one aqueous, for transport has been hypothesized by previous investigators to explain absorption patterns in the nasal mucosa (Hussain et al., 1985; Gibson and Olanoff, 1987; Corbo et al., 1989). The nasal absorption of small lipophilic or hydrophilic compounds is a complex

Table 1
Effect of pH on the residual percentage of MCP HCl in the rat nasal wash

Time (min)	Mean residual percentage MCP HCl at different pHs ± S.D.[a]				
	Control	pH 5	pH 6	pH 7	pH 8
3	90.95 ± 5.33	97.24 ± 2.88	94.66 ± 3.84	90.37 ± 5.92	86.24 ± 3.88
5	71.58 ± 8.51	89.69 ± 4.61	89.36 ± 2.65	85.19 ± 4.82	70.51 ± 4.51
10	62.19 ± 4.21	82.11 ± 4.56	69.65 ± 1.83	62.33 ± 4.96	60.03 ± 5.93
15	49.86 ± 4.50	75.57 ± 4.85	53.29 ± 2.94	51.01 ± 3.81	41.88 ± 7.15
20	38.08 ± 3.01	62.03 ± 6.31	38.12 ± 4.18	37.81 ± 3.54	32.09 ± 5.77
25	24.55 ± 5.13	56.38 ± 5.82	29.81 ± 3.54	24.69 ± 4.53	20.55 ± 3.81
30	20.33 ± 3.14	45.25 ± 4.97	24.21 ± 3.08	17.52 ± 5.63	12.97 ± 4.62
40	14.33 ± 2.05	36.98 ± 4.12	17.06 ± 2.68	12.39 ± 3.72	5.74 ± 2.13
60	9.68 ± 2.50	30.23 ± 3.68	10.33 ± 4.55	7.03 ± 2.44	ND[b]
K_{app}[c] (min^{-1}) ± S.D.	0.0452 ± 0.0017b,e	0.0223 ± 0.0019a,c,d,e	0.0425 ± 0.0048b,e	0.0495 ± 0.0017b,e	0.0666 ± 0.0048a,b,c,d

Different letters (a, b, c, d or e) significantly different from control, pH 5, 6, 7 or 8, respectively, at $P < 0.05$ using one-way ANOVA followed by LSD for multiple comparisons.

[a] S.D.: standard deviation.

[b] ND: not detected.

[c] K_{app}: apparent first-order absorption rate constant.

interaction between the ability of the compound to partition rapidly into the lipophilic cellular matrix or to utilize the aqueous pore (paracellular) pathway suitable for the transport of hydrophilic (ionized) compounds (Donovan and Huang, 1998). The aqueous pore pathway is more accessible to hydrophilic compounds and is limited primarily by the drug molecular size as a result of steric hindrance as well as its charge; being more selective for cationic drugs (Karlsson et al., 1999). However, McMartin et al. (1987) suggested that these pores must exist in a wide range of molecular sizes in order to account for the wide molecular weight range of hydrophilic compounds absorbed through the nasal mucosa. Hayashi et al. (1985) reported that the number of water channels is four times higher in the nasal membrane than in the jejunal membrane of rats.

By reviewing the data in Table 1, it is obvious that MCP HCl, in the control solution, is well absorbed from the nasal mucosa where the residual percentage was 9.68 in the lavage fluid 60 min after deposition i.e. almost 90% of the input solution is absorbed nasally.

Concerning the effect of pH on the nasal drug absorption, this was studied using phosphate buffer which is safe to the nasal mucosa compared to acetate and citrate buffers (Pujara et al., 1995).

Table 1 shows the percent of MCP HCl remaining in the nasal cavity after the deposition of 20 μl of different MCP HCl solutions at different times prior to rinsing. From the data, it is clear that the % drug remaining unabsorbed in the rat nasal cavity decreased as the pH of the instilled solution was increased over the pH range 5–8. The absorption data of MCP HCl in all the used solutions best fitted first-order kinetic rate (Eq. (1)), with r^2-values for all profiles ranging from 0.91 to 0.99. It is clear that the rate of drug absorption, expressed by K_{app}, increased as the pH was increased. The K_{app} of MCP HCl at pH 8 (0.0666 min^{-1}) was significantly greater ($P < 0.05$) than at other pHs reaching about a three-fold increase when compared to K_{app} (0.0223 min^{-1}) at pH 5. This increase could be interpreted on the basis of the pH-partition theory for absorption of weak electrolytes. MCP HCl being a salt of a weak base, its ionization and hence absorption are determined by its pK_a and the pH of the solution in which it is dissolved. At high pH, more drug will exist in the unionized form which has greater lipophilicity than the ionized form and the latter parameter is a prime determinant of membrane penetration. However, upon plotting the theoretical unionized percent of MCP HCl, calculated using Eq. (2), versus pH and compare the obtained profile with the experimentally-found profile of the absorption rate constant (K_{app}) versus pH (Fig. 2), we observed a deviation from the classical pH partition theory were the two curves did not coincide. There was increased drug absorption than would be explained solely by the pH partition hypothesis indicating the importance of the aqueous pore pathway for the diffusion of different ionized or hydrophilic drugs through the nasal mucosa. Previously reported, phosphate buffer with pH from 3 to 10 had no effect on the nasal mucosal integrity (Pujara et al., 1995) so it seems unlikely that the pH range used in this study could have effect on the nasal mucosal integrity/properties. Similar inconsistencies between nasal absorption of drugs and pH-partition theory were also reported by Hirai et al. (1981a), Huang et al. (1985), Kaneo (1983) and Su et al. (1984).

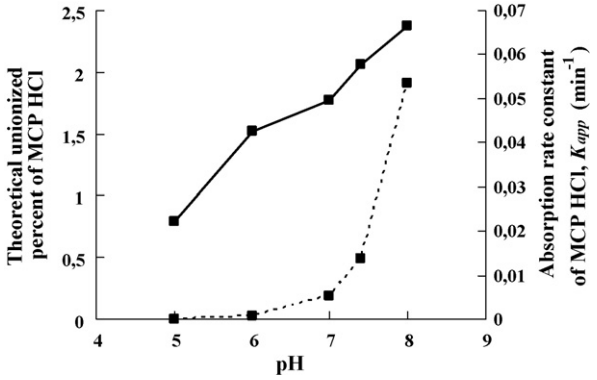

Fig. 2. Apparent absorption rate constant (solid line) and theoretical undissociated percent (dashed line) of MCP HCl through the nasal mucosa of rats at various pHs.

3.2. Effect of BAC

The FDA states that nasal preparations should be sterilized or preserved. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations and their safety has been investigated with respect to nasal mucociliary clearance and ciliary beat frequency (Batts et al., 1990, 1991; Romeijn et al., 1996). Van de Donk et al. (1980) have shown that mercury-containing preservatives have a fast and irreversible effect on ciliary movement and should not be used in nasal systems. Seeing that BAC caused no significant changes in the nasal mucociliary clearance after acute and long term administration in a concentration 0.02% to humans (Ainge et al., 1994), it has been chosen as a preservative in this study and its effect on the nasal absorption of MCP HCl was investigated at the relevant preservative concentration 0.01 and 0.02%.

The results of the MCP HCl nasal absorption as well as the first order absorption rate constant K_{app} for the drug are shown in Table 2. The absorption data revealed a decrease in the residual percentage drug (or an increase of drug absorbed) for solutions containing 0.01 or 0.02% BAC compared to control. The K_{app} for MCP HCl solution containing either 0.01 or 0.02% BAC were higher (0.0513 and 0.0487 min^{-1}) when compared to the control formulation (0.0452 min^{-1}). However, this higher K_{app} was only statistically significant ($P < 0.05$) in case of solutions containing 0.01% BAC. The absorption enhancement effect produced by 0.01% BAC could be attributed to its surface active properties. Previously, the in situ nasal perfusion studies (Hirai et al., 1981b) and in vitro systems (Wheatley et al., 1988) pointed to the surface active agents as being able to increase epithelial permeability via membrane disruption. Particularly speaking, BAC is a positively charged surface active agent; it may interact with negatively charged cell membrane via electrostatic binding to surface proteins, may cause structural destabilization of the epithelium and could thus increase membrane permeability. This is coherent with the results of Vora et al. (1993) who

Table 2
Effect of benzalkonium chloride on the residual percentage of MCP HCl in the rat nasal wash

Time (min)	Mean residual percentage MCP HCl with solutions containing different concentrations of BAC ± S.D.[a]		
	Control	0.01% BAC	0.02% BAC
3	90.95 ± 5.33	87.45 ± 4.11	91.83 ± 5.24
5	71.58 ± 8.51	68.45 ± 3.88	71.35 ± 4.67
10	62.19 ± 4.21	52.98 ± 5.21	59.67 ± 5.13
15	49.86 ± 4.50	43.03 ± 4.69	47.67 ± 6.23
20	38.08 ± 3.01	35.51 ± 2.55	35.94 ± 3.11
25	24.55 ± 5.13	28.22 ± 4.12	21.66 ± 2.84
30	20.33 ± 3.14	21.83 ± 4.52	19.29 ± 3.15
40	14.33 ± 2.05	11.36 ± 3.22	13.87 ± 2.41
60	9.68 ± 2.50	5.49 ± 1.56	7.45 ± 2.34
K_{app}[b] (min^{-1}) ± S.D.	0.0452 ± 0.0017 b	0.0513 ± 0.0036 a	0.0487 ± 0.0033

Different letters (a or b) significantly different from control or 0.01% BAC solutions, respectively, at $P < 0.05$ using one-way ANOVA followed by LSD for multiple comparisons.
[a] S.D.: standard deviation.
[b] K_{app}: apparent first-order absorption rate constant.

reported an increase in the intranasal availability of growth hormone releasing peptide and Azria (1991) who used BAC as an intranasal absorption promoter for salmon calcitonin incorporated in HPMC inserts.

3.3. Effect of HPMC

Higher nasal drug absorption might be achieved if the rate of nasal mucociliary clearance (MCC) could be slightly decreased or halted in a reversible manner without altering the functionality of the nose. Such retardation in the MCC would be useful in increasing the contact time between the active drug and the nasal mucosa, in so doing would increase the bioavailability. One approach to attain longer mucosal residence time is to increase formulation viscosity by incorporation of a suitable polymer. Different drug solutions containing 0.2, 0.4 and 0.8% (w/v) HPMC were used and their viscosities were measured to be 4.5 ± 0.5, 23.7 ± 3.2 and 81.3 ± 5.6 cps, respectively, using Ostwald viscometer.

Table 3 shows the % MCP HCl detected as unabsorbed in the nasal lavage fluid at different times. Results demonstrated that increasing the solution viscosity decreased the absorption of MCP HCl where the residual percentage of drug after 60 min was 9.68, 12.63, 34.12 and 50.98% for solutions containing 0, 0.2, 0.4 and 0.8% HPMC, respectively. The absorption rate constant data showed no significant retardation ($P < 0.05$) of MCP HCl absorption with solutions containing 0.2% HPMC compared to the control solution. Conversely, the K_{app} was significantly reduced ($P < 0.05$) with the higher viscosifier concentrations where it showed 2- and 3.5-fold decrease for solutions containing 0.4 and 0.8% HPMC, respectively, as compared to control. The slow down of drug absorption in viscous solutions might be attributed to a decrease in the diffusion of the drug through the formulation (Rawlins, 1982). Though the increased viscosity may prolong the retention time of the drug in the nasal cavity, whether this will result in improved absorption of drugs or not remains unclear. According to a study by Pennington et al. (1988) increasing solution viscosity may provide a means of

Table 3
Effect of hydroxypropylmethyl cellulose on the residual percentage of MCP HCl in the rat nasal wash

Time (min)	Mean residual percentage MCP HCl with solutions containing different concentrations of HPMC ± S.D.[a]			
	Control	0.2% HPMC	0.4% HPMC	0.8% HPMC
3	90.95 ± 5.33	91.47 ± 2.75	95.99 ± 3.12	98.25 ± 2.13
5	71.58 ± 8.51	72.46 ± 1.84	82.41 ± 4.83	90.09 ± 2.80
10	62.19 ± 4.21	60.34 ± 2.19	79.53 ± 5.61	81.42 ± 3.18
15	49.86 ± 4.50	52.03 ± 2.55	69.43 ± 4.25	76.39 ± 3.56
20	38.08 ± 3.01	41.11 ± 3.18	52.44 ± 3.92	70.55 ± 4.09
25	24.55 ± 5.13	29.16 ± 3.54	47.67 ± 5.66	66.48 ± 3.13
30	20.33 ± 3.14	22.39 ± 3.08	42.59 ± 4.11	63.51 ± 2.82
40	14.33 ± 2.05	16.01 ± 2.86	38.11 ± 5.61	56.15 ± 2.63
60	9.68 ± 2.50	12.63 ± 4.16	34.12 ± 2.88	50.98 ± 3.33
K_{app}[b] (min^{-1}) ± S.D.	0.0452 ± 0.0017c,d	0.0411 ± 0.0017c,d	0.0227 ± 0.0023a,b,d	0.0135 ± 0.0021a,b,c

Different letters (a, b, c or d) significantly different from control, 0.2, 0.4 or 0.8% HPMC solutions, respectively, at $P < 0.05$ using one-way ANOVA followed by LSD for multiple comparisons.
[a] S.D.: standard deviation.
[b] K_{app}: apparent first-order absorption rate constant.

Table 4
Effect of 0.5% solution of various enhancers on the residual percentage of MCP HCl in the rat nasal wash

Time (min)	Residual percentage MCP HCl at different pHs						
	Control	SC	SDC	CS L	CS H	Prot SO4	Poly-L-arg
3	90.95 ± 5.33	89.85 ± 4.22	89.25 ± 3.54	91.02 ± 6.19	90.45 ± 5.13	92.8 ± 4.63	88.56 ± 5.43
5	71.58 ± 8.51	70.17 ± 2.09	58.81 ± 2.84	76.94 ± 4.32	82.56 ± 4.86	76.61 ± 7.82	65.38 ± 4.19
10	62.19 ± 4.21	54.96 ± 5.31	35.87 ± 1.98	47.26 ± 3.86	45.29 ± 4.14	53.01 ± 2.45	55.17 ± 4.47
15	49.86 ± 4.50	34.46 ± 3.94	22.58 ± 4.19	38.05 ± 2.18	28.62 ± 3.81	40.49 ± 8.14	33.13 ± 3.05
20	38.08 ± 3.01	22.41 ± 2.06	12.41 ± 2.43	25.97 ± 3.07	24.34 ± 3.44	33.83 ± 10.24	29.51 ± 3.24
25	24.55 ± 5.13	16.81 ± 1.82	8.31 ± 2.54	19.83 ± 1.85	18.33 ± 3.76	22.93 ± 5.14	20.56 ± 5.18
30	20.33 ± 3.14	8.52 ± 2.70	ND[a]	10.35 ± 1.73	9.12 ± 2.11	17.69 ± 3.94	13.05 ± 2.95
40	14.33 ± 2.05	ND[a]	ND[a]	ND[a]	ND[a]	8.16 ± 3.02	7.5 ± 1.84
60	9.68 ± 2.50	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
K_{app}[b] (min^{-1}) ± S.D.	0.0452 ± 0.0017b,c,d,e,f,g	0.0761 ± 0.0077a,c,f	0.1017 ± 0.0113a,b,e,f,g	0.0698 ± 0.0053a,c	0.0752 ± 0.0081a,b,c,f	0.0612 ± 0.0090a,b,c,e	0.0658 ± 0.0053a,c

Different letters (a, b, c, d, e, f or g) significantly different from control, SC, SDC, CS L, CS H, Prot So4 or Poly-L-arg at $P < 0.05$ using one-way ANOVA followed by LSD for multiple comparisons.
[a] ND: not detected.
[b] NK_{app}: apparent first-order absorption rate constant.

prolonging the therapeutic effect of nasal preparations. Suzuki and Makino (1999) showed that a drug carrier such as hydroxypropyl cellulose was effective for improving the absorption of low molecular weight drugs but did not produce the same effect for high molecular weight peptides. In a study conducted by Kilian and Müller (1998) an increase in bioavailability of metoprolol was obtained by the inclusion of 2% methyl cellulose into the formula. On the contrary, 0.25% (w/v) methyl cellulose incorporated into desmopressin nasal formulation produced a slower and more sustained absorption with no apparent improvement in the bioavailability of the peptide (Harris et al., 1989). It should be emphasized that the anaesthetized rats used for this experiment have their esophagus ligated. As such, the issue of nasal MCC has already been circumvented that is why the increased viscosity revealed no improvement in the drug absorption.

3.4. Effect of absorption enhancers

Another formulation approach to improve MCP HCl nasal absorption is through the use of different absorption enhancers. The selected enhancers were the anionic bile salts: (SC) and (SDC), the cationic polysacharrides (CS H) and (CS L) as well as the cationic peptides: (Prot) and (Poly-L-arg). The above enhancers were used at conc. 0.5% which was frequently used in nasal drug delivery studies (Natsume et al., 1999).

Table 4 shows the percentages MCP HCl remaining unabsorbed after intranasal administration of the drug solutions containing the different enhancers and the rate constant, K_{app}, of MCP HCl absorption. The outcome of the absorption study illustrate that all promoters used enhanced the drug absorption to different extents compared to control. Behl et al. (1998b) reported that it is easier to achieve nasal drug absorption enhancement than some of the other mucosal routes due to the "porous" nature of the nasal mucosa that is well suited for opening up the pathways for improved absorption of many drugs.

On the basis of the promotion of MCP HCl intranasal absorption, the studied enhancers could be arranged in the following decreasing order: SDC > SC > CS H > CS L > Poly-L-arg > Prot > control.

Regarding the bile salts, their mechanism of action are quiet diverse and only partly understood, their influence could be at the level of the drug or at the level of nasal mucous membrane. Solubilization of the drug in the aqueous vehicle seems to be irrelevant since MCP HCl is freely soluble. At the level of the nasal mucosa there are three possible modes of action: (1) alteration of the mucus layer. The mucus layer covering the cell surface of the mucosa can be seen as an unstirred layer acting as a barrier to the diffusion of drug molecules. Anionic and cationic surfactants are able to reduce the mucus viscosity and elasticity and so the barrier function of the layer (Merkus et al., 1999; Marttin et al., 1995), (2) opening of the tight junctions between the epithelial cells (Inagaki et al., 1985) and (3) extraction of membrane components like proteins, LDH and cholesterol by co-micellization (Duchateau et al., 1986). This mechanism of absorption promotion effect of bile salts will be investigated in subsequent work carried out in our laboratory by performing

leaching studies of various nasal membrane components (Part II).

It is worthy to note that the dihydroxy unconjugated bile salt SDC produced significantly greater ($P < 0.05$) enhancing effect than SC which is a trihydroxy unconjugated bile salt as revealed by K_{app} of 0.1017 and 0.0761 min^{-1}, respectively. This observation would suggest that nasal absorption promotion increases with increase in the hydrophobicity of the bile salt and is in harmony with the results reported by Gordon et al. (1983) and Hirai et al. (1981b) working on nasal absorption of insulin and by Shao et al. (1994) working on nasal acyclovir absorption.

Pertaining to chitosans, these cationic polymers were also able to enhance MCP HCl absorption designated by an increase in K_{app} from 0.0452 min^{-1} with the control to 0.06983 and 0.07517 min^{-1} with CS L and CS H, respectively. The underlying mechanism was suggested to be a combination of mucoadhesion and transient widening of the tight junctions in the nasal membrane. The latter effect is owing to an ionic interaction between the positive charges of these polysaccharides and the negative charges on the surface of the epithelial cells and the subsequent neutralization of anionic sites on the cell surface (Natsume et al., 1999).

According to Schipper et al. (1996), a high molecular weight is necessary for chitosans to increase the epithelial permeability and to promote the absorption of hydrophilic drugs in the in vitro Caco-2 cell model. Likewise, Tengamnuay et al. (2000) suggested that chitosans should differ in their molecular weight by at least two-fold in order to have a clearly differentiating effect on the nasal absorption enhancement of a kyotorphin analogue. On the contrary, Aspden et al. (1996) found no correlation between the molecular weight of chitosans and their enhancing effect on the in vivo nasal absorption of insulin in rats. Similarly, in our study, the observed insignificant difference ($P < 0.05$) between K_{app} for MCP HCl obtained with CS H and CS L revealed that even though they differ in molecular weight by four-fold, yet they produce very similar nasal absorption enhancement effect for MCP HCl.

The other cationic promoters: Prot and Poly-L-arg, showed a significant enhancement ($P < 0.05$) of drug absorption where the K_{app} of MCP HCl increased from 0.0452 min^{-1} with the control solution to 0.0612 and 0.06577 min^{-1} with the two cationic polymers, respectively. As indicated by Miyamoto et al. (2001) the cationic peptides protamine and poly-L-arginine increased the permeability of cultured glomerular and tracheal epithelial cell monolayers to mannitol and albumin. Resembling chitosans, the ionic interaction between positive and negative charges and the subsequent neutralization of the anionic sites on the epithelial cell surface would cause the tight junctions to open transiently thereby promoting absorption.

In conclusion, the results of this study suggest that the rate of nasal absorption of MCP HCl can be enhanced by: adjusting the pH of the drug solution at 8, incorporation of 0.01% BAC and/or absorption enhancers. Of the various absorption enhancers tested, SDC gave the highest promoting effect and might represent a viable approach to achieving rapid systemic delivery of the antiemetic drug in emergency situations. The possible irritation effect of the different enhancers in addition to a bioavailability study are performed in subsequent work (Part II).

References

Aikawa, K., Mitsutake, N., Uda, H., Tanaka, S., Shimamura, H., Aramaki, Y., Tsuchiya, S., 1998. Drug release from pH-response polyvinylacetal diethylaminoacetate hydrogel, and application to nasal delivery. Int. J. Pharm. 168, 181–188.

Ainge, G., Bowles, J.A.K., McCormick, S.G., Richards, D.H., Scales, M.D.C., 1994. Lack of deleterious effects of corticosteroid sprays containing benzalkonium chloride on nasal ciliated epithelium. In vivo results in laboratory animals. Drug Invest. 8, 127–133.

Aspden, T.J., Illum, L., Skaugrud, Ø., 1996. Chitosan as a nasal delivery system: evaluation of insulin absorption enhancement and effect on nasal membrane integrity using rat models. Eur. J. Pharm. Sci. 4, 23–31.

Azria, M., 1991. Nasal pharmaceutical compositions. US Patent no. 4988512, Sandoz Ltd., Basel, Switzerland.

Batts, A.H., Marriott, C., Martin, G.P., Wood, C.F., Bond, S.W., 1990. The effect of some preservatives used in nasal preparations. J. Pharm. Pharmacol. 42, 145–151.

Batts, A.H., Marriott, C., Martin, G.P., Bond, S.W., Greaves, J.L., Wilson, C.G., 1991. The use of a radiolabelled saccharin solution to monitor the effect of the preservatives thiomersal, benzalkonium chloride and EDTA on human nasal clearance. J. Pharm. Pharmacol. 43, 180–185.

Behl, C.R., Pimplaskar, H.K., Sileno, J., deMeireles, J., Romeo, V.D., 1998a. Effects of physicochemical properties and other factors on systemic nasal drug delivery. Adv. Drug Deliv. Rev. 29, 89–116.

Behl, C.R., Pimplaskar, H.K., Sileno, J., Xia, W.J., Grie, W.J., deMeireles, J., Romeo, V.D., 1998b. Optimization of systemic nasal drug delivery with pharmaceutical excipients. Adv. Drug Deliv. Rev. 29, 117–133.

Corbo, C., Huang, Y.C., Chien, Y.W., 1989. Nasal delivery of progestational steriods in ovariectomized rabbits. II. Effect of penetrant hydrophilicity. Int. J. Pharm. 50, 253–260.

Davis, S.S., Illum, L., 2003. Absorption enhancers for nasal drug delivery. Clin. Pharmacokinet. 42, 1107–1128.

Dollery, C., 1999. Therapeutic Drugs, second ed. Churchill Livingstone, London, New York, Toronto, pp. M132–M136.

Donovan, M.D., Huang, Y., 1998. Large molecule and particulate uptake in the nasal cavity: the effect of size on nasal absorption. Adv. Drug Deliv. Rev. 29, 147–155.

Duchateau, G.S.M.J.E., Zuidema, J., Merkus, F.W.H.M., 1986. Bile salts and intranasal drug absorption. Int. J. Pharm. 31, 193–199.

Faraj, J.A., Hussain, A.A., Aramaki, Y., Iseki, K., Kagoshima, M., Dittert, L., 1990. Mechanism of nasal absorption of drugs. III. Nasal absorption of leucine enkephalin. J. Pharm. Sci. 79, 698–702.

Gibson, R.E., Olanoff, L.S., 1987. Physicochemical determinants of nasal drug absorption. J. Control. Release 6, 361–365.

Gizurarson, S., 1993. The relevance of nasal physiology to design of drug absorption studies. Adv. Drug Deliv. Rev. 11, 329–347.

Gordon, G.S., Moses, A.C., Silver, R.D., Flier, J.S., Carey, M.C., 1983. Nasal absorption of insulin: enhancement by hydrophobic bile salts. Proc. Natl. Acad. Sci. U.S.A. 82, 7419–7423.

Harrington, R.A., Hamilton, C.W., Brogden, R.N., Linkewich, J.A., Romankiewicz, J.A., Heel, R.C., 1983. Metoclopramide: an updated review of its pharmacological properties and clinical use. Drugs 25, 451–494.

Harris, A.S., Ohlin, M., Svensson, E., Lethagen, S., Nilsson, I.M., 1989. Effect of viscosity on the pharmacokinetics and biological response to intranasal desmopressin. J. Pharm. Sci. 78, 470–471.

Hayashi, M., Hirasawa, T., Muraoka, T., Shiga, M., Awazu, S., 1985. Comparison of water influx and sieving coefficient in rat jejunal, rectal and nasal absorptions of antipyrine. Chem. Pharm. Bull. 33, 2149–2152.

Hirai, S., Yashiki, T., Matsuzawa, T., Mima, H., 1981a. Absorption of drugs from the nasal mucosa of rats. Int. J. Pharm. 7, 317–325.

Hirai, S., Yashiki, T., Mima, H., 1981b. Mechanism for the enhancement of the nasal absorption of insulin by surfactants. Int. J. Pharm. 9, 173–184.

Huang, C.H., Kimura, R., Nassar, R.B., Hussain, A., 1985. Mechanism of nasal absorption of drugs. I. Physicochemical parameters influencing the rate of in situ nasal absorption of drugs in rats. J. Pharm. Sci. 74, 608–611.

Hussain, A.A., 1998. Intranasal drug delivery. Adv. Drug Deliv. Rev. 29, 39–49.

Hussain, A.A., Bawarshi-Nassar, R., Huang, C.H., 1985. Physicochemical consideration in intranasal drug administration. In: Chien, Y.W. (Ed.), Transnasal Systemic Medications. Elsevier, Amsterdam, pp. 121–137.

Illum, L., 2003. Nasal drug delivery possibilities, problems and solutions. J. Control. Release 87, 187–198.

Inagaki, M., Sakakura, Y., Itoh, H., Ukai, K., Miyoshi, Y., 1985. Macromolecular permeability of the tight junction of the human nasal mucosa. Rhinology 23, 213–221.

Kaneo, Y., 1983. Absorption from the nasal mucous membrane. I. Nasal absorption of hydralazine in rats. Acta. Pharm. Suec. 20, 379–388.

Karlsson, J., Ungell, A., Grasjo, J., Artursson, P., 1999. Paracellular drug transport across intestinal epithelia: influence of charge and induced water flux. Eur. J. Pharm. Sci. 9, 47–56.

Kilian, N., Müller, D.G., 1998. The effect of a viscosity and an absorption enhancer on the intra nasal absorption of metoprolol in rats. Int. J. Pharm. 163, 211–217.

Martin, A.N., 1993. Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, fourth ed. Lea and Febiger, Philadelphia and London, p. 342.

Marttin, E., Verhof, J.C., Romeijn, S.G., Merkus, F.W.H.M., 1995. Effects of nasal absorption enhancers on rat nasal epithelium in vivo: release of marker compounds in the nasal cavity. Pharm. Res. 12, 1151–1157.

McMartin, C., Hutchinson, E.F., Hyde, R., Peters, G.E., 1987. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. J. Pharm. Sci. 76, 535–540.

Merkus, F.W.H.M., Schipper, N.G.M., Hermens, W.A.J.J., Romeijn, S.G., Verhoef, J.C., 1999. Absorption enhancers in nasal drug delivery: efficacy and safety. J. Control. Release 24, 201–208.

Miyamoto, M., Natsume, H., Iwata, S., Ohtake, K., Yamaguchi, M., Kobayashi, D., Sugibayashi, K., Yamashina, M., Morimoto, Y., 2001. Improved nasal absorption of drug using poly-L-arginine: effect of concentration and molecular weight poly-L-arginine on the nasal absorption of FITC-dextran in rats. Eur. J. Pharm. Biopharm. 52, 21–30.

Natsume, H., Iwata, S., Ohtake, K., Miyamoto, M., Yamaguchi, M., Hosoya, K., Kobayashi, K., Sugibayashi, K., Morimoto, Y., 1999. Screening of cationic compounds as an absorption enhancer for nasal drug delivery. Int. J. Pharm. 185, 1–12.

Paget, G.E., Barnes, J.M., 1964. Interspecies dosage conversion scheme in evaluation of results and quantitative application in different species. In: Laurence, D.R., Bacharach, A.L. (Eds.), Evaluation of Drug Activities: Pharmacometric, vol. 1. Academic Press, London and New York, pp. 160–162.

Pennington, A.K., Ratcliffe, J.H., Wilson, C.G., Hardy, J.G., 1988. The influence of solution viscosity on nasal spray deposition and clearance. Int. J. Pharm. 43, 221–224.

Pitrè, D., Stradi, R., 1987. Metoclopramide Hydrochloride. Analytical Profiles of Drug Substances, vol. 16. Academic Press, New York, pp. 327–360.

Pujara, C.P., Shao, Z., Duncan, M.R., Mitra, A.K., 1995. Effects of formulation variables on nasal epithelial cell integrity: biochemical evaluations. Int. J. Pharm. 114, 197–203.

Rawlins, E.A., 1982. Diffusion. In: Bentley's Textbook of Pharmaceutics, eighth ed. Baillière Tindall, London, p. 119.

Romeijn, S.G., Verhoef, J.C., Marttin, E., Merkus, F.W.H.M., 1996. The effect of nasal drug formulations on ciliary beating in vitro. Int. J. Pharm. 135, 137–145.

Schipper, N.G.M., Vårum, K.M., Artursson, P., 1996. Chitosans as absorption enhancers for poorly absorbable drugs. 1. Influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelial (Caco-2) cells. Pharm. Res. 13, 1686–1692.

Shao, Z., Park, G., Krishnamurthy, R., Mitra, A., 1994. The physicochemical properties, plasma enzymatic hydrolysis and nasal absorption of acyclovir and its 2′-ester prodrugs. Pharm. Res. 11, 237–242.

Stoklosa, M.J., Ansel, H.C., 1996. Isotonic Solutions. In: Pharmaceutical Calculations, 10th ed. Williams and Wilkins, Philadelphia, pp. 144–149.

Su, K.S.E., Campanale, K.M., Gries, C.L., 1984. Nasal drug delivery system of a quaternary ammonium compound: clofilium tosylate. J. Pharm. Sci. 73, 1251–1254.

Suzuki, Y., Makino, Y., 1999. Mucosal drug delivery using cellulose derivatives as a functional polymer. J. Control. Release 62, 101–107.

Tengamnuay, P., Sahamethapat, A., Sailasuta, A., Mitra, A.K., 2000. Chitosans as nasal absorption enhancers of peptides: comparison between free amine chitosans and soluble salts. Int. J. Pharm. 197, 53–67.

Van de Donk, H.J.M., Muller Plantema, I.P., Zuidema, J., Merkus, F.W.H.M., 1980. The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas. Rhinology 18, 119–130.

Vora, J.K., Christensen, G.A., Reginato, M.J., Macia, R.A., Gochoco, C.H., Oh, C.K., 1993. Influence of formulation variables on the plasma growth hormone levels following intranasal administration of growth hormone-releasing peptide in anesthetized rats. J. Control. Release 24, 193–200.

Wheatley, M.A., Dent, J., Wheeldon, E.B., Smith, P.L., 1988. Nasal drug delivery: an in vitro characterization of transepithelial electrical properties and fluxes in the presence or absence of enhancers. J. Control. Release 8, 167–177.