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Rapid-onset intranasal delivery of metoclopramide hydrochloride Part II: Safety of various absorption enhancers and pharmacokinetic evaluation

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Abstract

In the present study, several nasal absorption enhancers, used in metoclopramide hydrochloride (MCP HCl) nasal solutions, have been screened for their possible damaging effect in the *in vitro* human erythrocytes lysis experiment. Moreover, the *in vivo* leaching of biological markers from the rat nasal epithelium was used as a quantitative assessment for possible nasal mucosal irritation whereby the extent of release of total protein and lactate dehydrogenase (LDH) in the nasal lavage fluid was determined. Results showed that insignificant hemolysis from normal saline ($P < 0.05$) occurred with the enhancer protamine sulphate while poly-L-arginine and sodium cholate demonstrated very low (<15%) hemolysis and caused insignificant protein and LDH release from the rat nasal mucosa. Conversely, sodium deoxycholate and chitosan polymers (either of low or high molecular weight) showed high (>60%) hemolysis *in vitro* and the release of the biological markers *in vivo* was significantly higher ($P < 0.05$) than the control solution (no enhancer).

A significant correlation ($P < 0.05$) existed between the enhancement effect of MCP HCl nasal absorption and the amounts of protein ($r = 0.85$) and LDH ($r = 0.88$). Furthermore, the pharmacokinetics of MCP HCl was determined after intravenous (IV), per-oral and intranasal administration of 10 mg drug dose in rabbits. The application of a nasal spray (NS) solution containing 0.5% sodium cholate resulted in a significant improvement ($P < 0.05$) in both the rate and extent of absorption of MCP HCl where the T_{max} achieved was 23.3 min as compared to 50 min in case of the oral solution while the area under the serum concentration–time curve ($AUC_{0-\infty}$) were 506.1, 434.9 and 278.7 μg/ml min for IV, NS and oral solutions, respectively. These values corresponded to absolute bioavailabilities of 87.21 and 55.61% for the NS and oral solutions, respectively. It could thus be concluded that NS of MCP HCl represents a viable approach to achieving rapid and high systemic drug absorption during the emergency treatment of severe emesis.

Keywords: Nasal absorption enhancers; Protein release; Hemolysis; Lactate dehydrogenase; Absolute bioavailability

1. Introduction

In numerous studies the efficiency of the nasal route for the systemic delivery of various medications has been proven. Both hydrophobic drugs, e.g. propranolol (Hussain et al., 1980) and hydrophilic drugs, e.g. clofilium tosylate (Su et al., 1984) are absorbed by the nasal mucosa. The absorption of drugs in the nasal cavity is facilitated by a relatively large surface area, rich vascularisation and, in effect, only a single layer of cells separating the application site from the blood vessels (Chien et al., 1989). In acute situations the rapid-onset of action can be of vital importance. In case of emesis, the use of a nasal spray may be preferred to intravenous or oral administration for practical reasons, it is amenable to self-medication, it is essentially painless and does not require sterile technique (Behl et al., 1998).

The oral antiemetic metoclopramide suffers from low and variable bioavailability due to first pass metabolism (Martindale, 1989; Dollery, 1999). To obviate the low oral bioavailability and the painful injections to cancer patients, nasal metoclopramide formulations have been formulated in a previous work and the nasal drug absorption from these formulae has been studied (Zaki et al., 2006). The rate of absorption was significantly improved by the use of different

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absorption enhancers, however, use of enhancers is not without risk because the leaching of membrane components such as protein and enzymes may cause local irritation to the nasal mucosa (Hosoya et al., 1994). Therefore, well-designed animal toxicological studies should be performed to characterize the formulation's effect on the mucous membrane.

The aim of the present study is to select, among the previously screened enhancers, the one with low toxicity and good enhancing properties by performing *in vitro* hemolytic study as well as leaching of different biological markers from the nasal cavity of anaesthetized rats. The *in vivo* model developed by Marttin et al. (1995) was adopted to study and compare the effects of different enhancers whereby the release of protein and enzymes in the nasal lavage was determined. The results of the previous efficacy Zaki et al. (2006) and the present safety studies were used to guide further bioavailability studies in rabbits.

2. Materials and methods

2.1. Materials

Metoclopramide hydrochloride (Lepetit, Italy), methyl paraben sodium urethane, sodium cholate, sodium deoxycholate, protamine sulphate, poly-L-arginine (MW 70–150 kDa), bovine serum albumin (Sigma, USA), chitosan low MW (150 kDa), chitosan high MW (600 kDa) (Fluka, Germany), sodium chloride, benzalkonium chloride analytical grade (ADWIC Co., Egypt), kits for assay of lactate dehydrogenase (Stanbio Laboratory, UK), methanol and water HPLC grade (Romil Limited, London, UK), acetonitrile and acetic acid (Prolabo, France).

2.2. Methods

2.2.1. Preparation of solutions

Control solution. Aqueous solution containing 9 mg/ml metoclopramide hydrochloride (MCP HCl) (Paget and Barnes, 1964).

Test solutions. The control solution to which 0.5% of different enhancers were dissolved. The absorption enhancers tested were: 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 (Stoklosa and Ansel, 1996).

2.2.2. Hemolysis study

Hemolysis experiments were done according to the method of Jabbal Gill et al. (1994) using human blood. Briefly, the blood was centrifuged at 3000 rpm for 5 min. The obtained erythrocyte pellets were washed four times with normal saline. Finally, after repeated washing and centrifugation, an adequate amount of normal saline was added to the erythrocyte pellets to obtain a 10% erythrocyte standard dispersion. Subsequently, 1.75 ml of distilled water, normal saline or 0.5% isotonic solution of different enhancers were mixed with 0.125 ml of 10% erythrocyte standard solution. The mixture was incubated for 10 min at 37 °C then centrifuged for 5 min at 2000 rpm. The absorbance of the supernatant was measured at 543 nm (Shimazu double beam spectrophotometer, Kyoto, Japan).

The absorbance of supernatant when using distilled water and normal saline was assumed to be 0 and 100%, respectively.

2.2.3. Release of biological marker compounds in vivo

2.2.3.1. Surgical technique. Twenty-one albino male rats, weighing 250–300 g each, were used and the surgical technique was carried out as described in Part I. Briefly, at the time of the experiment, the rats were anaesthetized with an intraperitonea injection of 1.3 g/10 ml urethane solution (in a dose of 1 ml/100 g rat body weight). 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 solution from the nasal cavity to the mouth. Prior to instillation of test solution, the nasal cavity was washed carefully with 10 ml normal saline solution.

2.2.3.2. Deposition of enhancers' solutions. The method described by Marttin et al. (1995) was adopted. In each rat 20 μl of the control or enhancer solution was administered in one nostril by means of a micropipette. After 20 min incubation period, the nasal cavity was perfused with 10 ml physiological saline at a flow rate 2 ml/min to wash out the biochemical markers released from the nasal epithelial membrane. The lavage fluid was divided into two fractions of 5 ml each and stored at −4 °C until analysis.

2.2.3.3. Analytical procedures.

1. *Total proteins*: The protein content of the first fraction of the nasal lavage fluid was determined at λ_{max} 540 nm by the method of Lowry et al. (1951) using bovine serum albumin as a standard.
2. *Lactate dehydrogenase activity*: The activity of the enzyme LDH in the second fraction of the nasal lavage fluid was determined spectrophotometerically at λ_{max} 340 nm against distilled water as a blank using the appropriate kit.

The results of the hemolytic study and the release of biochemical markers were expressed as the mean ± standard deviation of three measurements and the results were compared by one-way analysis of variance (ANOVA). To further compare the effects of different enhancers, multiple comparisons were performed using a least significant difference (LSD) test, with $P < 0.05$ as significance level.

2.2.3.4. Bioavailability study. The bioavailability of MCP HCl was determined from the following preparations:

1. Isotonic nasal spray solution composed of 73.53 mg MCP HCl, 0.5 mg sodium cholate, 0.2 mg HPMC and 0.01 mg benzalkonium chloride per ml of solution.

2. Oral solution composed of 2 mg/ml MCP HCl in distilled water.
3. Intravenous injection: Commercial Primperan® injection (10 mg/2 ml).

2.2.4. Animal handling, drug administration and analysis

Six male New Zealand white rabbits weighing 2.86 ± 0.12 kg (mean ± S.D.) were used. They were housed individually in stainless steel cages, fed a commercial laboratory rabbit diet and had free access to water.

In a crossover study and with a washout period of 1 week, the six rabbits received the developed nasal MCP HCl formulation as a spray, the oral solution as well as the intravenous bolus injection. The rabbits were fasted overnight before dosing. The animals were conscious throughout the duration of the experiments and were held in rabbit restrainers during blood sampling.

The rabbits received the developed spray solution in the right nostril using a commercial metered dose spray pump calibrated to deliver 0.1355 ml of solution (equivalent to 10 mg MCP HCl). In addition, the animals received 5 ml of the prepared oral solution by an oral tube as well as IV bolus of Primperan® injected into their marginal ear vein.

After intranasal (IN), per-oral (Peror) or intravenous (IV) administration of the formulations, blood samples (1.5 ml) were collected at time intervals of 5, 10, 20, 30, 45, 60, 90, 120, 180 and 240 min from the marginal ear vein of the rabbits. Blood samples were allowed to clot and then centrifuged at 3000 rpm for 10 min to obtain the serum. Serum samples were finally stored at −20 °C until analysis. A validated reversed phase isocratic HPLC method with UV detection at 254 nm was used as a modification of the method of Radwan (1998). The mobile phase consisted of methanol:water (65:35) v/v pH adjusted to 3.5 with acetic acid and delivered into the HPLC apparatus at flow rate of 2 ml/min. At time of analysis, 1 ml of methyl paraben (internal standard) solution in methanol (10 μg/ml) and 1 ml acetonitrile were added to 1 ml of each thawed serum sample. The tubes were vortex-mixed for 2 min and then centrifuged at 3000 rpm for 15 min. The upper layer was filtered through a nylon membrane filter (0.45 μm), degassed by ultrasonication for 10 min before use and 20 μl were injected into the HPLC apparatus (Schimadzu, LC10-AS liquid chromatograph) connected to an ultraviolet variable wavelength detector (Model SPD-10A) with a C-18 reversed phase column (Bondapack, 5 μm, 3.9 mm × 150 mm, Shimadzu, Japan) and isocratic pump (Model LC10-AS, Shimadzu, Japan).

2.2.5. Data treatment and statistics

Peak plasma concentration C_{max} and the time to achieve this peak T_{max} were the empirical maximum plasma concentrations and the time taken to achieve this peak, respectively. The elimination rate constant, K was determined by the linear regression analysis of the terminal linear part of the log serum concentration versus time curve. The areas under the concentration curves (AUC) were calculated by the linear trapezoidal rule. The AUC from 0 to 5 min for intravenous administration was determined by extrapolation of the zero value by using linear regression analysis on the concentrations at the first two sampling points. Extrapolation to infinite time was achieved by dividing the last concentration by the elimination rate constant. The $AUC_{0-\infty}$ values obtained from the MCP HCl serum concentration–time plots after IV, Peror and IN administration were used to calculate the absolute bioavailability using the standard equation:

$$\text{absolute bioavailability (\%)} = \frac{AUC_{Peror/IN}}{AUC_{IV}} \times 100$$

The biological half life $T_{1/2elim}$ was calculated from 0.693/K. The absorption rate constant K_a was estimated by the method of feathering (Gibaldi, 1984).

The non-compartmental parameters AUMC (area under first moment curve) was calculated using the linear trapezoidal rule with extrapolation to infinite time, MRT (mean residence time) was determined as AUMC/AUC and MAT (mean absorption time) was calculated as $1/K_a$ (Gibaldi, 1984). Results are expressed as mean ± standard error of the mean (S.E.M.) of six determinations. Statistical tests of significance were performed using one-way ANOVA with multiple comparisons by LSD method, and differences were considered significant when $P < 0.05$.

3. Results and discussion

3.1. Hemolysis study

Erythrocyte hemolysis is commonly used as a model to investigate membrane interactions, because erythrocytes are readily available and their lysis is easily measured. The interaction of different absorption enhancers with erythrocyte membranes can be compared with this *in vitro* method (Merkus et al., 1999).

Table 1 shows the hemolytic effect of 0.5% of the different tested enhancers on the human erythrocytes. From the data, the enhancers could be arranged in descending order according to the percentage hemolysis as follows: SDC > CS H > CS L > SC > Poly-L-arg > Prot ≈ control.

Statistical treatment of the data using one-way ANOVA followed by LSD test for multiple comparisons revealed that the percentage hemolysis of Prot was insignificantly different from control and the other enhancers pointing to the biocompatibility of this enhancer.

Table 1
Hemolytic effects of different absorption enhancers

Enhancer	Hemolysis (%)
Distilled water	100.00
Saline (control)	0.00 b,c,d,e,g
SC	15.19 ± 3.17 a,c,d,e,f,g
SDC	87.73 ± 7.14 a,b,d,e,f,g
CS L	62.74 ± 3.35 a,b,c,f,g
CS H	68.59 ± 3.22 a,b,c,d,f,g
Prot	1.96 ± 0.32 b,c,d,e,g
Poly-L-arg	8.95 ± 1.39 a,b,c,d,e,f

a, b, c, d, e, f or g: significantly different from control, SC, SDC, CS L, CS H, Prot, SO_4 or Poly-L-arg, respectively, at $P < 0.05$ using one-way ANOVA followed by LSD for multiple comparisons.

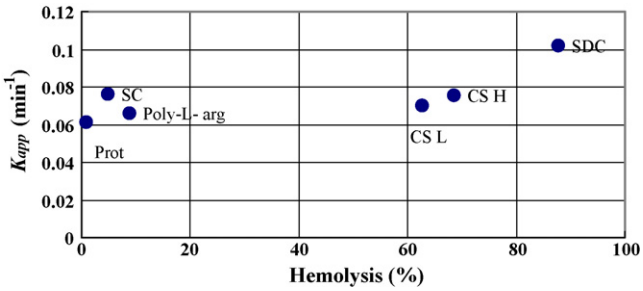

Fig. 1. Relationship between hemolytic and enhancing effects of different absorption enhancers.

The MCP HCl absorption rate constant values, obtained with each enhancer Zaki et al. (2006), were plotted against the respective hemolytic percentage as illustrated in Fig. 1. Results show that SDC, CS H and CS L had high enhancing effect (high absorption rate constant, K_{app}, values) yet the percentage hemolysis was also high ranging from 63% to about 88% indicating toxicity. On the other hand, SC, Poly-L-arg and Prot which significantly enhanced the nasal absorption of MCP HCl caused slight erythrocyte lysis (<20%). These findings are consistent with those reported by Natsume et al. (1999).

3.2. Release of marker compounds

The erythrocyte lysis model is too sensitive to extrapolate the obtained results to *in vivo* situations, hence it is not recommended to use the erythrocyte model to evaluate the toxicity of enhancers for nasal drug delivery without performing other; more relevant *in vivo* experiments such as the release of biological compounds from the nasal mucosa (Merkus et al., 1999).

The extent of release of total protein indicates directly the extent of damage and irritation to the nasal mucosa. The protein detected in the nasal lavages may come from several sources: glycoproteins of the mucus layer, membrane proteins and enzymes. Moreover, the release of the intracellular enzyme lactate dehydrogenase (LDH) indicates the amount of cell leaching and/or lysis of the nasal mucosa.

Table 2 shows the effect of 0.5% of different enhancers on the release of protein and LDH from the nasal membrane. From the results, the enhancers: SDC, CS H, CS L, SC and Poly-L-arg arranged in descending order exhibited more protein release than control. Conversely, the enhancer Prot resulted in protein levels lower than the control. The greatest protein leaching was produced by SDC where the observed protein release was 16.96 mg% as compared to control (4.135 mg%). Similar results were reported by Yamamoto et al. (1996) who demonstrated that among different enhancers, SDC caused the most extensive release of tissue protein. Statistical treatment of the data revealed an insignificant difference from the control solution (no enhancer) in case of the enhancers SC, Poly-L-arg and Prot. While SDC, CS L and CS H caused protein leaching significantly higher than the control ($P < 0.05$).

Table 2
Effect of different absorption enhancers on the amount of protein and LDH released from the rat nasal cavity

Enhancer	Protein (mg%)	LDH (U/l)
Control	4.14 ± 0.92 c,d,e	2518.00 ± 60.51 c,d,e
SC	4.37 ± 1.08 c	2577.50 ± 70.03 c,d,e
SDC	16.96 ± 3.56 abdefg	3098.24 ± 91.66 a,b,d,e,f,g
CS L	7.10 ± 0.32 ac	2697.30 ± 49.83 a,b,c,f,g
CS H	6.91 ± 0.97 ac	2736.80 ± 55.11 a,b,c,f,g
Prot	3.94 ± 0.99 c	2496.50 ± 44.72 c,d,e
Poly-L-arg	4.19 ± 0.65 c	2524.74 ± 48.88 c,d,e

a, b, c, d, e, f or g: significantly different from control, SC, SDC, CS L, CS H, Prot, SO_4 or Poly-L-arg, respectively, at $P < 0.05$ using one-way ANOVA followed by LSD for multiple comparisons.

The observed effects of the absorption enhancers on the release of protein from the nasal epithelium *in vivo* could be used to elucidate the mechanism behind their *modus operandi*. The observed significant increase in protein release caused by SDC and the chitosans could partly be attributed to the release of mucus glycoproteins from secretory cells. This finding agrees with previously reported histological studies that have shown that the application of sodium deoxycholate and chitosan polymers to the nasal epithelium of the rat resulted in the discharge of mucus (Tengamnuay et al., 2000; Merkus et al., 1993).

Concerning the lactate dehydrogenase release data, Table 2 demonstrates the activities of LDH released 20 min after installation of different enhancers' solutions in the rat nasal cavity. From the results it is clear that highest release were observed with SDC with a value of 3098.24 U/l as compared to 2736.8, 2697.3, 2577.5, 2524.74, 2496.5 and 2518 U/l for CS H, CS L, SC, Poly-L-arg, Prot and control, respectively. According to ANOVA, no significant difference existed between the control and either SC, Poly-L-arg or Prot indicating comparable effect, which would suggest that these substances are not destructive for the epithelial membrane. On the other hand, ANOVA revealed a significant difference from the control in case of SDC, CS H and CS L indicating the capability of these enhancers in creating leaky biological membranes.

A noteworthy finding is the significant difference between the two bile salts: SDC and SC in releasing LDH enzyme (considered as intracellular protein). These results are in harmony with those obtained by Shao and Mitra (1992) who demonstrated that different bile salts exhibited dramatic differences in releasing intracellular protein and they attributed the severity of membrane damage to the ability of the bile salts to penetrate the nasal membrane. In the rat *in situ* nasal perfusion technique, the disappearance of SDC from the rat nasal perfusate was faster than sodium glycocholate. The authors thus concluded that higher membrane penetration occurs in case of the more hydrophobic dihydroxy bile salt compared to the trihydroxy member.

The following rank order could be established for the tested enhancers, with increasing toxicity as judged by the protein and LDH release:

$$\text{Control} \approx \text{Prot} \approx \text{Poly-L-arg} \approx \text{SC} < \text{CS L} < \text{CS H} < \text{SDC}.$$

To discriminate between different enhancers with respect to efficacy and safety, a correlation was made between the K_{app}, i.e. apparent first order absorption rate constant of MCP HCl Zaki et al. (2006) and the released protein and LDH from the nasal

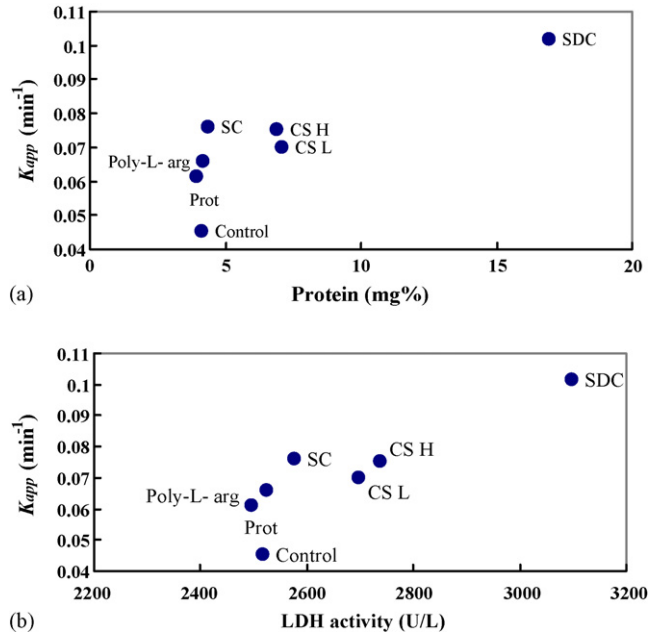

Fig. 2. Relationship between leaching and enhancing effects of different absorption enhancers: (a) protein leaching vs. K_{app} (apparent first-order absorption rate constant); (b) LDH leaching vs. K_{app} (apparent first-order absorption rate constant).

mucosa. Consequently, a decision on the optimum enhancer can be made based on the benefit to risk ratio.

Fig. 2a depicts the relationship between the protein release and the MCP HCl absorption rate constant where a significant correlation ($P \leq 0.05$ level) existed between the two parameters with Pearson correlation coefficient = 0.849. Similarly statistical analysis revealed that the LDH release was significantly correlated ($P \leq 0.01$ level) with the K_{app} with Pearson correlation = 0.879 as shown in Fig. 2b. These observations indicated that the effect of the various enhancers in increasing the permeability of the nasal epithelium was due to their ability to extract membrane components. Furthermore, the plot revealed that Prot, Poly-L-arg and SC are safe though they caused significant enhancement of MCP HCl absorption through the nasal membrane, as manifested by their high K_{app} (Part I). In contrast, SDC, CS H and CS L have very powerful enhancing effect but were harmful as judged by the release of the marker compounds. Consistently, in a similar *in vivo* model based on the leaching of biological markers from the rat nasal cavity. Marttin et al. (1995), Shao and Mitra (1992) also reported that SDC belongs to the category of enhancers with the most damaging effect. Furthermore, morphological and ciliotoxicity studies revealed the damaging effects of SDC on the nasal epithelium (Merkus et al., 1993; Ennis et al., 1990). However, the minor but significant damaging effect of the chitosan polymers are not in keeping with the results of Aspden et al. (1996) who found negligible release of the cell membrane and cytosol-bound enzymes but higher release of protein as compared with the control after perfusion for 90 min in a rat model. Moreover, Aspden et al. (1997) reported the safety and biocompatibility of two chitosan salts: the hydrochloride and the glutamate salts. The discrepancy could be attributed to the effect of the acetate species present as a solvent for the free basic chitosans used in this study. It was demonstrated that the acetate buffer species resulted in large protein release and damaged the nasal mucosa as compared to adipate, citrate and phosphate buffers (Pujara et al., 1995).

From the toxicological studies conducted in the present study as well as the previously performed formulation studies Zaki et al. (2006) SC, Poly-L-arg and Prot proved to be optimum enhancers for the nasal administration of MCP HCl. They have high absorption promoting effect together with low toxicity.

3.3. Bioavailability study

Nasal MCP HCl formulation containing SC as enhancer was chosen for bioavailability studies as it showed the highest efficacy compared to the other two safe enhancers Poly-L-arg and Prot.

The time courses of the serum levels of MCP HCl following the administration of IV, oral and NS solutions are presented in Fig. 3. The corresponding bioavailability data and other relevant pharmacokinetic parameters derived from the serum drug levels for IV, oral and NS solutions are summarized in Table 3.

Results showed that the peak serum drug concentration C_{max} after administration of the NS solution was significantly higher ($P < 0.05$) than that obtained after administration of the oral drug solution. The C_{max} was 4.39 ± 0.393 μg/ml for the NS solution as compared to 2.63 ± 0.152 μg/ml for the oral solution.

The T_{max} together with the $T_{1/2abs}$ (calculated as 0.693/Ka) offer independent criteria to evaluate the *in vivo* drug absorption rate. A significantly shorter T_{max} was achieved by the NS solution 23.33 min which was approximately one-half that obtained with the oral solution (50 min). The mean $T_{1/2abs}$ observed (and MAT) after the NS solution was five times lower than that observed after the oral solution and the differences were statistically significant. These results demonstrate the superiority of intranasal over oral administration for achieving faster MCP HCl absorption, with a potential of clinical application in acute situations such as severe nausea and vomiting.

Similar results were obtained with other drugs when administered nasally. The T_{max} of the antimigraine drug, sumatriptan,

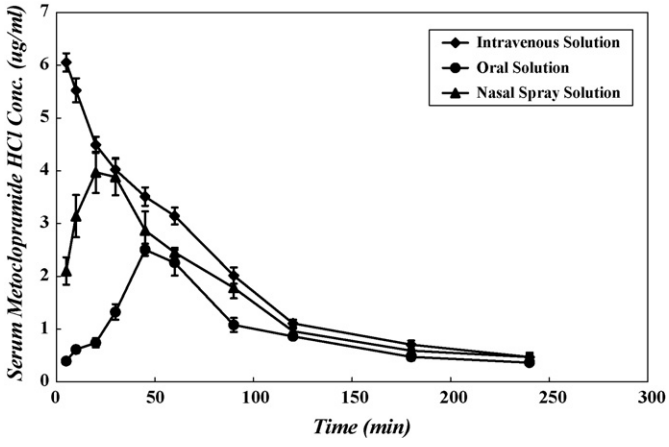

Fig. 3. Mean serum concentration–time profiles following administration of intravenous, oral or nasal spray solutions of metoclopramide HCl in six rabbits.

Table 3
Mean pharmacokinetics parameters of metoclopramide HCl following administration of intravenous, oral or nasal spray solutions in rabbits

Parameter	Intravenous solution	Oral solution	Nasal spray solution
C_{max} (μg/ml)	6.05 ± 0.173 b,c	2.63 ± 0.152 a,c	4.39 ± 0.393 a,b
T_{max} (min)		50.00 ± 3.16 c	23.33 ± 2.11 b
K_a (min^{-1})		0.0697 ± 0.0021 c	0.3403 ± 0.0093 b
K_{elim} (min^{-1})	0.0072 ± 0.00058	0.0076 ± 0.00161	0.0066 ± 0.00167
$T_{1/2elim}$ (min^{-1})	99.57 ± 7.81	114.84 ± 26.91	159.77 ± 50.24
$T_{1/2abs}$ (min^{-1})		9.94 ± 0.95 c	2.04 ± 0.344 b
MRT (min)	101.46 ± 3.98 b	143.02 ± 10.03 a	137.10 ± 14.91
MAT (min)		14.35 ± 1.66 c	2.94 ± 0.502 b
$AUMC_{0-\infty}$ (μg/ml min^2)	57240.1 ± 2244.77	39863.1 ± 2795.6	59633.9 ± 6485
AUC_{0-240} (ug/ml min)	439.6 ± 10.82 b,c	228.2 ± 7.98 a,c	355.1 ± 17.16 a,b
$AUC_{0-\infty}$ (ug/ml min)	506.1 ± 13.9 b,c	278.7 ± 9.95 a,c	435.0 ± 25.82 a,b
Absolute bioavailability (%)	100.00 ± 0 b	55.61 ± 3.29 a,c	87.21 ± 7.74 b

a, b or c: significantly different from intravenous, oral or nasal spray solutions, respectively, at $P < 0.05$ using one-way ANOVA followed by LSD for multiple comparisons.

now available as an intranasal spray, was 30 min as reported by Ayres et al. (1996) while the T_{max} of the narcotic analgesic oxymorphone was 24 min (Hussain and Aungst, 1997). However, Chou and Donovan (1997) reported T_{max} value of 15 min for procaine HCl, which is structurally related to MCP HCl but with lower MW 272.8.

The mean MRT value after IV bolus was shorter than after nasal or oral administration because the MRT calculated after IV bolus injection reflects only the elimination rate processes in the body. After oral and nasal administration of the drug, the MRT is the result of both absorption and elimination and is therefore higher. Contrary with what previously reported by Duchateau et al. (1986), an insignificant difference in the mean MRT was observed between the nasal and oral routes. The authors used oral tablet for comparison and attributed the longer MRT to the dissolution time of the tablet. However, we used an oral solution and not a tablet, which justifies the insignificant difference in the MRT.

Concerning the extent of absorption of MCP HCl from the developed nasal spray formulation and oral drug solution as compared to IV injection, Table 3 shows that the AUC_{0-240} and $AUC_{0-\infty}$ after IV bolus were 439.6 ± 10.82 and 506.1 ± 13.9, respectively, while after nasal administration, the mean values achieved were 355.1 ± 17.16 and 434.98 ± 25.82, respectively. However, the same parameters were 228.2 ± 7.98 and 278.73 ± 9.95, respectively, for the drug oral solution. Statistical treatment of the data revealed a significant difference ($P < 0.05$) between the three formulations.

The discernible superior AUC_{0-240} and $AUC_{0-\infty}$ (which corresponds to 156% relative bioavailability) and C_{max} of nasal *versus* oral clearly indicates higher nasal absorption of MCP HCl. Consequently, the absolute bioavailability (%) of the drug was estimated as 55.61 and 87.21 for the oral solution and NS solution, respectively. The statistical treatment of the absolute bioavailability values revealed that the difference between IN and IV route was insignificant ($P < 0.05$) while it was significant between the IN and oral as well as between IV and oral.

Bioavailability of MCP HCl after oral administration was low presumably due to first-pass metabolism because the drug is well absorbed orally (Martindale, 1989; Dollery, 1999). The high bioavailability of intranasal metoclopramide HCl administered as a spray indicates the circumvention of the first-pass effect. The nasal route has been found to give improved bioavailability compared to the oral route in different studies using other drugs. In view of the absence of pre-systemic metabolism, the absolute bioavailability of propranolol is 100% compared to oral (16–60%) (Hussain et al., 1980). Meclizine has an oral bioavailability of 22% in dogs and 8% in rats but up to 89% in dogs and 51% in rats when given intranasally (Chovan et al., 1985).

Kilian and Müller (1998) reported that the antihypertensive drug metoprolol suffering from first pass effect, had a significantly shorter T_{max} of 0.5 h following nasal administration as compared to a T_{max} of 0.75 h following per-oral administration and the AUC after oral intake was only 40% of the AUC observed with the nasal route of administration. The efficacy of intranasal midazolam as premedication and sedative prompted its potential use in the management of acute seizures (Lahat et al., 2000). Midazolam was rapidly absorbed after administration of a nasal spray solution, with a mean peak concentration reached after 14 ± 5 min and high absolute bioavailability (0.83 ± 0.19), that was significantly different from 1.0 (Knoester et al., 2002).

In conclusion, intranasal MCP HCl formulated as a spray solution containing sodium cholate represents a safe and viable approach to achieving rapid-onset systemic drug levels and higher bioavailability through bypass of the liver metabolism for the acute management of nausea and vomiting.

References

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.

Aspden, T.J., Mason, J.D.T., Jones, N.S., Lowe, J., Skaugrud, Ø., Illum, L., 1997. Chitosan as a nasal delivery system: the effect of chitosan solutions on *in vitro* and *in vivo* mucociliary transport rates in human turbinates and volunteers. J. Pharm. Sci. 86, 509–513.

Ayres, D.W., Barrow, A., Scully, N.L., Curtis, G.C., Hughes, H.M., 1996. Absorption, pharmacokinetics and metabolism of ^{14}C-sumatriptan following intranasal administration to the rat. Xenobiotica 26, 1273–1282.

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

Chien, Y.W., Su, K.S.E., Chang, S.F., 1989. Nasal Systemic Drug Delivery. Marcel Dekker, New York.

Chou, K.-J., Donovan, M.D., 1997. The distribution of local anesthetics into the CSF following intranasal administration. Int. J. Pharm. 168, 137–145.

Chovan, J.P., Klett, R.P., Rakietten, N., 1985. Comparison of Meclizine levels in the plasma of rats and dogs after intranasal, intravenous and oral administration. J. Pharm. Sci. 74, 1111–1113.

Dollery, C., 1999. Therapeutic Drugs, 2nd ed. Churchill Livingstone, Toronto, pp. M132–136.

Duchateau, G.S.M.J.E., Zuidema, J., Albers, W.M., Merkus, F.W.H.M., 1986. Nasal absorption of alprenolol and metoprolol. Int. J. Pharm. 34, 131–136.

Ennis, R.D., Borden, L., Lee, W.A., 1990. The effects of permeation enhancers on the surface morphology of the rat nasal mucosa: a scanning electron microscopy study. Pharm. Res. 7, 468–475.

Gibaldi, M., 1984. Biopharmaceutics and Clinical Pharmacokinetics. Lea and Febiger, Washington, pp. 17–28, 131–155.

Hosoya, K., Kubo, H., Natsume, H., Sugibayashi, K., Morimoto, Y., 1994. Evaluation of enhancers to increase nasal absorption using Ussing chamber technique. Biol. Pharm. Bull. 17, 316–322.

Hussain, A., Aungst, B.J., 1997. Intranasal absorption of oxymorphone. J. Pharm. Sci. 86, 975–976.

Hussain, A., Foster, T., Hirai, S., Kashihara, T., Batenhorst, R., Jones, M., 1980. Nasal absorption of propranolol in humans. J. Pharm. Sci. 69, 1240–1243.

Jabbal Gill, I., Illum, L., Farrai, N.F., De Ponti, R., 1994. Cyclodextrins as protection agents against enhancer damage in nasal delivery system. I. Assessment of effect by measurement of erythrocyte haemolysis. Eur. J. Pharm. Sci. 1, 229–236.

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

Knoester, P.D., Jonker, D.M., van der Hoeven, R.T.M., Vermeij, T.A.C., Edelbroek, P.M., Brekelmans, G.J., de Haan, G.J., 2002. Pharmacokinetics and pharmacodynamics of midazolam administered as a concentrated intranasal spray. A study in healthy volunteers. Br. J. Clin. Pharmacol. 53, 501–507.

Lahat, E., Goldman, M., Barr, J., Bistritzer, T., Berkovitch, M., 2000. Comparison of intranasal midazolam with intravenous diazepam for treating febrile seizures in children: prospective randomised study. Br. Med. J. 321, 83–86.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Martindale, 1989. In: Reynolds, J.E.F. (Ed.), The Extra Pharmacopoeia, 29th ed. The Pharmaceutical Press, London, pp. 1097–1100.

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.

Merkus, F.W.H.M., Schipper, N.G.M., Hermens, W.A.J.J., Romeijn, S.G., Verhoef, J.C., 1993. Absorption enhancers in nasal drug delivery: efficacy and safety. J. Control. Release 24, 201–208.

Merkus, F.W.H.M., Verhoef, J.C., Marttin, E., Romeijn, S.G., van der Kuy, P.H.M., Hermens, W.A.J.J., Schipper, N.G.M., 1999. Cyclodextrins in nasal drug delivery. Adv. Drug Deliv. Rev. 36, 41–57.

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, pp. 160–162.

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.

Radwan, M.A., 1998. Determination of metoclopramide in serum by HPLC assay and its application in pharmacokinetic study in rat. Anal. Lett. 31 (14), 2397–2410.

Shao, Z., Mitra, A.K., 1992. Nasal membrane and intracellular protein and enzyme release by bile salts and bile salt-fatty acid mixed micelles: correlation with facilitated transport. Pharm. Res. 9, 1184–1188.

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.

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.

Yamamoto, A., Uchiyama, T., Nishikawa, R., Fujita, T., Muranishi, S., 1996. Effectiveness and toxicity screening of various absorption enhancers in the rat small intestine: effects of absorption enhancers on the intestinal absorption of phenol red and the release of protein and phospholipids from the intestinal membrane. J. Pharm. Pharmacol. 48, 1285–1289.

Zaki, N.M., Awad, G.A.S., Mortada, N.D., Abd ElHady, S.S., 2006. Rapid-onset intranasal delivery of metoclopramide hydrochloride Part I. Influence of formulation variables on drug absorption in anesthetized rats. Int. J. Pharm. 327, 89–96.