Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 64 (2006) 246–254
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Nasal absorption of metoclopramide from different Carbopol® 981 based formulations: In vitro, ex vivo and in vivo evaluation ☆

Cetin Tas [a], Cansel Kose Ozkan [a], Ayhan Savaser [a], Yalcin Ozkan [a,*], Umut Tasdemir [b], Hikmet Altunay [c]

[a] Gülhane Military Medical Academy, Department of Pharmaceutical Technology, Etlik-Ankara, Turkey
[b] Lalahan Livestock Research Center Institute, Lalahan-Ankara, Turkey
[c] Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Antakya-Hatay, Turkey

Received 7 February 2006; accepted in revised form 16 May 2006
Available online 8 June 2006

Abstract

There is a need for nasal drug delivery of metoclopramide HCI (MTC) in specific patient populations where the use of commercially available intravenous and oral dosage forms may be inconvenient and/or unfeasible. In this perspective, nasal dosage forms (solution, gel and lyophilized powder) of MTC were prepared by using a mucoadhesive polymer Carbopol 981 (CRB 981). The drug release studies of formulations were performed by using a modified horizontal diffusion chamber with cellulose membrane and excised cattle nasal mucosa as diffusion barriers. After the ex vivo experiments, the morphological appearances of the nasal mucosa were analyzed with the light microscopic studies. In vivo experiments were carried on sheep model. The release of MTC from solution and powder formulations was found higher than gel formulation ($p < 0.05$) and no severe damage was found on the integrity of nasal mucosa after ex vivo experiments. The penetration enhancing effect of dimethyl-β-cyclodextrin (DM-β-CD) used in powder formulations was observed in ex vivo and in vivo experiments. In contrast to in vitro and ex vivo experiments the nasal bioavailability of gel formulation was found higher than those of the solution and powder ($p < 0.05$) and might represent a promising novel tool for the systemic delivery of MTC.

Keywords: Nasal drug delivery; Metoclopramide; Carbopol 981; Sheep; Powder; DM-β-CD

1. Introduction

Metoclopramide (MTC), a substituted benzamide, is a dopamin receptor antagonist active on gastrointestinal motility. Its antiemetic efficacy has been demonstrated for prevention of nausea and vomiting associated with cancer and radiation therapy, surgery, as well as pregnancy. Patients with difficulty to swallow are a population of choice for this kind of treatment, especially in the case of nausea. In this perspective nasal administration of MTC represents an interesting alternative administration route to others [1]. Systemic delivery of drugs via nasal cavity has a number of advantages. The relatively large area is well vascularized and the epithelium is relatively leaky. Metabolism in the gastrointestinal tract and during first pass through the liver can be avoided. This site also provides easy, inexpensive administration that is suitable for self-medication, and does not require any sterile equipment [2]. However, there are some problems such as mucociliary clearance (MCC) and low permeability of the nasal mucosa for the drugs, having high polarity and molecular size, that have a large influence on the efficiency of the nasal absorption of active substance [3]. Several strategies were tested to improve nasal absorption of drugs, one of them was the use of bioadhesive polymers. Bioadhesives can increase nasal absorption of drugs with several ways such as increasing

☆ Presented at the 4th International Postgraduate Research Symposium on Pharmaceutics (IPORSHIP-2004), September 20–22, 2004 Istanbul, Turkey.

* Corresponding author. Tel.: +90 312 304 60 73; fax: +90 312 223 82 43.
E-mail address: yozkan@gata.edu.tr (Y. Ozkan).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.05.017

the residence time of drugs in the nasal cavity, opening tight junctions between the epithelial cells [4]. CRB derivatives are bioadhesive polymers used in attempts to formulate mucoadhesive drug delivery systems for application to various mucosal sites such as nasal application [5,6]. Another approach is the use of absorption enhancers, such as surfactants, bile salts, fusidate derivatives, fatty acids, phospholipids and cyclodextrins [7,8].

The present experiments were designed to see whether intranasal administration of MTC could be an efficient route for systemic delivery. So, the mucoadhesive dosage forms of MTC were prepared such as lyophilized powder, solution and gel using CRB 981 and their in vitro and ex vivo release characteristics investigated. Histological examinations with light microscopy were used to assess the effect of formulations on the nasal mucosa after ex vivo experiments. In vivo experiments were carried on sheep model. The penetration enhancing efficacy of DM-β-CD used in powder formulation was also examined.

2. Materials and methods

2.1. Materials

MTC, propylene glycol (Sigma, USA), DM-β-CD (Capital Limited, England), CRB 981 (Goodrich, Belgium), triethanolamine (Carlo Erba, Italy) were used. All other chemicals were commercial products of reagent grade and were used without further purification.

2.2. Preparation of formulations

The contents of all prepared formulations are given in Table 1.

2.2.1. Solution formulations

CRB 981 was added to the mixture of 0.9% NaCl solution and propylene glycol. Benzalconium chloride was used as preservative at the concentration of 0.02%. After standing overnight, MTC was dissolved in the rest of the isotonic solution and was added slowly to the polymeric solution. The pH of the solution formulation was adjusted 6.5 with triethanolamine.

2.2.2. Gel formulations

Gel formulations were prepared as described before [5]. Briefly required amounts of water and propylene glycol were mixed together. This mixture was then divided into two equal parts. An appropriate amount of carbomer resin was added to one part. After standing overnight appropriate amount of triethanolamine was added and well mixed until the gel was formed. The MTC and benzalconium chloride were dissolved in the rest of the mixture and were added slowly to the gel formulations by mixing gently. The pH of the gel formulation was adjusted to 6.5 with triethanolamine.

2.2.3. Powder formulations

MTC was added to purified water in lyophilization vials and sonicated until complete dissolution. CRB 981 was dispersed into the same solution. The mixture was frozen for 1 h (−20 °C) and then lyophilized at 0 °C for 24 h. The powders were sieved (150 μm mesh) and stored at room temperature until used. The percentage of drug loading of the formulations was determined by HPLC with fluorescence detection.

2.3. In vitro release studies of MTC with cellulose membrane

MTC release from formulations was tested with a modified horizontal diffusion chamber with a receiver compartment volume of 32 ml and the effective diffusion area was 1 cm^2. Formulation containing 2 mg of MTC was placed at the donor compartment and complemented to 2 ml with Krebs–Henseleit Solution (KHS). The receptor phase (KHS) was continuously stirred and kept at a temperature

Table 1
Compositions of the formulations

Code of formulation	Pharmaceutical form	MTC [mg-(%w/v)]	CRB 981 [mg-(% w/v)]	PG (ml)	BZC Sol 5 mg/100 ml	0.9% NaCl solution (ml)	TEA	Distilled water (ml)	DM-β-CD (mg)
F1	Solution	1600 (2.67)	30 (0.05)	10	240 μl	60[a]	q.s.	–	–
F2	Solution	1600 (2.67)	60 (0.1)	10	240 μl	60[a]	q.s.	–	–
F3	Solution	1600 (2.67)	90 (0.15)	10	240 μl	60[a]	q.s.	–	–
F4	Gel	1250 (1.25)	500 (0.5)	15	400 μl	–	q.s.	100[a]	–
F5	Gel	1250 (1.25)	1000 (1)	15	400 μl	–	q.s.	100[a]	–
F6	Gel	1250 (1.25)	1500 (1.5)	15	400 μl	–	q.s.	100[a]	–
F7	Powder	20	20	–	–	–	–	2[b]	–
F8	Powder	40	20	–	–	–	–	2[b]	–
F9	Powder	20	40	–	–	–	–	2[b]	–
EF8	Powder	40	20	–	–	–	–	2[b]	3.16

PG, propylene glycol; BZC, benzalconium chloride; q.s, quantum sufficit; TEA, triethanolamine.

[a] q.s. to make % w/v: g/100 ml The weight/weight ratio of DM-β-CD used in powder formulation is 5%. The final concentration of BZC in the solution and gel formulations is 0.02%.

[b] Distilled water was evaporated durings the process of lyophilization.

of 37 ± 0.5 °C. A sample of 500 μl was taken from the receiver solution at predetermined time intervals and replaced with an equal amount of KHS.

2.3.1. Ex vivo release studies of MTC through nasal mucosa
2.3.1.1. Tissue preparation. The use of natural membranes is very important to predict the real drug release characteristic. So in this experimental section of the study cattle nasal mucosa was chosen for the following reasons. It is easy to obtain cattle nasal mucosa at slaughterhouse in Turkey. The area of the respiratory mucosa in the snout is relatively large, and this makes it possible to obtain more material from each animal. It also makes it easier to handle the tissue and increases the chance of getting intact, unstrained pieces of mucosa. On the other hand an ethical advantage is that material is obtained from slaughterhouse, and the sacrifice of animals for only a small piece of the animal thus avoided.

Cattle nasal cavity mucosa was obtained from the local slaughterhouse (Çubuk, Ankara, Turkey). Within 10 min of the killing of the animal, the cavity mucosa was carefully removed, then immediately immersed in KHS (approx 900 ml), placed on ice and O_2/CO_2 (95%/5%) was applied.

2.3.1.2. Permeation studies. Within 80 min of removal, the cavity mucosa was carefully cut with a scalpel and mounted in the diffusion chambers with the mucosal and serosal sides facing the donor and receiver phases, respectively. Both sides of the nasal mucosa were filled with the KHS solution and bubbled with 95% O_2/5% CO_2. Following a preincubation period of 45–60 min, used in order to reach 37 °C and electrophysiological equilibrium, permeability experiments were started by replacing the buffer on both sides of the tissue with prewarmed (37 °C) gassed buffer. The other experimental procedures and samples collection were performed in a same fashion as explained earlier.

2.3.1.3. Histological evaluation. Each piece of mucosa was carefully removed from the diffusion chamber, rinsed with KHS after the transport experiments. Mucosa samples were fixed in Bouien's fixative (picric acid:saturated formaldehyde:acetic acid, 15:5:1, v/v) for 6 h. The specimens were dehydrated gradually by incubating for 1 h in each of 70%, 80%, 96% and finally absolute alcohol. The samples were then incubated in methyl benzoate for 24 h in order to soften the materials. The samples were immersed first into benzene/paraplast (1:1) mixture, and then into pure paraplast® for 6 h in a vacuum oven and embedded in paraplast. The blocks were cut in sections 5-6 μm in thickness with a rotary microtom (Leica RM 2025, Germany). The sections were stained with Crossmon Modified Triple stain for light microscopic examinations (Karl-Zeiss, Germany). The mucosa, fixated directly after isolation at the slaughterhouse, was used as a control. The examined mucosa was then scored according to coarse ranking system based on observation where 0 = no damage, equal to control, 1 = less than 25% of epithelial cells lost, 2 = less than 50% of epithelial cells lost, 3 = less than 75% of epithelial cells lost, 4 = only basal cells left and 5 = all epithelial and basal cells lost. The results of this scoring are presented as the mean value of individual scorings from all mucosa exposed to the same formulation [9].

2.4. Nasal absorption of MTC in sheep model, sample collection and preparation

In vitro and in vivo correlation cannot be done because of the lack of the ability of mimicking MCC which is one of the most important parameters in nasal drug delivery. So the use of different species between ex vivo and in vivo can be considered. Sheep model was preferred for the following reasons. Sheep make an ideal model for evaluation of nasal delivery of drugs because of their large nares, the anatomical similarity to the human nasal cavity in terms of nasal cavity surface area per kg body weight, easy accessibility of veins for blood sampling and their mild temperament under experimental conditions. Furthermore results obtained from the sheep model have been found to correlate well to data obtained in humans [10]. Fifteen female sheep (Akkaraman, ~50 kg) were divided into three groups. Formulations (0.4 mg/kg of MTC) were divided equally between both nostrils and administered to unanesthetized, awake sheep with polyethylene tubes. The formulations were released from the tubes using a syringe containing compressed air. We also administered orally (simple solution) and IV dosage forms at the same dose. A wash-out period of 7 days was left between two administrations. After application, blood samples, approximately 5 ml, were taken from the two external jugular veins in an alternating fashion at predetermined time intervals. Sera were separated by centrifugation and stored frozen at −20 °C until required for analysis.

The sample preparation: 300 μl of sodium hydroxide and 30 μl propylparaben at concentration of 1,000,000 ng/ml were added to 600 μl of the sera sample in a conical test tube and vortexed for a few seconds. Diethyl ether (2.5 ml) was then added. The solution was vortexed for 15 s and centrifuged for 5 min, at 4400 rpm. Organic layer was taken and evaporated to dryness under a stream of nitrogen, then reconstituted with 300 μl of mobile phase.

In vivo and ex vivo experiments have been carried out under approval of Gulhane Military Medical Academy Animal Experiment Ethics Committee with the decision number of 07/64.

2.5. Analytical procedure, data analysis and statistics

The amount of MTC in the samples was determined using a HPLC system consisting of the following components: a Hewlett-Packard (Avondale, USA) Model 1100 series with a Model Agilent series G-13159 fluorescence detector and Model Agilent 1100 series G-1329 ALS auto sampler and HP chemstation. The chromatographic

separation was performed using an isocratic elution. The mobile phase components were acetonitrile:0.05 M KH_2PO_4 (20:80, v/v) adjusted to pH 6.2 with 0.1 N *o*-phosphoric acid and delivered at a flow rate of 1 ml min^{-1}. The separation was carried out at 20 °C, on a reversed phase Waters® Bondopak C18 column (300 × 3.9 mm, 5 μm particle size). A fluorescence detector was set at an excitation wavelength of 272 nm and emission wavelength of 355 nm. An injection volume of 20 μl was used for in vitro and ex vivo experiments and 100 μl for in vivo studies. Propyl paraben was used as an internal standard.

In in vitro and ex vivo experiments, the cumulative of MTC permeated per unit area was plotted against time, and the slope of the linear portion of the plot was used as steady state flux (J_{SS}). The permeability coefficient (K_p) was calculated with Eq. (1), in which C_V is the total donor concentration of the formulation

$$K_p = J_{SS}/C_V \quad (1)$$

In in vivo studies, a non-compartmental model analysis was performed using pharmacokinetic software Pharmacologic Calculation System (version 4.1) to estimate the pharmacokinetic parameters of MTC in sera. The area under the curves (AUC) from time zero to the last experimental data point of 4 h were used in calculating the bioavailability of each formulation. The C_{max} and T_{max} were also obtained from Pharmacologic Calculation System. The bioavailability of each formulation was calculated with Eq. (2) according to Shargel and Yu [11]:

$$F = [(AUC_{in}/AUC_{iv})(Dose_{iv}/Dose_{in}) \times 100 \text{ or}$$
$$F = [(AUC_{oral}/AUC_{iv})(Dose_{iv}/Dose_{oral}) \quad (2)$$

Statistical analysis of the data obtained was performed using SPSS (Version 11). For comparisons of data one-way analysis of variance using Duncan's multiple comparison test and independent Samples *t* test were used. The level of statistical significance was chosen as less than 0.05 (i.e. $p < 0.05$).

3. Results and discussion

3.1. In vitro release studies of MTC with cellulose membrane

It was observed that the increasing polymer content decreased the release of the active substance. The steady state flux (J_{ss}) and permeability coefficient (K_p) values were also found to be lower at high polymer contents (Table 2). It is possible that at the higher polymer contents the active substance is trapped in smaller polymer cells and it is structured by its close proximity to the polymer molecules. This increase for the diffusional resistance was found to be more than expected [5,12]. Also, the density of chain structure augmentation at high polymer contents limits the active substance's movement area [3,13].

The release of MTC from gel formulations was found significantly lower as compared with solution ones (Table 2). This can be explained with the inverse relation between viscosity and drug release property. The apparent viscosity or macroviscosity of the formulation influences the diffusion of particles, when the characteristic length is larger than the length scale of the structure elements in the formulation. Molecules, on the other hand, are influenced by the so-called microviscosity, i.e., the environment that the solute molecule is affected by, such as physical properties of the polymer. It was indicated that thermodynamic activity and viscosity had a dominant effect on the release of the active ingredient from the vehicle [14].

Following the studies with cellulose membrane, one optimal formulation in each dosage form group, should have been chosen according to maximum drug release profile. But some critical points must be paid attention especially in nasal drug delivery studies.

For nasal formulations, viscosity enhancers (usually polymers) may be necessary in order to prevent drainage of the formulation. However, a simple boost in viscosity of the nasal formulation is disadvantageous due to inconvenience associated with delivering high viscosity with ease and consistency [15].

Table 2
Polymer content, viscosity and drug release parameters (by using cellulose membrane at the end of a 4-h period) of the formulations

Code of formulation	Polymer content[a] (mg)	Viscosity (cps)	Spindle number	Amount of MTC released (%)	J_{ss} (μg/cm² h)	K_p (cm/h)
F1	0.038	5.02 ± 0.37	2	34.64 ± 1.07	158.19 ± 3.61	79.09 ± 1.81
F2	0.075	6.01 ± 0.13	2	31.95 ± 0.79	145.05 ± 4.21	72.53 ± 2.11
F3	0.113	10.07 ± 0.81	2	30.49 ± 1.67	137.97 ± 6.33	68.96 ± 3.17
F4	0.8	1566 ± 103	3	19.84 ± 0.32	93.75 ± 2.72	46.88 ± 1.36
F5	1.6	5298 ± 149	4	17.68 ± 0.89	89.85 ± 4.53	44.93 ± 2.27
F6	2.4	6819 ± 153	4	15.01 ± 0.20	77.57 ± 1.97	38.79 ± 0.99
F7	2			23.75 ± 0.91	119.32 ± 1.79	59.66 ± 0.91
F8	1			29.51 ± 2.24	145.68 ± 3.65	72.84 ± 1.83
F9	4			19.26 ± 1.65	85.79 ± 2.99	42.89 ± 1.49
EF8	1			29.89 ± 1.99	150.90 ± 2.75	75.45 ± 1.38

Measurements were done at 23 ± 1 °C with the spindle speed of 10 rpm.
Note: Each reading is an average of five determinations ± SD.
[a] Polymer content of the formulation to apply 2 mg of MTC to the donor compartment of modified horizontal diffusion chamber (calculated according to Table 1).

The maximum polymer content for solution formulations (F3) to prevent drainage and result in an increase in the retention time of the drug in contact with the mucus membranes (Table 2) was preferred. Among the gel formulations medium viscosity one (F5) was chosen to provide ease of delivery (Table 2). For powder formulations we paid attention to the ratio of active substance/polymer ratio and chose the 2/1 (F8) to apply maximum drug amount at minimum powder mass (Table 1).

The polymer content of the three formulations at the applied dose (2 mg MTC) to the donor cell is as follows: 0.113, 1.6 and 1 mg for solution, gel and powder, respectively (Table 2). So it is apparent that the release of the active substance from the formulations must increase as the following orders: gel, powder, solution according to above explanations. The low release of MTC from gel formulation agrees with this hypothesis (Fig. 1, $p < 0.05$). The unexpected result of the similar release profiles of solution and powder formulations can be explained with the ionization of the active substance in the donor phase for powder formulation (Fig. 1, $p > 0.05$). CRB 981 is an acidic polymer and its neutralization was not done before lyophilization process in powder formulations. So the final product is acidic structure and this affects the pH of the donor medium. MTC has the pK_a value of 9.0 and at lower pH is found as ionic structure [16,17]. Cellulose membrane is a hydrophilic membrane so it is more permeable to ionic substances. Also acidic medium of the donor phase can affect the structure of the cellulose membrane and make it more permeable.

The penetration enhancing effect of DM-β-CD was not observed in this part of the study (Fig. 1). Because DM-β-CD might have increased the penetration of MTC in three ways, briefly enhancing its solubility, protecting from enzymatic degradation and direct effect on membrane. The solubility problem is not valid for MTC and enzymatic degradation can be important for peptide and protein drugs. The last choice, membrane effect, can only be exerted on natural membranes [18–20].

3.2. Permeation studies with excised cattle concha mucosa

As it was observed with cellulose membrane, the gel formulation gave the minimum drug release profile (Fig. 2, $p < 0.05$). The release of the MTC from solution formulation was found to be higher when compared with the powder formulation (Table 3, $p < 0.05$). On the other hand when cellulose membrane was used these two formulations presented the same release profile (Fig. 1, $p > 0.05$). This difference arises from the diffusional barriers' structure. As it is known that nasal mucosa has lipophilic structure and is more permeable for unionized substances [21]. Many drugs are either weak acidic or basic compounds and depending on the pH value, they are ionized or un-ionized. The un-ionized species are more lipid-soluble and hence more readily absorbed. The conditions which suppress ionization favor the absorption. The relative concentrations of un-ionized and ionized forms of weakly acidic or basic drugs in a formulation at a given pH can be readily calculated using the Henderson–Hasselbach equation (3).

$$\text{pH} = pK_a + \log[\text{un-ionized form}]/[\text{ionized form}] \quad \text{for basic compounds} \tag{3}$$

It is apparent from the Henderson–Hasselbach equations that for basic compounds the relative concentration of the ionized form would increase with a decrease in the pH of a formulation [17]. MTC, as a weak basic drug, will be in ionized form at low pH value in powder formulations. As a result the low release of active substance from the powder formulation compared to the solution one is due to the ionization of MTC in the donor phase.

The penetration of active substance through nasal mucosa was found very low when compared with cellulose membrane (Fig. 3, $p < 0.05$). This could be attributed to the fact that the complexity of the composition of the mucosa offered more resistance to the penetrating drug molecules during the diffusion process [5].

DM-β-CD enhanced the penetration of active substance (Fig. 2, $p < 0.05$). The penetration enhancing activity of DM-β-CD can be explained with its membrane effects on nasal mucosa and this is consistent with previous reports [7,19,22].

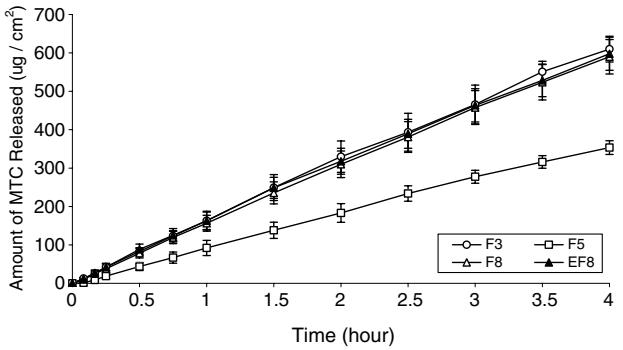

Fig. 1. Comparison of in vitro drug release from different dosage forms (F3 solution, F5 gel, F8 and EF8 powder) prepared with CRB 981 by using cellulose membrane (F3–F5, F5–F8: $p < 0.05$).

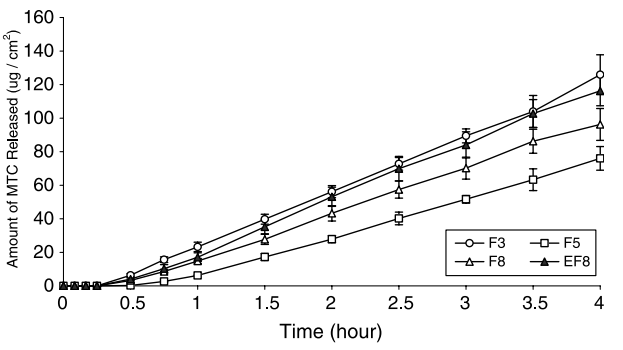

Fig. 2. Comparison of ex vivo drug release from different dosage forms (F3 solution, F5 gel, F8 and EF8 powder) prepared with Carbopol 981 by using nasal mucosa (F3–F5, F3–F8, F5–F8, F8–EF8: $p < 0.05$).

Table 3
Drug release parameters of the formulations in ex vivo experiments by using nasal mucosa ($n = 3$) and the mean scoring[a] values from the light microscopy study subsequent to the transport study of formulations

Code of formulation	Amount of MTC released%	J_{ss} (μg/cm^2 h)	K_p (cm/h)	Scoring 0–5[a]
F3	6.29± 0.29	33.94 ± 0.97	16.97 ± 0.49	3
F5	3.80 ± 0.25	23.52 ± 0.84	11.76 ± 0.42	1
F8	4.81 ± 0.21	28.31 ± 0.45	14.16 ± 0.23	4
EF8	5.37 ± 0.19	33.73 ± 0.98	16.86 ± 0.49	4

Each reading is the average of three determinations ± S.D.
[a] Where 0 = no damage, equal to control, 1 = less than 25% epithelial cells lost, 2 = less than 50% epithelial cells lost, 3 = less than 75% epithelial cells lost, 4 = only basal cells left, 5 = all epithelial and basal cells lost.

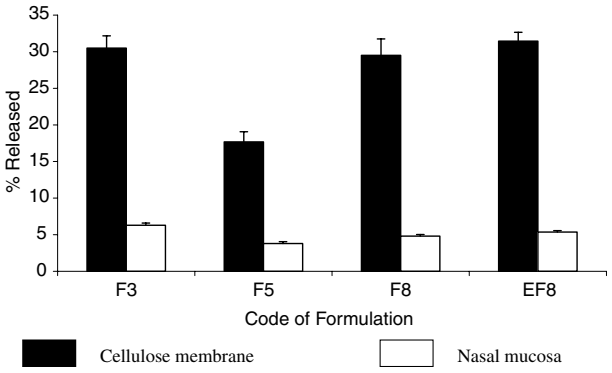

Fig. 3. Comparison of in vitro and ex vivo release of MTC from different dosage forms (F3 solution, F5 gel, F8 and EF8 powder) by using cellulose membrane and nasal mucosa at the end of 4 h period.

3.3. Histological study

The microscopy pictures show the full 240 min period of exposure to formulations including washing. For all formulations no severe damage was found on the integrity of nasal mucosa (Table 3, Fig. 4). The observed changes on nasal mucosa can be summarized as epithelium disruption and complete loss of some parts of the epithelium (Fig. 4). Morphological changes in the nasal epithelia exposed to gel formulation were milder than those exposed to solution and powder ones (Table 3, Fig. 4). The difference seems to lie in the formulation, i.e., there would be negative effects when the CRB is applied in the form of a powder as opposed to when the application is in gel form. Also this result reveals the fact that the mucosa remains viable after the gel exposure and retains a good morphology. The untoward effect of powder formulation may be due to its acidic structure [23,24]. The findings of the epithelia exposed to EF8 coded formulation were similar that of the formulation F8 (Fig. 4). So the effects of DM-β-CD on nasal mucosa are negligible and the results obtained here are in reasonable agreement with the results of other workers [8,19,25].

3.4. Nasal absorption of MTC in sheep model

The mean sera concentration-time curve of MTC after nasal, intravenous and oral administrations to five sheep is shown in Fig. 5. The pharmacokinetic parameters and the values of absolute bioavailabilities of three different nasal preparations of MTC are summarized in Table 4.

In contrast to in vitro and ex vivo experiments, intranasal administration of the gel formulation resulted in increased sera levels of MTC as compared to those for the powder and liquid formulation (Fig. 5, $p < 0.05$). This result is consistent with the findings that gel formulations are suitable carriers for nasal applications of drugs [26–29]. Gel formulations can reduce MCC and this might have provided the longer residence time of the formulation at the site of absorption [9,30].

Adhesion of polymers to nasal mucosa is influenced by interaction between polymer molecules and mucosa, and exclusion of the polymers by the ciliary movement. Previous reports reveal the fact that chemically or physically gel formulation exhibits stronger adhesion to nasal mucosa than powder and solution ones [9,31].

It is well known that the nasal mucosa has many secretory glands, and water transport through such secretory glands may occur. A preliminary experiment showed that the polyacrylic acid gel increased the water influx in rat rectum. Therefore, it is considered that the water absorption promoted by this gel base would increase the absorption of MTC [32].

There is no statistical difference between nasal gel application and oral solution administration (Table 4, Fig. 5). So nasal gel application of MTC can be an alternative to oral administration.

DM-β-CD enhanced the absorption of MTC by affecting the nasal epithelium's structure (Fig. 5, $p < 0.05$). This membrane effect can be explained with the mechanism of opening tight junctions. Polar drugs, such as MTC, are believed to pass through the epithelium via gaps or pores between the cells (the tight junctions) [21]. DM-β-CD facilitates paracellular transport by altering tight junctions either through chelating divalent cations present in tight junction or through interaction with macromolecules involved in the formation of the tight junction [19,22].

Powder formulation was found numerically effective than solution formulation (Fig. 5). This result agrees with the other workers' studies [33–35]. This result can be explained with faster drainage of intranasally applied solution may have reduced its bioavailability. On the other hand the supposed mechanism for an increase in nasal

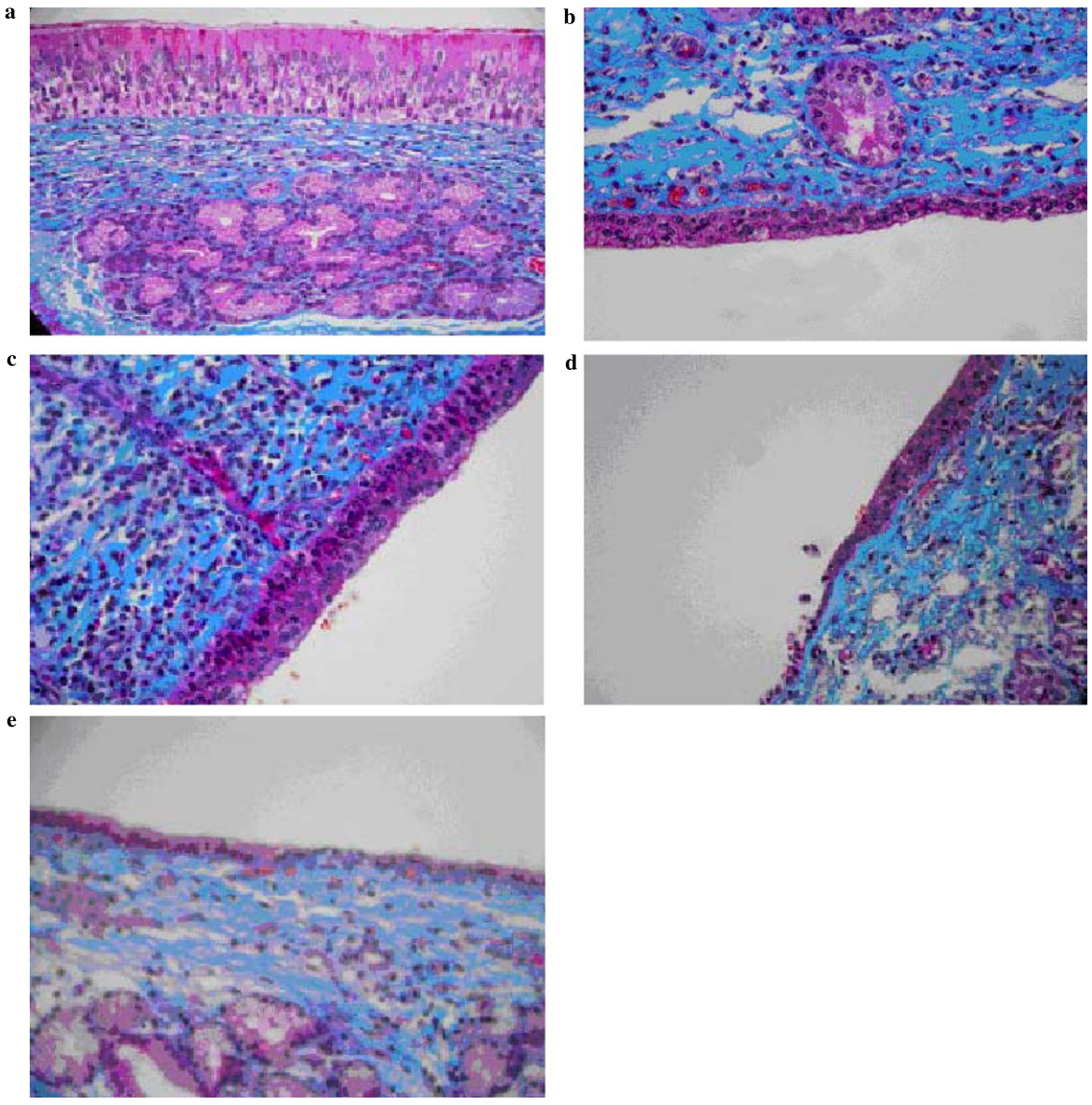

Fig. 4. Images from the histological evaluation of nasal mucosa; control (a), solution (b;F3), gel (c;F5), powder (d;F8) and powder + DM-β-CD (e;EF8).

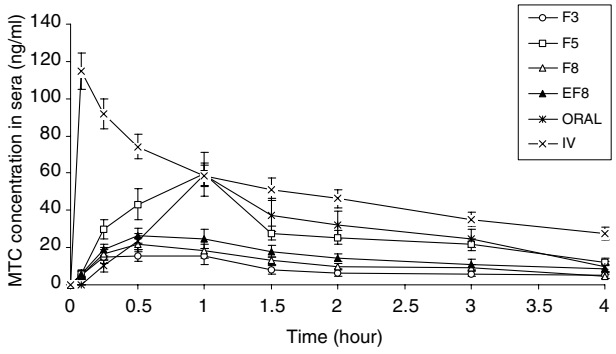

Fig. 5. Mean sera concentration–time profiles in sheep after oral, IV and intranasal administrations of different formulations at a dose of 0.4 mg/kg applied to five sheep (F5–F3, F8, EF8: $p < 0.05$; F8–EF8: $p < 0.05$).

bioavailability for powder formulation was ascribed to an enhanced residence time in the absorption site and an increased topical concentration of the active substance by attaching to the powder particle [35,36].

4. Conclusions

The different results of gel formulations between in vitro, ex vivo and in vivo experiments show that it is necessary to make in vivo experiments for nasal drug delivery studies. The penetration enhancing effect of DM-β-CD was observed in ex vivo and in vivo experiments. Histological examinations after ex vivo experiments revealed that all formulation gave no serial

Table 4
Pharmacokinetic parameters after intravenous, oral and nasal administration of MTC in sheep ($n = 5$)

Code of formulation	t_{max} (h)	C_{max} (ng/ml)	AUC_{0-4} (ng h/ml)	k_a (h)	k_e (h)	F (%)
F3	0.58 ± 0.08	15.73 ± 2.99	34.92 ± 6.01	5.29 ± 0.87	0.27 ± 0.05	17.48 ± 3.12
F5	1.04 ± 0.09	59.43 ± 6.21	113.47 ± 15.39	4.98 ± 0.63	0.32 ± 0.04	56.79 ± 5.77
F8	0.49 ± 0.05	21.68 ± 3.84	46.53 ± 6.32	5.86 ± 0.29	0.41 ± 0.07	23.29 ± 3.31
EF8	0.53 ± 0.05	26.41 ± 4.05	62.27 ± 8.77	4.67 ± 0.31	0.37 ± 0.03	31.16 ± 3.49
ORAL	1.15 ± 0.16	59.29 ± 11.58	113.19 ± 17.64	1.37 ± 0.44	0.51 ± 0.06	56.65 ± 6.01
İV	5 ± 00	114.54 ± 9.78	199.81 ± 20.02	–	0.29 ± 0.03	100

Each value represents the mean ± SD ($n = 5$), k_a=absorption constant, k_e=elimination constant.

damage to nasal mucosa. İn vivo results suggest that nasal gel formulation might represent a promising novel tool for the systemic delivery of MTC and can be used instead of oral administrations for people suffering from nausea and vomiting.

Acknowledgments

This work was supported by Gülhane Military Medical Academy Research and Development Center with the project number AR2003-38.

References

[1] N. Vivien, P. Buri, L. Balant, S. Lacroix, Nasal absorption of metoclopramide administered to man, Eur. J. Pharm. Biopharm. 40 (1994) 228–231.
[2] K. Östh, J. Grasjö, E. Björk, A new method for drug transport studies on pig nasal mucosa using a horizontal ussing chamber, J. Pharm. Sci. 91 (2002) 1259–1273.
[3] C. Hascicek, N. Gonul, N. Erk, Mucoadhesive microspheres containing gentamicin sulfate for nasal administration: preparation and in vitro characterization, IL Farmaco 58 (2003) 11–16.
[4] C. Callens, J.P. Remon, Evaluation of starch-maltodextrin-Carbopol® 974 P mixtures for the nasal delivery of insulin in rabbits, J. Control. Release 66 (2000) 215–220.
[5] C. Tas, Y. Ozkan, A. Savaser, T. Baykara, In vitro and ex vivo permeation studies of chlorpheniramine maleate gels prepared by carbomer derivatives, Drug. Dev. Ind. Pharm. 30 (2004) 637–647.
[6] M.I. Ugwoke, R.U. Agu, H. Vanbilloen, J. Baetens, P. Augustijns, N. Verbeke, L. Mortelmans, A. Verbruggen, R. Kinget, G. Bormans, Scintigraphic evaluation in rabbits of nasal drug delivery systems based on Carbopol® 971 P and carboxymethylcellulose, J. Control. Release 68 (2000) 207–214.
[7] G. Yetkin, N. Çelebi, İ. Agabeyoglu, N. Gökçora, The effect of dimethyl-β-cyclodextrin and sodium taurocholate on the nasal bioavailability of salmon calcitonin in rabbits, S.T.P. Pharm. Sci. 9 (1999) 249–252.
[8] F.W.H.M. Merkus, J.C. Verhoef, E. Marttin, S.G. Romeijn, P.H.M. Van der Kuy, W.A.J.J. Hermens, N.G.M. Schipper, Cyclodextrins in nasal drug delivery , Adv. Drug. Del. Rev. 36 (1999) 41–57.
[9] K. Östh, M. Paulsson, E. Björk, K. Edsman, Evaluation of drug release from gels on pig nasal mucosa in a horizontal ussing chamber, J. Control. Release 83 (2002) 377–388.
[10] Y-H. Cheng, P. Watts, M. Hinchcliffe, R. Hotchkiss, R. Nankervis, N.F. Faraj, A. Smith, S.S. Davis, L. Illum, Development of a nasal nicotine formulation comprising an optimal pulsatile and sustained plasma nicotine profile for smoking cessation, J. Control. Release 79 (2002) 243–254.
[11] L. Shargel, A.B.C. Yu, Applied Biopharmaceutics and Pharmacokinetics, second ed., Appleton-Century-Crofts, Norwalk, 1985, 231-243.
[12] K.I. Al-Khamis, J. Hadgraft, Microviscosity and drug release from gels, Pharm. Res. 4 (1986) 214–217.
[13] C. Tas, Y. Ozkan, A. Savaser, T. Baykara, In vitro release studies of chlorpheniramine maleate from gels prepared by different cellulose derivatives, IL Farmaco 58 (2003) 605–611.
[14] K. Welin-Berger, J.A.M. Neelissen, B. Bergenstahl, The effect of rheological behaviour of a topical anaesthetic formulation on the release and permeation rates of the active compound, Eur. J. Pharm. Sci. 13 (2001) 309–318.
[15] J.S. Chu, R. Chandrasekharan, G.L. Amidon, N.D. Weiner, A.H. Goldberg, Viscometric study of polyacrilic acid systems as mucoadhesive sustained-release gels, Pharm. Res. 8 (1991) 1408–1412.
[16] A.C. Moffat, J.V. Jackson, M.S. Moss, B. Widdop, E.S. Greenfield, Clarke's Isolation and Identification of Drugs, second ed., London, 1986, 776–777.
[17] D.A. Wadke, A.T.M. Serajuddin, H. Jacobson, Pharmaceutical Dosage Forms: Tablets, vol. 1, in: H.A. Liebermann, L. Lachman, J.B. Schwartz (Eds.), Marcel Dekker, New York and Basel, 1989, pp. 28–29.
[18] F. Ahsan, J.J. Arnold, E. Meezan, D.J. Pillion, Mutual inhibition of the insulin absorption-enhancing properties of dodecylmaltoside and dimethyl-β-cyclodextrin following nasal administration, Pharm. Res. 18 (2001) 608–614.
[19] T. Irie, K. Wakamatsu, H. Arima, H. Aritomi, K. Uekama, Enhancing effects of cyclodextrins on nasal absorption of insulin in rats, Int. J. Pharm. 84 (1992) 129–139.
[20] E. Marttin, J.C. Verhoef, F. Spies, J. Van der Meulen, J.F. Nagelkerke, H.K. Koerten, F.W.H.M. Merkus, The effect of methylated β-cyclodextrins on the tight junctions of the rat nasal respiratory epithelium: electron microscopic and confocal laser scanning microscopic visualization studies, J. Control. Release 57 (1999) 205–213.
[21] L. Illum, Nasal drug delivery: new developments and strategies , DDT 7 (2002) 1184–1189.
[22] C. Agerholm, L. Bastholm, P.B. Johansen, M.H. Nielsen, F. Elling, Epithelial transport and bioavailability of intranasally administered human growth hormone formulated with the absorption enhancers didecanoyl-L-α-phosphatidylcholine and α-cyclodextrin in rabbits, J. Pharm. Sci. 83 (1994) 1706–1711.
[23] C. Callens, E. Adriaens, K. Dierckens, J.P. Remon, Toxilogical evaluation of a bioadhesive nasal powder containing a starch and Carbopol® 974 P on rabbit nasal mucosa and slug mucosa, J. Control. Release 76 (2001) 81–91.
[24] M.I. Ugwoke, R.U. Agu, M. Jorissen, P. Augustijns, R. Sciot, N. Verbeke, R. Kinget, Nasal toxilogical investigations of Carbopol 971 P formulation of apomorphine: effects on ciliary beat frequency of human nasal primary cell culture and in vivo on rabbit nasal mucosa, Eur. J. Pharm. Sci. 9 (2000) 387–396.
[25] K. Asai, M. Morishita, H. Katsuta, S. Hosoda, K. Shinomiya, M. Noro, T. Nagai, K. Takayama, The effects of water-soluble cyclodextrins on the histological integrity of the rat nasal mucosa, Int. J. Pharm. 246 (2002) 25–35.
[26] A.R. Najafabadi, P. Moslemi, H. Tajerzadeh, Intranasal bioavailability of insulin from carbopol-based gel spray in rabbits, Drug Deliv. 11 (2004) 295–300.
[27] K. Morimoto, H. Tabata, K. Morisaka, Nasal absorption of nifedipine from gel preparations in rats, Chem. Pharm. Bull. 35 (1987) 3041–3044.

[28] Y. Ozsoy, T. Tuncel, A. Can, N. Akev, S. Birteksoz, A. Gerceker, In vivo nasal studies on nasal preparations of ciprofloxacin hydrochloride, Pharmazie 55 (2000) 607–609.
[29] T. Tuncel, G. Otuk, I. Kuscu, S. Ates, Nasal absorption of roxitromycin from gel preparations in rabbits, Eur. J. Pharm. Biopharm. 40 (1994) 24–26.
[30] P. Arora, S. Sharma, S. Garg, Permeability issues in nasal drug delivery, DDT 7 (2002) 967–975.
[31] F. Nakamura, R. Ohta, Y. Machida, T. Nagai, In vitro and in vivo nasal mucoadhesion of some water-soluble polymers, Int. J. Pharm. 134 (1996) 173–181.
[32] K. Morimoto, K. Morisaka, A. Kamada, Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel, J. Pharm. Pharmacol. 37 (1985) 134–136.
[33] W.A. Lee, B.A. Narog, T.W. Patapoff, J. Wang, Intranasal bioavailability of insulin powder formulations: effect of permeation enhancer-to-protein ratio, J. Pharm. Sci. 80 (1991) 725–729.
[34] K. Nakamura, Y. Maitani, K. Takayama, The enhancing effect of nasal absorption of FITC-dextran 4,400 by β-sitosterol β-D-glucoside in rabbits, J. Control. Release 79 (2002) 147–155.
[35] M.I. Ugwoke, S. Exaud, G.V.D. Mooter, N. Verbeke, R. Kinget, Bioavailability of apomorphine following intranasal administration of mucoadhesive drug delivery systems in rabbits, Eur. J. Pharm. Sci. 9 (1999) 213–219.
[36] F. Ishikawa, M. Murano, M. Hiraishi, T. Yamaguchi, I. Tamai, A. Tsuji, Insoluble powder formulation as an effective nasal drug delivery system, Pharm. Res. 19 (2002) 1097–1104.