Effect of solubility enhancement on nasal absorption of meloxicam

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ABSTRACT

Besides the opioids the standard management of the World Health Organization suggests NSAIDs (non-steroidal anti-inflammatory drugs) alone or in combination to enhance analgesia in malignant and non-malignant pain therapy. The applicability of NSAIDs in a nasal formulation is a new approach in pharmaceutical technology.

In order to enhance the nasal absorption of meloxicam (MX) as an NSAID, its salt form, meloxicam potassium monohydrate (MXP), registered by Egis Plc., was investigated in comparison with MX. The physico-chemical properties of the drugs (structural analysis, solubility and dissolution rate) and the mucoadhesivity of nasal formulations were controlled. In vitro and in vivo studies were carried out to determine the nasal applicability of MXP as a drug candidate in pain therapy.

It can be concluded that MX and MXP demonstrated the same equilibrium solubility at the pH 5.60 of the nasal mucosa (0.017 mg/ml); nonetheless, MXP indicated faster dissolution and a higher permeability through the synthetic membrane. The animal studies justified the short T_{max} value (15 min) and the high AUC of MXP, which is important in acute pain therapy. It can be assumed that the low mucoadhesivity of MXP spray did not increase the residence time in the nasal cavity, and the elimination from the nasal mucosa was therefore faster than in the case of MX. Further experiments are necessary to prove the therapeutic relevance of this MXP-containing innovative intranasal formulation.

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1. Introduction

Intranasal and pulmonary administration are an effective way to deliver drugs into the systemic circulation as an alternative to the oral and parenteral routes for some therapeutic agents (Pacławski et al., 2015). Nasal dosage forms of drugs (spray, gel or powder) have gained importance in recent years because of the rapid onset of action, the circumvention of the first-pass elimination by the liver and the gastrointestinal (GI) tract, the non-invasiveness and the simple daily administration. Nasal transmucosal absorption is affected by the physicochemical properties of the drugs (such as charge, molecular weight, solubility, pKa, logP and permeability, etc.) and formulation factors like dosage form, excipients, pH, viscosity, volume or osmolality (Arora et al., 2002; Illum, 2002).

Intranasal formulations are well known in pain therapy, in particular in the case of chronic malignant pain (Striebel et al., 1993). The opioids (e.g. morphine, butorphanol, fentanyl, etc.) have been formulated as intranasal sprays, reaching T_{max} within 25 min, and in the bloodstream their bioavailability is high (in general, ˜50%) as compared with opioids administered intravenously with 100% bioavailability (Veldhorst-Janssen et al., 2009).

The World Health Organization (WHO) has developed a protocol to guide the treatment of different forms of malignant and non-malignant pain therapy (WHO, 2007). Fig. 1 summarizes the ladders for pain management. In this standard management, besides the opioids, non-steroidal anti-inflammatory drugs (NSAIDs) are suggested for acute pain therapy or co-administered to enhance analgesia.

NSAIDs, which belong in BCS Class 2 with poor solubility and high permeability (Tsume et al., 2012), are really important drugs in pain therapy. Their solubility is pH-dependent (low solubility in acidic medium) and their permeability is influenced by various sections of the GI tract. An increase of the solubility of the NSAID can therefore result in faster absorption, e.g. from the gastric region, to reach an analgesic

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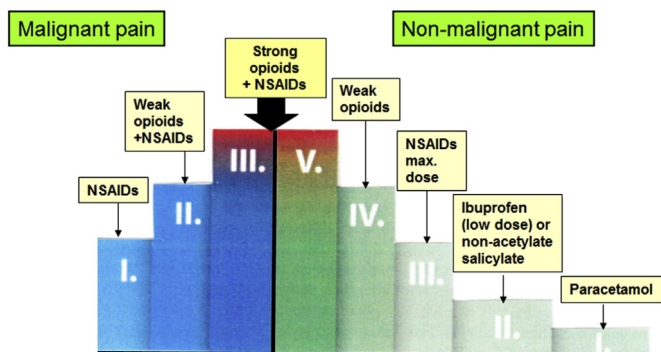E-mail address: revesz@pharm.u-szeged.hu (P. Szabó-Révész).

Fig. 1. Management for malignant and non-malignant pain therapy (WHO, 2007).

effect. On this basis, primarily solid and semi-solid dosage forms (tablets, capsules and suppositories) are on the market.

The intranasal application of NSAIDs may be an alternative route for acute pain therapy, with quick transcellular transport, a high plasma concentration and co-administration with other pain killer to enhance analgesia. Nonetheless, NSAID-containing nasal products as pain killers are not available in therapy. One reason may be a low pH value of the nasal liquid (pH: 5.60) and consequently a low solubility of the NSAID in this medium, as well as the dose amount, irritation, efflux mechanism, *etc.* The applicability of a NSAID in a nasal formulation is therefore a new approach in pharmaceutical technology. A dissolved MX-containing nasal formulation was patented by Castile et al. (2005). The aqueous compositions used co-solvents and contained the dissolved MX in high concentration, which was well tolerated when administered intranasally and provided rapid and effective systemic drug absorption in an animal study. Unfortunately, the composition was found to be unstable in long-term stability tests (precipitation was observed). Another analgesic NSAID agent (a ketorolac tromethamine-containing solution) was successfully administered intranasally to elicit a systemic effect (Li et al., 2015).

In our previous work, MX was chosen as NSAID for intranasal administration in order to attain an analgesic effect. MX has poor aqueous solubility (4.4 µg/ml at 25 °C) (Ambrus et al., 2009), and we therefore used a "top-down" method with the aim of reducing the particle size into the micro or the nano-range and hence improving its bioavailability, such as dry ball-milling (Kürti et al., 2011), high-pressure homogenization (Pomázi et al., 2013) and combined wet milling technology (Bartos et al., 2015). Nanosuspensions, as potential drug formulations, can be achieved by combined wet milling technology (Liua et al., 2011). The results indicated that the reduction of the MX particle size into the nano-range led to increased saturation solubility and dissolution rate, and an increased adhesiveness to surfaces as compared with micronized MX particles. In our earlier studies, MX proved not to be toxic in a cell culture model of the nasal epithelium and did not influence the paracellular pathway (Kürti et al., 2013).

In order to enhance the bioavailability of MX, salt formation may be a new approach to increase its solubility and dissolution rate and to attain fast absorption through the nasal membrane to reach the blood stream. One-salt form of MX is meloxicam potassium monohydrate (MXP), which is a new agent registered by Egis Plc. (Budapest, Hungary) - patent number: US8097616 B2 (Mezei et al., 2012).

The novel meloxicam potassium salt monohydrate is a valuable intermediate in the synthesis of high-purity MX drug substance. The key intermediate of this protocol is the new potassium salt monohydrate of meloxicam, which makes possible the efficient removal of impurities, resulting in an environmentally friendly manufacturing process of the high-purity (>99.90%) drug substance (Mezei et al., 2009).

MXP-containing dosage forms have not been described to date. Our aim was therefore to investigate the physicochemical properties of MXP in comparison with those of MX and to prepare intranasal liquid formulations with both agents. *In vitro* and *in vivo* studies were carried out to determine the nasal applicability of MXP as a drug candidate in pain therapy.

2. Materials and methods

2.1. Materials

MX (4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2H-benzothiazine-3-carboxamide-1,1-dioxide) and MXP (4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2H-benzothiazine-3-carboxamide-1,1-dioxide potassium monohydrate) were obtained from Egis Plc. (Budapest, Hungary) (Fig. 2). Both of the raw materials are yellow. The melting point of MX is 267 °C and that of MXP is 253 °C (Hughey et al., 2011). Sodium hyaluronate (HA) (M_w = 1400 kDa) as viscosity enhancer and mucoadhesive agent was obtained as a gift from Gedeon Richter Plc. (Budapest, Hungary). For the rheological measurements, mucin (porcine gastric mucin type II) and reagents were purchased from Sigma Aldrich (Sigma Aldrich Co. LLC, St. Louis, MO, USA).

2.2. Investigation of raw materials

Measurement of micrometric properties (SEM, particle size analysis) and equilibrium solubility were carried out to compare MX and MXP before the preparation of the nasal formulations.

2.2.1. Scanning electronmicroscopy (SEM)

SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan) was used to visualize the shape and surface characteristics of the samples. The samples were sputter-coated with gold–palladium under an argon atmosphere, using a gold sputter module in a high-vacuum evaporator, and the samples were examined at 10 kV and 10 µA; the air pressure was 1.3–13 MPa.

2.2.2. Particle size analysis

The particles of MX and MXP were measured with the Leica Image Processing and Analysis System (Leica Q500MC, LEICA Cambridge Ltd., Cambridge, UK). The particles were described in terms of their length, breadth, perimeter, roundness and surface area. The roundness was calculated from the ratio of the perimeter squared to the area (1). An adjustment factor of 1.064 corrected the perimeter for the effect of the corners produced by the digitization of the image. The mean values were determined by the examination of 500 particles from each sample.

$$Roundness = \frac{Perimeter^2}{4 \cdot \pi \cdot Area \cdot 1.064} \tag{1}$$

2.2.3. Equilibrium solubility of raw materials

The equilibrium solubilities of MX and MXP were determined by a standardized saturation shake-flask (SSF) method. The specifications of the method were published earlier (Baka et al., 2008).

First, 3–15 ml of different media (phosphate buffers (PBs) with a pH of 5.60 or 7.40 and water with a pH of 5.50) and 5–80 mg of MX or MXP were measured in a glass container to ensure an excess of the solid material. After waiting for 1 h, the pH values of the samples were adjusted with 1 M NaOH or 1 M HCl, depending whether there was a slight shift
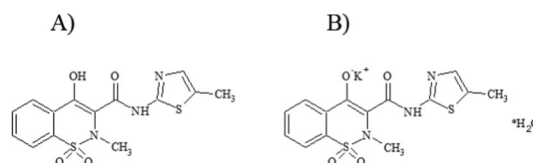

Fig. 2. Chemical structures of MX (A) and MXP (B).

in the acid (for MX) or in the alkali (MXP) direction, so as to restore the original pH of the media. The heterogeneous samples with two phases were placed into the thermostat at 37 °C for 6 (MX) or 12 h (MXP) under intensive stirring to ensure solubility equilibrium. The mixers were then turned off so that the sedimentation of the samples was constant during 18 h (MX) or 36 h (MXP) at 37 °C. Three parallel aliquots (10–250 μl) were taken out with a Hamilton syringe and diluted (2–1000 fold) with the current medium if necessary. The concentrations of the samples were determined by UV spectrophotometry (Jasco V-550 UV/VIS) at 364 nm. All solubility measurements were carried out in two parallels, and the value of the solubility was calculated from six measurement points.

2.2.4. Dissolution test

The paddle method (USP dissolution apparatus, type II Pharma Test, Hainburg, Germany) was used to examine the rates of dissolution of MX and MXP. The medium was 100 ml PBs of pH 5.60 and 7.40 at 37 °C. The paddle was rotated at 50 rpm and the sampling was performed up to 60 min. After filtration, the drug contents of the aliquots were determined by spectrophotometry (Unicam UV/VIS Spectrophotometer, Cambridge, UK) at 364 nm.

2.3. Preparation and examination of intranasal formulations

2.3.1. Preparation of intranasal sprays

Intranasal formulations, sprays as dosage forms (MX spray or MXP spray) were developed with 2 mg/ml MX or MXP and 1 mg/ml HA, where the dispersion media was 100 ml PBs of pH 5.60 at 37 °C. HA-containing liquids with concentrations of 1 mg/ml were prepared, allowing 24 h for swelling in the media and these viscous liquids served as vehicles for the distribution of MX and MXP. Intranasal formulations contain the drug in suspended form with a suggested particle size from 5 to 40 μm (Billotte et al., 1999). One PB (pH = 7.40) was made from NaCl (8.0 g/l), KCl (0.20 g/l), $Na_2HPO_4*1H_2O$ (1.44 g/l) and KH_2PO_4 (0.12 g/l), diluted up to 1000 ml with distilled water. The other PB (pH = 5.6) was a mixture of stock solutions A and B. 100 ml PB (pH = 5.6) was made from 94.4 ml stock solution A (containing 9.08 mg/l KH_2PO_4) and 5.6 ml stock solution B (containing 11.61 mg/l K_2HPO_4 concentration).

2.3.2. Examination of intranasal sprays

2.3.2.1. In vitro permeability study.
In vitro permeability studies were carried out on a modified horizontal Side-Bi-Side™ cell model (Grown Glass, New York). The two chambers were divided by an impregnated (with isopropyl myristate) synthetic membrane (PALL Metricel membrane with 0.45 μm pores). The volumes of the donor and the acceptor phase were the same (3.0 ml) with a 0.69 cm² diffusion area. 3.0 ml of nasal spray was used as donor phase and PB (pH 7.40) served as an acceptor phase. The temperature of the phases was 37 °C (Thermo Haake C10-P5, Sigma, Aldrich Co.) and the rotation rate of the stir-bars was set to 100 rpm. Aliquots (2.0 ml) were taken from the acceptor phase by pipette and were replaced with fresh receiving medium at 5, 10, 15 and 60 min of the measurement. The amount of MX or MXP diffused was determined spectrophotometrically (Unicam UV/VIS) at 364 nm; each sample was measured three times.

The flux (*J*) of the drug was calculated from the quantity of MX which has permeated through the membrane after 60 min, divided by the surface of the membrane insert and the duration [μg/cm²/h]. The permeability coefficient (K_p) was determined 2) from *J* and the initial drug concentration in the donor phase (C_d [μg/cm³]):

$$K_p\left(\frac{cm}{h}\right) = \frac{J}{C_d} \qquad (2)$$

2.3.2.2. Rheology and mucoadhesion.
Rheological measurements were taken at 37 °C with a Physica MCR101 rheometer (Anton Paar GmbH, Graz, Austria). A concentric cylindrical measuring device with a diameter of 10.835 mm was used for the experiment. Viscosity curves were plotted to determine the viscosity of the samples. In the shear rate interval from 0.1 to 100 1/s, viscosity values were plotted. The method was based on earlier studies by Bartos et al. (2015). To clarify the roles of MX and MXP in mucoadhesion, samples were prepared with and without mucin; the samples containing mucin were stirred for 3 h before the measurements (the final mucin concentration was 5% w/w). The mucoadhesivity was determined on the basis of the rheological synergism between the polymer and the mucin. The synergism parameter (bioadhesive viscosity component, η_b) can be calculated from the following Eq. (3):

$$\eta_b = \eta_t - \eta_m - \eta_p, \qquad (3)$$

where η_t is the viscosity of the mucin and polymer-containing samples, and η_m and η_p are the viscosities of the mucin and nasal spray, respectively (Hassan and Gallo, 1990). Three parallel measurements were used to determine the viscosity values (η_t, η_m and η_p) and the standard deviations.

2.3.2.3. In vivo study.
Each intranasal formulation contained 2 mg/ml MX or MXP and 1 mg/ml HA in phosphate buffer at pH 5.6. A dose of 60 μg API per animal was administered into the nostrils of male Sprague–Dawley rats (b.w. 160–180 g, $n = 5$) *via* a micropipette. The animals were anaesthetized with isoflurane before the drug administration. The viscous liquid was slowly ejected into the left nostril and some seconds later into the right nostril (at approximately 45 degree angle). Blood samples were taken from the tail vein before and 5, 15, 30 and 60 min after the drug administration. The experiments performed conformed to the European Communities "Council directive for the care and use of laboratory animals" and were approved by the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013). The calculated area under the time–concentration curve (AUC) was analysed by means of PK Solver 2.0 software (Zhang et al., 2010) through non-compartmental analysis of plasma data, using the extravascular input model. The AUCs of the time (min) – concentration (mg/ml) curves of each animal were fitted with a linear trapezoidal method.

2.3.2.4. Determination of MX and MXP from the blood samples.
The drug contents of blood samples were quantitated with an Agilent 1260 HLPC system (QP, DAD, ALS). The method was published earlier (Bartos et al., 2015). MX, MXP and piroxicam (PIR) as internal standard were separated on a C18 column (Phenomenex Inc., Torrance, CA, USA). Isocratic elution was performed with 45:55 (v/v) acetonitrile–potassium phosphate buffer solution (0.05 M) (pH adjusted to 2.7 with orthophosphoric acid) at a flow rate of 1.1 ml/min. All the samples were filtered through a 0.20 μm PES syringe membrane filter (Phenomenex Inc., Torrance, CA, USA). The sample injection volume was 10 μl. The total run time was 12 min, and the column temperature was 30 °C. Concentration was measured through the UV absorbance at 254 ± 4 nm. Qualitative determination was carried out by comparison of the spectra of standards. Primary stock 0.1 mg/ml solutions of MX, MXP and PIR were prepared in methanol and stored at -8 °C. Calibration plots of MX, MXP and PIR were freshly prepared and were linear ($R^2 > 0.9996$ and 0.999, respectively) in the concentration range 0.25–10.0 mg/ml ($n = 3$). During the separation, the active substances were eluted with distinct retention times: 10.12 ± 0.01 (MX and MXP) and 7.36 ± 0.03 min (PIR). The limits of quantification (LOQ) were calculated by working standards with values of 0.171 (MX and MXP) and 0.275 μg/ml (PIR).

The animal blood samples (200 μl) were diluted with 500 μl of extraction liquid (potassium phosphate buffer, 0.03 M, pH 2.7) and spiked
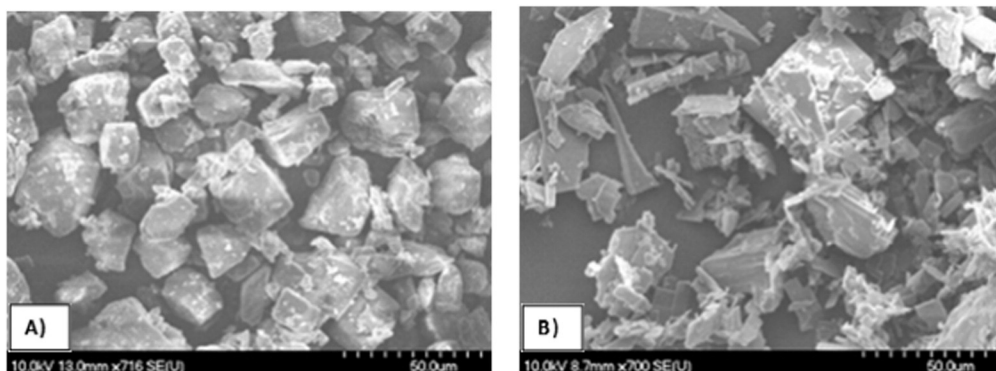
Fig. 3. SEM images of MX (A) and MXP (B).

Table 1
Particle size and roundness of the raw materials.

Sample	Length (μm)	Breadth (μm)	Perimeter (μm)	Roundness	Area (μm²)
MX	6.451 ± 4.741	3.970 ± 2.577	21.481 ± 18.045	2.081 ± 0.774	22.037 ± 36.102
MXP	12.372 ± 5.894	6.370 ± 2.743	38.300 ± 19.314	2.353 ± 1.007	52.495 ± 46.096

with 10 μl of the working internal standard (IS) solution at a final plasma concentration of 1.3 mg/ml. The solid phase extraction (SPE) cartridges used (Strata-X-C 33 mm Polymeric Strong Cation tubes, Phenomenex Inc., Torrance, CA, USA) were conditioned with 0.5 ml of methanol, followed by 0.5 ml of extraction liquid. The prepared blood samples were allowed to run through the SPE cartridge at a flow rate of 0.8 ml/min. The cartridges were rinsed with 0.5 ml of extraction liquid and 0.5 ml of methanol (5%) and dried in vacuum for 5 min. Elution was then performed with 0.5 ml of 5:95 (v/v) ammonium hydroxide–methanol elution solution and dried in a vacuum oven (Binder, Germany) at 20–30 mbar and 45 °C for 2–3 h. The dried residue was reconstituted in 3 ml of eluent and then mixed (60 s), sonicated (2 min) and centrifuged at 12,000g for 5 min. 20 ml of supernatant was injected onto the C18 column.

2.3.2.5. Statistical analyses. Data were expressed as means ± SD, and groups were compared by using Student's *t*-test. Differences were considered statistically significant when p < 0.05.

3. Results

3.1. Investigation of raw materials

3.1.1. SEM

The SEM images clearly showed the difference between the two samples (Fig. 3). MX has well-developed crystals with a smooth surface. In contrast, the crystals of MXP are misaligned, and therefore have a different habit (form, surface and size).

3.1.2. Particle size analysis

The results of particle size analysis did not reveal much difference between MX and MXP (Table 1), which had been anticipated by the SEM investigations. The crystals of MXP were twice as large as those of than MX, but the larger surface (area) of MXP could be explained by the presence of small crystals. The roundness value of the drugs was the same, but the different habit of the MXP crystals was not established by Leica investigations.

3.1.3. Equilibrium solubility

The solubilities of the active ingredients influence the absorption, and therefore media with different pH values were used in the solubility

testing. PB at pH 5.60 simulated the pH of the nasal mucosa, while the PB with pH 7.40 and distilled water as vehicles imitated optional pH values for the preparation of nasal spray, and the pH of the acceptor phase was 7.40 in the diffusion tests.

MX is a representative NSAID "oxicam". It has acidic (enol) with a pKa of 3.43 and basic (thiazole ring) functional groups. The N basicity of the thiazole ring prevails in acidic medium (pKa < 1). The pH-dependent solubility of MX is connected to the formation of the anionic form of the drug due to dissociation of the enolic OH group. Due to the polarity of the anionic form, its solubility shows a large difference as compared with the non-ionic neutral form. The results indicate that the solubilities of the non-dissociated free acid (MX) and the salt form (MXP) of the drug are the same at the same pH value of the medium (Table 2). However, a large difference was detected in distilled water. MXP was alkali-hydrolysed in water, and the pH value of the saturated aqueous solution was therefore 8.15, which resulted in a 350-times higher solubility than that of MX at pH 5.80. This is associated with the difference in the degree of ionization.

3.1.4. Dissolution testing

The rates of dissolution of MX and MXP were investigated in the media with pH 5.60 (Fig. 4). Although the equilibrium solubilities of MX and MXP are the same at pH 5.60 (0.017 mg/ml), the difference in their rates of dissolution is considerable. This is due to the faster dissolution of the salt form and the larger surface of MXP than that of MX (see Table 1), and consequently the MXP crystals reach saturation

Table 2
Equilibrium solubilities of the raw materials at different pH (37 °C).

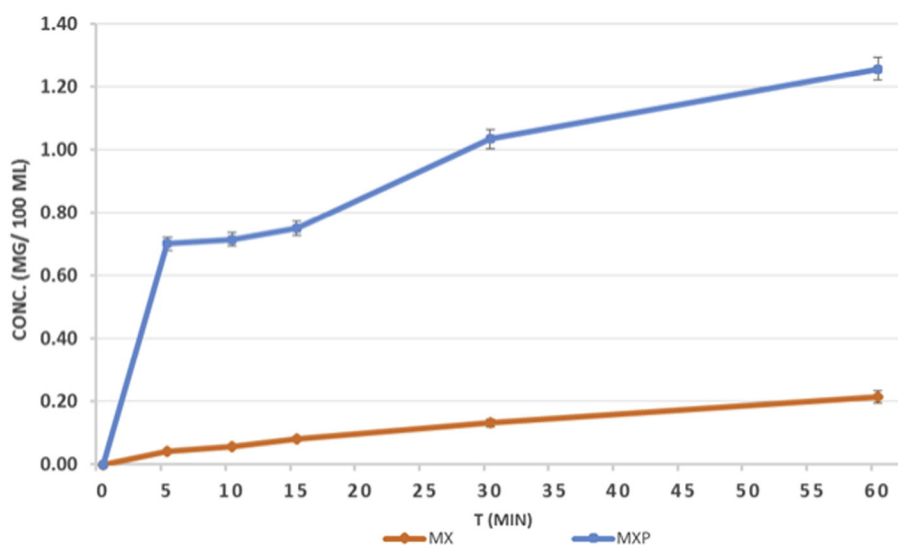| Solubility medium	MX		MXP	
	Final pH of solubility test	Solubility [mg/ml]	Final pH of solubility test	Solubility [mg/ml]
Phosphate buffer pH = 5.60	5.60	**0.017 ± 0.001**	5.60	**0.017 ± 0.001**
Phosphate buffer pH = 7.40	7.32	**0.933 ± 0.054**	7.33	**0.729 ± 0.001**
Distilled water pH = 5.50	5.80	**0.040 ± 0.040**	8.15	**13.10 ± 0.015**

Fig. 4. Extent of dissolution of MX and MXP at pH 5.60 ($n = 3$).

solubility faster than MX. Fig. 4 shows the amount of MXP dissolved during 1 h (0.013 mg/ml), which is in good agreement with its equilibrium solubility at pH 5.60.

3.2. Examination of intranasal sprays

Intranasal sprays were developed with 2 mg/ml MX or MXP and 1 mg/ml HA. The dispersion media was phosphate buffer with pH 5.60. The results of the solubility of MX and MXP allow the conclusion that both were really in suspended form (dispersed microcrystals) in the media.

3.2.1. In vitro permeability

The horizontal cell model (Side-Bi-Side™) was used to measure the cumulative amounts of MX and MXP that diffused through a synthetic membrane from nasal sprays against time. Application of this model provided the continuous stirring of the donor phase because of the homogeneous distribution of the suspended drugs (Horváth et al., 2015). Fig. 5 shows that MX spray at pH 5.60 permeate at higher rate through the membrane than MXP spray, which indicated a faster diffusion and a higher drug concentration. This could be explained by the difference in pH on the two sides of the membrane (acceptor phase at pH 7.40), which generated a driving force in the system.
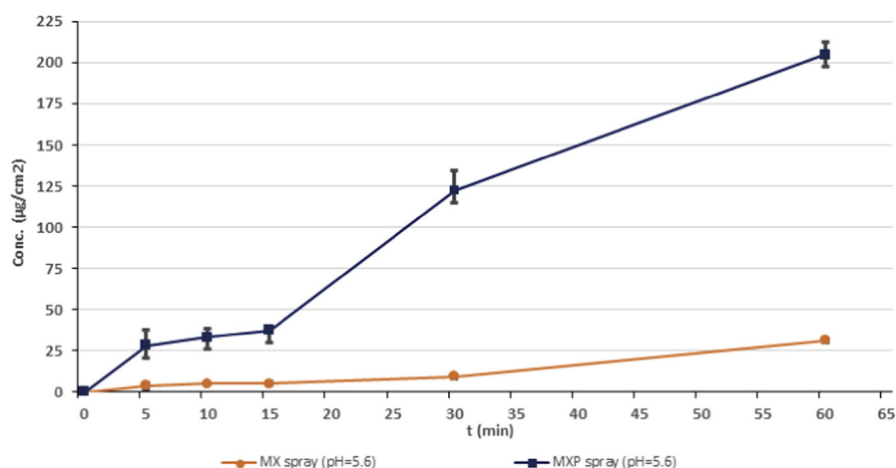
The flux (J), which shows the amounts of MX and MXP that permeate through 1 cm2 of the membrane within 1 h, was significantly higher in the case of the MXP spray (pH = 5.60) as compared with the MX spray. The permeability coefficient (K_p) calculated from the flux data for the MXP spray (pH = 5.60) was also significantly higher than in the MX case (Table 3). The data in the table show the growth trend in the flux and permeability coefficients for MXP, which are connected to the salt form, but no connection was found between the *in vitro* permeabilities of MX and MXP and the mucoadhesivities of the sprays.

3.2.2. Mucoadhesion

In our earlier study, intranasal formulations with a low concentration of HA exhibited a viscoelastic character (Bartos et al., 2015), which was not influenced by micro- and nanoparticles of MX. As a viscosity enhancer, HA aids the homogeneous distribution of suspended drug in the nasal formulation and acts as a mucoadhesive agent, resulting in a longer residence time on the mucosa. For the rheological investigation of mucoadhesivity, the nasal formulations were mixed with 5% mucin and the synergism parameter was calculated from the viscosity at a shear rate of 100 1/s. Simulating the mucosal surface, the mucoadhesivities of the sprays containing HA were measured in PB at pH 5.60, without drugs as with MX or MXP.

HAs are mucoadhesive polymers, which was verified in our experiments (positive synergism value) (Fig. 6). When suspended drug

Fig. 5. *In vitro* permeability of the sprays through a synthetic membrane containing MX and MXP ($n = 3$).

Table 3
Flux (J) and permeability coefficient (K_p) values of nasal sprays.

|	J [μg/cm²/h]	K_p [cm/h]
MX spray (pH = 5.60)	31.30	0.0157
MXP spray (pH = 5.60)	204.60	0.1023
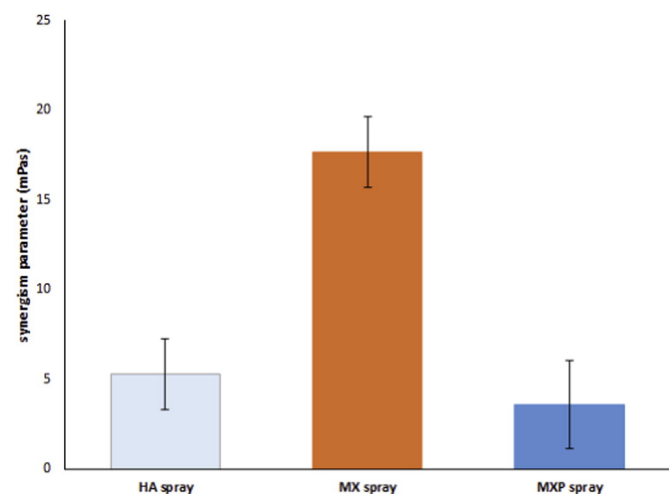
Fig. 6. Calculated synergism parameters of the samples at a shear rate of 100 1/s ($n = 3$).

(MX) was used in the formulation, the synergism was increased (MX spray). The suspended drug improved and promoted the format of netpoints between the HA and the mucin polymers, result in marked mucoadhesivity. Addition of the ionic drug (MXP) decreased the synergism, which can be explained by the interaction of the salt and the HA polymers (Krüger-Szabó et al., 2015).

3.2.3. In vivo permeability

The plasma concentration of the drug in rats is shown in Fig. 7. In the event of the MXP-containing spray, a 3 times higher plasma level was observed after 5 min as compared with the formulation containing MX. This is in accordance with the faster dissolution and faster absorption of MXP from the medium at pH 5.6. The difference in mucoadhesivity of the MX and MXP-containing sprays at pH 5.60 (see Fig. 4) does not significantly influence the drug absorption through
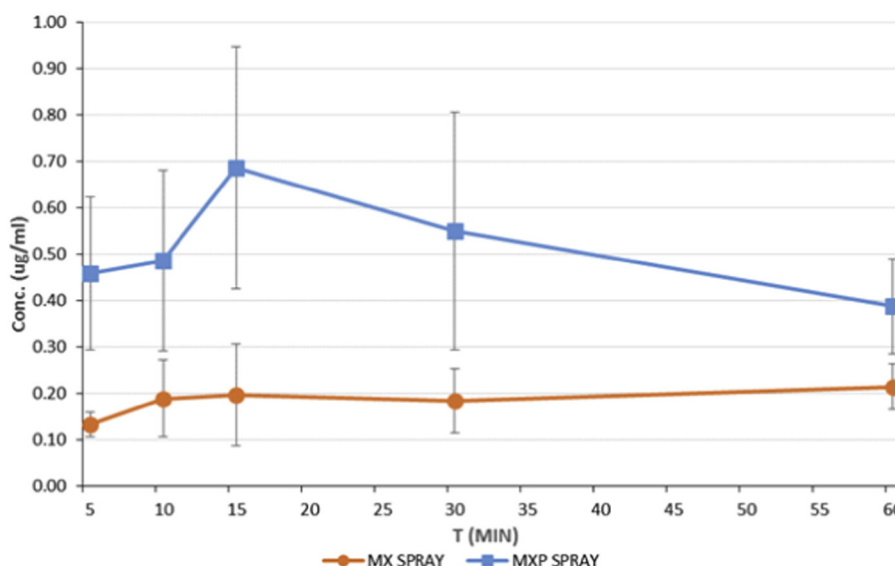
the nasal membrane. In the case of the MXP spray, the maximum concentration (T_{max}) was reached at 15 min, after the elimination had become predominant.

The AUC is proportional to the amount of drug absorbed during the investigated time interval (Fig. 8). The calculated AUC values were gradually increased by using the salt form (from AUC_{MX}: 10.927 min∗μg/ml to AUC_{MXP}: 29.738 min∗μg/ml). Our results demonstrated a correlation between the value of AUC and the non-dissociated free acid (MX) and salt (MXP) forms of the drug.

4. Discussion

The applicability of NSAIDs in a nasal formulation is a new approach in pain therapy. MX was the first enolic acid oxicam derivative patented for intranasal administration (Castile et al., 2005). MX has poor water solubility and is relatively well-permeable, and different strategies were therefore used to increase its dissolution rate and solubility (Ambrus et al., 2009, Kürti et al., 2013).

MX is applied in solid dosage forms, indicating prolonged absorption (the C_{max} value of MX is within 4–5 h), (Busch et al., 1998), which cannot use for rapid analgesia. According to our pervious results, the pharmacokinetics of nasally applied MX nanoparticles was similar to that after intravenous injection: the C_{max} was reached within 5 min (Kürti et al., 2013). Nasal absorption can be improved through the higher mucoadhesivity of the MX-containing formulation, which increases the residence time in the nasal cavity, and the formation of a well-structured system can ensure the controlled release of MX without C_{max} (Bartos et al., 2015).

In order to enhance the bioavailability of MX, salt formation was a new approach to increase its solubility, dissolution rate and fast absorption through the nasal membrane to reach the blood stream. The potassium salt MXP was a new agent registered by Egis Plc. (Budapest, Hungary) (patent number: US8097616 B2). MXP-containing dosage forms have not been described to date, and therefore we investigated the physico-chemical properties of MXP in comparison with MX and prepared intranasal liquid formulations with both agents. In vitro and in vivo studies were carried out to determine the nasal applicability of MXP as a drug candidate in pain therapy.

Our results demonstrated that both of the raw materials consist of yellow crystals, but different habits. MX and MXP have high melting points (MX = 267 °C and MXP = 253 °C). The solubilities of the non-dissociated free acid (MX) and salt form (MXP) of the drug are equal at the same pH of the medium (PB pH = 5.60 or 7.40). The equilibrium
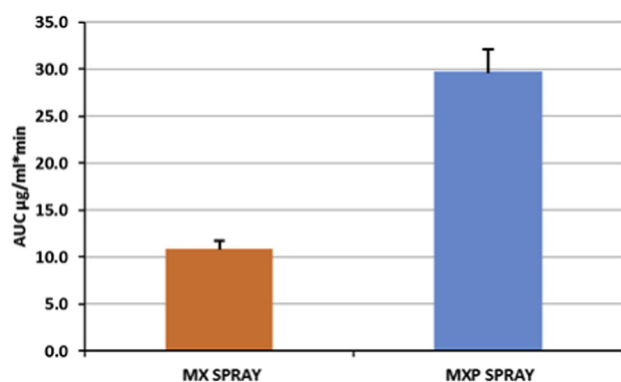

Fig. 7. Plasma drug concentration vs. time profiles in rats after intranasal administration of the sprays containing MX and MXP ($n = 5$).

Fig. 8. AUCs of the sprays containing MX and MXP ($n = 5$).

solubilities of MX and MXP are higher in the medium with higher pH value (pH = 7.40), but a considerable difference was detected in distilled water at own pH. MXP was alkali-hydrolyzed in water and the pH value of saturated aqueous solution was 8.15, which resulted in a 350-times higher solubility than that of MX in water at pH 5.80. This was associated with the difference in degree of ionization.

Although the solubilities of MX and MXP are the same at pH 5.60 (0.017 mg/ml), the difference in their rates of dissolution is considerable. This difference stems from the faster dissolution and larger surface of MXP, and consequently the MXP crystals can reach saturation solubility faster than as MX. The amount of MXP dissolved during 1 h (0.013 mg/ml) is in good agreement with its equilibrium solubility at pH 5.60.

The *in vitro* permeability results on a synthetic membrane suggest the potential usefulness of the MXP spray (pH = 5.60) for nasal delivery because of the higher permeability value in comparison with the MX spray (pH = 5.60). Experiments where MXP spray was administered nasally into rats (as compared with MX spray) showed that the maximum concentration T_{max} was reached at 15 min, and the calculated AUC values gradually increased in use of the salt form. It was found that the difference in mucoadhesivity of MX and MXP-containing sprays did not significantly influence the drug absorption through the nasal membrane.

It is known that intranasal administration can allow the drug absorption not only in the bloodstream but also in the central nervous system (CNS). The direct pathways as the olfactory nerve and the olfactory epithelial are known for transfer of drugs into the CNS (Huston and Schwarting, 1997). Intranasal administration of NSAIDs as MX and MXP is new approach in the pain therapy, therefore the study of direct transport can assist the understanding of the rapid analgesia.

5. Conclusion

In conclusion we demonstrated that using salt form can result a faster dissolution and enhance the bioavailability. Nasal delivery, as an alternative way, could offer a great solution for drug administration. Both the *in vitro* and the *in vivo* results indicated that MXP could be suggested for the development of an intranasal liquid dosage form for use in rapid pain management, but further experiments are necessary to prove the therapeutic relevance of this MXP-containing innovative intranasal formulation.

Conflicts of interest

The authors declare no conflict of interest.

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