International Journal of Pharmaceutics 379 (2009) 67–71

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Effect on the nasal bioavailability of co-processing drug and bioadhesive carrier via spray-drying

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ARTICLE INFO

Article history:
Received 4 March 2009
Accepted 9 June 2009
Available online 17 June 2009

Keywords:
Co-spray-drying as all-in-one process
Nasal administration
Amioca®/Carbopol® 974P powder formulation
Metoprolol tartrate

ABSTRACT

A mucoadhesive combination of a maize starch (Amioca®, mainly consisting of amylopectine) and a cross-linked acrylic acid-based polymer (Carbopol® 974P) was spray-dried with metoprolol tartrate (used as model molecule) in order to develop a powder suitable for nasal drug delivery via a one-step manufacturing process. The bioavailability of metoprolol tartrate after nasal administration of this powder to rabbits was compared with powders manufactured via other procedures: (a) freeze-drying of a dispersion prepared using the co-spray-dried powder, (b) freeze-drying of a dispersion prepared using a physical mixture of drug and mucoadhesive polymers. After co-processing via spray-drying a low bioavailability (BA $10.8 \pm 2.3\%$) was obtained, whereas manufacturing procedures based on freeze-drying yielded a higher BA: $37.9 \pm 12.8\%$ using the co-processed powder and $73.6 \pm 24.9\%$ using the physical mixture. The higher bioavailability was due to the deprotonation of poly(acrylic acid) during neutralisation of the dispersion prior to freeze-drying. This induced repulsion of the ionised carboxyl groups and a lower interaction between poly(acrylic acid) and starch, creating a less compact matrix upon hydration of the polymer and allowing an easier escape of metoprolol tartrate from the matrix. This study showed that co-processing of a mucoadhesive Amioca®/Carbopol® 974P formulation with metoprolol tartrate via co-spray-drying did not provide any added value towards the bioavailability of the drug after nasal administration of the mucoadhesive powder.

1. Introduction

The nasal cavity offers great opportunities for systemic drug delivery as the nasal epithelium has a large surface area which is thin and highly vascularized (Hinchcliffe and Illum, 1999). The nasal route is also suitable for self-medication and absorbed drugs pass directly into the systemic circulation thereby avoiding hepato-gastrointestinal first-pass metabolism (Chang and Chien, 1984; Chien and Chang, 1985). In spite of the potential of nasal drug delivery, some limitations have to be overcome: (a) only a low drug dose can be administered as the amount of liquid or powder delivered to the nose is limited to 150 μl and 20 mg, respectively (Hinchcliffe and Illum, 1999; Teshima et al., 2002), (b) enzyme activity in the nasal cavity, (c) residence time of the formulation is limited by mucociliairy clearance and (d) the mucus layer and nasal epithelium are barriers for drug absorption (Ugwoke et al., 2005). This study focussed on the development of a bioadhesive nasal delivery system that provides a prolonged contact between the formulation and the absorptive sites in the nasal cavity by reducing the mucociliairy clearance rate.

The mucoadhesive powder formulation used in this study was a spray-dried combination of a maize starch (Amioca®, mainly consisting of amylopectine) and a cross-linked acrylic acid-based polymer (Carbopol® 974P) that amplified the mucoadhesive capacity of the formulation (Callens and Remon, 2000). Ameye et al. (2005) showed that co-processing of the powder mixture via spray-drying yielded a product with a higher buccal bioadhesive capacity and bioavailability compared with a physical mixture of Amioca® starch and Carbopol®. When this powder carrier was used in previous studies for nasal delivery (Callens and Remon, 2000; Pringels, 2005), post-processing of spray-dried Amioca®/Carbopol® powder was required to incorporate the drug into the formulation: the drug was mixed with an aqueous dispersion prepared from the mucoadhesive powder and after freeze-drying the solid cake was sieved to obtain a powder with particle size suitable for nasal delivery.

In this study, it was investigated if this time-consuming procedure could be replaced by a one-step manufacturing process using spray-drying to co-process an aqueous dispersion of Amioca®, Carbopol® and drug into a mucoadhesive powder. This single-step manufacturing technique would not only reduce the number of unit

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doi:10.1016/j.ijpharm.2009.06.008

operations, improve production efficiency and reduce costs, it also yields a homogeneous and free-flowing powder (Gonnissen et al., 2008) with an ideal particle size for nasal delivery.

A mucoadhesive powder containing Amioca®, Carbopol® and metoprolol tartrate (used a model drug) was manufactured via different procedures to evaluate the effect of processing on the bioavailability of metoprolol tartrate after nasal administration of the mucoadhesive powder formulations to rabbits: the formulations were co-processed via spray-drying or prepared via freeze-drying of an aqueous dispersion of the different ingredients.

2. Materials and methods

2.1. Materials

Metoprolol tartrate was kindly donated by EQ Esteve (Barcelona, Spain). The spray-dried mixture of Amioca® starch and Carbopol® 974P (ratio: 25/75, w/w) (SD 25/75) (batch: 13724-8E) was prepared by National Starch and Chemical Company (Bridgewater, NJ, USA). All other chemicals were of analytical grade.

2.2. Preparation of metoprolol tartrate formulations

2.2.1. Intravenous and nasal PBS formulations

A metoprolol tartrate solution of 2 mg/ml was prepared in a phosphate buffered saline solution (PBS, pH 7.4) (2.38 g $Na_2HPO_4{\cdot}2H_2O$, 0.19 g KH_2PO_4 and 8.0 g NaCl per liter distilled water), of which 300 μl was intravenously administered to rabbits ($n = 6$).

A metoprolol tartrate solution of 64 mg/ml was prepared in PBS (pH 7.4), of which 50 μl per nostril was nasally administered to the rabbits ($n = 6$).

2.2.2. Nasal powder formulations

2.2.2.1. Co-processing of Amioca®, Carbopol® and metoprolol tartrate via spray-drying. A dispersion of 12 g metoprolol tartrate and 30 g SD 25/75 was prepared in 1 l distilled water and homogenised with a rotor-stator mixer (L4RT, Silverson, East Longmeadow, USA) during 5 min. This aqueous dispersion was diluted to a volume of 3 l and spray-dried in a lab-scale Mobile Minor spray-dryer (GEA NIRO, Copenhagen, Denmark). The powders were spray-dried using the following process conditions: feed rate of 28.4 g/min, inlet and outlet drying air temperature of 160 and 80 °C, respectively, drying gas rate of 80 kg/h, atomising air pressure of 2 bar and compressed air flow of 60%. The solution was fed to a two-fluid nozzle (diameter: 2 mm) at the top of the spray-dryer by means of a peristaltic pump, type 520U (Watson Marlow, Cornwall, UK). The spray-dryer operated in co-current airflow. The spray-dried particles were collected in a reservoir attached to a cyclone, cooled down to room temperature. The powder had a concentration of 5.7 mg metoprolol tartrate per 20 mg (w/w). This powder (further identified as Formulation M1) was stored in a sealed vial (room temperature, ambient relative humidity) prior to its further use.

2.2.2.2. Freeze-drying of a co-processed mixture of Amioca®, Carbopol® and metoprolol tartrate. 500 mg M1 powder was dispersed in 15 ml distilled water and the pH of this dispersion was adjusted to 7.4 using 2 M NaOH. After neutralisation a concentration of 4.8 mg metoprolol tartrate per 20 mg powder (w/w) was obtained. The aqueous dispersion was freeze-dried using an Amsco-Finn Aqua GT4 freeze-dryer (Amsco, Germany). The dispersion was frozen to 228 K within 175 min at 1000 mbar. Primary drying was performed at 258 K and at a pressure varying between 0.8 and 1 mbar during 13 h, followed by secondary drying at elevated temperature (283 K) and reduced pressure (0.1–0.2 mbar) for 7 h. After freeze-drying the powder was sieved (63 μm) at low relative humidity (20%) and ambient temperature. The fraction below 63 μm was stored in a sealed vial (room temperature, ambient relative humidity) until use. The powder prepared using formulation M1 as starting material is further identified as formulation M2.

Table 1
Overview of the different powder formulations evaluated in this study and their production method.

Formulation	Procedure
M1	Co-processing of SD 25/75 and MT via spray-drying
M2	Freeze-drying of dispersion of formulation M1 and subsequent sieving
M3	Freeze-drying of SD/25 dispersion and MT and subsequent sieving

2.2.2.3. Freeze-drying of a physical mixture of Amioca®/Carbopol® and metoprolol tartrate. To obtain the powder identified as M3 an aqueous dispersion of 500 mg SD 25/75 and 201 mg metoprolol tartrate in 15 ml distilled water was neutralised to pH 7.4 with NaOH (2 M) and the resulting gel (containing 4.8 mg MT per 20 mg powder (w/w)) was freeze-dried under the same conditions as described in Section 2.2.2.2. After lyophilisation the powder was sieved (63 μm) at low relative humidity (20%) and ambient temperature. This fraction below 63 μm was stored in a sealed vial (room temperature, ambient relative humidity) until use.

For sake of clarity an overview of the different formulations evaluated in this study and their production procedure is shown in Table 1.

2.3. Metoprolol tartrate assay in bioadhesive powders

Metoprolol tartrate concentration in the powder formulations was determined by means of a HPLC-method (concentration range: 0–3.5 mg MT/ml distilled water). To extract metoprolol tartrate from the powder, 10 mg of each powder formulation (M1–M3) was dissolved in 1 ml distilled water before addition of 100 μl hydrochloric acid (37%). Next, the suspension was centrifuged (700 × *g*, 5 min) and the supernatant was filtered over a cellulose membrane (Spartan 30/0.2 μm, Whatman GmbH, Dassel, Germany). Afterwards, the filtrate was 200-fold diluted and injected on the HPLC column (HPLC-method see Section 2.4.2).

2.4. Nasal bioavailability study

2.4.1. Study design

The protocol of the animal experiments was approved by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO) (Merelbeke, Belgium). New Zealand white rabbits (3.0 ± 0.5 kg) were fasted 16 h prior to the experiment. Water was available ad libitum. The rabbits were sedated with an intramuscular injection of 0.05 ml/kg Placivet® (Codifar, FL, USA) immediately after administration. A first group of six rabbits received 0.6 mg MT intravenously. Ten milligrams powder (M1–M3) was administered in each nostril of a second group of six rabbits using polyethylene tubes (Medisize, Hillegom, The Netherlands). The powder was released from the tubes using a syringe containing 1 ml compressed air (2.5 bar). This device was based on a system developed by Sørensen (1991).

The tubes were filled under conditions of low relative humidity (20%) and ambient temperature. Blood samples were collected from the ear veins at—5, 1, 2, 5, 10, 15, 20, 30, 40, 50 and 60 min after intravenous administration and at—5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after nasal delivery of the powder formulations. After adding heparin (LEO Pharma, Wilrijk, Belgium) to the test tubes, plasma samples were separated by centrifugation

(700 × g, 5 min) and samples were stored frozen at −20 °C until HPLC-analysis.

2.4.2. HPLC analysis of metoprolol tartrate in plasma

The metoprolol tartrate plasma concentrations were determined by a validated HPLC-fluorescence method. All chemicals were of analytical grade.

Samples were prepared by adding twenty microliters of an internal standard solution (5.6 μg/ml of alprenolol in water for analysis of blood samples after nasal administration and 1.9 μg/ml alprenolol in water for analysis of the intravenous samples) and 680 μl PBS to 300 μl plasma sample. The drug was extracted using a solid phase extraction (SPE) method. The SPE columns were conditioned consecutively with 1 ml methanol, 1 ml water and 1 ml PBS. Next, the plasma samples were spiked on the SPE columns. Columns were rinsed with 1 ml water and metoprolol tartrate was eluted with 1 ml methanol. The eluates were evaporated to dryness under a nitrogen flow, the residue was dissolved in 150 μl water and 20 μl was injected. The plasma concentrations were determined via a calibration curve. The standards for the calibration curve (20 μl of internal standard solution, 20 μl of a standard solution with known MT concentration in water, 280 μl blank plasma and 680 μl PBS) underwent the same treatment as the plasma samples. The concentrations of the standard solutions were 0.375; 0.5625; 0.75; 2.25; 5.625; 7.5; 15.0; 22.5 μg/ml metoprolol tartrate in water for analysing the samples after nasal administration and 0.275; 0.550; 0.875; 1.1; 1.925; 2.75; 4.4 μg/ml MT in water for analysing the intravenous samples.

The HPLC equipment (Hitachi, Darmstadt, Germany) consisted of a solvent pump (L-7110 pump) set at a constant flow rate of 0.800 ml/min, a fluorescence detector (L-7480) set at 275 nm as excitation wavelength and 300 nm as emission wavelength, a LiChrosper® 100 CN column (5 μm, 250 mm × 4 mm) and pre-column LiChrosper® (5 μm, 4 mm × 4 mm), an autosampler and injector (Gilson 234 autoinjector, Wisconsin, USA) with an injection loop of 50 μl (Valco Instruments Corporation, Houston, TX, USA). The area under the curve was calculated with the softwarepack D-7000 Multi-Manager (Merck, Darmstadt, Germany). The SPE-equipment consisted of OASIS HLB (1 ml, 30 mg) cartridges (Waters, Brussels, Belgium) and a 16-port vacuum manifold (Alltech Europe, Laarne, Belgium). The eluens had the following composition: 5 ml of a 2 M NaH_2PO_4 buffer solution, 50 ml acetonitrile and 945 ml water, adjusted to pH 3 with 150 μl phosphoric acid.

2.4.3. Data analysis

The individual serum concentration–time profiles were analysed by MW/Pharm version 3.15 (Medi-ware, Utrecht, The Netherlands) and the maximum metoprolol tartrate serum concentrations (C_{max}) and t_{max} values were determined from the individual serum concentration–time profiles. Data were normalised (C_{max} and AUC) in order to adjust for differences in administered dose (due to differences in the initial drug concentration of the formulations and degradation of the active ingredient during spray-drying). The influence of the powder formulations on the absolute bioavailability, C_{max} and t_{max} of metoprolol tartrate was analysed using one-way ANOVA. Normal distribution of the data was tested using the Kolmogorov–Smirnov test and the homogeneity of variances was tested using the Levene's test. If the distribution of the data was not normal or the variances were not homogeneous, the data were transformed (logarithm). The software program SPSS version 15.0 was used for statistical analysis.

2.5. FT-IR analysis

ATR FT-IR spectra were recorded for Amioca®, Carbopol®, metoprolol tartrate, SD 25/75 and M1–M3 samples using a Bruker Vertex 70 FT-IR spectrometer equipped with a Hyperion IR microscope. A Ge ATR crystal was pressed against the powder for obtaining the ATR FT-IR spectrum (4 cm^{-1} resolution, 50 scans).

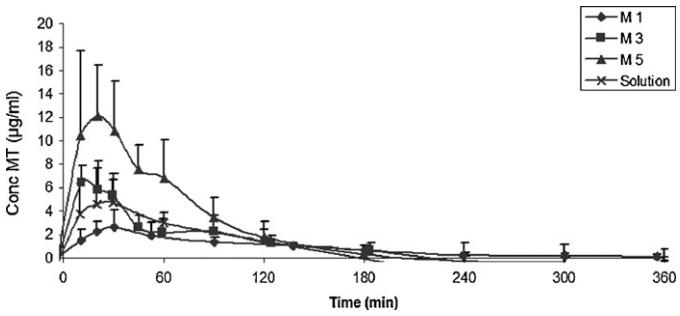

Fig. 1. Plasma concentration profiles of metoprolol tartrate after nasal delivery of MT in Amioca®/Carbopol® 974P 25/75 using 20 mg different powder formulations (M1 (♦), M2 (■) and M3 (▲)) to rabbits. Data were normalised to compensate for the difference in administered dose per formulation.

3. Results and discussion

In this study, co-processing of Amioca®/Carbopol® 974P 25/75 with the model molecule metoprolol tartrate via spray-drying was investigated to develop a nasal drug delivery system via an all-in-one process. Bioadhesive Amioca®/Carbopol® 974P (ratio 25/75) carriers containing metoprolol tartrate were prepared using different procedures (Table 1) and the absolute bioavailability of all formulations was evaluated after nasal administration in rabbits. To evaluate the effect on nasal bioavailability of neutralisation of poly(acrylic acid) a dispersion of the co-processed formulation was neutralised with NaOH and the resulting gel was freeze-dried. Neutralisation of the carboxylic groups of Carbopol® 974P prior to spray-drying was not possible as the viscosity of the dispersion was too high for spray-drying. Dilution of such a viscous dispersion was not recommended due to the low process yield when spray-drying a highly diluted dispersion.

In former studies Coucke et al. (2009, in press) used the mucoadhesive Amioca®/Carbopol® powder formulation as carrier for nasal delivery of peptides (insulin, calcitonin, human growth hormone) and proteins (inactivated influenza vaccine). Because of the heat-liability of these active components and the high temperature required for the spray-drying process, a heat-stabile small model molecule (metoprolol tartrate) was selected for the study. Metoprolol tartrate is a cardioselective β_1-blocker and is one of the first-line drugs for the management of systemic hypertension and angina pectoris (Frishman and Alwarshetty, 2002). When orally administered, metoprolol tartrate is completely absorbed but due to an intensive first-pass effect, only 50% of the given dose is found in the systemic circulation. Therefore, metoprolol tartrate is an interesting molecule to incorporate in a formulation for nasal delivery.

The plasma concentration profiles of metoprolol tartrate after nasal delivery of M1, M2 and M3 powders and a control solution are shown in Fig. 1, the corresponding pharmacokinetic parameters are detailed in Table 3. All pharmacokinetic data were normalised to compensate for differences in delivered dose due to a variable initial drug content in the formulations and drug loss during processing (Table 2).

The co-processed M1 powder formulation had a significantly lower bioavailability (10.8 ± 2.3%) and slower metoprolol tartrate release rate (Fig. 1) compared with the neutralised samples: 37.9 ± 12.8% ($0.01 \geq P > 0.001$) and 73.6 ± 20.1% ($P \leq 0.001$) for formulations M2 and M3, respectively. Formulation M3, which was neutralised, resulted in significantly higher metoprolol tartrate plasma concentrations ($P \leq 0.001$) compared to co-spray-dried M1

Table 2
Metoprolol tartrate content (expressed as a percentage of the theoretical concentration) in formulations M1–M3.

Formulation	Metoprolol tartrate content (%) ± SD
M1	80.5 ± 0.2
M2	71.9 ± 0.7
M3	99.0 ± 1.4

Table 3
Absolute bioavailability, C_{max}- and T_{max}-values after nasal delivery of 20 mg powder formulation (M1–M3, see Table 2 in rabbits. Data were normalised to compensate for the difference in administered dose per formulation.

Formulation	BA (%)	C_{max} (μIU/ml)	t_{max} (min)	n
M1	10.8 ± 2.3[a]	3.1 ± 1.3[c]	33.6 ± 13.5	6
M2	37.9 ± 12.8	7.0 ± 2.1	12.3 ± 3.9	6
M3	73.6 ± 24.9	13.3 ± 4.9	20.8 ± 9.2	6
MT solution	20.6 ± 17.5[b]	6.0 ± 3.2[d]	22.5 ± 5.1	6

[a] Significantly lower than M2 ($0.01 \geq P > 0.001$) and M3 ($P \leq 0.001$).
[b] Significantly lower than M3 ($0.01 \geq P > 0.001$).
[c] Significantly lower than M2 ($0.01 \geq P > 0.001$) and M3 ($P \leq 0.001$).
[d] Significantly lower than M3 ($0.01 \geq P > 0.001$).

powder. These differences in pharmacokinetic parameters can be explained by changes in molecular interactions between the different components of the mucoadhesive formulation as identified by the FT-IR spectra of metoprolol tartrate, SD 25/75, M1 and M3 shown in Fig. 2 (M2 powder had a similar spectrum as M3). The specific absorption band of metoprolol tartrate was found at 1513 cm^{-1}, while the carbonyl absorption band of poly(acrylic acid) appeared at 1700 cm^{-1}. This band was very prominent in formulation M1, indicating that the carboxylic acid groups were not ionized. This observation in combination with the broad hydroxyl absorption band at 3500–3000 cm^{-1} (due to the O–H function of Carbopol® 974P) indicated that strong interaction via H-bridges between poly(acrylic acid) and starch was possible. As a result a compact matrix is obtained (even after hydration of the polymer upon nasal administration) in which MT is entrapped. Hence release of MT from the mucoadhesive matrix was hindered, yielding a low bioavailability. This was in contrast to the dispersions of formulations M2 and M3 which were neutralised to pH 7.4 with NaOH prior to freeze-drying. The carboxylate (band positions at 1551 and 1400 cm^{-1}) formed in the carbopol-polymer at higher pH induced repulsion between the negatively charged deprotonated carboxylate groups and reduced the H-bridges between Amioca® starch and poly(acrylic acid). In addition there was a strong interaction

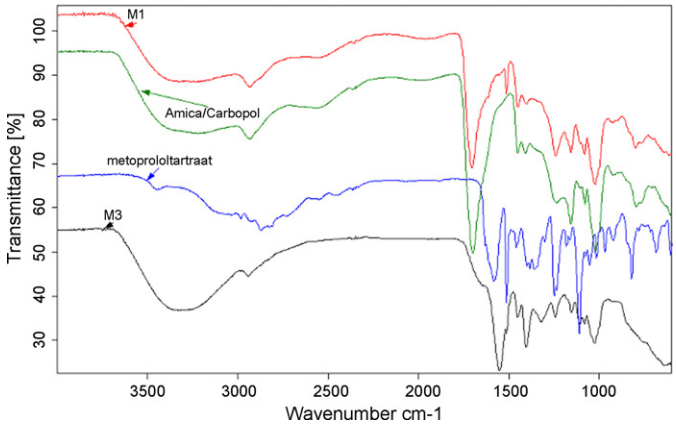

Fig. 2. IR-spectra of metoprolol tartrate, Amioca®/Carbopol® 974P powder and formulations M1 and M3.

between the carboxylate groups and water. These factors all contributed to the formation of a less compact matrix from which metoprolol tartrate needs to escape, yielding a faster release and higher bioavailability of metoprolol tartrate after nasal administration for the formulations M2 and M3. The effect of a less compact mucoadhesive matrix is probably even partly negated by the higher viscosity of the neutralised formulations upon hydration of the polymers. The broad hydroxyl absorption band between 3500 and 3000 cm^{-1} in the spectrum of M1, due to the stretch vibrations of carboxylic O–H functions of Carbopol® 974P, was no longer present in the spectrum of M3. The low wavenumber shoulder, between 3500 and 3000 cm^{-1} in the spectrum of M3, arises from metoprolol tartrate while the Amioca® starch hydroxylic O–H vibrations appear in the explicit band around 3350 cm^{-1}. Although release of metoprolol (pK_a 9.68, i.e. positively charged in the mucoadhesive powder, independent if the powder was neutralised) could be hampered by complex formation with negatively charged carboxylate, interaction between both is unlikely as metoprolol is strongly surrounded by H-bridges of tartrate. Formulation M3 had a significantly higher ($0.01 \geq P > 0.001$) bioavailability than the nasal control solution and a higher, but not significantly different bioavailability compared to powder M2 since M3 was prepared via physical mixing of the ingredients in combination with freeze drying, whereas M2 particles were spray-dried prior to freeze drying ensuring a stronger entrapment of the active in the polymer matrix.

Another factor that could have contributed (although to a lesser extent) to the difference in bioavailability between the formulations is the particle size of the nasally administered formulations since particles smaller than 10 μm have the potential to be deposited in the lower respiratory tract when nasally administered, thus reducing the dose fraction reaching the intended delivery site (Hinds, 1999; Garmise et al., 2006): 3.4% of the co-spray-dried M1 particles were smaller than 10 μm versus only 0.5% of the formulation which was freeze-dried after spray-drying.

Limited research has been done in the field of nasal delivery of metoprolol tartrate. Rajinikanth et al. (2003) investigated the nasal administration of metoprolol tartrate incorporated in bioadhesive sodium alginate microspheres. In vivo studies in rabbits showed a significant improvement of an isoprenaline-induced tachycardia in comparison with the oral and nasal administration of a MT solution. Absolute bioavailabilities of 30% were obtained after nasal delivery in microspheres.

Kilian and Müller (1998) studied the effect of a viscosity enhancer (methyl cellulose) and a surfactant (polysorbate-80) incorporated in a nasal metoprolol tartrate solution given to rats. The viscosity-enhancing capacities of methyl cellulose increased contact time of the drug with the absorption surface which reflected in higher AUC values. The nasal use of a surfactant did not improve the bioavailability, probably because of inclusion of the drug in micelles.

Bioavailabilities obtained after nasal administration of the co-spray-dried Amioca®/Carbopol® 974P-metoprolol tartrate formulation and the physical mixture of Amioca®/Carbopol® 974P combined with metoprolol tartrate were remarkably higher than those in the cited experiments.

4. Conclusion

The present study demonstrated that co-processing of a mucoadhesive Amioca®/Carbopol® 974P powder formulation with metoprolol tartrate (used as model drug) via spray-drying to obtain a powder for nasal drug delivery via a one-step process, did not have an added value towards the bioavailability of metoprolol tartrate after nasal delivery. Bioavailability improved using a mucoadhesive matrix that was neutralised to pH 7.4 prior to manufacturing via freeze-drying since, due to repulsion of the carboxylate groups

of poly(acrylic acid), a less dense matrix was formed upon hydration.

Acknowledgements

Daniel Tensy and Liselotte Coorevits are acknowledged for their excellent technical assistance during the animal experiments.

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