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Intranasal bioavailability of apomorphine from carboxymethylcellulose-based drug delivery systems

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Abstract

Carboxymethyl cellulose (CMC) powder formulation of apomorphine was prepared by lyophilization and characterized with respect to the in vitro and intranasal in vivo release of apomorphine in rabbits. This was compared to apomorphine release from degradable starch microspheres (DSM) and lactose, as well as in vivo absorption after subcutaneous injection. In vitro apomorphine release from CMC was sustained, unlike that of DSM and lactose. Changing the drug loading of CMC from 15 to 30% (w/w) influenced drug release rate, which increased with increased drug loading. In vivo absorption of apomorphine from lactose, DSM and subcutaneous injection were rapid and not sustained. Slower absorption rates of apomorphine occurred from CMC. The fastest absorption rate was obtained with lactose and the slowest with CMC of 15% (w/w) drug loading. The T_{max} from the CMC dosage forms were significantly prolonged compared to the immediate release forms. Plasma drug levels were sustained with CMC. The plasma concentration was maintained within 50% of the C_{max}, longer (15% (w/w), 70 min; 30% (w/w), 40 min) compared to the rest (lactose, 20 min; DSM, 25 min, subcutaneous injection, 35 min). The sustained plasma level of apomorphine by CMC was achieved with relative bioavailabilities equivalent to subcutaneous injection.

Keywords: Apomorphine; Parkinson's disease; Nasal drug delivery; Controlled release; Carboxymethylcellulose; Microspheres

1. Introduction

Nasal mucociliary clearance is one of the most important limiting factors to nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. However, certain polymers (mucoadhesive polymers) can adhere onto the nasal mucosa for reasonably prolonged periods, preventing rapid nasal clearance. Their use is therefore an avenue for improving nasal drug absorption as well as prolonging the duration of action of intranasally administered drugs.

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Cellulose derivatives are a class of polymer, some of which can be used in nasal mucoadhesive drug delivery formulation. Their widespread use in the formulation of different types of dosage forms makes them quite attractive. Also there are several pharmaceutical grade derivatives of cellulose that can provide several varieties of polymer characteristics. Hydroxypropyl methylcellulose, hydroxypropyl cellulose and carboxymethylcellulose sodium have all been investigated for nasal drug administration (Nagai et al., 1984; Vidgren et al., 1992; Nakamura et al., 1996). Hydroxypropyl methylcellulose improved the nasal absorption of insulin (Nagai et al., 1984). Carboxymethylcellulose (CMC) is another cellulose derivative which has yet to be investigated for use in nasal drug delivery.

Degradable starch microspheres (DSM) are mucoadhesive and biocompatible microspheres that have been used to enhance nasal absorption of insulin (Farraj et al., 1990; Edman et al., 1992), gentamicin (Illum et al., 1988) and human growth hormone (Illum et al., 1990). The use of DSM even in chronic diseases may not pose severe toxicological problems given that it is well tolerated both in experimental animals and man (Björk et al., 1991; Holmberg et al., 1994). It was thought interesting to investigate whether DSM could sustain nasal absorption of a drug such as apomorphine that is well absorbed after intranasal administration.

Apomorphine is a very effective drug in the management of 'on-off' motor response fluctuations in the treatment of Parkinson's disease. Apomorphine is administered clinically by subcutaneous injections, as its bioavailability after peroral administration is less than 5%, which is attributed to extensive first-pass metabolism (Gancher et al., 1991). Unfortunately, as a result of the short duration of therapeutic effect (less than 1 h) (Sam et al., 1995), high injection frequencies (up to 10–15 times a day) are often required. Therefore there is a need for a sustained drug delivery system for apomorphine.

The objective of this study was to formulate a nasal powder delivery system of apomorphine using CMC as the polymeric carrier and to characterize it with respect to in vitro and in vivo drug release. Furthermore, DSM was similarly investigated. This would provide information as to its usefulness in nasal delivery of a drug such as apomorphine that is well absorbed intranasally. Both dosage forms were compared to subcutaneous injection, and intranasally administered lactose/apomorphine powder mixture.

2. Materials and methods

2.1. Dosage forms preparation

Apomorphine.HCl (Alpha Pharma, Zwevegem, Belgium) was added to purified water (Elgastat Maxima SC, ELGA, High Wycombe, UK) in lyophilization vials and sonicated (Branson 2200, Branson Ultrasonics, Danbury, CT) until complete dissolution. Carboxymethyl cellulose (Blanose, type 7H3SXF, Aqualon, Surrey, UK) and degradable starch microspheres (Pharmacia and Upjohn, Milan, Italy) were dispersed, and anhydrous lactose (DC Lactose 20, DMV, Veghel, The Netherlands) dissolved in the solution. They were frozen overnight (-35°C) and first lyophilized (Alpha, Christ, Osterode, Germany) at -10°C for 24 h and then at 0°C for 24 h. The powders were sieved (180 μm mesh) and stored at room temperature until used. The amounts of these excipients were varied according to the final drug loading needed to give a total formulation weight of 1 g for CMC and lactose, and 0.5 g for DSM. The percentage drug loading of the formulations was determined by HPLC with electrochemical detection (Sam et al., 1994). The solution for s.c. injection was prepared to contain apomorphine.HCl and sodium metabisulfite (1:0.5) in water for injection and filtered through a sterilized 0.2-μm filter prior to injection.

2.2. In vitro dissolution testing

The in vitro release study was carried out with the modified rotating basket (Ugwoke et al., 1999) placed in a USP XXII dissolution vessel of the SR8-Plus dissolution test station (Hanson Research, Chatsworth, CA). Twenty milligrams of each formulation was weighed on a membrane

filter (0.45 μm) and secured firmly between the filter holder and the cup (both surfaces smeared with silicone grease) with clamps. Two grooves on the side of each filter-holder facing the filter were air vents allowing complete contact between release medium and filter. The release medium, USP phosphate buffer of pH 6.0 (500 ml) containing 0.01% 2-mercaptoethanol as anti-oxidant (Sam et al., 1994), was maintained at 37°C. The acceptor compartments were connected to a flow-through cell and the medium circulated with a peristaltic pump (Gilson Minipuls 3, Gilson, Villiers Le Bel, France). The modified baskets were rotated at 50 rev./min and released drug analyzed spectrophotometrically at 272 nm (HP 84752 A, Hewlett Packard, Santa Clara, CA).

2.3. Drug administration, sample collection and analysis

Six male New Zealand white rabbits were used. The animals were procured from and cared for throughout the period of the experiment by the Animalium Department of the Katholieke Universieit Leuven (Leuven, Belgium). The rabbits weighed (mean $\pm$ S.D.) 2.7 ± 0.18 kg at the beginning and 3.3 ± 0.17 kg at the end of the study period.

The percentage drug loading of powder forms of apomorphine.HCl studied were about 15% (w/w) in all cases and 30% (w/w) for CMC. The dose of apomorphine was 0.50 mg/kg body weight for CMC (both drug loading) and 0.25 mg/kg body weight for the rest.

Subcutaneous injection was administered at the lower half back with 26-guage needles. The nasal formulations were insufflated with a home-made device (Fig. 1) consisting of an air-filled 10-ml syringe compressed to 2.5 ml and an electrically-actuated valve expelling the drug through a plastic tip (internal diameter 2.3 mm, external diameter 3.0 mm) inserted 1–1.5 cm into one nostril. The formulations were placed about 1 cm from the end of the plastic tip for easy administration, to prevent drug loss and clogging of the plastic tip due to polymer swelling during the process. Cases with incomplete administration, which can be determined from the weight of the tip, rendered the experiment void and were repeated at a later date. Blood samples were taken from the marginal ear vein at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360 420, and 480 min except for the immediate release formulations where additional samples were taken at 1.5, 7 and 12 min, and stopped after 240 min. Sample handling and HPLC analysis were carried out as described in Sam et al. (1994).

2.4. Pharmacokinetic data treatment

Computation of the elimination rate constant (K_e) was carried out iteratively with the software TOPFIT Pharmacokinetics and Pharmacodynamics data analysis system software, version 2.0 (Gustav Fischer Verlag, Stuttgart, Germany). The absorption rate constant (K_a) was calculated using the Wagner–Nelson method since the data fitted a $(1+1)$ compartment model. The secondary model parameters like the elimination half-life ($T_{1/2,elim}$) and the noncompartmental parameters such as the mean residence times (MRT_{abs}, and MRT_{disp}) were generated with the same software.

The area under the curve (AUC) was calculated by the trapezoidal rule without extrapolation to infinity. Peak plasma concentration (C_{max}) and the time to achieve this peak (T_{max}) were reported as the mean values from all the rabbits. These values were not read from the average concentration

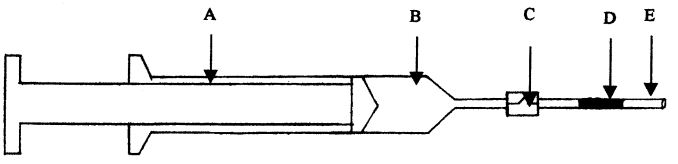

Fig. 1. Schematic diagram of a home-made device used for intranasal drug administration: A, airtight 10-ml syringe; B, compressed air; C, electrically actuated valve; D, powder or solution; E, plastic tip.

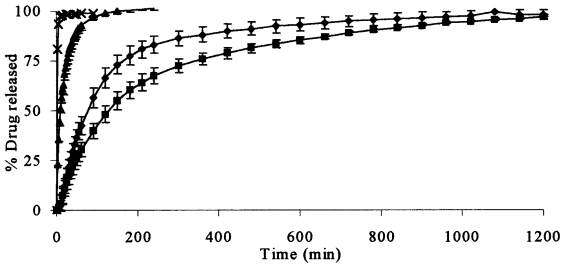

Fig. 2. In vitro release profiles (mean ± S.D., $n = 3$) of apomorphine.HCl from CMC 15% (w/w) (filled squares) and 30% (w/w) (filled diamonds), DSM (filled triangles) and lactose mixture (crosses, error bars not shown for clarity).

curves shown in the figure. The T_{max} together with the $T_{1/2abs}$ (calculated from the absorption rate constant, K_a) and mean residence time of absorption (MRT_{abs}) were used for comparison since they all offer independent criteria to evaluate the in vivo drug absorption rate. The number of data sets was six for in vivo experiments and three for in vitro release studies, and results are reported as mean ± S.E.M. for in vivo and S.D. for in vitro results. Statistical tests of significance were performed with Instat®, version 1.13 (Graphpad Software, San Diego, CA) using one-way ANOVA with multiple comparisons, and differences were considered statistically significant when $P < 0.05$.

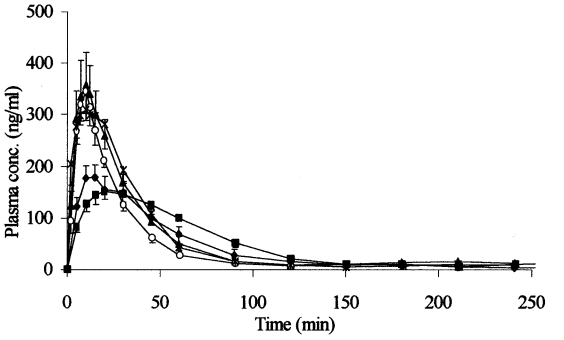

Fig. 3. Mean plasma concentration–time profiles (mean ± S.E.M., $n = 6$) of apomorphine in rabbits after administration of s.c. injection (stars), CMC (15% w/w, filled squares), CMC (30% w/w, filled diamonds), lactose mixture (open circles) and DSM (filled triangles). The time axis was plotted only up to 250 min.

3. Results and discussion

3.1. Drug content and in vitro release of apomorphine.HCl

The actual apomorphine.HCl concentrations in the different formulations were: CMC, 14.15% (w/w) and 28.43% (w/w); DSM, 29.42% (w/w); lactose, 28.1% (w/w); and solution for s.c. injection, 5.82 mg/ml.

The in vitro release profiles of apomorphine.HCl from CMC, DSM and lactose mixture performed with the rotating cups are shown in Fig. 2. Drug release from CMC was sustained in vitro. Drug loading affected the release rate, with 30% (w/w) drug loaded powder releasing more quickly than the 15% (w/w) loaded powder. Sustained release of apomorphine from DSM was not obtained compared to the lactose. The times taken for the release of 50 and 85% of the drug ($t_{50\%}$ and $t_{85\%}$ in min) were 129 ± 19.1 and 440 ± 69.3, respectively for CMC (15% w/w), and 76 ± 13.8 and 212 ± 54.8, respectively for CMC (30% w/w). This was unlike 10 ± 0.6 and 31 ± 3.2, respectively for DSM or the complete release of apomorphine from a lyophilized lactose mixture within 10 min.

3.2. Pharmacokinetics

3.2.1. Time profiles of apomorphine in plasma

The mean plasma drug concentration–time profiles from all the rabbits are shown in Fig. 3, and the mean of salient pharmacokinetic parameters in Table 1. Immediate absorption of apomorphine from all the dosage forms, without lag times, was observed. The initial absorption of apomorphine from both drug loading of CMC was rapid, and only slightly slower compared to lactose or DSM. The shortest $T_{1/2abs}$ was obtained with lactose. The ranked order of the mean $T_{1/2abs}$ was CMC (15% w/w) > CMC (30% w/w) ≅ subcutaneous injection ≅ DSM > lactose. The mean $T_{1/2abs}$ of CMC (15% w/w) was twice that of CMC 30% (w/w) and about fivefold longer than lactose. The difference between the two drug loadings of CMC was however not significant ($P = 0.0661$), as well as between other data pairs

except CMC 15% (w/w) and lactose ($P = 0.0114$). Correspondingly, the MRT_{abs} obtained with lactose was the shortest, and the longest was that of CMC (15% w/w), which was three times higher compared to the former. The ranked order was CMC (15% w/w) > CMC (30% w/w) > DSM > subcutaneous injection ≅ lactose. There were statistically significant differences between the following data pairs of the MRT_{abs}: CMC 15% (w/w) and lactose ($P = 0.0079$), CMC 15% (w/w) and DSM ($P = 0.0346$), CMC 15% (w/w) and subcutaneous injection ($P = 0.0126$), and CMC 15% (w/w) and CMC 30% (w/w) ($P = 0.0493$).

Comparing the in vivo release of apomorphine from the different dosage forms by their T_{max} shows that the peak plasma drug concentration was attained the fastest with lactose and the slowest with CMC (15% w/w), which took about twice as long as that of lactose. The ranked order of the mean T_{max} was CMC (15% w/w) > CMC (30% w/w) > DSM ≅ subcutaneous injection ≅ lactose. There was no significant difference between the two drug loadings of CMC ($P = 0.1917$). There were significant differences between the mean T_{max} from CMC (15% w/w) and the immediate release forms: subcutaneous injection ($P = 0.0111$); lactose ($P = 0.0063$) and DSM ($P = 0.0116$). Complete release of apomorphine occurred quickly as it was detectable (above the detection limit) for up to 4 h and longer in only 50% of the cases of both sustained release CMC dosage forms. With the immediate release forms, 20% of them were detectable for up to 4 h or more. It is noteworthy that while a lot of research effort towards intranasal administration of insulin has been very disappointing, requiring an absorption enhancer to yield only ≈10% bioavailability in man (Moses et al., 1983), equally so is the fact that only very few reports (Ugwoke et al., 1999) have been provided showing significantly improved intranasal T_{max} from mucoadhesive delivery systems compared to intranasal solutions.

There was a rapid nasal absorption of apomorphine from all the formulations. The immediate release preparations, lactose and DSM, showed mean T_{max} values of about 10 min, which were equivalent to that of subcutaneous injections. This also confirms the utility of an intranasal immediate release dosage form in the management of emergency cases in diseases like migraine, epilepsy, diabetes, parkinsonism, etc. The T_{max} after intranasal lactose and DSM were similar. This shows that the DSM has no sustained drug release capability, in spite of its proven mucoadhesive and absorption enhancement capabilities (Illum et al., 1987, 1988; Farraj et al., 1990; Illum et al., 1990; Edman et al., 1992; Vivien et al., 1994), suggesting that a mucoadhesive formulation may not always sustain nasal drug absorption. Given the rapid release of apomorphine from DSM, its utility in nasal drug delivery could further be exploited in administering emergency drugs such as apomorphine, as well as nasal administration of poorly absorbed drugs as already proven with insulin (Farraj et al., 1990; Edman et al., 1992), gentamicin (Illum et al., 1988) and human growth hormone (Illum et al., 1990). On the other hand, since lactose exhibits drug plasma profiles similar to the expensive DSM, it may be

Table 1
Mean ($n = 6$) pharmacokinetic parameters for each formulation and/or route of administration[a]

	S.c. injection	Lactose mixture	DSM	CMC 15%	CMC 30%
AUC (ng/ml × min)	12 680 (855)	10 273 (679)	14 287 (1892)	14 502 (1166)	10 361 (1464)
Rel. bioavail. (%)	100 (0)	82 (7.0)	96 (7.8)	102 (15.6)	82 (9.7)
$T_{1/2elim}$ (min)	18.7 (1.68)	16.7 (2.80)	18.6 (1.70)	18.8 (5.23)	25.2 (3.18)
C_{max} (ng/ml)	352.5 (16.7)	363.1 (33.4)	376.9 (64.3)	165.7 (17.1)	196.4 (25.5)
T_{max} (min)	10.7 (4)	9.5 (0.7)	10.8 (1.7)	21.8 (3.1)	16.8 (1.7)
$T_{1/2abs}$ (min)	4.6 (2.49)	1.8 (0.42)	4.2 (1.45)	9.6 (2.49)	4.4 (1.10)
MRT_{abs} (min)	5.7 (2.39)	4.9 (0.57)	7.4 (1.87)	4.46 (3.27)	8.0 (0.80)
MRT_{disp} (min)	25.6 (4.47)	19.8 (4.13)	20.3 (2.84)	52.4 (11.08)	42.4 (9.86)

[a] S.E.M. in parentheses.

worthwhile investigating the nasal toxicity of lactose for intranasal administrations of drugs that are well absorbed from this site. Its low cost is particularly appealing compared to other powders for nasal administration such as DSM or cyclodextrins.

3.2.2. Extent of absorption

High nasal absorption of apomorphine was observed as shown in Table 1. The C_{max} and AUC of CMC formulations in Table 1 were normalized for an equivalent dose of apomorphine as administered with all the other preparations. The AUC after subcutaneous injection was not statistically different compared to lactose, DSM and CMC (15 and 30% w/w drug loading). There were significant differences between CMC (15% w/w) and CMC (30% w/w) ($P = 0.0356$), and between lactose and DSM ($P = 0.0404$).

Very high plasma drug concentrations were rapidly attained following s.c. injections and intranasal insufflation of lactose and DSM. This was followed by a rapid decline in plasma drug concentrations. However, even though rapid absorption occurred with the CMC formulations, the decline rates in plasma drug concentration were more gradual compared to the rest. In a few cases, plateaus of plasma drug levels were even obtained. The highest C_{max} was obtained from DSM. However, there were no statistically significant differences between the C_{max} of the other immediate release forms. This was different when compared with the sustained release preparations: s.c. injection/CMC (15% w/w) ($P = 0.0043$); s.c. injection/CMC (30% w/w) ($P = 0.0.0115$); lactose/CMC (15%) ($P = 0.0031$); lactose/CMC (30% w/w) ($P = 0.0083$); DSM/CMC (15% w/w) ($P = 0.0020$); DSM/CMC (30% w/w) ($P = 0.0052$). On the other hand, no significant difference exists between the two formulations of CMC ($P = 0.5685$).

4. Conclusions

Previous studies on nasal drug delivery using cellulose derivatives have shed some light on their usefulness. The release of cromolyn sodium was sustained in vitro by carboxymethyl cellulose sodium. This was proposed to be due to factors such as properties of the polymer network, polymer concentration and polymer–drug interaction (Vidgren et al., 1992). In vivo enhancement of nasal insulin absorption was obtained by hydroxypropyl cellulose. Compared to lactose, the nasal absorption was also sustained (Nagai et al., 1984). Our results with CMC conforms with results reported previously with other cellulose polymers.

Plasma profiles following administration of the CMC powders are interesting in a number of ways. First, a rapid initial drug absorption makes it very useful in intranasal administration of drugs where rapid therapeutic effects are desired. Additionally, the plasma drug concentration was sustained, especially with CMC (15% w/w) for which it was maintained within 50% of the C_{max} for about 70 min. This is noticeably better than with lactose (20 min) and s.c. injection (35 min). The clinical relevance of this is that the frequency of drug administration could be reduced by as much as half. For patients requiring up to 10–15 injections per day, this is good news. Also, one should be aware that too long an inhibition of mucociliary clearance would be harmful. Compared to lactose, DSM neither sustained apomorphine release in vitro nor in vivo.

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