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Intranasal administration of melatonin starch microspheres

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Abstract

Using melatonin as model drug, starch microspheres for intranasal administration were prepared by an emulsification-crosslinking technique using a uniform design to optimize preparation conditions. The entrapment ratio of melatonin in the microspheres was 11.0% and particle sizes ranged from 30 to 60 μm. Melatonin was released from the microspheres in a sustained manner in vitro. Nasal clearance of ^{99m}Tc labeled starch microspheres was investigated using gamma scintigraphy. It was revealed that >80% of the starch microspheres could be detected in the nasal tissue 2 h after administration, compared to 30% for a solution. The pharmacokinetics of melatonin starch microspheres was investigated after intranasal administration. The absorption rate was rapid (T_{max} = 7.8 min), and the absolute bioavailability was high, 84.07%. A good correlation was found between in vitro release and in vivo absorption data.

Keywords: Melatonin; Starch microspheres; Intranasal administration

1. Introduction

For systemic therapy, drugs are traditionally administered by oral and parenteral routes. However, in many instances, oral administration is unsuitable when the drug undergoes significant degradation in the gastrointestinal tract or is metabolized to a high degree via the first-pass effect in the liver. In addition, the parenteral route can be undesirable or impractical if a drug is intended for the treatment of chronic diseases. Therefore, an alternative route of administration would be preferred. In recent years, intranasal administration has been extensively evaluated. The nasal dosage forms involved include solutions, sprays, microspheres, gels and liposomes (Mao et al., 1998). Although solutions are easy to use, they achieve a poor bioavailability, due to their short residence time in the nasal mucus. A drug solution is cleared from the nasal cavity into the nasopharynx with an average speed of 6 mm min^{-1}. The average half-life of clearance is found to be 15 min. It had been demonstrated that a significant improvement in bioavailability could be achieved when drugs were administered as bioadhesive microspheres without absorption enhancers (Ryden and Edman, 1992). Clearance half-lives in the order of 4 h (Illum et al., 1987) could also be observed. Starch microspheres are not only biodegradable, but also show a high degree of swelling when in contact with aqueous media. They then form a gel-like system, which prolongs residence

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time in the nose and contact with the nasal mucus significantly. In addition, starch microspheres do not stimulate an albumin-like antigen response in vivo. Starch microspheres are most promising for intranasal administration.

Melatonin (MT), an indole amide neurohormone secreted by the pineal gland in a circadian fashion, has been shown to play a critical role in the synchronization of body rhythms with night and day cycles (Arendt, 1996). In addition, it can be used to improve sleep quality and latency, especially in elderly people (Garfinkel et al., 1995; Zhdanova et al., 1995). The sleep-inducing effects of melatonin have advantages over conventional hypnotics, since it is not a hypnotic drug itself and only induces a state of sleepiness without the adverse side effects of conventional hypnotics. However, its oral bioavailability is below 20%, due to an extensive first-pass hepatic metabolism and variable absorption rates (Lane and Moss, 1985; Lee et al., 1995). Transdermal delivery systems of melatonin, which avoid a first-pass metabolism, have previously been described (Lee et al., 1994; Yates et al., 1996; Benes et al., 1997); however, the lag time and depot-effect limit the usefulness of this route. Buccal delivery has been shown to achieve sufficient systemic drug levels with less variability than the oral or transdermal delivery routes (Benes et al., 1997). Still, other routes of administration, in particular nasal administration, have been considered. However, due to the low solubility of melatonin in water, its delivery presents several problems. In an early attempt to develop a nasal formulation, the administration of 1.7 mg of melatonin in ethanol was described (US Patent, 1998). Due to the serious local irritation and painful administration, this formulation was found to be unsuitable for clinical use. In another recent study, intranasal administration of melatonin with or without sodium glycocholate (GC) was investigated (Bachgaard et al., 1999); the bioavailability was 94 and 55%, respectively.

The purpose of this study was to develop melatonin starch microspheres for intranasal administration, which could be capable of providing a fast onset and controlled released behavior without toxicity. The deposition and subsequent clearance of the drug from the nose was investigated by gamma scintigraphy and the absolute bioavailability calculated.

2. Materials and methods

2.1. Materials

Melatonin was purchased from Zhejiang Melatonin Company Ltd. (Huangyan, China). Soluble cornstarch was purchased from Tianjin Pharmaceutical Excipent Company (Tianjin, China). All other chemicals used were of analytical grade.

2.2. Preparation of starch microspheres

Starch microspheres incorporating melatonin were produced by an emulsification-crosslinking technique. The main factors influencing sphere formation, i.e. starch concentration, water phase pH, amount of cross-linking agent, and cross-linking time, were investigated on the basis of single-factor studies. Subsequently, the four factors mentioned above were divided into seven levels. A U_7 (7^6) uniform design table was used to coordinate the experiment. Microsphere appearance and extent of cohesion were regarded as the appraising standard and the results were optimized using a uniform-design data-processing program. The experimental method has been published elsewhere in detail (Mao et al., 2003). Briefly, a 14% soluble starch solution in phosphate buffer of pH 8.0 was heated in a water bath to approximately 62 °C (water phase). Thirty milliliter of cyclohexane was mixed with 1.5 ml Span-80 under magnetic stirring for 5–10 min (oil phase). Subsequently, 2 ml of the water phase was added drop-wise to the oil phase under magnetic stirring, leading to the immediate formation of microspheres. After 10 min, 1 ml of 3% w/v terephthaloyl chloride solution was added drop-wise to solidify the microspheres and the solution was stirred for another 5 h at room temperature. The microspheres were eventually isolated by centrifugation, washed with ethanol, filtered through a Millipore filter, washed again with ethanol and frozen overnight. The entrapment ratio of melatonin in the starch microspheres was calculated to be 11.01%.

2.3. Characterization of the starch microspheres

Microsphere size was determined by light microscopy. Particle morphology was examined by scanning electron microscopy (Amray-1000b, Peking).

2.4. Evaluation of in vitro melatonin release

Release of the drug was carried out using the small beaker method, as described in the Chinese Pharmacopoeia 2000, at 37 °C and 100 rpm min^{-1}. Phosphate buffer (pH 7.4) (0.1 l) was used as the dissolution medium. A dynamic dialysis technique was employed. Fifty milligram of MT-SMS was dispersed in a pretreated dialysis bag with 5 ml phosphate buffer pH 7.4. The dialysis bag was sealed and suspended in 95 ml phosphate buffer solution in a dissolution beaker. Samples of 5 ml were removed at selected time intervals, filtered through a Millipore filter (pore size 0.8 μm), and assayed spectrophotometrically at a wavelength of 278 nm for drug content according to the method reported previously (Chen et al., 2000). Five milliliter buffer was added to the release medium immediately after sampling. All experiments were performed in quadruplicate. The cumulative mass of drug released was calculated according to the following equation:

$$Q = \frac{C \times V \times D}{W_s \times F \times 10}$$

where C (μg ml^{-1}) is drug concentration, V (ml) is medium volume, D is dilution, W_s (mg) is the weight of the sample, F (%) is entrapment ratio.

The release of the pure melatonin was determined with the same method.

2.5. Nasal clearance of MT-SMS in rabbit

2.5.1. SMS ^{99m}Tc labeling

Four-hundred milligram of SMS was suspended in 4 ml saline. 1 ml 5 mg ml^{-1} $SnCL_2{\cdot}2H_2O$ (in 1 mol l^{-1} HCL), 4 ml 100 mg ml^{-1} NaAc, and technetium-99m pertechnetate eluate containing approximately 370 MBq of activity were added and mixed. The suspension was washed with saline three times to eliminate unlabeled pertechnetate eluate. Subsequently, the microspheres were freeze-dried overnight. The Tc labeling efficiency was calculated to be 86.20%.

2.5.2. Starch solution ^{99m}Tc labeling

Twenty milligram of soluble starch was dissolved in 2 ml saline solution. 0.5 ml 5 mg ml^{-1} $SnCL_2{\cdot}2H_2O$ (in 1 mol l^{-1} HCL), 2 ml 100 mg ml^{-1} NaAc, and technetium-99m pertechnetate eluate containing about 740 MBq of activity were added and mixed. This solution was regarded as a control.

2.5.3. Nasal clearance studies

Six healthy, male rabbits weighing between 1.5 and 2.5 kg were selected for the clearance studies. All of them were anesthetized by intra-abdominal injection of sodium pentobarbital at a dose of 25 mg kg^{-1}. First of all, a 3.7 MBq ^{99m}Tc soluble starch solution was placed in a microinjector equipped with soft polyethylene tube. The tube was carefully inserted 6 mm into the nasal cavity and the solution was administered. The 3.7 MBq ^{99m}Tc SMS were administrated as a spray. Immediately after administration the animals were laid on their backs under the monitor of Sophy DSX-NXT Spect system. The deposition and subsequent clearance of different nasal delivery systems were followed by gamma scintigraphy. Regions of interest were drawn around the site of deposition and the total activity in this area was characterized.

2.5.4. In vivo studies

Twelve healthy rabbits weighing between 1.5 and 2.5 kg were divided into three groups. A melatonin solution was administered both intravenously and intranasally, while the melatonin starch microspheres were administered intranasally as a spray. The administered dose was 0.1 mg kg^{-1}. At selected time intervals, 2 ml blood samples were withdrawn from the marginal ear vein into 10% EDTA-2Na pretreated tubes. Plasma was obtained by centrifugation at 3000 rpm for 5 min and stored at −80 °C until analysis. The plasma samples were analyzed by HPLC (LC-6A, Shimadzu, Japan) according to the method previously reported by our group (Chen et al., 2000).

3. Results and discussion

3.1. Physicochemical characterization of the microspheres

In previous studies, Illum (Illum et al., 1987) found that particle size was related to intranasal drug absorption. Particles below 10 μm were carried into the tracheobronchial region with the air stream, whereas larger particles deposited mainly in the anterior unciliated portion of the nose. A size range of 40–60 μm

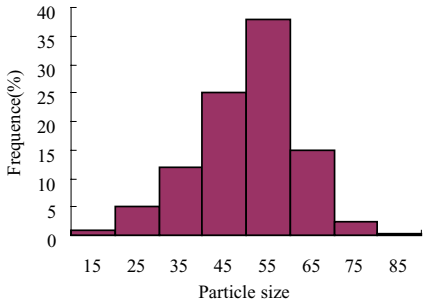

Fig. 1. Particle size distribution of melatonin starch microspheres.

was suitable for intranasal administration. The size of the starch microspheres prepared in this study was in the range of 30–60 μm (Fig. 1), which is favorable for intranasal absorption. The microspheres were of spherical shape and their surface was smooth with some visible cracks (Fig. 2).

3.2. In vitro release

The release profiles are showed in Fig. 3. The $t_{1/2}$ of melatonin was 5.6 min and 12.3 min for MT-SMS. The release profile of MT-SMS can be described by a first-order kinetic equation:

$$\ln(100 - P) = 4.1029 - 0.01548t, \quad r = 0.9954.$$

It was observed that melatonin is released from the microspheres in a sustained manner, which is a desirable property for formulations treating conditions involving an inability to remain asleep.

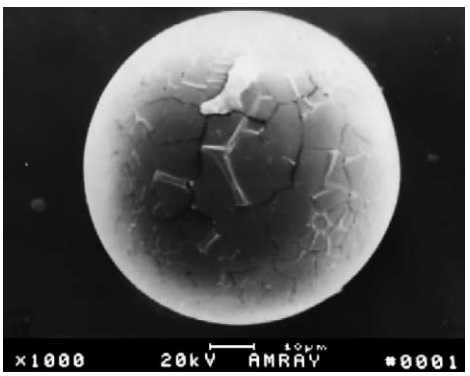

Fig. 2. Morphology of melatonin starch microspheres by SEM (240×).

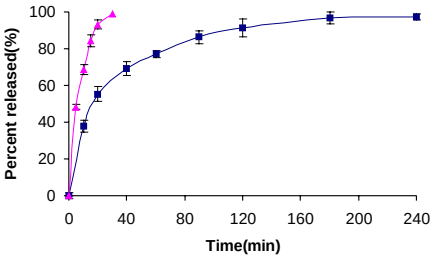

Fig. 3. In vitro release profiles of melatonin in phosphate buffer pH 7.4 (37 °C): melatonin(▲), melatonin starch microspheres(■).

3.3. Nasal clearance studies

There are many methods to measure drug clearance rates from nasal mucosa. Generally, they can be divided into three groups according to the detected substance. The first method involves labeling the substance with a radioactive nuclide (Harris et al., 1986; Illum et al., 1987), with which the deposition and subsequent clearance of the drug can be followed by gamma scintigraphy. The second is to mix a fluorescent dye with the drug and determine the cumulative amount of tracer in the pharynx at selected time intervals (Maureen and Zhou, 1995). The third involves mixing saccharin with the drug (Van de Donk and Van den Heuvel, 1982; Batts and Marriottc, 1991). Drug clearance rate is determined by the sweet taste. Among the three methods, gamma scintigraphy is the most exact, because it can collect photos successively and, thus, reflect drug clearance directly. Furthermore, it can avoid such errors occurring in the second method caused by inaccurate sampling in the pharynx, as well as entrance of the fluorescent dye into the gastrointestinal tract from the pharynx through the esophagus. Gamma scintigraphy can also avoid the subjective error of the third method. Consequently, gamma scintigraphy is employed to study the nasal clearance of melatonin starch microspheres in this paper.

Fig. 4 shows the clearance of the tracer from the nasal cavity after administration of the melatonin starch microspheres and a melatonin solution. It was revealed that >80% of radioactivity from the starch microspheres remained in the nasal mucosa 2 h after administration, as compared to only 30% after application of the solution.

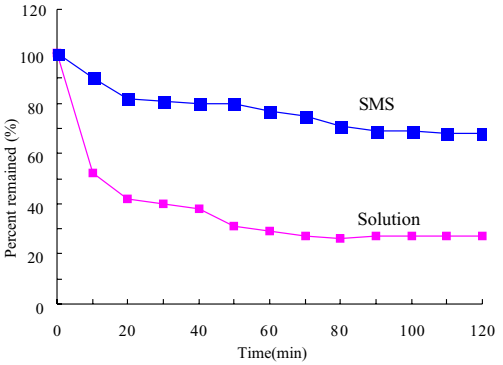

Fig. 4. Clearance of tracer from the rabbit's nasal cavity after administration of melatonin starch microspheres compared to that of a solution.

It is noteworthy that the formulations are cleared from the site of deposition quite rapidly during the first 20 min, after which clearance becomes slower. This can be explained by the drug distribution on the nasal mucosa. Drugs deposited in the ciliary area are cleared quickly, while drugs deposited in the unciliated portions are cleared slowly. The profiles (Fig. 4) reveal that the clearance rate of starch microspheres from the rabbit nose is slower than that of the solution. After 2 h, 80% of the initial activity from the starch microspheres is still at the site of deposition, while only 30% from the control solution remains. The slow clearance of the microspheres can most probably be attributed to the fact that the microspheres undergo a process of water uptake and swelling (its swelling rate was 69.14% when in contact with aqueous medium), thereby forming a mucoadhesive system through hydrogen bonding. The swelling of the spheres is, therefore, critical for the enhancement of drug transport across the nasal mucosa. Compared with gelatin microspheres (Chen et al., 2000), starch microspheres show a much longer deposition time in nose.

3.4. In vivo studies

Plasma concentrations of different times are presented in Table 1. Fig. 5 depicts the corresponding concentration–time profiles.

The data were analyzed using the 3P87 pharmacokinetics program and compartment models based

Table 1
Melatonin plasma concentrations after administration of different dosage forms in rabbits

Dosage form	Plasma concentration (ng ml^{-1})										
	5 min	10 min	20 min	40 min	60 min	90 min	120 min	180 min	270 min	360 min
Intravenous MT	87.22 ± 8.24	58.02 ± 9.31	27.68 ± 6.84	10.41 ± 2.27	5.68 ± 1.63	2.87 ± 0.70	1.21 ± 0.39	0.89 ± 0.31	0.57 ± 0.17	0.29 ± 0.08
Intranasal MT	50.38 ± 6.59	38.84 ± 8.94	21.72 ± 5.26	11.34 ± 3.63	5.88 ± 1.97	3.20 ± 0.46	1.44 ± 0.56	0.84 ± 0.08	0.56 ± 0.07	0.31 ± 0.08
Intranasal SMS	27.84 ± 1.58	34.24 ± 2.36	18.52 ± 1.07	11.72 ± 0.44	9.04 ± 0.54	4.88 ± 0.41	2.96 ± 0.18	2.68 ± 0.23	1.92 ± 0.17	1.08 ± 0.09

($X \pm S$) ($n = 4$).

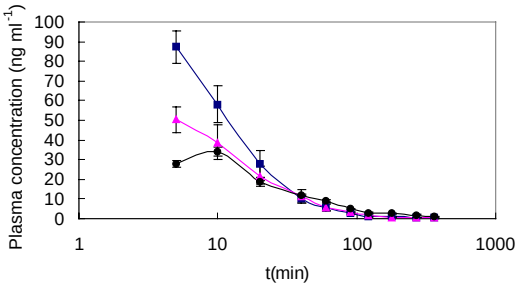

Fig. 5. Mean plasma concentration–time profiles of melatonin after administration of different formulations (0.1 mg kg^{-1}): intravenous solution (■), intranasal solution (▲), intranasal starch microspheres (●).

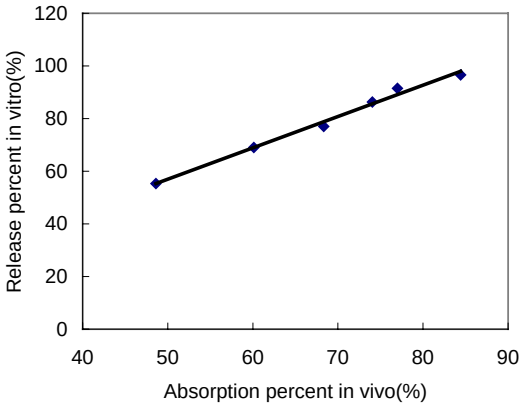

Fig. 6. Relationship between absorption percentages of melatonin starch microspheres in vivo (*f*) and release percentages in vitro (*F*).

on AIC variance and *F*-values. It has been reported that a two-compartment model better describes the in vivo process than a one-compartment model (Bachgaard et al., 1999). Consequently, the pharmacokinetic parameters were calculated according to a two-compartment model. The mean retention time (MRT) of the drug was calculated using the statistical square method, whereas the area under concentration–time curve (AUC) was obtained using the trapezoidal rule. The absolute bioavailabilities of the two dosage forms were also calculated, as presented in Table 2.

3.5. Correlation between in vivo and in vitro

The Loo–Riegelman method was employed to calculate the absorption percentage of melatonin SMS in vivo (*f*) according to the plasma concentrations at different times (Table 1). A relatively good correlation exists between *f* and drug release percentage in vitro (*F*), as shown in Fig. 6. The regression equation is as follows: $F = 1.1919 - 2.6407$, $r = 0.995$. Statistical significance is calculated according to covariance analysis, $F = 314.386$, $P < 0.01$, indicating that *f* and *F* correlate significantly at every time point.

4. Conclusions

Melatonin starch microspheres for intranasal administration were prepared by an emulsification-crosslinking technique. Particle sizes were in the range of 30–60 μm, which is optimal for intranasal administration. In vitro release experiments showed that melatonin was released from the microspheres in a sustained manner; $t_{1/2}$ of the pure melatonin was 5.6 min, whereas a value of 12.3 min was observed for melatonin starch microspheres. Nasal clearance studies showed that >80% of the radioactivity from the starch microspheres was present in the nasal mucosa 2 h after administration, compared to only 30% radioactivity from the solution. The absolute bioavailability of melatonin starch microspheres after intranasal administration was 84.07%. A significant correlation between in vivo and in vitro was disclosed.

Table 2
Pharmacokinetic parameters of different dosage forms of melatonin in rabbits

Formulations	T_{max} (min)	C_{max} (ng ml^{-1})	$AUC_{0\rightarrow\infty}$ (ng min ml^{-1})	MRT (min)	*F* (%)
Intravenous	–	–	2203	45.41	–
Intranasal	4.70	50.10	1536	64.91	69.72
Intranasal microspheres	7.80	32.16	1852	150.58	84.07

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