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Intranasal absorption of melatonin in vivo bioavailability study

Erik Bechgaard *, Karsten Lindhardt, Lise Martinsen

The Royal Danish School of Pharmacy, Department of Pharmaceutics, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark

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Abstract

The bioavailability of melatonin in rabbits after nasal administration of two formulations has been studied. In each case, a total amount of 1.5 mg melatonin in 50 μl was administered and compared with 1.5 mg i.v. The test solutions were formulated with 40% polyethylene glycol 300 (PEG 300), one with 1% sodium glycocholate (+ GC) and one without (− GC). The bioavailability for + GC was 94% (S.D. ± 29%, $n = 4$), whereas the bioavailability for − GC was 55% (S.D. ± 17%, $n = 6$). These results indicate that GC has an enhancer effect ($P < 0.05$). However, the relatively high bioavailability without GC shows that it might not be necessary to use an enhancer for a clinical relevant formulation. The pharmacokinetics of melatonin could be described by a one-compartment model, and the serum half-life was about 13 min. The absorption rate for both formulations was very fast ($t_{max} = 5$ min) and C_{max} mean was 493 ± 290 ng/ml ($n = 4$) and 249 ± 125 ng/ml ($n = 6$) for + GC and − GC, respectively.

Keywords: Bioavailability; Nasal; Melatonin; Rabbit; Sodium glycocholate; Polyethylene glycol 300.

1. Introduction

Melatonin, a hormone secreted by the pineal gland, seems to play a critical role in the synchronisation of body rhythms with the environments' night and day cycle. Several studies have shown melatonin to relieve passengers' subjective feelings of jet lag and tiredness after long-haul flights (Arendt, 1986, 1988, 1996; Petrie et al., 1993). Additionally, it is shown to improve sleep quality and latency especially in the elderly, without affecting REM sleep (Garfinkel et al., 1995; Zhdanova et al., 1995). A controlled study (Vollrath et al., 1981) has shown that nasal administration of 1.7 mg melatonin induced sleep. However, no kinetic plasma data were registered, and the vehicle used (ethanol) can be questioned with respect to local toxicity and irritation.

The current knowledge of melatonin pharmacokinetics in humans is limited. Lane and Moss (1985) estimated a low oral bioavailability, typi-

* Corresponding author. Tel.: + 45-35-370-850; fax: + 45-35-371-277.

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cally below 10%, due to huge first-pass hepatic metabolism. This estimate is based on already existing plasma data after oral dosing of 2.5 mg ($n = 1$), 80 mg ($n = 5$) and 100 mg ($n = 1$) (Wetterberg, 1978; Matthews et al., 1981; Waldhauser et al., 1984) and intravenous data (10 μg) from Iguchi and Ibayashi (1982). Lee et al. (1995) estimated a bioavailability lower than 20% after oral dosing of a 0.5 mg controlled release formulation.

Recent studies performed in Beagle dogs given an oral dose of 0.98 mg/kg resulted in a bioavailability of 16.9% (Yeleswaram et al., 1997). However, at extreme doses of 10 mg/kg, the bioavailabilities of both Beagle dogs and Cyno monkeys were about 100%, which indicate that the metabolism of melatonin is a saturable process. The relevance of such high doses is questionable, because the maximal effect of melatonin is likely to be found at much lower doses.

The knowledge of melatonin dosing, however, is still very limited, especially concerning oral application. Up to 1.2 g melatonin has been dosed to humans orally (Vollrath et al., 1981), but Zhdanova et al. (1995) have shown that even an oral dose of 0.3 mg has a significant effect. Martindale (1996) recommends an oral dose of 5 mg based on Petrie et al. (1993). As a nasal dose of 1.7 mg was found to be effective in humans (Vollrath et al., 1981) and as a clinical relevant formulation was preferred for the experiment, a dose of 1.5 mg was applied for the rabbits. Additionally, a high mg/kg dose allows a more precise analysis of the blood levels. Furthermore, the solubility is very critical for intranasal application, since one clinical dose needs to be dissolved in a volume not exceeding approximately 150–300 μl for administration into two nostrils (Bechgaard et al., 1997a). However, a practical volume is 50 μl, thus a concentration of 30 mg/ml would be convenient. As the solubility of melatonin in water is only 2 mg/ml, an addition of 40% polyethylene glycol 300 (PEG 300) was necessary. Forty percent PEG 300 used for nasal formulations was studied on Caco-2 cells, and in defiance of hypertonicity, no toxicity was found (unpublished data). Furthermore, low intranasal irritation was observed for PEG 300 in humans (Bechgaard et al., 1998a), and low toxicity was found in rabbits after 14 days of repeated nasal application three times a day of pure PEG 200 (Hjortkjær et al., 1999).

Based on the expected low and variable bioavailability after oral administration of melatonin, an alternative route of administration, like intranasal, would be of interest. Using nasal administration, first-pass hepatic metabolism is avoided and absorption into the systemic circulation can occur rapidly (Bechgaard et al., 1997a). The nasal mucosa also seems to have less proteolytic activity than the gastrointestinal tract (Zhou and Po, 1990). Sodium glycocholate (GC) is an enhancer with relatively low toxicity (Gizurarzon et al., 1990) and good enhancing properties (Pontiroli et al., 1989); consequently, this enhancer is chosen.

The purpose of the present study was to estimate the nasal bioavailability of melatonin, when administered from a nasal formulation with possible clinical relevance (40% PEG 300) and, furthermore, to study the benefit of combining this vehicle with a well known absorption enhancer (1% GC).

2. Materials and methods

2.1. Chemicals

Melatonin for both i.v. and nasal administration was from Bachem (Bubendorf, Switzerland). Demineralized water was used throughout the experiment. Sterile sodium chloride solution 0.9% was obtained from Nycomed DAK (Roskilde, Denmark). PEG 300 was obtained from Union Carbide Chemicals and Plastics Company (Danbury, USA). Sodium glycocholate (approximately 99%) was purchased from Sigma Chemicals (St. Louis, USA).

2.2. Preparations

A stock solution of melatonin in PEG 300 (30 mg/ml), making it hypertonic, was prepared and shaken overnight, because of a low solubility rate. This solution was diluted in sterile isotonic sodium chloride to a final concentration of 3

mg/ml (10% PEG 300) prior to i.v. administration. For the nasal administration, a stock solution of melatonin in PEG 300 (75 mg/ml) was prepared and diluted with demineralized water to a final concentration of 30 mg/ml (40% PEG 300) just before nasal administration. A formulation of 30 mg/ml melatonin in 40% v/v PEG containing 1% GC was also prepared.

2.3. In vivo study

Six male New Zealand White rabbits, obtained from Hvidesten (Allerød, Denmark) with a mean weight of about 3.6 kg, were used in a modified cross-over design ($n = 6$) with a wash-out period of 7 days. In the first leg, all rabbits had melatonin administered nasally, three in the absence of enhancers and three with enhancer. In the second leg, all six rabbits were given melatonin i.v. In the third and final leg, which consisted of only a nasal administration of melatonin, the six rabbits were crossed in relation to the first leg. The i.v. test was carried out between the two nasal administrations. Melatonin solution (0.5 ml) was administered intravenously in the marginal ear vein over a period of 30 s. All nasal preparations were administered with the rabbit in a supine position; the rabbit was kept in this position for 1 min after administration. Nasal solution (50 μl) was administered with a Eppendorf Multipipette; into the left nostril in the first leg and in the third leg into the right nostril.

Blood samples of 1.5 ml withdrawn from the marginal ear vein were collected in microcentrifuge tubes just before administration and 5, 10, 15, 30, 45 and 60 min after administration of melatonin. Serum was obtained after centrifugation at $3300 \times g$ and 4°C for 20 min and stored at -20°C until analysis.

2.4. High-performance liquid chromatography analysis of melatonin

The concentration of melatonin in rabbit serum was determined by high-performance liquid chromatography (HPLC) analyses with on-line column enrichment (Bechgaard et al., 1997a,b). Prior to injection, the serum was diluted 1:1 with water. Before each serum injection, the enrichment column was washed with 2×1 ml 95% methanol in water followed by 4×1 ml water. Next, 100 μl of sample was applied followed by 2×1 ml water and 1 ml 20% methanol in water as described by Bechgaard et al. (1998b). The concentration of melatonin in serum (5–1500 ng/ml for i.v and i.n.) was calculated from a standard curve carried out every day of sample analysing.

2.5. Calculation

The area under the concentration–time curve from 0 to 60 min ($AUC_{0-60\ min}$) was calculated using the trapezoidal rule (the serum concentrations were corrected with respect to body weight). AUC from 0 to 5 min for i.v. administration was determined by extrapolation to zero using linear regression analysis on the initial two concentrations. On average, $AUC_{0-5\ min}$ accounted for 30% of the $AUC_{0-60\ min}$ (range 21.5–36.0%).

Plasma concentrations are corrected for differences in body weight during the test period by a factor $f = W/W_{mean}$, where W is the body weight of the individual rabbit and W_{mean} is average body weight of the rabbits ($n = 6$). Student's t-tests were used for statistical calculation of bioavailability and C_{max}.

2.6. Results and discussion

The i.v. data describes a one-compartment model with a half-life ($t_{1/2}$) of 13 ± 2 min. The serum half-life, after nasal application, is similar ($t_{1/2} = 14 \pm 3$ min) for both formulations. In comparison, $t_{1/2}$ in humans is found to be approximately 30 min (Mallo et al., 1990). As seen from Fig. 1 and Table 1, the bioavailability after nasal administration of melatonin is 55% (S.D. $\pm$ 17 %, $n = 6$) for $-$GC and 94% (S.D. $\pm$ 29%, $n = 4$) for $+$GC. The relatively high bioavailability without enhancer was expected due to the high lipofilicity and small size of melatonin. However, these results indicate that GC has ($P < 0{,}05$) a significant enhancing effect on melatonin absorption. Unfortunately, two $+$GC rabbits had to be excluded from the calculations due to sneezing after nasal application. However, the relatively high

Table 1
Mean time (t_{max}) to maximal serum concentration (C_{max}, weight corrected), area under the curve (AUC) from 0 to 60 min and bioavailability of intranasal melatonin formulations (−GC and +GC) after administration of 1.5 mg melatonin (results expressed as mean ± S.D.)

Formulation	t_{max} (min)	C_{max} (ng/ml)	$AUC_{0-60\ min}$	Bioavailability (%)
Intravenous	5	478 ± 105	9228 ± 916	
−GC ($n=6$)	5	249 ± 125	4834 ± 2004	55 ± 17*
+GC ($n=4$)[a]	5	493 ± 290	7637 ± 2581	94 ± 29*

[a] Two rabbits were excluded from the calculations due to sneezing after nasal application.
* Significant difference ($P<0.05$).

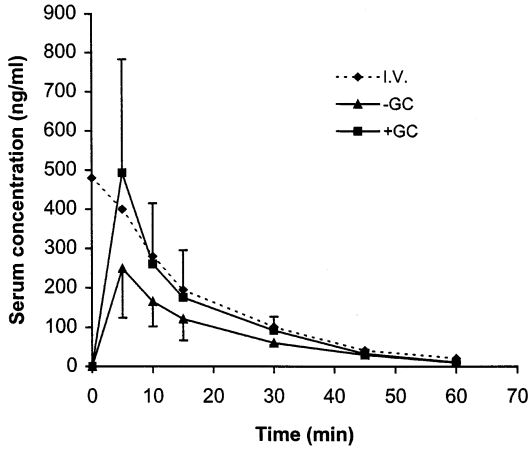

Fig. 1. Mean serum concentration–time profiles of melatonin after intranasal administration of 1.5 mg melatonin in 40% PEG 300 without (▲) and with (■) 1% sodium glycocholate. The i.v. curve is represented by the dotted line with symbol (●).

bioavailability without GC shows that it might not be necessary to use an enhancer (with an additional irritating potential) for a clinical relevant formulation. The absorption rate was very fast ($t_{max}=5$ min) and C_{max} was 493 ± 290 ng/ml ($n=4$) and 249 ± 125 ng/ml ($n=6$) for +GC and −GC, respectively.

The bioavailability for oral administered melatonin is typically given as < 10% for humans (Lane and Moss, 1985). In this study, the bioavailabilities in rabbits show much higher values, which indicate a potential advantage of using nasal delivery for melatonin. The limited knowledge, however, about extrapolation of animal data to humans, as mentioned by Verhoef et al. (1994), should be kept in mind.

The high nasal bioavailability suggests that it is possible to make a clinically relevant nasal formulation. However, an initial study of melatonin intranasally administered to two subjects suggests that 1.5 mg melatonin in itself is moderately irritating after nasal application. Therefore, it may be wise to use a lower dose; for example, 0.3 mg as described by Zhdanova et al. (1995).

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