Uptake of Melatonin into the Cerebrospinal Fluid After Nasal and Intravenous Delivery: Studies in Rats and Comparison with a Human Study

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Purpose. To investigate the possibility of direct transport of melatonin from the nasal cavity into the cerebrospinal fluid (CSF) after nasal administration in rats and to compare the animal results with a human study.

Methods. Rats (n = 8) were given melatonin both intranasally in one nostril (40 μg/rat) and intravenously by bolus injection (40 μg/rat) into the jugular vein using a Vascular Access Port. Just before and after drug administration, blood and CSF samples were taken and analyzed by HPLC.

Results. Melatonin is quickly absorbed in plasma (T_{max} = 2.5 min) and shows a delayed uptake into CSF (T_{max} = 15 min) after nasal administration. The melatonin concentration-time profiles in plasma and CSF are comparable to those after intravenous delivery. The AUC_{CSF}/AUC_{plasma} ratio after nasal delivery (32.7 ± 6.3%) does not differ from the one after intravenous injection (46.0 ± 10.4%), which indicates that melatonin enters the CSF via the blood circulation across the blood-brain barrier. This demonstrates that there is no additional transport via the nose-CSF pathway. These results resemble the outcome of a human study.

Conclusions. The current results in rats show that there is no additional uptake of melatonin in the CSF after nasal delivery compared to intravenous administration. This is in accordance with the results found in humans, indicating that animal experiments could be predictive for the human situation when studying nose-CSF transport.

KEY WORDS: cerebrospinal fluid; human; intranasal; intravenous; melatonin; rat.

INTRODUCTION

The main problem in the development of neuroactive compounds is the passage of these drugs across the blood-brain barrier (BBB). This tight barrier protects the brain from exogenous compounds including drugs (1). Several methods have been investigated to open or manipulate the BBB (2) to enable drugs passing from the blood circulation into the brain. Nevertheless, these methods did not give a satisfying solution to the problem of brain targeting. Circumventing the BBB by targeting via the nose to brain pathway has been suggested as a possible alternative way to reach the brain and the surrounding cerebrospinal fluid (CSF) (3,4).

The neuronal connection between the nasal cavity and the CSF and brain has been extensively investigated on the possibility for brain targeting of drugs. Animal and human studies have been performed providing pharmacokinetic (PK) (5–7) and pharmacodynamic (PD) data (8–13), respectively. In human studies, hormones and peptide drugs were tested, mainly monitoring PD effects. Arginine-vasopressin (9), cholecystokinin-8 (13), adrenocorticotropin (ACTH) 4-10 (10), and insulin (12) increased brain potentials after nasal delivery compared to intravenous administration. Nasal delivery of angiotensin II increased both norepinephrine and vasopressin release, which was opposite to the effects after intravenous administration (11). These effects after nasal angiotensin II administration show similarities with the results after intracerebroventricular delivery in rats (14,15), suggesting that nasal administration of angiotensin II induces a direct central effect.

Animal studies give a PK support for drug targeting via the nose-brain/CSF pathway. The influence of physicochemical factors like molecular weight, ionization degree, and lipophilicity on nose-brain transport has been investigated in animals (5). A large number of animal studies with low-molecular-weight drugs as hydroxyzine (6), dopamine (16), cephalexin (17), anti-HIV agents as D4T (18) and zidovudine (19), metals (20), viruses (21,22), steroid hormones (23), and polypeptides (24,25) claim that the nasal route of drug administration offers direct access to the brain and CSF in animals.

The key question is still whether this direct transport route is really effective or not. To verify the actual feasibility of this novel approach, it is necessary to compare animal studies with human data. In order to extrapolate the results from animals to humans, the studies mentioned above need to be complemented with human PK and animal PD data. This difference in available data between animals and men is due to practical reasons. It is more difficult to sample human CSF than to monitor PD effects in human subjects, whereas the contrary holds for animal studies. A recent *Neurology* paper describes for the first time the uptake of two model compounds in blood and CSF after nasal and intravenous delivery in the same human being. In neurosurgery patients with a CSF drain, it was possible to investigate the nose-CSF pathway of the low-molecular-weight and lipophilic substance melatonin and the high-molecular-weight and hydrophilic molecule hydroxocobalamin, both serving as model compounds (26). Due to the strict inclusion and exclusion criteria, only three subjects could be investigated. In order to substantiate the results of this human study, in the current paper the same melatonin formulation was investigated in rats (n = 8) using a comparable experimental setup. Furthermore, such a comparison can provide a basis for extrapolating the results of nose-CSF studies from animals to men.

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ABBREVIATIONS: $AUC_{CSF, in}$, area under the melatonin concentration-time curve in CSF after nasal delivery; $AUC_{CSF, iv}$, area under the melatonin concentration-time curve in CSF after intravenous injection; $AUC_{plasma, in}$, area under the melatonin concentration-time curve in plasma after nasal delivery; $AUC_{plasma, iv}$, area under the melatonin concentration-time curve in plasma after intravenous injection; C_{max}, maximal concentration; HPLC, high-performance liquid chromatography; PD, pharmacodynamic(s); PK, pharmacokinetic(s); T_{max}, time to reach the maximal concentration.

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MATERIALS AND METHODS

Materials

Melatonin [logP = 1.2 (27)] was from Biosynth AG (Staad, Switserland), povidone iodine from Sigma Chemical (St. Louis, MO, USA), and β-cyclodextrin from Wacker-Chemie (Krommenie, The Netherlands). Ethanol (96%) of analytical grade was from Merck (Darmstadt, Germany). Sterile saline (0.9% NaCl) and heparin (400 IU/ml) were obtained from the Hospital Pharmacy of Leiden University Medical Center (Leiden, The Netherlands). Janssen Pharmaceutica (Beerse, Belgium) supplied Hypnorm (fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml). Dormicum (midazolam, 5 mg/ml) was from Genthon B.V. (Nijmegen, The Netherlands). Nembutal (pentobarbital sodium, 60 mg/ml) was purchased from Sanofi Sante Nutrition Animale (Libourne, France) and Temgesic (buprenorphine, 0.3 mg/ml) from Schering-Plough (Maarssen, The Netherlands). Dichloromethane and KH_2PO_4 were from J. Baker (Deventer, The Netherlands), and acetonitrile was from Biosolve LTD (Valkenswaard, The Netherlands). All other reagents were of analytical grade.

Melatonin Formulations

The melatonin formulation for nasal delivery consisted of melatonin (2.0 mg/ml) and β-cyclodextrin (7.5 mg/ml) dissolved in saline (28). This formulation also contained benzalkonium chloride (0.01% w/v) and EDTA (0.1% w/v) as preservatives. A 10-fold lower concentration was used for intravenous bolus injection.

Animals

Male Wistar rats (Charles River, Someren, The Netherlands) were used, weighing 330–465 g at the start of the experiments. The animals (n = 8) were housed (2 per cage) with free access to food and water with a 12-h light/dark cycle. At the end of the experiments, the animals were euthanized with an overdose of Nembutal (1–2 ml, intraperitoneally). All animal experiments were approved by the Ethical Committee for Animal Experiments (Leiden University).

Nasal and Intravenous Delivery of Melatonin

Prior to drug administration, rats were anaesthetized with Hypnorm (0.5 ml/kg) and Dormicum (0.5 ml/kg) intramuscularly and fixed in a stereotaxic frame (model 51600, Stoelting, Wood Dale, IL, USA) using the supine-70° angle position (29). For intranasal administration of the melatonin formulation, a polyvinylchloride (PVC) tube (ID 0.5 mm, OD 1.0 mm) attached to a Hamilton syringe was inserted into the left nostril of the rat for about 2 cm. The nasal melatonin dose (40 μg/20 μl/rat) was delivered by gently pushing the plunger of the syringe. After delivery of the formulation, the PVC tube was removed.

For the intravenous bolus injection, the rats were provided with a Vascular Access Port (VAP) as described before (30). The intravenous melatonin formulation (40 μg melatonin/200 μl/rat) was administered using a 1-ml syringe attached to a Huberpoint needle. Subsequently, the VAP was rinsed with 500 μl saline to make sure that the entire formulation had entered the blood stream.

Prior to and following melatonin delivery, blood and CSF samples were taken until 120 min after administration. Each rat received both the nasal and intravenous treatment. Between experiments the animals were allowed to recover for one week.

Blood and CSF Sampling

Blood samples (200 μl) were taken from the tail vein using heparinized tubes (Microvette CB 100/200, Sarstedt, Nümbrecht, Germany), and the samples were stored at 4°C until analysis.

For CSF sampling, a cisternal puncture was performed as described before (29). Briefly, rats were anesthetized and fixed in a stereotaxic frame as mentioned above. The cisternal puncture was performed 5.2–6.5 mm ventrally from the occipital crest, depending on the rat's weight. After the puncture, one drop of CSF was microscopically examined on erythrocyte contents; the experiment was continued when the erythrocyte contamination was less than 500 cells/μl (<0.01% of normal blood content). Following intranasal or intravenous drug administration, CSF samples (about 30 μl) were taken and directly collected in pre-weighed HPLC vials, and the volume was added up to 180 μl with Millipore water. All samples were analyzed the same day.

Melatonin Analysis

Blood samples were pretreated as follows. Blood samples were centrifuged (15 min at 14,000 rpm; ambient temperature) and the obtained plasma (100 μl) was extracted with dichloromethane (2 ml) by shaking at 1000 rpm (Vibrax, type VXR; Fisher Scientific, Hertogenbosch, The Netherlands) for 10 min. The two-phase system was centrifuged (5 min at 3000 rpm; ambient temperature), and the organic phase was pipetted into other tubes. Then dichloromethane was evaporated under a mild nitrogen stream at 35°C, and the residue was dissolved in 250 μl mobile phase [10 mM KH_2PO_4 (pH 3.0): acetonitrile = 73: 27]. Plasma and CSF samples were analyzed on melatonin as previously described (31). Briefly, samples were analyzed by isocratic HPLC consisting of a Jasco PU-980 pump (Jasco, B&L Systems, Zoetermeer, The Netherlands), a chromspher C_{18} column (100 × 3.0 mm) with 5-μm-sized particles (Varian BV, Houten, The Netherlands) using a flow of 1.0 ml/min and fluorescence detection (λ_{ex} = 224 nm, λ_{em} = 348 nm; Jasco 821, B&L Systems) with a detection limit of 8 pg/ml.

Data Analysis

The area under the concentration-time curve (AUC) values (0–120 min) were calculated using the trapezoidal rule. The CSF ratio was determined according to Eq. 1. This ratio is a measure for CSF uptake after nasal delivery related to the uptake after intravenous administration (26). All AUC values and CSF ratios were calculated per individual animal before determining the mean value. Data were analyzed according to the paired Student's *t* test, using the computer program SPSS version 8.0 for Windows.

$$\text{CSF ratio} = \frac{AUC_{CSF,in}}{AUC_{plasma,in}} \Big/ \frac{AUC_{CSF,iv}}{AUC_{plasma,iv}} \qquad (1)$$

RESULTS

In eight rats, melatonin (40 μg/rat) was administered intranasally and subsequently intravenously. Following intranasal administration, the plasma C_{max} for melatonin was observed in the first sample taken after delivery (t = 2.5 min) which was similar after intravenous bolus injection. Both routes showed comparable plasma concentration-time profiles of melatonin (Fig. 1). The uptake of melatonin into the CSF was delayed for about 10–15 min compared to the absorption in plasma after intranasal and intravenous delivery (for both routes: T_{max} = 15 min; Fig. 1). In CSF, the uptake phase was similar for the intranasal and the intravenous route of administration, reaching mean C_{max} values of 18 ng/ml. This value was 3.5- to 5-fold lower than the C_{max} found in plasma (64 ± 37 and 87 ± 30 ng/ml after intravenous and intranasal administration, respectively; Fig. 1).

Table I gives an overview of the AUC values in plasma and CSF after intranasal and intravenous melatonin delivery, the AUC_{CSF}/AUC_{plasma} ratios, and the CSF ratio. The calculated CSF ratio (0.76 ± 0.31) shows that the relative uptake of melatonin into the CSF after nasal delivery is not significantly different from the uptake after intravenous injection. This ratio is smaller than 1, which indicates that there is no additional transport from the nasal cavity into the CSF. The CSF ratio found in rats is similar to that obtained in humans, as is also shown in Table I.

DISCUSSION

The current study demonstrates that nasal delivery of melatonin in rats does not result in additional uptake of this lipophilic/low-molecular-weight drug (MW = 232 g/mol) into the CSF via the nose-CSF pathway compared to intravenous administration. This is in contrast to some earlier reported rat studies with low-molecular-weight lipophilic and hydrophilic compounds (16,17,23). In these studies, the drug concentrations in CSF after intranasal and intravenous delivery were determined at 1–2 time points only, which gives limited information about the CSF uptake of a drug and may therefore be misleading. Possible discarding of CSF samples contaminated with blood was also not reported. Blood contamination in CSF may lead to false-positive conclusions. Nevertheless, in a previous study from our laboratory, another lipophilic and low-molecular-weight drug, hydrocortisone, was evaluated for nose-CSF transport in rats (30). When comparing the AUC_{CSF}/AUC_{plasma} ratios after intranasal and intravenous delivery for this steroid hormone, no direct nose-CSF transport was observed. These findings are supported by studies with other lipophilic drugs such as the serotonin antagonist (S)-UH-301 (7), a cognition enhancer (32), and the antihistamine triprolidine (6). A lack of direct nose-CSF transport was also reported for the hydrophilic vitamin B_{12} analog hydroxocobalamin, which was studied in the same rat model as described here (33).

The current rat studies show results similar to a human study [Table I; (26)], in which the same melatonin formulation is tested. The administered melatonin dose in rats is relatively high in comparison with the human study on a mg/kg basis: about 20- and 40-fold higher for intranasal and intravenous administration, respectively. If the same dose (mg/kg) for humans would be used for rats, the melatonin concentrations in plasma and particularly in CSF would have been below the limit of detection of the used HPLC assay. Therefore, in the current rat study the same melatonin formulation but at a higher dose (40 μg/rat) was used. Similar to this rat study, all human subjects received two melatonin treatments [intranasally and intravenously (26)], and in both species melatonin is rapidly absorbed in the blood circulation after nasal delivery (T_{max} = 2.5 and 5 min for rats and humans, respectively). The relative uptake of melatonin into the CSF after nasal delivery compared to intravenous administration is comparable in rats and humans, which is evident from the calculated CSF ratios (Table I).

It should be noted that large interspecies differences exist in the anatomy, especially with respect to the shape of the nasal cavity and the relative sizes of the olfactory and respiratory epithelia. In rats, about 50% of the nasal cavity is covered with olfactory epithelium, whereas in humans this is only 8% (34). Therefore, for compounds that are taken up via

Table I. AUC_{CSF}/AUC_{plasma} Ratios and the CSF Ratio of Melatonin

	Intranasal	Intravenous
Rats*		
AUC_{CSF} (ng · min/ml)	774 ± 133	1069 ± 313
AUC_{plasma} (ng · min/ml)	2429 ± 576	2310 ± 400
AUC_{CSF}/AUC_{plasma} (%)	32.7 ± 6.3	46.0 ± 10.4
CSF ratio (Eq. 1)	0.76 ± 0.31	
Humans†		
CSF ratio (Eq. 1)	0.71 ± 0.30	

Data are presented as mean ± SD.
* (n = 8).
† (n = 3) (26).

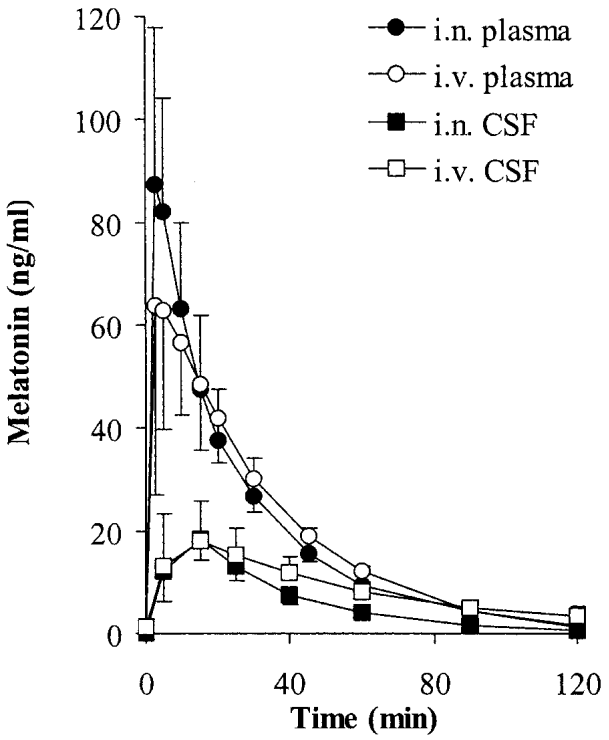

Fig. 1. Plasma and CSF concentrations after intranasal (i.n.) and intravenous (i.v.) delivery of melatonin (40 μg/rat) in rats. Results are expressed as mean ± SD (n = 8).

the olfactory epithelium, a difference in CSF ratio between rats and humans can be expected. Our study shows however no direct or extra transport of melatonin from the nose to the CSF. Obviously, there is no transport via the olfactory area and in both species the observed fast nasal absorption takes place via the respiratory epithelium that is highly vascularized and easily permeable for the low-molecular-weight lipophilic compound melatonin.

In conclusion, no additional transport from the nasal cavity to the CSF is found after intranasal and intravenous administration of melatonin in rats. Furthermore, the results of the current rat studies and the reported human study (26) offer an opportunity to compare animal and human PK data, obtained by using the same drug formulation and a resemblance in experimental methods. Comparison of these two studies demonstrates that for nose-CSF transport of melatonin, rat experiments can be predictive for human studies. To strengthen the basis for extrapolation from animal data to the human situation, more nasal drug formulations need to be investigated in both animals and men.

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