Research Article

Pharmacokinetics and safety of intravenous, intravesical, rectal, transdermal, and vaginal melatonin in healthy female volunteers: a cross-over study

Dennis Zetner[1], Lars Peter Kloster Andersen[2], Rasmus Alder[1], Majken Lyhne Jessen[1], Anders Tolstrup[1], Jacob Rosenberg[1]

1: Center for Perioperative Optimization, Department of Surgery, Herlev Hospital, University of Copenhagen, Denmark

2: Department of Anesthesiology Z, Bispebjerg Hospital, University of Copenhagen, Denmark

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Corresponding Author:

Dennis Zetner

Center for Perioperative Optimization, Department of Surgery

Herlev Hospital, University of Copenhagen

Borgmester Ib Juuls Vej 1

2730 Herlev, Denmark

Tel: +45 2729 1376

E-mail: dennis.zetner@gmail.com

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Abstract

Introduction: We aimed to investigate the pharmacokinetic properties and safety of melatonin administered by different routes of administration.

Methods: The study employed a cross-over design in healthy female volunteers. Twenty-five mg of melatonin was administered intravenously, intravesically, rectally, transdermally, and vaginally. Blood samples were collected at specified time-points up to 24 hours following administration by intravenous, intravesical, rectal, and vaginal routes, and up to 48 hours following transdermal administration. Plasma melatonin concentrations were determined by radioimmunoassay. Sedation was evaluated by a simple reaction-time test, and sleepiness was assessed by the Karolinska Sleepiness Scale. Adverse events were registered for each route of administration.

Results: Ten participants were included. We documented a mean (SD) tmax of 51 (29) min for intravesical, 24 (20) min for rectal, 21 (8) h for transdermal, and 147 (56) min for vaginal administration. Mean (SD) t½ elimination was 47 (6) min for intravenous, 58 (7) min for intravesical, 60 (18) min for rectal, 14.6 (11.1) hours for transdermal, and 129 (17) min for vaginal administration. Mean (SD) bioavailability was 3.6 (1.9)% for intravesical, 36.0 (28.6)% for rectal, 10.0 (5.7)% for transdermal, and 97.8 (31.7)% for vaginal administration. No significant changes in reaction times were observed following administration of melatonin by any of the administration routes. Increased tiredness was documented following transdermal administration only. No serious adverse effects were documented.

Conclusion: This study estimated the pharmacokinetic parameters for the intravenous, intravesical, rectal, transdermal and vaginal administration of melatonin. Melatonin administered by these routes of administration was safe.

Introduction

The pharmacokinetic properties of oral and intravenous melatonin have previously been thoroughly investigated in humans [1, 2]. However, the literature regarding the pharmacokinetics of melatonin administered by alternative routes of administration is limited [3]. Correspondingly, knowledge concerning its safety and possible adverse effects when given by these routes is sparse. Thus, this study aimed to investigate the pharmacokinetic properties and safety of melatonin when administered intravenously, intravesically, rectally, transdermally, and vaginally.

Materials and Methods

The study is reported according to the ClinPK-statement [4]. It was performed in accordance with the Helsinki II declaration, and was approved by the local ethics committee of the Capital Region of Denmark (record no.: H-17036312), the Danish Medicines Agency (EudraCT no.: 2017-000997-13), and the Danish Data Protection Agency (record no.: HGH-2017-104, no. 05981). The study was registered at clinicaltrials.gov (NCT03519750). Oral and written informed consent was obtained from all subjects.

This study employed a cross-over design. Melatonin was administered intravenously, intravesically, rectally, transdermally, and vaginally to all participants with a wash-out period of at least 7 days between each study session. The eligibility criteria are outlined in Table 1.

For intravenous administration, 25 mg of melatonin was dissolved in 2 mL of 99.9% ethanol and 23 mL of 0.9% saline. The melatonin solution was administered during a 10 minutes period (infusion rate 2.5 mL/min). For intravesical administration, 25 mg of melatonin was dissolved in a 50 mL of 50% w/w dimethyl sulfoxide (DMSO) in 0.9% saline solution. Participants were instructed not to urinate within the first hour following instillation. The dose of DMSO was based on Rimso 50, an intravesical formulation approved by the US Food and Drug Administration [5]. The rectal formulation consisted of 25 mg of melatonin dissolved in 2.5 mL of 20% w/w glycofurol, 40% w/w DMSO, and 40% w/w 0.9% saline. The dose of DMSO was based on a previous study applying DMSO rectally in combination with lidocaine [6]. For the application to the skin, 1 g of standard skin lotion containing 25 mg melatonin and 150 mg DMSO was administered to cover a 20x20 cm area on the chest of each participant. The chest area was outlined with a measuring tape. Dose of DMSO was based on DOLOBENE® SportsGel [7], a gel containing heparin and DMSO applied topically for local inflammation, tendinitis and sprains. The vaginal administration consisted of 25 mg of melatonin dissolved in a suppository consisting of 2.2 mL of hard fat produced by IOI Oleochemical (WITEPSOL® H 15) [8]. The intravenous and intravesical formulations were produced by Skanderborg Pharmacy,

Denmark. The rectal, vaginal, and transdermal melatonin formulations were produced by Glostrup Pharmacy, Denmark. All melatonin products were developed and manufactured according to Good Manufacturing Practice standards [9].

Primary outcome

Blood samples were collected at baseline (prior to melatonin administration) and at 0, 10, 20, 30, 40, 50, 60 minutes, and 2, 3, 4, 6, 8, and 24 hours following administration of intravenous, intravesical, rectal, and vaginal melatonin. Blood samples were collected at baseline and at 0, 30, and 60 min, and 2, 4, 6, 8, 10, 12, 16, 24, and 48 hours following application of melatonin to the skin.

Blood samples were centrifuged at 3000 rpm and stored at -80˚C until analyses were performed. Radioimmunoassay (RIA) was employed to analyze melatonin plasma concentrations (Melatonin Direct RIA BA R-3300, Labor Diagnostika Nord, Nordhorn, Germany). The characteristics of the assay were as follows: intra-assay coefficient of variation (CV) 9.8-13.4%; interassay CV 8.0-13.3%; limit of detection was 2.3 pg/mL; linearity of the RIA-kit ranged between 8.5 and 529.0 pg/mL. If plasma concentrations exceeded test kit linearity levels, samples were diluted in accordance with the manufacturer's guidelines. Plasma samples were analyzed in duplicate and the mean value was reported.

Secondary outcomes

A simple reaction-time (SRT) test was applied to evaluate the sedative effects of each melatonin formulation by means of an online-based test [10, 11]. Evaluations were performed at baseline and at 1, 2, 3, 4, 5, 6, 7, 8, 24 hours following administration of intravenous, intravesical, rectal and vaginal melatonin formulations. SRT evaluations were performed at baseline and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 24, and 48 hours following application of melatonin to the skin.

The Karolinska Sleepiness Scale (KSS) was employed to evaluate subjective sleepiness. The scale has previously been validated against psychomotor performance and EEG-variables [12]. KSS evaluations were performed at baseline and at 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours following administration of intravenous, intravesical, rectal, and vaginal melatonin. Correspondingly, evaluations were performed at baseline and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 24, and 48 hours following application of melatonin to the skin.

Documentation of adverse events included pre-specified self-reported symptoms of anxiety, confusion, depressed mood, dizziness and headache (yes/no). Garlic breath and localized burning

sensation from the site of administration/application (yes/no) were also included, since DMSO may induce these symptoms [13]. Finally, participants were asked to report additional symptoms of adverse reactions, if any (yes/no + description).

Statistical and pharmacokinetic analyses

Normality of data was assessed by visual inspection of residual plots and histograms. Data are presented as either mean (SD) or median (range) unless stated otherwise. Parametric or non-parametric statistical tests were employed according to data distribution. A p-value below 0.05 was considered statistically significant. Data were analyzed with IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA) and Graph Pad Prism version 7.0 (Graph Pad Software Inc., La Jolla, CA, USA).

Time to maximal concentrations (t_{max}) and maximal plasma concentrations (C_{max}) were assessed directly from relevant time points. Mean (SD) t_{max} was calculated based on the assessed t_{max} of each participant. Mean (SD) C_{max} was calculated from the C_{max} of each participant not from the mean t_{max}. We calculated a mean (SD) t_{max} from the t_{max} assessed from each participant. We estimated individual absorption rate constants (k_a) and elimination rate constants (k_e) by linear regression of ln-transformed melatonin plasma concentrations. Absorption half-life ($t_{½}$ absorption) and elimination half-life ($t_{½}$ elimination) were calculated as: $t_{½}\ absorption = \frac{\ln(2)}{k_a}$ and $t_{½}\ elimination = \frac{\ln(2)}{k_e}$. Areas-under-the-curve (AUC) of plasma concentrations were calculated applying the trapezoidal rule [14]. $AUC_{0\text{-}\infty}$ was estimated as $AUC_{0\text{-}24\ hours}$ + (C_{24} hours/k_e) for the intravenous, intravesical, rectal and vaginal administrations, whereas $AUC_{0\text{-}\infty}$ for the transdermal administration was estimated as $AUC_{0\text{-}48\ hours}$ + (C_{48} hours/k_e). Bioavailability (*f*) was calculated as:

$$f = 100 \times \frac{AUC_{\ 0-\infty\ (rectal,\ \ intravesical,\ \ vaginal\ or\ transdermal)}}{AUC_{\ 0-\infty\ (iv)}}$$

Changes in SRT and KSS were evaluated by comparing baseline values with the time point value obtained at C_{max}. Regarding the intravenous administration, baseline values were compared with the time point value 1 hour following administration.

Results

Demographic data are presented in Table 2. No participants dropped out or were lost to follow-up. Missing data occurred in two participants. Due to a dysfunctional venous access catheter in one

participant, blood samples could not be drawn at 20 and 30 minutes following rectal administration. Hence, we chose to exclude data for this administration route from further analyses in this participant. In addition, the intravenous administration of melatonin failed in one participant. Therefore, data regarding this administration route and related data, such as AUC estimates could not be performed for this participant.

Melatonin plasma concentrations following intravenous administration are presented in Figure 1. Plasma concentrations following intravesical, rectal, transdermal, and vaginal administration are depicted in Figure 2. The pharmacokinetic parameters of the individual administration routes are shown in Table 3. Intravenous melatonin demonstrated first-order elimination, with a $t_{½}$ elimination of approximately 47 min. The intravenous administrations demonstrated a large variation in C_{max} between participants, as well as AUC. Intravesical administration had a t_{max} of 51 minutes and demonstrated a bioavailability of 3.6%. Rectal administration had a $t_{½}$ absorption of 5 min, a $t_{½}$ elimination of 60 min, and demonstrated a bioavailability of 36%. Transdermal administration reached t_{max} between 16 and 24 hours, most participants showing t_{max} at 24 hours, giving a mean t_{max} of 20.5 hours. Further, transdermal administration demonstrated a $t_{½}$ elimination of 14.6 h, and a bioavailability of 10%. Vaginal administration reached t_{max} at 147 min and had a $t_{½}$ elimination of 129 min. The vaginal bioavailability varied extensively, with three participants reaching a bioavailability of over 100%. Mean bioavailability was 97.8%.

Pre- and post-administration SRT scores did not differ for any of the routes of administration ($p > 0.05$). Nor did pre- and post-administration KSS-scores differ in the intravenous, intravesical, rectal or vaginal administration routes ($p > 0.05$). The KSS score following transdermal administration was significantly increased compared to baseline values ($p = 0.028$), see Table 4.

Pre-defined adverse events are displayed in Table 5. In addition to the pre-defined adverse events, one participant reported transient nausea after receiving the intravenous dose of melatonin. No other adverse events were reported.

Discussion/Conclusion

This cross-over study in healthy female volunteers estimated standard pharmacokinetic parameters of melatonin administered intravenously, intravesically, rectally, transdermally, and vaginally. Data relating to intravenous administration of melatonin documented first-order elimination with an

estimated half-life of approximately 47 min. Intravesical administration was characterized by a very limited bioavalability. Rectal administration demonstrated rapid absorption and a moderate bioavailability. Transdermal melatonin displayed an extended but limited absorption. Vaginal administration displayed an extensive bioavailability compared to all other routes of administraion. No serious adverse events were observed. Increased tiredness assessed by KSS was observed only after transdermal administration.

Melatonin has potential widespread clinical actions [15-17]. Optimal drug delivery relating to the specific patient and treatment may prove pivotal to improve clinical effects. Hence, pharmacokinetic properties are needed to describe drug distribution in detail e.g. if local and/or systemic effects can be expected. Correspondingly, safety evaluations are needed for the different administration routes, evaluating both local and systemic harms. Currently, an increasing clinical interest relates to the radioprotective [18-22], anti-oxidative [23, 24] and anti-cancer [25-28] properties of melatonin. Localized radiation therapy may be combined with local melatonin administration regimens, e.g. administered transdermally, rectally, vaginally, or intravesically, thus, increasing local tissue concentration gradients and potentially limiting drug-related systemic adverse effects. However, the clinical impact of this strategy needs to be established in future studies. Moreover, alternative routes of administration, such as rectally or vaginally administered formulations could be employed when fasting, or when gastroparesis or GI-tract dysfunction inhibits oral intake. Both routes exhibit an improved bioavailability and augmented absorption compared with standard oral melatonin [2].

Our data documented no significant differences between pre- and post-administration SRT values in any routes of administration. This is consistent with previous studies documenting maintained psychomotor function following melatonin administration [11]. The KSS score was only significantly higher 24 hours following transdermal administration. Interestingly, increased subjective sleepiness was not observed at any other time point or with other routes of administration. This finding is unexpected due to fact that melatonin is a well-documented hypnotic [29-31]. Following intravesical administration, 8 of 10 participants experienced a local burning sensation related to the urinary bladder region. This mild adverse reaction is in agreement with a previous study employing similar urinary bladder-administered formulations [5]. Correspondingly, halitosis was experienced by

4 and 1 participants following intravesical and rectal administrations, respectively. Halitosis is related to the limited quantity of DMSO being excreted va the lungs as dimethyl sulfide [32].

This study has several strengths. This is the first study to investigate intravesical, rectal, and vaginal administration of melatonin in humans [3]. It is also the first study to estimate bioavailability following transdermal administration of melatonin [3]. We chose to include a sufficient number of participants and measuring points, increasing the generel quality of data. Plasma samples were analyzed according to previous studies [1]. Finally, we adhered to the ClinPK-statement [4], and employed a cross-over design to minimize reporting bias and reduce inter-individual variation.

Our study also has a number of limitations that needs to be adressed. First, a limited number of missing data occurred. In one participant, two blood samples following the rectal administration were missed due to a dysfunctional venous access. In another participant, intravenous melatonin was not administered due to human error. It is, however, unlikely that these missed data would change outcomes significantly. Second, even though plasma concentrations are thoroughly described in this study, local tissue concentrations of melatonin have not been measured. This issue could be adressed in future studies, e.g. by microdialysis techniques. Third, the bioavailability of intravesical melatonin demonstrated very low values. We emphazised the need to avoid urinary voiding the first hour following administraion. It is, however possible that a quantity of melatonin was excreted externally (with the urine) following this period. Fourth, three participants demonstrated a vaginal bioavailability of over 100%. This inaccuracy may result from suboptimal timing of the sampling points, not describing the exact course of plasma concentrations curves, potentially over- or underestimating AUC data. Another reason could be the extended 10 min intravenous bolus of melatonin. It may be speculated that a fraction of melatonin may already have been eliminated during the period of infusion, reducing the estimated AUC of intravenous melatonin. This reduction of the intravenous AUC, would lead to an over-estimation of the bioavailability of the other administration routes. The reasoning, however, for this administration regimen was safety relating to the exstensive intravenous dose of administered melatonin. Fifth, data concerning KSS scores at 24 hours following transdermal administration should be interpreted with care since participants received less than 8 hours of sleep due to the blood sampling frequency. Also, participants slept in a hospital enviroment. Hence, the significant increase of KSS scores at 24 hours post-administraion may also partly be attributed to poor quality of sleep, rather than hypnotic effects of melatonin.

This cross-over study in healthy female volunteers estimated the pharmacokinetic parameters of melatonin administered intravenously, intravesically, rectally, transdermally, and

vaginally. Melatonin administed by alternative routes of administration was safe, and only mild transient adverse effects were observed.

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Statement of Ethics

The study was conducted in accordance with the Helsinki II declaration. Ethical approval was obtained prior to study initiation from the local ethics committee of the Capital Region of Denmark (record no.: H-17036312), oral and written consent was obtained from all articipants.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The study was conceptualized and designed by DZ, LPKA and JR. Data were acquired by DZ, RA, MLJ and AT. Data were analyzed by DZ. The manuscript was drafted by DZ and critically revised by LPKA, RA, MLJ, AT and JR. All authors gave final approval of the manuscript prior to submission and have agreed to be accountable for all aspects of the work.

References

1. Andersen LP, Werner MU, Rosenkilde MM, Harpsoe NG, Fuglsang H, Rosenberg J, et al. Pharmacokinetics of oral and intravenous melatonin in healthy volunteers. BMC Pharmacol Toxicol. 2016;17:8.
2. Harpsoe NG, Andersen LP, Gogenur I, Rosenberg J. Clinical pharmacokinetics of melatonin: a systematic review. Eur J Clin Pharmacol. 2015;71:901-9.
3. Zetner D, Andersen LP, Rosenberg J. Pharmacokinetics of alternative administration routes of melatonin: a systematic review. Drug Res (Stuttg). 2016;66:169-73.
4. Kanji S, Hayes M, Ling A, Shamseer L, Chant C, Edwards DJ, et al. Reporting guidelines for clinical pharmacokinetic studies: the ClinPK Statement. Clin Pharmacokinet. 2015;54:783-95.
5. Rawls WF, Cox L, Rovner ES. Dimethyl sulfoxide (DMSO) as intravesical therapy for interstitial cystitis/bladder pain syndrome: a review. Neurourol Urodyn. 2017;36:1677-84.
6. Kravchick S, Peled R, Ben-Dor D, Dorfman D, Kesari D, Cytron S. Comparison of different local anesthesia techniques during TRUS-guided biopsies: a prospective pilot study. Urology. 2005;65:109-13.
7. Grovet. Dolobene Sport-Gel 100 mg. [Cited 2020 Mar 24] Available from: https://www.grovet.com/dolobene-gel-100-g.html.
8. IOI Oleochemical. WITEPSOL® H 15. [Cited 2020 Mar 24] Available from: https://www.ioioleo.de/en/products/pharma/witepsol-h-15.php.
9. Eurpoean Medicines Agency - EMA. Good Manufacturing Practice (GMP) guidelines 2020. [Cited 2020 Mar 9] Available from: https://ec.europa.eu/health/documents/eudralex/vol-4_en.
10. Allen J. The online reaction time test 2002. [Cited 2020 Mar 3] Available from: http://getyourwebsitehere.com/jswb/rttest01.html.
11. Andersen LP, Werner MU, Rosenkilde MM, Fenger AQ, Petersen MC, Rosenberg J, et al. Pharmacokinetics of high-dose intravenous melatonin in humans. J Clin Pharmacol. 2016;56:324-9.
12. Kaida K, Takahashi M, Akerstedt T, Nakata A, Otsuka Y, Haratani T, et al. Validation of the Karolinska sleepiness scale against performance and EEG variables. Clin Neurophysiol. 2006;117:1574-81.
13. Kollerup Madsen B, Hilscher M, Zetner D, Rosenberg J. Adverse reactions of dimethyl sulfoxide in humans: a systematic review. F1000Res. 2018;7:1746.
14. Matthews JN, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. BMJ. 1990;300:230-5.
15. Gonzalez Gonzalez A, Rueda Revilla N, Sanchez-Barcelo E. Clinical uses of melatonin: evaluation of human trials on cancer treatment. Melatonin Res. 2019;2:47-69.
16. Tordjman S, Chokron S, Delorme R, Charrier A, Bellissant E, Jaafari N, Fougerou C. Melatonin: pharmacology, functions and therapeutic benefits. Curr Neuropharmacol. 2017;15:434-443.
17. Hansen MV, Halladin NL, Rosenberg J, Gögenur I, Møller AM. Melatonin for pre- and postoperative anxiety in adults. Cochrane Database Syst Rev. 2015;4:CD009861.
18. Wang Q, Sun Z, Du L, Xu C, Wang Y, Yang B, et al. Melatonin sensitizes human colorectal cancer cells to gamma-ray ionizing radiation in vitro and in vivo. Int J Mol Sci. 2018;19.
19. Zetner D, Andersen LP, Rosenberg J. Melatonin as protection against radiation injury: a systematic review. Drug Res (Stuttg). 2016;66:281-96.
20. Zetner D, Kamby C, Mahmood F, Rosenberg J. MELADERM-trial: melatonin cream against acute radiation dermatitis in patients with early breast cancer. Melatonin Res. 2019;2:32-43.
21. Farhood B, Goradel NH, Mortezaee K, Khanlarkhani N, Salehi E, Nashtaei MS, et al. Melatonin as an adjuvant in radiotherapy for radioprotection and radiosensitization. Clin Transl Oncol. 2019;21:268-79.
22. Ben-David MA, Elkayam R, Gelernter I, Pfeffer RM. Melatonin for prevention of breast radiation dermatitis: a phase II, prospective, double-blind randomized trial. Isr Med Assoc J. 2016;18:188-92.

23. Zhang HM, Zhang Y. Melatonin: a well-documented antioxidant with conditional pro-oxidant actions. J Pineal Res. 2014;57:131-46.
24. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: under promises but over delivers. J Pineal Res. 2016;61:253-78.
25. Sanchez-Hidalgo M, Guerrero JM, Villegas I, Packham G, de la Lastra CA. Melatonin, a natural programmed cell death inducer in cancer. Curr Med Chem. 2012;19:3805-21.
26. Sainz RM, Mayo JC, Rodriguez C, Tan DX, Lopez-Burillo S, Reiter RJ. Melatonin and cell death: differential actions on apoptosis in normal and cancer cells. Cell Mol Life Sci. 2003;60:1407-26.
27. Cabrera J, Negrin G, Estevez F, Loro J, Reiter RJ, Quintana J. Melatonin decreases cell proliferation and induces melanogenesis in human melanoma SK-MEL-1 cells. J Pineal Res. 2010;49:45-54.
28. Di Bella G, Mascia F, Gualano L, Di Bella L. Melatonin anticancer effects: review. Int J Mol Sci. 2013;14:2410-30.
29. Stone BM, Turner C, Mills SL, Nicholson AN. Hypnotic activity of melatonin. Sleep. 2000;23:663-9.
30. Arendt J, Skene DJ. Melatonin as a chronobiotic. Sleep Med Rev. 2005;9:25-39.
31. Paul MA, Gray G, Kenny G, Pigeau RA. Impact of melatonin, zaleplon, zopiclone, and temazepam on psychomotor performance. Aviat Space Environ Med. 2003;74:1263-70.
32. Parkin J, Shea C, Sant GR. Intravesical dimethyl sulfoxide (DMSO) for interstitial cystitis--a practical approach. Urology. 1997;49:105-7.

Figure Legends

Fig. 1. Logarithmic transformation of melatonin plasma concentration following intravenous administration.

Fig. 2. Melatonin plasma concentration following intravesical, rectal, transdermal, and vaginal administration.

Table 1: Inclusion and exclusion criteria

Inclusion criteria
Healthy female
20-40 years old
BMI 18-30 kg/m^2
Exclusion criteria
Inability to understand Danish, written or spoken
Current use of melatonin or other hypnotics/sedatives
Current pregnancy [a]
Current breast feeding
Current alcohol or drug abuse [b]
Mental illness [c]
Serious comorbidity [d]
Participation in other clinical trials less than 1 month prior to current study
Nightshift work within the last 14 days prior to study
Planned nightshift work within the study period
Known and diagnosed sleep-disorder [e]

a) Urine human chorionic gonadotropin was tested on every study day. b) Defined as over 5 units of alcohol per day, or any usage of illegal drugs. c) Defined as having a diagnosis and being in medical treatment. d) American Society of Anesthesiologists (ASA) physical status 3-4. e) Defined as being in current medical treatment.

Table 2: Demographic variables

Variable	Median (Range)
Age (years)	23 (22-27)
Height (cm)	172 (163-184)
Weight (kg)	64 (54-71)
BMI (kg/m^2)	21.1 (18.7-23.1)
Ethnicity	***n***
Caucasian	9
Asian	1

Table 3: Pharmacokinetic variables of 25 mg melatonin

Administration route	C_{max} pg mL^{-1}	t_{max}	$t_{½\ absorption}$	$t_{½\ elimination}$	$AUC_{0-\infty}$ pg mL^{-1} min	*f*
Intravenous	752,616 (334,359)	0 (0)	-	47 min (6 min)	371,328 (164,858)	-
Intravesical	6,987 (6,113)	51 min (29 min)	11 min (11 min)	58 min (7 min)	13,691 (9,048)	3.6 (1.9)
Rectal	62,449 (33,816)	24 min (20 min)	5 min (4 min)	60 min (18 min)	117,742 (73,222)	36.0 (28.6)
Transdermal	897 (551)	20.5 h (8.0 h)	5.4 h (1.4 h)	14.6 h (11.1 h)	32,644 (10,046)	10.0 (5.7)
Vaginal	50,828 (22,813)	147 min (56 min)	17 min (4 min)	129 min (17 min)	377,237 (163,559)	97.8 (31.7)

Bioavailability, *f*; time to maximum concentration, t_{max}; maximal plasma concentration, C_{max}; elimination half-life, $t_{½}$ elimination; area-under-the-curve, AUC; absorption half-life, $t_{½}$ absorption; minutes, min; hours, h. Values are shown as mean (SD).

Table 4: Simple reaction time (SRT) test and Karolinska sleepiness scale (KSS) at t_{max}

Administration route	t_{max}	SRT baseline (sec)	SRT t_{max} (sec)	*p*	KSS baseline	KSS t_{max}	*p*
Intravenous	0 min	0.265 (0.018)	0.263 (0.018)	0.891	3 (1-6)	3 (1-4)	0.887
Intravesical	51 min	0.264 (0.018)	0.271 (0.029)	0.524	3 (2-5)	2.5 (2-5)	0.739
Rectal	24 min	0.258 (0.023)	0.262 (0.025)	0.711	3 (1-6)	3.5 (2-6)	0.863
Transdermal	20. 5 h	0.233 (0.030)	0.256 (0.018)	0.078	3 (2-5)	4.5 (3-5)	0.028
Vaginal	147 min	0.245 (0.023)	0.253 (0.018)	0.297	3 (2-6)	4 (3-6)	0.196

Time to reach maximum concentration, t_{max}; minutes, min; hours, h; seconds, sec; SRT-scores are presented as mean (SD); KSS scores are presented as median (range)

Table 5: Adverse events

Administration route	Confusion *n*	Depressed mood *n*	Dizziness *n*	Headache *n*	Garlic breath[a] *n*	Local burning[b] *n*
Intravenous	0	1	0	1	0	0
Intravesical	0	0	0	2	4	8
Rectal	0	0	0	0	1	1
Transdermal	0	0	0	4	0	0
Vaginal	0	0	0	0	0	0

a) garlic-like breath or odor b) local burning sensation at drug application site.

Figure 1: Logarithmic transformation of melatonin plasma concentration following intravenous administration.

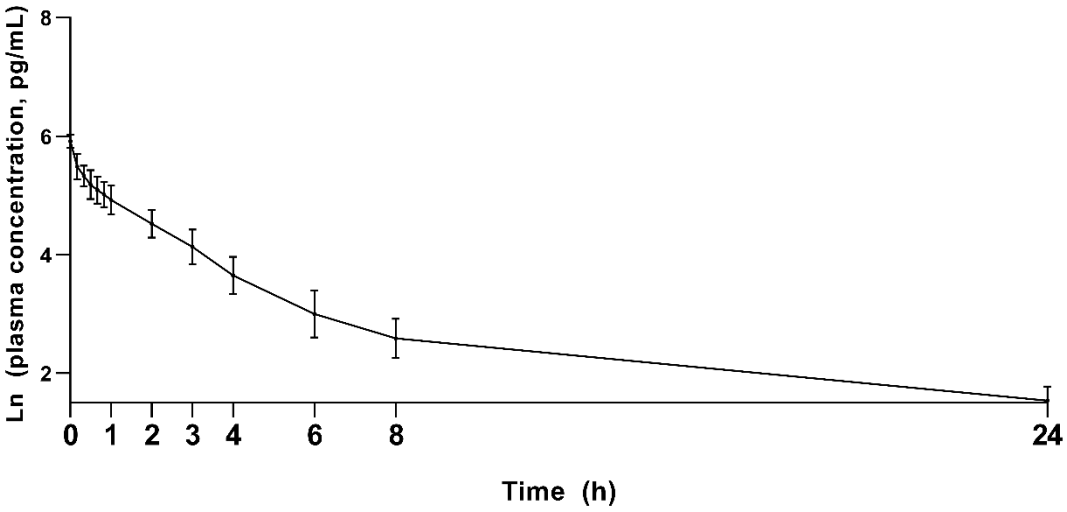

Figure 2: Melatonin plasma concentration following intravesical, rectal, transdermal, and vaginal administration.