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Pharmacokinetics and Safety of Intravenous, Intravesical, Rectal, Transdermal, and Vaginal Melatonin in Healthy Female Volunteers: A Cross-Over Study

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Keywords
Melatonin · Pharmacokinetics · Adverse effects

Abstract
Introduction: We aimed to investigate the pharmacokinetic properties and safety of melatonin administered by alternative routes of administration. ***Methods:*** This study employed a cross-over design in healthy female volunteers. Twenty-five milligrams of melatonin was administered intravenously, intravesically, rectally, transdermally, and vaginally. Blood samples were collected at specified time points up to 24 h following intravenous, intravesical, rectal, and vaginal administration, and up to 48 h 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) time to maximal concentration of 51 (29) min for intravesical, 24 (20) min for rectal, 21 (8) h for transdermal, and 147 (56) min for vaginal administration. The mean (SD) elimination half-life was 47 (6) min for intravenous, 58 (7) min for intravesical, 60 (18) min for rectal, 14.6 (11.1) h for transdermal, and 129 (17) min for vaginal administration. The 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:*** Rectally and vaginally administered melatonin may serve as relevant alternatives to standard oral melatonin therapy. Transdermal delivery of melatonin displayed an extended absorption and can be applied if prolonged effects are intended. Intravesical administration displayed, as expected, a very limited bioavailability. Melatonin administered by these routes of administration was safe.

Introduction

Melatonin is an indolamine primarily produced in the pineal gland of mammals [1]. Synthesis of the hormone is regulated by the light-dark cycle of the surrounding environment, detected by photosensitive retinal ganglion

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cells and transmitted through a complex neural network via the suprachiasmatic nucleus to the gland [1, 2]. The physiological melatonin plasma levels peak between midnight and 3:00 a.m. and display low levels during daytime [3]. Melatonin regulates various circadian rhythms, including the sleep/awake cycle in humans [4], and is involved in widespread physiological processes such as glucose homeostasis, and reproductive, thermodynamic, immune, oncostatic, and cardiovascular functions [4–7]. Also, melatonin is a free radical scavenger and possesses potent antioxidant effects [8, 9]. Exogenous melatonin is applied therapeutically for sleep disturbances, such as age-associated insomnia [10], and for circadian rhythm regulation, such as for jet lag [11] and in shift work [12]. With these indications, between 2 and 10 mg of melatonin is administered [12]. Currently, alternative indications are also being evaluated, such as the radioprotective effects in patients receiving radiotherapy as part of oncological treatments [13–16]. Interestingly, in oncological trials, increased doses up to 40 mg are administered [17]. The pharmacokinetic properties of oral and intravenous melatonin have previously been thoroughly investigated in humans [18, 19]. A systematic review documented an elimination half-life ($t_{½}$ elimination) of approximately 45 min [19]. However, the literature regarding the pharmacokinetics of melatonin administered by alternative routes of administration is still limited [20]. Correspondingly, detailed knowledge concerning the possible adverse effects and safety is sparse. Thus, this study aimed to investigate the pharmacokinetic properties and adverse effects of melatonin when administered intravenously, intravesically, rectally, transdermally, and vaginally.

Materials and Methods

The study is reported according to the ClinPK statement [21]. 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. A dose of 25 mg of melatonin was chosen since this was the dose to be used in another clinical trial [16]. This dose is in coherence with other studies investigating the protective effects of melatonin in cancer therapy [13–15, 17]. The eligibility criteria are outlined in Table 1.

Table 1. Inclusion and exclusion criteria

Inclusion criteria
Healthy female[a]
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[b]
Current breast feeding
Current alcohol or drug abuse[c]
Mental illness[d]
Serious comorbidity[e]
Participation in other clinical trials <1 month prior to the current study
Night-shift work within the last 14 days prior to the study
Planned night-shift work within the study period
Known and diagnosed sleep disorder[f]

ASA, American Society of Anesthesiologists. [a] Defined as having no medical illnesses requiring medication. [b] Urine human chorionic gonadotropin was tested on every study day. [c] Defined as over 5 units of alcohol per day, or any usage of illegal drugs. [d] Defined as having a diagnosis and being in medical treatment. [e] ASA physical status 3–4. [f] Defined as being in current medical treatment.

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-min period (infusion rate 2.5 mL/min). For intravesical administration, 25 mg of melatonin was dissolved in 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 [22]. 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 [23]. For application to the skin, 1 g of standard skin lotion containing 25 mg melatonin and 150 mg DMSO was administered to cover a 20 × 20-cm area on the chest of each participant. The chest area was outlined with a measuring tape. The dose of DMSO was based on DOLOBENE® SportsGel [24], 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) [25]. The melatonin used in the trial was produced by Flamma S.p.A. (Chignolo d'Isola, Italy). The intravenous and intravesical formulations were prepared by Skanderborg Pharmacy, Denmark. The rectal, vaginal, and transdermal melatonin formulations were prepared by Glostrup Pharmacy, Denmark. All melatonin products were developed and manufactured according to Good Manufacturing Practice standards [26].

Primary Outcome

Blood samples were collected at baseline (prior to melatonin administration) and at 0, 10, 20, 30, 40, 50, and 60 min, and 2, 3, 4, 6, 8, and 24 h 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 h following application of melatonin to the skin. Participants slept in the hospital in relation to the 16- and 24 -h samples following transdermal application of melatonin.

Blood samples were centrifuged at 3,000 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 9.8–13.4%; interassay coefficient of variation 8.0–13.3%; limit of detection 2.3 pg/mL; and linearity of the RIA-kit 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 [27, 28]. Evaluations were performed at baseline and at 1, 2, 3, 4, 5, 6, 7, 8, and 24 h 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 h 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 [29]. KSS evaluations were performed at corresponding intervals as the SRT evaluations.

Documentation of adverse events included prespecified 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 [30]. 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) depending on the distribution of data. Parametric or nonparametric 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 GraphPad Prism version 7.0 (GraphPad Software Inc., La Jolla, CA, USA). The pharmacokinetic analyses were performed using GraphPad Prism version 7.0 and Microsoft Excel (Microsoft Corporation, Microsoft Office 365 ProPlus, version 16.0.11929.20776).

Time to maximal concentration (t_{max}) and maximal plasma concentration (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 and not from the mean t_{max}. We calculated the 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 follows: $t_{½}$ absorption = [ln − (2)]/k_a and $t_{½}$ elimination = [ln − (2)]/k_e. Areas under the curve (AUCs) of plasma concentrations were calculated by applying the trapezoidal rule [31]. $AUC_{0-\infty}$ was estimated as $AUC_{0-24\ h}$ + (C_{24} h/k_e) for the intravenous, intravesical, rectal, and vaginal administrations, whereas $AUC_{0-\infty}$ for the transdermal administration was estimated as $AUC_{0-48\ h}$ + (C_{48} h/k_e). Bioavailability (f) was calculated as follows:

$$f = 100 \times (AUC)\left({}_{0-\infty}(\textit{rectal, intravesical, vaginal or transdermal})\right) / (AUC)_{-}\left({}_{0-\infty}(iv)\right)$$

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 h following administration.

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

Results

Demographic data are presented in Table 2. No participants dropped out or were lost to follow-up. Missing data occurred in 2 participants. Due to a dysfunctional venous access catheter in 1 participant, blood samples could not be drawn at 20 and 30 min following rectal administration. Hence, we chose to exclude data for this administration route from further pharmacokinetic analyses in this participant. Since the participant received melatonin, we still employed the SRT and KSS data for further analyses. In addition, the intravenous administration of melatonin failed in 1 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, intravesical, rectal, transdermal, and vaginal administrations are presented in Figure 1.The pharmacokinetic parameters of the individual administration routes are shown in Table 3. Intravenous melatonin demon-

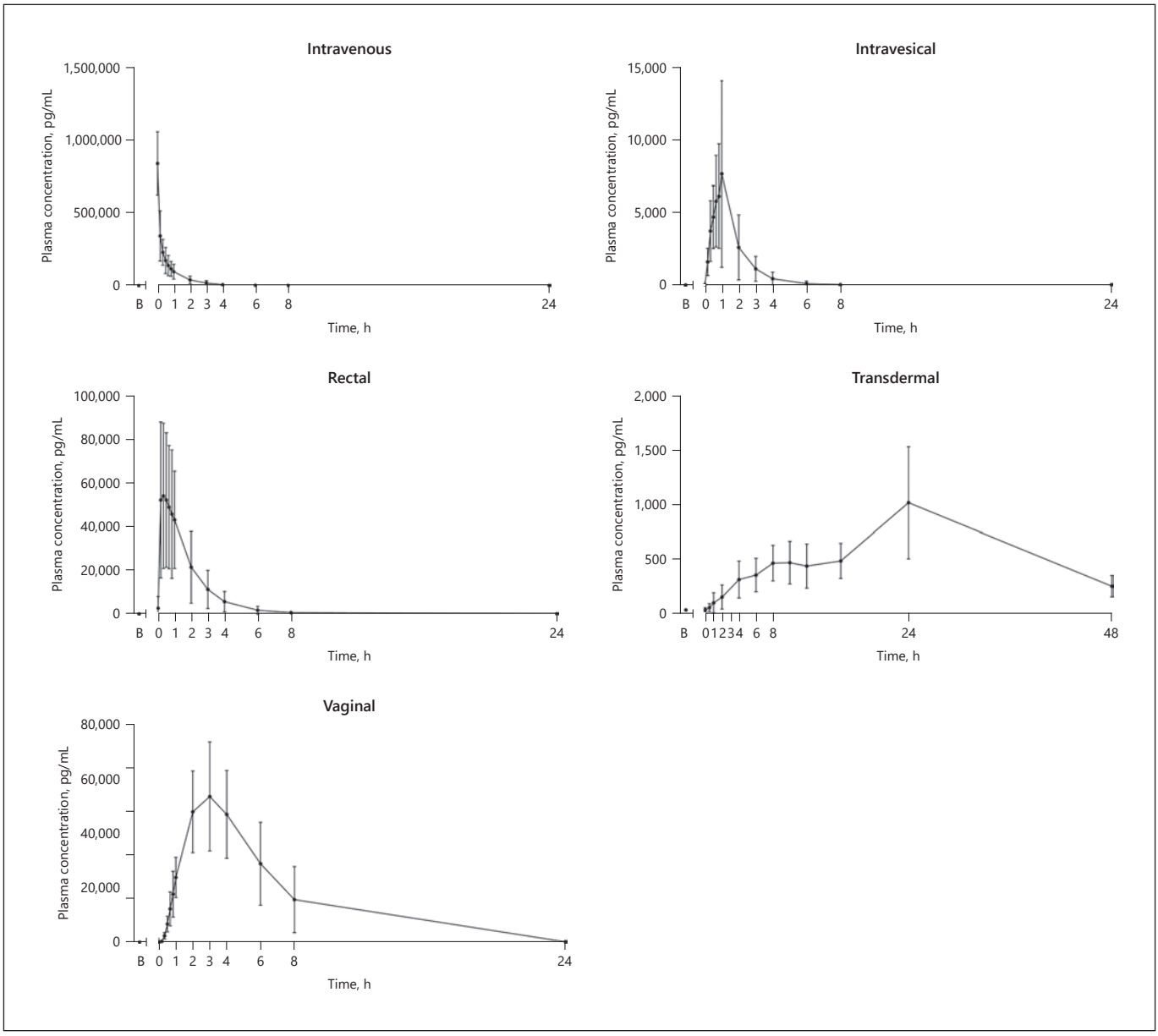

Fig. 1. Melatonin plasma concentrations following intravenous, intravesical, rectal, transdermal, and vaginal administration.

strated 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 min and demonstrated a bioavailability of 3.6%. Rectal administration had a $t_{½}$ absorption of 5 min and a $t_{½}$ elimination of 60 min, and demonstrated a bioavailability of 36%. Transdermal administration reached t_{max} between 16 and 24 h, with most participants showing t_{max} at 24 h, giving a mean t_{max} of 20.5 h. 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 3 participants reaching a bioavailability of over 100%. The 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

Table 3. Pharmacokinetic variables of 25 mg melatonin

Administration route	*n*	C_{max}, pg mL^{-1}	t_{max}	$t_{½}$ absorption	$t_{½}$ elimination	$AUC_{0-\infty}$, pg mL^{-1} min	*f*,[a] %
Intravenous	9	752,616 (334,359)	0 (0)	–	47 min (6 min)	371,328 (164,858)	–
Intravesical	10	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	9[a]	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	10	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	10	50,828 (22,813)	147 min (56 min)	17 min (4 min)	129 min (17 min)	377,237 (163,559)	97.8 (31.7)

Values are shown as mean (SD). *f*, bioavailability; t_{max}, time to maximal concentration; C_{max}, maximal plasma concentration; $t_{½}$ elimination, elimination half-life; AUC, area under the curve; $t_{½}$ absorption, absorption half-life. [a] For the bioavailability calculations, $n = 9$, apart from rectal where $n = 8$.

Table 4. SRT test and KSS at t_{max}

Administration route	*n*	t_{max}	SRT baseline (s)	SRT t_{max} (s)	*p* value	KSS baseline	KSS t_{max}	*p* value
Intravenous	9	0 min	0.265 (0.018)	0.263 (0.018)	0.891	3 (1–6)	3 (1–4)	0.887
Intravesical	10	51 min	0.264 (0.018)	0.271 (0.029)	0.524	3 (2–5)	2.5 (2–5)	0.739
Rectal	10	24 min	0.258 (0.023)	0.262 (0.025)	0.711	3 (1–6)	3.5 (2–6)	0.863
Transdermal	10	20.5 h	0.233 (0.030)	0.256 (0.018)	0.078	3 (2–5)	4.5 (3–5)	0.028
Vaginal	10	147 min	0.245 (0.023)	0.253 (0.018)	0.297	3 (2–6)	4 (3–6)	0.196

SRT scores are presented as mean (SD); KSS scores are presented as median (range). t_{max}, time to maximal concentration; SRT, simple reaction time; KSS, Karolinska Sleepiness Scale.

Table 5. Adverse events

Administration route	Included, *n*	Confusion, *n*	Depressed mood, *n*	Dizziness, *n*	Headache, *n*	Garlic breath,[a] *n*	Local burning,[b] *n*
Intravenous	9	0	1	0	1	0	0
Intravesical	10	0	0	0	2	4	8
Rectal	10	0	0	0	0	1	1
Transdermal	10	0	0	0	4	0	0
Vaginal	10	0	0	0	0	0	0

[a] Garlic-like breath or odor. [b] Local burning sensation at the drug application site.

routes ($p > 0.05$). The KSS score following transdermal administration was significantly increased compared to baseline values ($p = 0.028$) (see Table 4).

Predefined adverse events are displayed in Table 5. In addition to the predefined adverse events, 1 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 bioavailability. 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 administration. No serious adverse events were observed. Increased tiredness assessed by KSS was observed only after transdermal administration.

Melatonin has potential widespread clinical actions [32–34]. Optimal drug delivery relating to the specific pa-

tient and treatment may prove pivotal to improve clinical effects. Hence, pharmacokinetic properties are needed to describe drug distribution in detail, for example, 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 [16, 35–38], antioxidant [39, 40], and anticancer [41–44] properties of melatonin. Localized radiation therapy may be combined with local melatonin administration regimens, for example, 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 [19].

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 [28]. The KSS score was only significantly higher 24 h 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 [45–48]. 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 [22]. Correspondingly, halitosis was experienced by 4 and 1 participant following intravesical and rectal administrations, respectively. Halitosis is related to limited quantity of DMSO being excreted via the lungs as dimethyl sulfide [49]. The number of participants experiencing mild transient adverse effects, such as confusion, depression, dizziness, or headaches, was lower than that in, or in accordance with previous studies [50].

This study has several strengths. This is the first study to investigate intravesical, rectal, and vaginal administrations of melatonin in humans [20]. It is also the first study to estimate bioavailability following transdermal administration of melatonin [20]. We chose to include a sufficient number of participants and measuring points, increasing the general quality of data. Plasma samples were analyzed according to previous studies [18]. Finally, we adhered to the ClinPK statement [21] and employed a cross-over design to minimize reporting bias and reduce interindividual variation.

Our study also has a number of limitations that need to be adressed. First, a limited number of missing data occurred. In 1 participant, 2 blood samples following the rectal administration were missed due to a dysfunctional venous access catheter. 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 addressed in future studies, for example, by microdialysis techniques. Third, the bioavailability of intravesical melatonin demonstrated very low values. We emphasized the need to avoid urinary voiding the first hour following administration. It is, however, possible that a quantity of melatonin was excreted externally (with the urine) following this period. Fourth, 3 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 concentration 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 overestimation of the bioavailability of the other administration routes. The reasoning, however, for this administration regimen was safety relating to the extensive intravenous dose of administered melatonin. Fifth, data concerning KSS scores at 24 h following transdermal administration should be interpreted with care since participants received <8 h of sleep due to the blood sampling frequency. Also, participants slept in a hospital environment. Hence, the significant increase of KSS scores at 24 h post-administration may also partly be attributed to poor quality of sleep, rather than the hypnotic effects of melatonin. Finally, the study was not powered to evaluate KSS and SRT as pharmacodynamic parameters, and hence findings should be interpreted with care.

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

dermally, and vaginally. Melatonin administered 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 participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The study was conceptualized and designed by D.Z., L.P.K.A., and J.R. Data were acquired by D.Z., R.A., M.L.J., and A.T. Data were analyzed by D.Z. The manuscript was drafted by D.Z. and critically revised by L.P.K.A., R.A., M.L.J., A.T., and J.R. All authors gave final approval of the manuscript prior to submission and have agreed to be accountable for all aspects of the work.

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