Pharmacokinetics of intravenous, rectal, intravesical, vaginal, and transdermal administration of exogenous melatonin in healthy female volunteers: a crossover study

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Background

Melatonin is a neurohormone, primarily produced by the pineal gland. The compound regulates circadian rhythms in mammals [1]. Exogenous melatonin is most often administered orally, but the drug displays poor and variable bioavailability due to an extensive first pass metabolism [2-4]. Previous human experimental and clinical trials have administered large doses of melatonin: up to 100 mg intravenously in adults, and 10 repeated intravenous doses of 10 mg/kg in neonates without any adverse effects [5, 6].

Experimental studies have documented that melatonin is a potent free radical scavenger [7]. The anti-oxidant effects of melatonin may possibly protect against the damaging effects of ionizing radiation applied in clinical cancer treatment and radiological imaging procedures [7]. A study demonstrated a significantly increased 30 day survival in mice receiving a lethal dose of ionizing radiation after injecting melatonin, as compared with controls [8]. Another study demonstrated significantly less radiation-induced enteritis in mice that received melatonin compared to a placebo [9]. Furthermore, melatonin has been documented to possess oncostatic and pro-apoptotic effects in various cancers in experimental studies [10, 11]. Finally, melatonin has also been demonstrated to potentiate the effect of ionizing radiation in cell cultures of human breast cancer cells [12, 13].

Radiation injury is a common complication leading to serious adverse reactions resulting from treatment of various cancers with ionizing radiation [14-21]. In the treatment of breast cancer, the most common complication of radiotherapy is radiation dermatitis [22]. A study has reported that 59% of patients with prostate cancer report abdominal problems, e.g. diarrhea, abdominal pain and blood in the stool, due to radiation injury [21]. Melatonin could potentially mitigate some of these adverse reactions to radiotherapy. This requires randomized controlled clinical trials with the administration of melatonin, including various forms of local administration.

Before any studies investigating the radioprotective effects of locally administered melatonin can be undertaken in humans, it is imperative that the pharmacokinetic properties of melatonin are investigated when administered in this way. No previous studies investigating the pharmacokinetics of vaginal, intravesical, or rectal administration of melatonin have been performed [23]. The few studies investigating transdermal administration of melatonin have been insufficient [24-26]; only one study has been able to estimate a $T_{½}$, and only in two volunteers [27], and no studies have investigated transdermal bioavailability [23].

Study design

Objective

The aim of the study is to determine the pharmacokinetic properties of, and possible adverse reactions of, melatonin when administered intravenously, rectally, intravesically, vaginally, and transdermally, in healthy female volunteers.

Design

This study is a crossover study, including five individual study sessions investigating five different routes of administration of melatonin. Four of the study sessions (intravenous, rectal, intravesical, and vaginal) will consist of one study day and one plasma sample the following day. The transdermal application will consist of one study day, followed by plasma samples at 24 and 48 hours following application. Between each study session, the subjects will have a washout period of a minimum of 7 days. The study will include a total of ten healthy female volunteers.

The subjects will participate in study sessions in the following order: intravenous, rectal, intravesical, vaginal, and transdermal administration. The study will be concluded after the

48-hour plasma sample after transdermal administration. Should a participant be menstruating at the time of the vaginal administration study session, the session will be postponed for one week.

Study course

Study session 1: Intravenous administration

The melatonin will be administered intravenously, followed by plasma samples over the following 24 hours. The intravenous melatonin will be dissolved in an ethanol/saline solution (2 ml 99.9 % ethanol/23 ml 0.9 % saline) containing 25 mg of melatonin. The infusion of melatonin will be performed over 10 minutes, the infusion rate being 2.5 mL per minute.

Study session 2: Rectal administration

The melatonin will be administered rectally in a fluid, followed by plasma samples over the following 24 hours. The melatonin formulation applied for rectal administration will be a 2.5 ml fluid containing 25 mg of melatonin. The delivery of the fluid is to be performed via an enema. The subjects will be asked to defecate, if possible in the morning, prior to the administration of melatonin.

Study session 3: Intravesical administration

The melatonin/dimethyl sulfoxide (DMSO) aqueous solution will be administered intravesically, followed by plasma samples over the following 24 hours. The melatonin used in the study will be applied intravesically through a catheter at a dose of 25 mg in 50 ml of a solution of 50% (w/w) DMSO in water, the solution containing melatonin at a concentration of 0.5 mg/ml. The subjects will be asked not to consume fluids three hours prior to the start of the study day, and will be asked to void their bladder prior to administration of melatonin.

Study session 4: Vaginal administration

The vaginal suppository containing melatonin will be administered vaginally, followed by plasma samples over the following 24 hours. The melatonin used in the study will be applied at a dose of 25 mg in a suppository (Witepsol S58) vaginally, by the participant themselves.

Study session 5: Transdermal administration

The melatonin will be administered topically as 1 g of a 2.5% (w/w) melatonin cream over a skin area of 20 x 20 cm (400 cm^2), followed by plasma samples over the following 48 hours. The dose of melatonin used in the study will thus be 25 mg. The participants will be asked to refrain from using any other creams or lotions one day prior to the study session, and for the following 48 hours.

Outcomes

The outcomes are identical for four of study sessions of the trial (intravenous, rectal, intravesical, and vaginal). The transdermal study session will have a follow-up of 48 hours rather than 24 hours. These differences are outlined below.

Primary outcome

Plasma concentration of melatonin at baseline, 0 min, 10 min, 20 min, 30 min, 40 min, 50 min, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, and 24 hours. Blood samples are drawn from a peripheral venous catheter within the first 8 hours, and the 24-hour samples will be taken as a single venipuncture. Each blood sample will have a volume of 6 ml, totaling 84 ml for each of the four first study sessions. For the blood samples drawn from a peripheral venous catheter, 4 ml of blood

will be drawn as “waste”, to ensure that the following 6 ml consist of undiluted blood, totaling another 52 ml of blood. In total, the subjects will have 136 ml of blood samples each study session.

For the transdermal study session, blood samples are drawn at baseline, 0 min, 30 min, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 16 hours, 24 hours and 48 hours. Intervals are based on data from previous pharmacokinetic studies of transdermal melatonin [23]. Blood samples are drawn from a peripheral venous catheter within the first 16 hours, and the 24- and 48-hour samples will be sampled through a single venipuncture each. Each plasma sample will have a total volume of 6 ml, totaling 78 ml for the transdermal study session. For the blood samples drawn from a peripheral venous catheter, 4 ml of blood will be drawn as “waste”, to ensure that the following 6 ml consist of undiluted blood, totaling another 44 mL of blood. In total, the subjects will have 122 ml of blood drawn samples for the transdermal study session.

Plasma samples will be kept in a locked freezer (with a temperature-alarm) at the Department of Surgery, Herlev Hospital. The plasma concentration will be assessed by radioimmunoassay (RIA)-technique at Gastrolab, Herlev Hospital. RIA-technique is a validated method of determining melatonin in plasma that has been used for years [28]. A bio-bank will be created, containing solely the plasma samples from this study (see Data Management for further details). In the case report forms (CRF), the collection time of each sample will be noted. The plasma concentrations will be delivered to the investigator digitally from the laboratory, and will afterwards be noted in the through double entry CRF, by independently two investigators.

Secondary outcomes

Sedative effects and other adverse effects

A Karolinska sleepiness scale (KSS)-score will be performed at baseline and once every hour for 8 hours, as well as at 24 hours, and noted in the CRF. For the transdermal study session, the score will

be performed at every hour on the first day until 16 hours, as well as at 24 and 48 hours. The KSS is frequently used to evaluate subjective sleepiness. The scale has been validated against performance and EEG-variables [29].

Simple reaction time (SRT) test: Subjects will be assessed by an online-based test (http://getyourwebsitehere.com/jswb/rttest01.html). Each volunteer will be instructed to press the mouse button as soon as possible, when a stoplight displayed on a computer screen changes from red to green. During the four first study sessions, each volunteer will be tested at baseline (before administration of melatonin), and once every hour for 8 hours after administration of melatonin, as well as 24 hours after administration of melatonin. For the transdermal study session, the test will be performed at every hour on the first day until 16 hours, as well as at 24 and 48 hours.

Other adverse reactions include pre-specified self-reported symptoms of nervousness, confusion, depressed mood, dizziness, headache, garlic breath and burning sensation from application area (yes/no). Volunteers will also be asked to report additional symptoms of adverse reactions, if any (yes/no + description). Results will be noted in the CRF.

When the subjects are included, a set of baseline demographics data will be recorded in the CRF, including date of birth, ethnicity, weight, height, and BMI.

Subjects/population

Inclusion criteria:

- Healthy female
- 20-40 years old
- BMI 18-30

Exclusion criteria:

- Inability to understand Danish, written or spoken.

- Current use of melatonin or other hypnotics/sedatives
- Current pregnancy (a positive urine-HCG; the subjects will be tested prior to each study session)
- Breast feeding
- Current alcohol or drug abuse (defined as over 5 units of alcohol per day, or any usage of illegal drugs)
- Mental illness (defined as being in medical treatment)
- Serious comorbidity (American Society of Anesthesiologists (ASA) physical status 3-4)
- Participation in other clinical trials less than 1 month prior to current study
- Night-shift work within the last 14 days prior to study
- Planned night-shift work within the study period
- Known and diagnosed sleep-disorder (defined as being in current medical treatment)
- Plasma hemoglobin <7.8 mmol/L (measured when screening participants)

Withdrawal and dropout criteria

The volunteers can withdraw their consent at any point during the trial. The dropout criteria of the study are if a subject at some point during the study starts to fulfill any of the exclusion criteria, or if a subject is unable to participate in all five study sessions. Furthermore, the investigator can at any point exclude participants from the trial, if the investigator deems it necessary for the safety of the participant. The reason for a subject withdrawing their consent will be reported in the CRF. Data from dropouts will not be used in the statistical analysis.

Conclusion of the study

Each volunteer will have concluded the study after finishing all five study sessions. A total of ten subjects will be included in the study. In case of dropouts, the study will be concluded after a total of eight subjects have completed the study. If more than two subjects drop out, we will include further subjects until a minimum of eight subjects have completed the study.

Handling of melatonin formulations

Storage

Melatonin alcohol/saline solution

Dry melatonin will be packaged according to EU GCP/GMP-guidelines in containers with 25 mg melatonin. All containers with dry melatonin will be given a batch number. The 99.9% ethanol solvent will be packaged in ampoules and also be given a batch number. The melatonin, the 0.9% saline, and the 99.9% ethanol solvent will be acquired via Skanderborg Apotek.

Melatonin rectal fluid

The melatonin fluid, consisting 25 mg of melatonin dissolved in 2.5 mL of a 20% w/w glycofurol, and 40% w/w DMSO solution in water. This fluid has been demonstrated by Bioneer A/S to have no degradation of melatonin while being stored at 25°C for 45 days. Therefore, the fluid will be stored at room temperature. This fluid will be produced and packaged by Glostrup Apotek, according to GMP-guidelines. The fluid will be packaged in single dose mini enemas, and each box will contain 12 mini enemas.

Melatonin/DMSO solution

The melatonin will be dissolved into a 50/50% w/w DMSO and water solution at a concentration of 0.5 mg/g. This solution has been demonstrated by Bioneer A/S to have no degradation of melatonin while being stored at 25°C for 45 days. Therefore, the fluid will be stored at room temperature. The fluid will be packaged in single dose vials (50 ml), and each box will contain 12 vials. The melatonin/DMSO solution will be produced and packaged by Skanderborg Apotek, according to GMP-guidelines.

Melatonin suppository

The suppository will consist of a Witepsol S58 base, containing 25 mg of melatonin. The Witepsol S58 base contains hard fat, polyoxyethylene (25) cetyl stearyl ether and glyceryl ricinoleate [30]. The suppositories will be stored at room temperature. The melatonin will be packaged as single dose suppository in blister packs. The sizes of the blister packs are to be decided. An applicator will possibly be included. The suppositories will be produced and packaged by Glostrup Apotek, according to GMP-guidelines.

Melatonin cream

The cream for transdermal application will consist of 25 mg/g melatonin as well as 150 mg/g DMSO, in a cream base. The dose for each application will be 1 g, and the cream will be packaged in 30 g tubes. The tubes will be stored at room temperature. The cream will be produced and packaged by Glostrup Apotek, according to GMP-guidelines.

Administration

A dose of 25 mg melatonin for local administration has been chosen. This dosage was based on the smallest doses that have demonstrated radioprotective effects in animals [7, 31-33]. The dose has been scaled for humans, taking into account that the effect aimed to achieve should be local, rather than systemic. All administrations of melatonin to the healthy volunteers will be performed by an investigator, except for the vaginal suppository and transdermal cream which will be administered by the subjects themselves.

Study session 1: Intravenous administration

On the day of the study, the intravenous solution will be prepared by an investigator: Two ml of ethanol (99.9%) will be aspirated into a 50 ml syringe. Two ml ethanol will be mixed into a glass of 25 mg dry melatonin, and aspirated back into the 50 ml syringe. Another 23 ml of saline 0.9% will be aspirated into the syringe. The solution will contain 25 mg melatonin in a 4 ml ethanol/46 ml saline solution (1 mg/ml). This solution will then be administered as a bolus through a venous line at an infusion rate of 2.5 ml/minute over 10 minutes. This method for delivering intravenous melatonin has been used in a previous pharmacokinetic study investigating exogenous melatonin administration [5, 34].

Study session 2: Rectal administration

The 25 mg melatonin will be administered as 2.5 ml of a 10 mg/ml fluid, as an enema via a plastic tube with a stud with an opening. The fluid used will be a 20% w/w glycofurol and 40% w/w DMSO aqueous solution. The plastic stud on the enema will be inserted into the rectum, and the fluid will be administered as a bolus. The enema is then removed, and a clock is started to measure time from administration.

Study session 3: Intravesical administration

50 ml of melatonin 0.5 mg/ml solution in the 50% (w/w) DMSO/water solvent will be aspirated into a 50 ml syringe, totaling 25 mg melatonin. A Clean Intermittent Catheter (CIC) is inserted into the subject through the urethra, into the bladder, and the bladder of the subject will be emptied. Afterwards, the 50 ml syringe will be connected to the CIC, and the 50 ml solution will be administered into the subject at a rate of 25 ml/minute over 2 minutes. The catheter is then removed, and a clock is started to measure time from administration. Subjects will be required not to void their bladder until after the 1-hour blood sample has been collected.

Study session 4: Vaginal administration

The 25 mg of melatonin will be administered in a suppository, vaginally. The subjects will be handed a suppository, which they will themselves take out of the blister pack, and insert vaginally. As soon as the suppository is administered, a clock is started to measure time from administration.

Study session 5: Transdermal administration

The melatonin cream will be administered over a skin area of 20 x 20 cm. This area will be measured and outlined with a marker on the subject's chest, starting at the medial end of the left clavicle, horizontally towards the mid-axillary line, and vertically parallel to the sternum towards the stomach. Afterwards, 1g of melatonin cream will be measured on a scale, on a piece of non-stick paper by the investigator. The subject will then apply the melatonin cream over the 20 x 20 cm area. The subject will be wearing a glove and only use the fingertips in order to maximize the dose applied. After the melatonin cream has been adequately applied, a clock is started to measure time from administration.

Adverse reactions

Adverse reactions to melatonin

Melatonin is non-toxic in both physiological and pharmacological doses [35]. In mice, rabbits, cats, and dogs, melatonin doses of 800 mg/kg administered intravenously demonstrated no toxicity [36].

A study administered 1 g of melatonin orally to patients for 25-30 days, with drowsiness as the only adverse reaction [37]. These patients had substantial clinical tests (hematological and blood chemistry tests, urine analysis, heart rate and blood pressure monitoring and ECG) performed, and no reactions to melatonin were demonstrated [37]. Another study investigated the effect of intravenously administered melatonin in healthy subjects, as well as patients with Parkinson's disease and epilepsy. They applied 1.25 mg/kg melatonin as a bolus injection. One of the patients, who had 1.25 mg/kg melatonin intravenously, received further two doses of 0.5 mg/kg. Correspondingly, melatonin was non-toxic and no adverse reactions were observed [38].

Furthermore, melatonin has been used as an intravenous infusion in neonates with sepsis, in neonates undergoing abdominal surgery, and in preterm infants [6, 39, 40]. A total of 224 patients were included (of whom 105 received melatonin). No adverse reactions were reported at a dose of 10 mg/kg intravenously [6, 39, 40].

Melatonin has also been given in very large doses up to 100 mg intravenously in adults, without adverse reactions [5]. Other studies investigated melatonin when used as a pre-medication for gynecological laparoscopic surgery, and demonstrated no adverse reactions at a dose of 5-20 mg of sublingual melatonin [41, 42]. No adverse reactions were reported in a study investigating pre-medication with 5 mg melatonin prior to laparoscopic cholecystectomy [43].

Drowsiness, confusion and depression have been described as adverse reactions of melatonin in a study examining whether melatonin could augment antidepressant treatment in patients with depression [44]. Furthermore, a few studies report headache [45] and fatigue as side-effects of melatonin [46].

A comprehensive review of the safety of melatonin concludes that no studies have indicated that exogenous melatonin should induce any serious reactions effects [47]. Long-term safety of melatonin in children and adolescents requires further investigation. Due to a lack of studies, pregnant and breast-feeding women should refrain from taking exogenous melatonin [47].

Adverse reactions of DMSO

DMSO is regularly used in the treatment of interstitial cystitis, either as monotherapy, or serving as a vehicle for anti-inflammatory agents [48-50]. DMSO has previously been applied as a solvent for intravesical drug administration in the treatment of bladder cancer [51, 52]. An adverse reaction of DMSO is a garlic-like breath odor and taste, due to pulmonary elimination of a small percentage of the DMSO as dimethyl sulfide. One study demonstrated that up to 48% of patients with interstitial cystitis reported adverse reactions, including urethral irritation and nausea [49]. However, these adverse reactions are self-limiting, lasting up to 24-48 hours [48-50].

Adverse reactions of glycofurol

Glycofurol has previously been used as a solvent for rectal administration of methadone [53, 54]. Furthermore, glycofurol is used as an ingredient in Stesolid® (diazepam) rectal fluid [55]. Glycofurol is generally perceived as a non-toxic and non-irritant material, being nevertheless irritating if injected undiluted [56]. In this study, the glycofurol will be diluted to a 20% w/w

glycofurol and 40% w/w DMSO in water, the solution containing 10 mg/g melatonin. Therefore any irritating effects from the glycofurol should be avoided.

Adverse effects of plasma sampling

During the study a total of 136 ml of blood will be sampled from the subjects per study session the first four study sessions (intravenous, rectal, intravesical, and vaginal). A total of 122 ml of blood will be sampled from the subject during the transdermal study session. The drawn volume is in no way a health risk for the subjects. Drawing of blood samples can leave the subjects with small hematomas and the patients will feel a small sharp scratch when the needle is inserted.

Adverse events

The subjects will be questioned about adverse events during each study session and at the 24-hour plasma sample (as well as at 48 hours for the transdermal study session). We have defined five pre-specified self-reported symptoms (fatigue, confusion, depressed mood, or headache), based on the most commonly reported adverse reactions of melatonin available in the literature [See attached Investigators Brochure]. Volunteers will also be asked to report additional symptoms of adverse effect, if any. Both adverse reactions and adverse events will be reported in the CRF. An adverse reaction is defined as harm or unwanted reaction to a drug, no matter the dosage. An adverse event is defined as any medical event in a patient or healthy volunteer in a clinical trial after treatment with a drug, without any necessary connection between the treatment and the undesirable event.

Serious adverse events (SAE) are defined as any medical event that result in death, are life-threatening, lead to hospitalization, result in disability or permanent damage, or any other important medical event. All serious adverse events will be registered and reported to the Danish

Medicines Agency (Lægemiddelstyrelsen) and the Danish Committee on Health Research Ethics (Videnskabsetisk Komité) in a final report.

Serious adverse drug reactions (SARs) will be reported annually (most likely irrelevant to this study, as it will not continue for a full year).

Suspected unexpected serious adverse reactions (SUSARs) will be reported immediately to the Danish Medicines Agency and the Danish Committee on Health Research Ethics.

Summary of Product Characteristics and Investigators Brochure (both are attached to this document) will be used to evaluate SUSARs. Adverse event registration will commence on the first study day and be finalized after the last study day. In case of serious adverse reactions the sponsor will decide if the study is to be terminated.

Setting

The setting is identical for study sessions one, two, three and four (intravenous, rectal, intravesical and vaginal) of the study. The subjects will be greeted and placed in a room where both the research assistant and the subject can sit comfortably without disturbance. Here the subject will be fitted with a single intravenous access and the baseline sample will be drawn. For the intravenous session, the subject will have two intravenous lines established (one in each arm). Afterwards, the subject will be led to a nearby lockable disturbance free room with a bed for the intravenous, rectal, intravesical, and vaginal study sessions. For the intravenous session, the melatonin will be administered in the arm contralateral to that from which the blood samples are drawn, after which the intravenous access will be removed. The administration of intravenous, rectal and intravesical melatonin will be performed by an investigator. The subjects will administer the vaginal melatonin themselves. Afterwards the subject will be led back to the first room, for the

remainder of the study day for continuous blood sampling and be evaluation of sedative effects and other adverse effects. After the blood sample at 8 hours, the intravenous access will be removed (later for the transdermal study, as described below). The subjects will be offered to have a chaperone present for the rectal, intravesical and vaginal administration of melatonin.

For the transdermal administration, the subjects will be greeted and placed in a room where both the research assistant and the subject can sit comfortably without disturbance. The subject will be fitted with a single intravenous access. The baseline blood sample will be drawn, and the melatonin cream will be administered over a 20 x 20 cm skin area of the thorax by the investigator and subject as described above. The subjects will be evaluated for sedative and other adverse effects. The subjects will remain in this room for the remainder of the study day. After the blood sample at 16 hours, the intravenous access will be removed. The subjects will be offered to have a chaperone present for the administration of the melatonin cream.

Following each study day, a blood sample will be drawn at 24 hours (and 48 hours for the transdermal study session) after administration of melatonin with a regular blood sampling kit. The setting for the 24- and 48-hour blood sample is identical for all five sessions of the study.

Statistics

The number of patients needed for this study is not based on a power calculation. We have decided that 8 subjects are needed to collect information of how the plasma concentrations develop over time. Because of the risk of dropouts, we have chosen to include a total of 10 subjects.

Data will be presented as either mean (SD) or median (IQR), unless stated otherwise. Data will be analyzed using 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}) will be assessed directly at the relevant time points. The pharmacokinetic variables: absorption constant (k_a), absorption half-life ($t_{½}$ absorption), elimination rate constant (k_e) and elimination half-life ($t_{½}$ elimination) will be estimated by "the method of residuals" [57]. Areas-under-the-curve (AUC) of plasma concentrations will be calculated by applying the trapezoidal rule [58]. $AUC_{0-\infty}$ will be estimated as $AUC_{0\text{-}8\ hours}$ + (C_8 hours/k_e). Bioavailability (*f*) will be calculated as follows:

$f = 100 \times \frac{AUC_{\ 0-\infty\ (rectal,\ \ intravesical,\ \ vaginal\ or\ transdermal)} \times D\ iv}{AUC_{\ 0-\infty\ (iv)} \times D\ rectal,\ \ intravesical,\ \ vaginal\ or\ transdermal}$ where D represents the dose administered.

Data monitoring and management

This study will be conducted in compliance with the protocol, the ICH-GCP guidelines and the applicable regulatory requirements. The standard procedures for quality control and quality assurance are followed in compliance with the ICH-GCP guidelines, and investigator/sponsor allows direct access to data/documents for monitoring, auditing, and inspection from both the Danish Medicines Agency (Lægemiddelstyrelsen) and the Good Clinical Practice (GCP)-unit. The GCP-unit will monitor the study. Data will be stored electronically on a secure server until 10 years

after the end of the study, after which all person-sensitive data are deleted. Blood samples will be destroyed after analysis, at a maximum of one year after sampling. A biobank will be created, solely containing these blood samples. Until analysis, the blood samples are kept in a locked freezer at -80°C (with a temperature-alarm) in a locked room at Gastroenheden D, Herlev Hospital. The blood samples will be analyzed with the RIA-technique at Gaslab, Herlev Hospital.

Approvals

Approval from Danish Data Protection Agency (Datatilsynet) will be sought in accordance with The Act on Processing of Personal Data (Persondataloven), and the recruitment of subjects for the study will not begin until this approval has been given. The same applies to approvals by the local ethics committee and the Danish Medicines Agency. Permission will be sought to register and store data manually and electronically from participants in the study. Data will be registered prospectively in CRF.

Enrollment of participants

The healthy female volunteers will be recruited through ads on www.forsoegspersoner.dk, ads in Medicinerorganisationernes Kommunikationsorgan (www.mok.dk) as well as on www.facebook.com. The participants will contact the investigator by email or telephone, and an information meeting where a companion is allowed, will be arranged.

Subjects eligible for inclusion will be informed about the study by the investigator at a meeting prior to the study-sessions. At this meeting they are allowed to bring a companion. The subject will receive both verbal and written information at this meeting. The subject will, before the information regarding the study is given, be informed that:

- It is an enquiry about willingness to participate in the study.

- Be informed that the study has been approved by the Danish Data Protection Agency, the Danish Committee on Health Research Ethics, and the Danish Medicines Agency.
- The subject has the right to a minimum of 24 hours for consideration.

The information will be given in a room where both the investigator and the subject can sit comfortably without disturbances. The subject will be given enough time to read and listen to the information and ask questions.

Insurance

For this study no additional insurance policies have been arranged that exceed the *Danish Act on the Right to Complain and Receive Compensation within the Danish Health Service* (Patientforsikringsloven). Volunteers participating in this study will be covered by the mentioned Act in accordance with "Bekendtgørelse af lov om klage- og erstatningsadgang inden for sundhedsvæsenet" in the same way as patients that are treated otherwise in the hospital. This is normal practice for investigator-initiated trials in Denmark.

Assurance of quality

The present procedures for quality control and assurance will be followed according to the EU GCP guidelines through planned and systematic agreements on monitoring before, during, and after the trial. A written authority for a third party (e.g. GCP monitor, the Danish Medicines Agency, etc.) for the access to medical records will be collected together with the informed, written consent.

Payment of participants

Participants will be paid DKK 150.00 every hour for the nine hours of study day 1-4, plus DKK 150.00 for each of the 24-hour blood samples. Participants will be paid DKK 150.00 every hour for the 17 hours of the transdermal study day, plus DKK 150.00 for the 24- and 48-hour blood samples. In total, the participants will be paid DKK 8,850.00. The participants will receive the entire DKK 8,850.00 upon finishing all five study-days and the 24- and 48-hour blood samples. Should a participant withdraw their consent or be excluded from the trial at any point, they will receive compensation corresponding to the amount of hours they have participated. There will be no reimbursement of transportation for the participants.

Ethics

The study will be performed in accordance with the Helsinki II declaration. Subjects will be included after oral and written consent. Subjects can withdraw from the study at any time without stating a reason. All information regarding participants in this study will be kept protected and no personal data will be published. The anonymity of all participants will be secured and data kept confidential. This study will only be initiated after approval from the ethics committee and the National Board of Health and will be carried out in accordance with the Helsinki declaration.

Risks and benefits of the study

There are minimal risks for the subjects, which are the discomfort of the five different routes of administration, as well as the plasma sampling, and the small risk of any adverse event.
The benefit of the study is clarification of the pharmacokinetics of melatonin when established rectally and intravesically. This knowledge is essential before studies investigating the radioprotective effects of locally administered melatonin in patients receiving ionizing radiation.

The benefit for the subjects is the financial compensation they receive, as described above.

Financing

The sponsor and investigators from Herlev Hospital have taken the initiative for this study. The funding of the study is paid through grant applications. The company RepoCeuticals ApS has agreed to donate an unrestricted grant of DKK 270,293.50 for the study to the Department of Surgery, Herlev Hospital. It will be administered at a research account by the hospital. All costs will be covered by the research account, and will be fully transparent. Neither the sponsor or investigators have any interests in RepoCeutical ApS; financial or otherwise.

Publication policy

The study will be published in an international peer-reviewed scientific journal. Both negative, positive, and inconclusive results will be published. The determination and documentation of authorship will be based on the authorship criteria and recommendations formulated by the International Committee of Medical Journal Editors (ICMJE).

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