The analgesic effects of exogenous melatonin in humans

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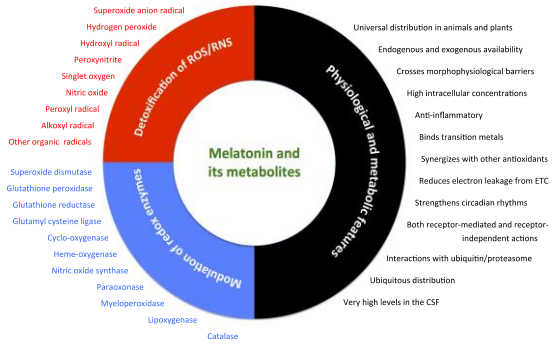
THE 5 ORIGINAL PAPERS ARE

1. Andersen LP, Werner MU, Rosenberg J, Gögenur I. A systematic review of peri-operative melatonin. Anaesthesia 2014;69:1163-71.
2. Andersen LP, Gögenur I, Fenger AQ, Petersen MC, Rosenberg J, Werner MU. Analgesic and anti-hyperalgesic effects of melatonin in a human inflammatory pain model: a randomized, double-blind, placebo-controlled, three-arm crossover study. Pain 2015;156:2286-94.
3. Andersen LP, Werner MU, Rosenkilde MM, Harpsøe NG, Fuglsang H, Rosenberg J, Gögenur I. Pharmacokinetics of oral and intrave-nous melatonin in healthy volunteers. BMC Pharmacol Toxicol 2016;17:8
4. Andersen LP, Werner MU, Rosenkilde MM, Fenger AQ, Petersen MC, Rosenberg J, Gögenur I. Pharmacokinetics of high-dose intravenous melatonin in humans. J Clin Pharmacol. 2016 Mar;56:324-9.
5. Andersen LP, Rosenberg J, Torup H, Gögenur I, Werner MU. Assessment of post-operative analgesic drug efficacy: method of data analysis is critical. *Manuscript*.

INTRODUCTION

The pineal gland (Glandula Pinealis) was first described by the Greek medical physician, surgeon and philosopher Galen (131 C.E. - 201 C.E.). He named the gland from the resemblance in shape and size of the seeds found in the cones of the stone pine (*Pinus Pinea*). Later, in the renaissance, the French mathematician Descartes (1596 – 1650) described the function of the gland as "the principal seat of the soul". The secretory product, *melatonin* was first isolated from bovine pineal glands, and named by the North-American dermatologist Aaron B. Lerner (September 21, 1920 – February 3, 2007) in 1958 [1]. Later in 1975, Lynch and colleagues described the circadian secretion pattern of melatonin in humans [2]. The following decades, evidence was gathered that melatonin controls photo-periodically mediated endocrine events, such as seasonal reproduction competence through interaction with the hypothalamo-pituitary-gonadal axis in non-human photoperiodic mammals [3]. Similarly, it is now widely accepted that endogenous melatonin controls circadian rhythms, both daily and seasonally [4]. A major discovery was made in 1993, when the anti-oxidative- and free-radical scavenger effects of melatonin were first described [5]. Another scientific breakthrough came in 1995 when melatonin was demonstrated in plants, indicating a universal function and ubiquitous distribution of the compound (see figure 1) [6].

Figure 1:



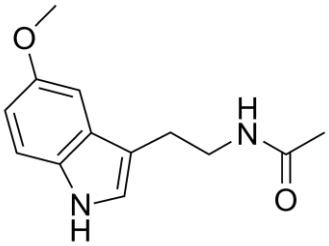
The widespread functions and ubiquitous distribution of melatonin.

BACKGROUND

The physiology and metabolism of endogenous melatonin in humans

The human pineal gland is located in the midline behind the third ventricle [7]. The gland measures 12 x 8 x 4 mm, weighing approximately 100 mg. The gland consists of pinealocytes, interstitial cells, phagocytes and neurons [7]. The gland is supplied by aa. Pineales (aa. Choroideae posteriores) [7]. Capillaries are fenestrated, and, hence, no blood-brain barrier exists [7].

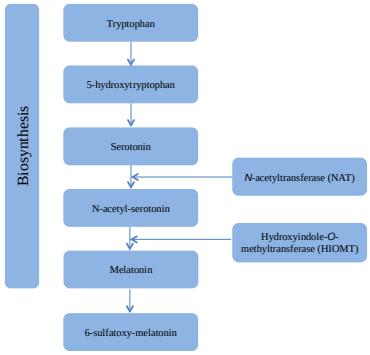
Figure 2:



The chemical structure of melatonin.

The main secretory product is the indoleamine, melatonin (N-acetyl-5-methoxytryptamine) (see figure 2). Melatonin is produced from tryptophan, which is converted to serotonin via 5-hydroxytryptophan [8]. Serotonin is then converted to N-acetyl-serotonin by the enzyme N-acetyltransferase (NAT). Finally, the rate-limiting enzyme, hydroxyindole-O-methyltransferase (HIOMT) converts N-acetyl-serotonin to melatonin (see figure 3) [8].

Figure 3:



The biosynthesis of melatonin.

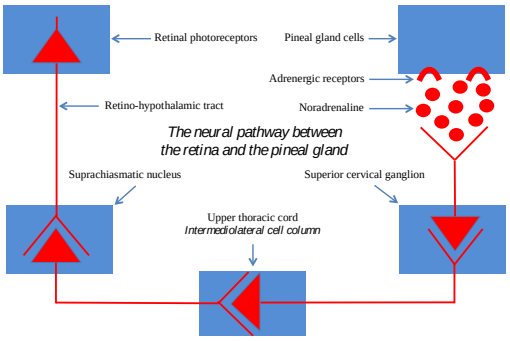
In mammals (including humans), melatonin is primarily produced in the pineal gland, however, other tissues, such as the retina, bone marrow, lymphocytes, skin, uterus and gastrointestinal-tract also contribute to a peripheral production of the compound [9].

Regulation of melatonin production is complex, however, main external input is mediated through the retino-hypothalamic tract [7, 8]. Retinal photoreceptors transmit to the suprachiasmatic nucleus (SCN) of the hypothalamus, further by descending pathways to the intermediolateral cell column of the upper thoracic cord, and finally to the superior cervical ganglion, which projects sympathetic neurons to the pineal gland (see figure 4) [7, 8].

Activated surface alpha$_1$- and beta$_1$-adrenergic receptors increase intracellular cAMP concentrations, inducing transcription of key enzymes in the melatonin biosynthesis. External light/dark-stimulation and the endogenous circadian rhythm of the SCN control the synthesis [8].

The physiological functions of endogenous melatonin in humans are widespread, denoted by specific melatonin receptors located widely in the human body [10]. Evidence shows that melatonin provides an endogenous synchronizer, stabilizing circadian rhythms [10]. These include direct- and indirect modulations of blood pressure, body temperature, cortisol rhythm, sleep-wake-cycle, immune function and anti-oxidative defence [10]. Furthermore, preliminary evidence indicates that human reproduction function may also be modulated by melatonin input [11].

Figure 4:



The neural pathways between the retina and the pineal gland.

Melatonin plasma levels in human adults range from 0-5 pg/mL during daytime to peak levels of 50-100 pg/mL at 2-4 a.m. [12]. Melatonin is hydroxylated by cytochrome P-450 (CYP)-enzymes (mainly CYP 1A2) in the liver yielding 6-hydroxy-melatonin, which is conjugated to sulphuric- or glucuronic acid, and excreted in the urine. The 24-hour excretion of the main urine metabolite, 6-sulfatoxymelatonin correlates to the area-under-the-curve plasma profile of endogenous melatonin [13].

The clinical use of exogenous melatonin

In the U.S., exogenous melatonin is sold as a non-prescription drug/food supplement without FDA-approval. In the E.U. countries, melatonin is categorized as a prescription drug, and approved for medical treatment of primary insomnia in people over 55 years of age. The Danish Health and Medicines Agency reports that 72.400 packages, containing 30 tablets of Circadin® (melatonin 2 mg, slow-release tablet) were prescribed in 2013 (Latest data; http://www.medstat.dk). An additional 29.700 packages, containing 100 tablets of 3 mg melatonin (magistral production) were also prescribed.

Melatonin has been investigated for a number of clinical conditions. A recent meta-analysis investigated sleep latency, overall sleep quality and total sleep time in patients suffering from primary sleep disorders [14]. The analysis included 19 randomized placebo-controlled studies (n = 1683). Patients received melatonin from 7 days up to 182 days in doses ranging from 0.1 mg - 5 mg and 0.05 mg/kg - 0.15 mg/kg. Melatonin reduced sleep onset latency, improved overall sleep quality and increased total sleep time. Other meta-analyses indicate similar beneficial hypnotic effects in jet lag [15] and shift work [16], though evidence levels are limited. Symptoms associated with neurological- and psychiatric diseases, such as Alzheimer's disease [17], ADHD [18] and depression [19], have also been treated effectively using melatonin. The literature is, however, limited, and findings have been inconsistent. Preliminary, randomized studies have also indicated clinical- and biochemical improvements of melatonin in various medical diseases, such as hypertension [20], metabolic syndrome [21], and chronic obstructive pulmonary disease (COPD) [22]. Finally, a number of low-volume studies have documented improved prognosis in terminal cancer patients [23]. In perioperative medicine, a number of randomized studies have indicated anxiolytic, analgesic and sleep-regulating effects of exogenous melatonin [24]. Finally, in critical care studies investigating sleep quality and certain types of delirium, exogenous melatonin has demonstrated promising, but preliminary results [25].

The analgesic action of melatonin in animals

A large number of experimental studies have evaluated the anti-nociceptive, anti-hyperalgesic, anti-inflammatory and anti-allodynic actions of exogenous melatonin [26-28]. The studies on pain-like behaviour have primarily included rodents, such as mice and rats. Exogenous melatonin was administered intraperitoneally, intrathecally, intravenously, subcutaneously, and orally, in doses ranging from 0.1 to 300 mg kg^{-1} BW [26-28]. Several pain models were employed, mimicking clinical pain states, such as *acute pain*, *inflammatory pain* and *chronic/neuropathic pain*. *Acute pain* was assessed by e.g. tail-flick test to hot water [29], hot-plate test [30], electrical tail stimulation [31] and various pinch/clamp procedures [32]. *Inflammatory pain* was provoked by injection of substances, such as carrageenan [31, 33] or formalin [34]. Finally, *chronic/neuropathic pain* was provoked by e.g. nerve ligation [35]. Exogenous melatonin produced significant dose-dependent anti-nociceptive effects in all presented experimental models of pain [26-28].

The mechanisms of action probably result from activation of specific MT_1 and MT_2 receptors (see figure 5) [36]. In mammals, these G-protein-coupled receptors are densely located in the suprachiasmatic nucleus and in pars tuberalis of the pituitary gland [37]. Other locations include dorsomedial and ventromedial hypothalamic nuclei, anterior hypothalamus, medial preoptic area, paraventricular thalamic nuclei, hippocampus, cerebral cortex, area postrema, amygdale and retina [37]. Interestingly, melatonin-receptors are also located in the dorsal horn of the spinal cord [38]. Activation of these receptors generally reduces intracellular formation of cyclic AMP, however, a number of second messenger molecules, such as cGMP, diacylglycerol, inositol triphosphate, arachidonic acid and intracellular Ca^{+2} are also regulated by these receptors [39].

Exogenous melatonin does not interact directly with opioid-receptors; however, several experimental studies indicate significant interactions between the endogenous opioid system and melatonin. Lakin and colleagues demonstrated that the anti-nociceptive effects of opioids were reduced in pinealectomized mice [40]. Correspondingly, the opioid-receptor antagonist, naloxone reversed the anti-nociceptive effects of exogenous melatonin [40]. Also, opioid-fibers- and receptors have been demonstrated in the pineal gland of mammals [41, 42]. Finally, experimental cell studies document, that exogenous melatonin increases the release of endogenous opioid compounds [43]. An interaction with the GABAergic system has also been established [44]. GABA-currents are potentiated by melatonin in cultured spinal cord neurons [45]. Furthermore, Golombek and colleagues demonstrated that the anti-nociceptive effects of melatonin could be reversed by the administration of flumazenil, a specific benzodiazepine antagonist [30]. Other receptor systems, such as the N-methyl-D-aspartate (NMDA) receptors may also be involved. Studies demonstrated that NMDA-activity and long-term potentiation in the hippocampus was reduced by exogenous melatonin [46].

Furthermore, the anti-nociceptive effects of melatonin in a formalin test were inhibited by the administration of various potassium ion-channel-blockers, indicating that anti-nociceptive effects of melatonin required potassium channel activation [47]. Similarly, melatonin inhibited Ca^{+2} channel currents in cultured dorsal root ganglion neurons [48].

Finally, melatonin has well-established anti-oxidative and anti-inflammatory properties [49]. Melatonin acts as a direct free radical scavenger (e.g. $OH^{\bullet}$, $NO^{\bullet}$), and activates anti-oxidative enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, gamma-glutamylcysteine synthetase and glutathione reductase [50]. Also, melatonin regulates inflammatory pathway signalling substances (e.g. cyclooxygenase [COX]), attenuating inflammatory processes, as documented in histological analyses following carrageenan injection in rats [33]. These combined effects may potentially reduce the peripheral inflammatory reactions, and subsequent hyperalgesia in relation to tissue damage.

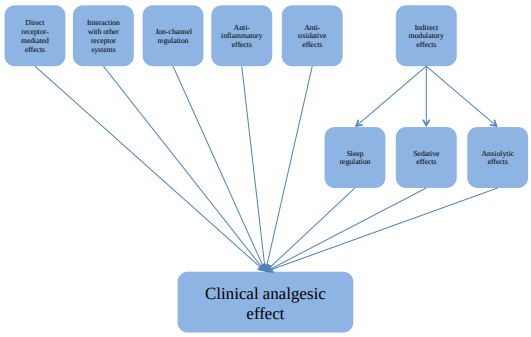
The analgesic effect of melatonin in humans

A recent randomized, double-blind, placebo-controlled, dose-response experimental study investigated the analgesic effect of exogenous melatonin in humans [51]. The study included 60 volunteers, and employed quantitative sensory testing (QST) to measure pressure pain threshold/tolerance and heat pain threshold/tolerance. Sublingual melatonin (0.05 mg/kg; 0.15 mg/kg; 0.25 mg/kg) or placebo was administered 30 min before test procedures. QST-variables were significantly increased dose-dependently by exogenous melatonin. Furthermore, melatonin plasma levels correlated to analgesic effects.

A number of clinical studies have assessed analgesic effects of exogenous melatonin [24]. Melatonin was administered as part of the analgesic regimen before intubation procedures in pre-term infants [52]. The study included 60 randomized patients. Ten mg kg^{-1} BW of intravenously administered melatonin significantly reduced pain scores up to 72 hours after administration, assessed by the premature infant pain profile (PIPP) score. Nociceptive reflexes during intubation, assessed by the neonatal infant pain scale (NIPS) did not differ between the groups. Interestingly, pro-inflammatory cytokine levels (interleukin (IL) 6, 8, and 12) were also significantly reduced in patients receiving melatonin.

In adult surgical patients, a randomized placebo-controlled study including 52 patients administered 6 mg x 2 of oral melatonin before open prostatectomy (see table 1) [53]. The study documented reduced intra- and postoperative analgesic consumption, and reduced postoperative pain intensity ratings [53]. Similarly, two randomized studies including 33 and 59 patients, administered 5 mg x 2 of oral melatonin before open hysterectomy [54, 55]. Melatonin reduced postoperative analgesic consumption, and postoperative pain intensity ratings compared to placebo [54, 55]. In ophthalmological procedures, a randomized study in 40 patients administered 10 mg of oral melatonin [56]. The study documented reduced intraoperative analgesic consumption, and reduced intra- and postoperative pain intensity ratings compared to placebo [56]. Another randomized placebo-controlled study in 40 patients undergoing hand surgery, receiving 10 mg of oral melatonin, documented reduced intra- and postoperative analgesic consumption, and reduced intraoperative pain intensity ratings [57]. Finally, a randomized placebo-controlled study in 53 patients undergoing laparoscopic cholecystectomy, administering 3 mg x 2 of sublingual melatonin before surgery, documented reduced intraoperative analgesic consumption [58]. However, nine randomized studies in different surgical procedures did not document any clinical effects of exogenous melatonin with respect to either analgesic consumption or pain intensity ratings compared to placebo [59-66].

Figure 5:



Possible analgesic actions of melatonin in humans.

A limited number of randomized studies have indicated that exogenous melatonin can be administered as analgesic treatment in complex chronic pain states, such as fibromyalgia [67, 68], inflammatory bowel syndrome [69, 70], cluster headache [71], endometriosis [72] and temporomandibular pain disorder [73].

OBEJCTIVES OF THE THESIS

Hypothesis of the thesis

We hypothesized that exogenous melatonin would possess analgesic effects in humans. Correspondingly, that the drug could be incorporated as a safe and effective analgesic drug in clinical practice.

Aims of the thesis

The aim of the thesis was to provide a systematic review of the literature concerning melatonin treatment in the perioperative period (see figure 6). Furthermore, we aimed to investigate the analgesic, anti-hyperalgesic and anti-inflammatory effects of exogenous melatonin in a validated experimental pain model in humans. Also, detailed pharmacokinetic analyses of oral and intravenous melatonin should be performed in order to estimate pharmacokinetic variables of exogenous melatonin. Finally, a novel assessment method for postoperative analgesic efficacy is presented and compared to existing statistical methods, potentially providing methods applicable for future analgesic drug trials.

METHODS

The human burn model (study I)

The human first degree burn provides the template for a validated inflammatory pain model [74-76]. In the present experimental setup, a computerized contact thermode (MSA Thermal Stimulator, Somedic AB, Hörby, Sweden; 5.0 x 2.5 cm^2) delivered a 47.0 ºC, 420 s burn, located at the medial part of the calf. Analgesic and anti-hyperalgesic effects were assessed by a standardized psychophysiological test battery. Pain ratings during the burn injury, and quantitative sensory testing (QST) including assessments of secondary hyperalgesia areas, mechanical- and thermal thresholds in the burn injury area, and pressure algometry, were performed.

The pain model has previously documented analgesic and anti-hyperalgesic effects of adenosine [77], non-steroidal anti-inflammatory drugs (NSAIDs) [78], lidocaine [79], ketamine [80] and opioids [81]. The model provides a well-documented, anatomically, spatially and temporally well-defined first-degree burn injury [74]. The induced inflammatory response is accompanied by significant changes in evoked pain responses, and in skin colour characteristics, consistent with the principal components of acute inflammation [74]. Furthermore, QST-outcomes have been correlated to analgesic efficacy and clinical pain [82]. During the burn injury, A-delta-fibers (myelinated; conduction velocities = 12 – 30 m/s) and C-fibers (unmyelinated; conduction velocities = 0.5 – 2.0 m/s) are activated [83]. After the burn, two distinctly separate pain phenomena are observed, *primary*- and *secondary hyperalgesia* [74]. Hyperalgesia refers to a state of "increased pain from a stimulus that normally provokes pain" [84]. *Primary* and *secondary* refers to skin areas inside- and adjacent to the burn area [85]. In the burn area, *primary hyperalgesia* is characterized by lowered mechanical- and thermal thresholds [74]. These changes are caused by sensitization of A-fiber mechano-heat–sensitive (MHS) nociceptors and C-fiber MHS nociceptors [86]. *Secondary hyperalgesia* is characterized by lowered mechanical thresholds (*but not thermal thresholds*) in the adjacent undamaged skin area [74]. This process requires a subset of the A-fiber nociceptors, that are neither capsaicin-sensitive nor heat-sensitive, and result from complex central processes in the dorsal horn of the medulla, involving wide-dynamic range (WDR) neurons, as well as descending modulatory inputs [86].

Inflammation analysis of the skin (study I)

Skin-related inflammation was assessed as changes in skin erythema and dermal thickness in the burn area using a combined light absorption skin analyser and high-frequency ultrasound scanner (Dermascan, Cortex Technology, Hadsund, Denmark). Skin erythema was measured by light absorption analysis using the erythema/melanin colour system. This technique has been used in a number of studies investigating inflammatory responses in the human burn model [87-89]. Dermal thickness was measured by a 20 MHz transducer, providing a 2D high-resolution ultrasound image (micrometers; 60 x 200 microns; penetrance = 23 mm) of the dermis [90]. Our study is the first to employ this non-invasive, but in-depth assessment of the inflammatory skin changes following an experimental burn.

Pharmacokinetic analyses (study III and IV)

Melatonin plasma levels were determined by radioimmunoassay technique (RIA) (Melatonin Direct RIA, DIAsource, Louvaine-La-Neuve, Belgium) in accordance to previous studies [91]. The pharmacokinetic analyses in study III and IV were performed employing Graph Pad Prism 6 (Graph Pad Software Inc., La Jolla, CA, USA).

For oral melatonin, time to maximal concentrations (t_{max}) and maximal plasma concentrations (C_{max}) were assessed directly. The pharmacokinetic variables: absorption constant (k_a), absorption half-life ($t_{1/2\ absorption}$), elimination rate constant (k_e) and elimination half-life ($t_{1/2\ elimination}$) were estimated by "the method of residuals" [92]. Areas-under-the-curve (AUC) of plasma concentrations were calculated by applying the trapezoidal rule [93]. $AUC_{0-\infty}$ was estimated as $AUC_{0\text{-}420\ min} + (C_{420\ min}/k_e)$. Bioavailability was calculated as ($AUC_{0-\infty\ oral}$ / $AUC_{0-\infty\ IV}$) x 100.

For intravenous melatonin, C_{max} was assessed directly at the time point 0 minutes after bolus injection. The pharmacokinetic variables: $t_{1/2\ elimination}$, volume of distribution (V_d) and clearance (CL) were estimated from individual linear regression lines of log-transformed (natural logarithm) plasma concentrations. Following standard equations were applied: $t_{1/2\ elimination} = \ln(2) / k_e$, V_d = dose / $C_{0\ min}$, $CL = k_e \times V_d$. $AUC_{0-\infty\ IV}$ was estimated as described above.

All pharmacokinetic variables were estimated individually for each volunteer, and summarized according to the distribution, as mean (SD) or median (IQR).

In study III, "Goodness of fit" of the individual linear regression lines (ln(melatonin plasma concentrations) vs. time) were estimated by the coefficient of determination (R^2). R^2 ranges from 0 and 1, and has no units (0 indicates a horizontal line going through the mean of all Y-values; 1 represents a perfect fit with no scatter). R^2 is calculated from the sum of the squares of the distances of the data points from the best-fit curve determined by regression analysis [94], applying the formula $R^2 = 1 - (SS_{res} / SS_{tot})$.

Assessment of sedative effects (Study IV)

Sedative effects of melatonin were evaluated as simple reaction times. Simple reaction times measure the cognitive integration of visual input and psychomotor function, and have previously been applied to assess the sedative effects of other psychoactive drugs [95, 96]. We applied a web-based test (http://getyourwebsite-here.com/jswb/rttest01.html). Each volunteer was instructed to press the mouse button as soon as possible, when a stoplight displayed on a computer screen, changed from red to green.

Assessment of analgesic drug efficacy (study V)

Analgesic drug efficacy was assessed by an integrated endpoint including longitudinally measured Pain Intensity and Opioid Consumption, *the PIOC*. A related, but less detailed method was originally presented by Silverman and colleagues [97]. Visual analogue scale (VAS) pain intensity ratings were employed as direct, simple and informative indices of patient pain, corroborating previous *melatonin-specific* analgesic drug trials [98]. Area-Under-the-Curve (AUC) of VAS pain intensity ratings were calculated in order to provide a 'weighted' and dynamic measure of patient pain [93]. Correspondingly, opioid consumption was included as a simple indirect analgesic outcome. Collectively, the PIOC integrates two standard inter-dependent analgesic outcomes, lowers the risk of mass significance, increases the statistical power, and provides a more comprehensive representation of analgesic drug efficacy and postoperative pain.

STUDIES

Study I

Abstract

Aim

We aimed to review all randomized studies investigating perioperative melatonin.

Methods

The review was performed according to the PRISMA-guidelines. Patient material, surgical procedure, anaesthetic technique, melatonin regimen and perioperative outcomes were evaluated for each of the included studies.

Results

24 studies (1,794 participants), reporting eight perioperative outcomes: anxiety; analgesia; sleep quality; oxidative stress; emergence behaviour; anaesthetic requirements; "steal induction"; and safety, were included. Melatonin reduced the standardized mean difference (95% CI) pre-operative anxiety score by 0.88 (0.44-1.33) and postoperative pain score by 1.06 (0.23-1.88), compared to placebo. The statistical heterogeneity (I^2) was 87% and 94%, respectively. Qualitative reviews indicated that melatonin may improve sleep quality and emergence behaviour, and possibly reduce oxidative stress and anaesthetic requirements.

Conclusions

Melatonin may reduce preoperative anxiety and postoperative pain. However, future high-powered studies are needed to determine a definite clinical effect. Also, optimal dosage, dosing intervals and administration routes should be investigated further.

Strengths

The review provides the first systematic review of randomized clinical trials investigating the collective clinical effects of melatonin in the perioperative period. Furthermore, the review included meta-analyses of the outcomes, *preoperative anxiety and postoperative pain*. Meta-analyses were performed in accordance to the standards described by the Cochrane Handbook for Systematic Reviews of Interventions [99]. Method quality assessments tools, such as the "Jadad" score and Cochrane's risk of bias tool were applied [99, 100]. Finally, clear definition of primary outcomes and *pre-study* sample size estimations were evaluated for each of the included studies.

Limitations

Although a systematic literature search was performed in five major medical literature databases, a hypothetical risk of missing studies, which are not indexed or written in a non-English language, cannot be excluded. Another limitation was the extensive methodological differences between the included studies. These differences comprised variable melatonin dosages, administration routes, timing of administrations, surgical procedures, anaesthetic techniques and perioperative outcomes. Hence, quantitative evaluations, in terms of meta-analyses, were only considered feasible with the outcomes, *preoperative anxiety* and *postoperative pain* [98]. Further quantitative estimations e.g. of possible publication bias by funnel plots could have been included [99]. However, due to the low number of heterogeneous studies, and the preliminary nature of our quantitative analyses, further data-synthesis, would not add further knowledge in these issues in our opinion. Conclusively, the study heterogeneity and the variable ratings in the method quality assessments, indicate that the results of the review should be interpreted with caution. Hence, no final recommendations concerning neither analgesic nor anxiolytic effects of melatonin can be established from our data.

Study II

Abstract

Aim

The aim of this study was to investigate the analgesic, anti-hyperalgesic and anti-inflammatory properties of melatonin, employing a validated human burn injury model.

Methods

The study was designed as a randomized, double-blind, placebo-controlled, three-arm crossover study. Each volunteer participated in three identical study sessions, receiving intravenous administration of 10 mg melatonin, 100 mg melatonin and placebo. Sixty minutes after administration of study medication (bolus injection), a burn injury was performed employing a computerized contact thermode (47.0°C, 420 s, 5.0 x 2.5 cm^2). Pain was rated during the burn injury, and quantitative sensory testing (QST) was performed at baseline, and, 1, 2, 4 and 6 hours after the burn injury. QST encompassed assessments of secondary hyperalgesia areas, mechanical and thermal thresholds in the burn injury area, and pressure algometry. Also, skin-reflectance spectrophotometry and high-resolution ultrasonography were employed to measure skin erythema and dermal thickness in the burn injury area.

Results

Twenty-nine healthy male volunteers completed the study. No significant effects of melatonin were observed with respect to primary or secondary outcomes compared to placebo. Melatonin was not associated with any adverse effects.

Conclusions

Melatonin did not demonstrate any analgesic, anti-hyperalgesic or anti-inflammatory effects in the human burn injury model.

Strengths

Study II investigated the analgesic, anti-hyperalgesic and anti-inflammatory effects of melatonin employing a validated inflammatory pain model in humans. The study was designed as a randomized, double-blind, placebo-controlled crossover study. Two separate dosages of melatonin, 10 mg and 100 mg, respectively, were administered intravenously in order to ensure optimal tissue delivery, and to document a possible analgesic dose-response relationship. The outcomes included a test battery of standard QST outcomes.

Limitations

Experimental animal studies document dose-dependent anti-nociceptive effects in dosages up to 300 mg kg^{-1} BW [26-28]. Based on the findings of these studies, it may be speculated that 10 mg and 100 mg of intravenous melatonin, respectively are insufficient to produce significant analgesic effects. Furthermore, four of six clinical studies demonstrating positive results, administered two separate preoperative dosages of melatonin [53-55, 58]. Hence, melatonin regimens including multiple dosages, administered up to 12 hours before the surgical trauma may be needed to induce significant clinical analgesic effects. Furthermore, the present study only included healthy male volunteers in order to standardize the subject sample. Consequently, the applied experimental setup may limit the external validity of our data. Previous experimental studies, however, did not document significant differences with respect to QST outcomes (*primary*) in the human burn model [101]. Correspondingly, no evidence indicates that melatonin provides differential analgesic effects between genders. Another limitation is the restricted tissue damage (*spatial and temporal*) induced by the burn injury, which may be insufficient to document significant analgesic effects of melatonin. Correspondingly, previous studies investigating other well-established analgesics, e.g. local anaesthetics [102], NSAIDs [88] and glucocorticoids [89], have been unable to demonstrate analgesic efficacy, employing the present pain model. Finally, our experimental test-paradigm may not be ideal to demonstrate the analgesic effects of melatonin. The collective anxiolytic, sleep-regulating and analgesic effects of melatonin may be needed to document significant clinical analgesic effects of the drug.

Study III

Abstract

Aim

The aim was to investigate the pharmacokinetics of oral and intravenous melatonin in healthy volunteers.

Methods

The study was designed as a crossover study**.** On two separate study days, each volunteer received 10 mg oral melatonin or 10 mg intravenous melatonin. Blood samples were collected at specified time points before and after administration of oral and intravenous melatonin (bolus). Plasma melatonin concentrations were determined by RIA technique. Pharmacokinetic analyses included "the method of residuals" and compartmental analysis. k_a, $t_{1/2\ absorption}$, t_{max}, C_{max}, $t_{1/2\ elimination}$, $AUC_{0-\infty}$, and bioavailability were estimated for oral melatonin. C_{max}, $t_{1/2\ elimination}$, V_d, CL and $AUC_{0-\infty}$ were estimated for intravenous melatonin.

Results

Twelve male volunteers completed the study. Baseline melatonin plasma levels were not significantly different between the study days ($P > 0.05$). Mean (SD) $t_{1/2\ absorption}$ of oral melatonin was 6.0 (3.1) minutes. Mean t_{max} was 40.8 (17.8) minutes with a median (IQR) C_{max} of 3,551 (2,501 - 8,058) pg ml^{-1}. Mean $t_{1/2\ elimination}$ was 53.7 (7.0) minutes. Median absolute bioavailability was 2.5 (1.7 - 4.7) %. Median C_{max} after iv bolus injection of melatonin was 389,875 (174,775 - 440,363) pg ml^{-1}. Mean $t_{1/2\ elimination}$ was 39.4 (3.6) minutes, mean V_d 1.2 (0.6) l kg^{-1} and mean CL 0.0218 (0.0102) l min^{-1} kg^{-1}.

Conclusions

The study estimated pharmacokinetic variables of oral and intravenous melatonin, in healthy male volunteers. Bioavailability of oral melatonin was only 3%.

Strengths

Study III estimated pharmacokinetics of oral and intravenous melatonin in dosages administered in perioperative clinical trials. The study applied standard pharmacokinetic methods, such as "feathering method" and compartmental analysis. A crossover study design was employed to reduce inter-individual difference in pharmacokinetic variables.

Limitations

The present study only included healthy male volunteers and, hence, the external validity of data may be compromised by potential gender differences in pharmacokinetic variables. Currently, however, no such differences genders have been reported [103]. Also, the included volunteers had a mean (SD) age of 27.1 (5.2) years, and it is possible that pharmacokinetic variation between other age groups may exist. Furthermore, preliminary studies indicate that external factors, such as smoking [104], caffeine [105] and oral contraceptives [106] may alter pharmacokinetic variables. Finally, our data documented a bioavailability of approximately 3%. Other experimental studies have reported mean values ranging between 9% and 30% [107]. We chose to imitate a clinical premedication scenario, and hence only a limited volume of tap water (5 cl) was allowed to facilitate intake of study medication. It could be speculated, that the administered dose of melatonin was incompletely absorbed. However, an easily dissoluble gelatine-capsule was employed, making incomplete absorption unlikely. Furthermore, naturally occurring gastric- and intestinal fluid secretion would facilitate the dissolving process. Finally, t_{max} values correlated between subjects, indicating an adequate physiological absorption process.

Study IV

Abstract

Aim

The aim was to investigate the pharmacokinetics and adverse effects of high-dose intravenous melatonin.

Methods

The study was designed as a crossover study. Volunteers participated in three identical study sessions, receiving an intravenous bolus of 10 mg melatonin, 100 mg melatonin and placebo. Blood samples were collected at baseline, and, 0, 60, 120, 180, 240, 300,

360, 420 min after bolus injection. Plasma melatonin concentrations were determined using RIA-technique. Pharmacokinetic variables were estimated by compartmental analysis. Registration of adverse effects included assessments of sedation, and other symptoms of possible adverse effects. Sedative effects, defined as simple reaction times, were measured at baseline, and, 120, 180, 300 and 420 minutes after administrations of study medication.

Results

Twelve male volunteers completed the study. Median (IQR) C_{max} after bolus injection of 10 mg and 100 mg of melatonin were 221,500 (185,638 – 326,176) pg ml^{-1} and 1,251,500 (864,375 – 1,770,500) pg ml^{-1}, respectively; mean (SD) $t_{1/2}$, 42.3 (5.6) minutes and 46.2 (6.2) minutes; mean (SD) V_d, 1.6 (0.9) l kg^{-1} and 2.0 (0.8) l kg^{-1}; mean (SD) *CL*, 0.0253 (0.0096) l min^{-1} kg^{-1} and 0.0300 (0.0120) l min^{-1} kg^{-1}; and median (IQR) $AUC_{0\text{-inf}}$, 8,634,813 (6,071,696 – 11,602,812) pg ml^{-1} min and 54,685,979 (36,028,639 – 105,779,612) pg ml^{-1} min. Sedation was not significantly different between the treatment groups. No other adverse effects were reported.

Conclusions

The study provides pharmacokinetic data of high-dose intravenous melatonin in healthy volunteers. Melatonin did not induce sedation or other adverse effects.

Strengths

Study IV estimated pharmacokinetic variables of high-dose intravenous melatonin in healthy male volunteers. Furthermore, the study provided data concerning a possible dose-response relationship with respect to sedative effects and self-reported adverse effects. The study applied standard pharmacokinetic compartmental analysis. A crossover study design was employed to reduce possible inter-individual difference of the pharmacokinetic variables.

Limitations

Due to the experimental setup, plasma samples were not collected between 0 and 60 minutes. It is possible that the estimated pharmacokinetic variables would appear differently, if additional plasma samples were collected. Another limitation refers to the fact that the study only included healthy male subjects. The external validity of pharmacokinetic variables is, therefore, limited with respect to gender and age. No significant gender difference in the pharmacokinetics of melatonin has, however, been reported so far [103]. However, it is possible that the pharmacokinetic variables estimated in our study would have appeared differently, if other age groups were examined. With respect to estimations of sedative effects, the present study did not document any difference between the treatment groups. The study included a subgroup of volunteers from study II and, hence, no pre-study sample size was estimated from the evaluated outcomes (sedative effects/other adverse effects). A post-hoc sample size calculation applying mean (SD) AUC sedation scores (placebo group vs. 10 mg melatonin group / placebo group vs. 100 mg melatonin group), a significance level of 5% (α) and a power of 80% (1-β), demonstrated a requirement of 13 and 44 volunteers, respectively. Our study did not fulfil these criteria, and it is therefore possible that our inability to document significant differences may result from a lack of statistical power (type II error). Still, as displayed in figure 3, the 10 mg melatonin dose appeared to induce more sedation than the 100 mg dose, which would seem unreasonable, if a direct dose-response relationship of melatonin is assumed. Moreover, only a small, clinically irrelevant difference of up to 0.02 seconds in mean sedation scores was observed between the treatment groups. These non-significant numerical differences are therefore likely to be coincidental, rather than representing an actual clinical difference between the groups.

Study V

Abstract

Background

The aim of this study was to re-analyse original data obtained from a postoperative analgesic drug trial, employing a collection of standard statistical methods in analgesic outcome assessments. Also, a modified integrated assessment method was evaluated.

Methods

Data originated from a randomized, double blind, placebo-controlled trial investigating the analgesic efficacy of a regional anaesthetic block following a major elective surgical procedure. The original data included measurements of pain intensity (visual analogue scale [VAS]) *at rest* and *during mobilization* ($VAS_{0/2/4/6/12/18/24\ hrs}$) and opioid consumption ($OC_{0\text{-}6/0\text{-}24\ hrs}$) administered by patient-controlled analgesia (PCA). The statistical analyses included: comparisons of discrete pain intensity scores ($VAS_{0/2/4/6/12/18/24\ hrs}$); summary measures of pain intensity (AUC-$VAS_{0\text{-}6/0\text{-}24\ hrs}$; 'summed' $VAS_{0\text{-}6/0\text{-}24\ hrs}$; two-way repeated measures (2W-RM) $ANOVA_{0\text{-}6/0\text{-}24\ hrs}$; and opioid consumption ($OC_{0\text{-}6/0\text{-}24\ hrs}$). Also, analyses included an integrated assessment of longitudinally measured pain intensity and opioid consumption ($PIOC_{0\text{-}6/0\text{-}24\ hrs}$).

Results

Sixty-one patients were included in the final analysis. Discrete pain intensity ratings differed significantly between the treatment groups at specific postoperative time points, however, correction for multiple comparisons abolished some of these differences. AUC-$VAS_{0\text{-}6\ hrs}$ differed significantly *at rest* and *during mobilization*, though, no difference was found for AUC-$VAS_{0\text{-}24\ hrs}$. Conversely, 'summed' $VAS_{0\text{-}6\ hrs}$ and $VAS_{0\text{-}24\ hrs}$ differed significantly between treatment groups *at rest* and *during mobilization*. 2W-RM $ANOVA_{0\text{-}6/0\text{-}24\ hrs}$ analyses documented significant interactions between treatment and time with respect to pain intensity scores *at rest* and *during mobilization*. $OC_{0\text{-}6/0\text{-}24\ hrs}$ were significantly different between the treatment groups. Finally, $PIOC_{0\text{-}6/0\text{-}24\ hrs}$ differed significantly *at rest* and *during mobilization*.

Conclusion

The employed statistical methods may alter the statistical significance of analgesic outcomes in postoperative pain trials. An integrated assessment method of longitudinally measured pain intensity and opioid consumption combines two inter-dependent analgesic outcomes, lowers the risk of mass significance, increases the statistical power, and provides more accurate and dynamic representation of analgesic drug efficacy and postoperative pain.

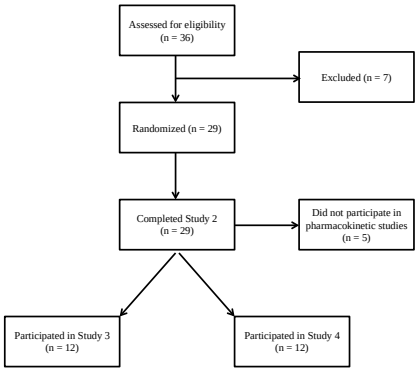
Strengths

The methods study compared standard statistical methods for assessing postoperative analgesic efficacy. Furthermore, a novel edition of an integrated assessment method combining postoperative pain intensity ratings and opioid consumption, *the PIOC* was introduced. We employed AUC as a summary measure of pain intensity in order to provide a temporal and "weighted" description of postoperative pain. Finally, the presented method provided improved statistical power, and lowered the risk of mass significance.

Limitations

The PIOC requires specific sample- and effect size calculations, employing alternative, but simple statistical formulas. We addressed this issue by adding a step-by-step guideline to the manuscript (appendix 1). Another vital issue relates to the included outcomes of the PIOC. We discussed the relevance of each of the current outcomes in detail in the original manuscript. However, additional related outcomes/adverse effects may potentially be included in PIOC. For example, postoperative nausea and vomiting (PONV) data would add valuable information concerning the clinical impact of reduced/increased opioid consumption. Finally, the study only included data material from a single analgesic drug trial. Hence, the conclusions may not apply *in general*. A previous method study addressed this issue by performing complex statistical simulations of the original version of the PIOC [108]. These mathematical techniques may provide detailed power analyses of each statistical test. It is, however, important to underline that simulations are computed from theoretical inferences, and, hence, do not represent actual patient-data, as opposed to our study.

Figure 6:



Volunteer flow of Study II, III and IV, respectively.

DISCUSSION

The present thesis aimed to investigate the analgesic effects of exogenous melatonin in humans. *First*, we presented a systematic review of randomized clinical trials administering melatonin in the perioperative period. Meta-analyses demonstrated significant analgesic and anxiolytic effects of melatonin, equating reductions of 20 mm and 19 mm, respectively on a VAS, compared with placebo. Qualitative reviews indicated that exogenous melatonin also improved sleep quality and emergence behaviour. Furthermore, melatonin may also reduce oxidative stress and anaesthetic requirements. *Second*, we aimed to investigate the analgesic, anti-hyperalgesic or anti-inflammatory effects of exogenous melatonin in a validated experimental pain model in humans. We were not able to demonstrate any significant effects of melatonin compared to placebo. *Third*, we estimated pharmacokinetic variables of oral and intravenous melatonin, respectively in two separate cross-over studies in healthy volunteers. One study also included evaluation of sedative effects, assessed as simple reaction times, and other self-reported adverse effects. No sedative effects or other adverse effects of melatonin were reported. *Finally*, in order to improve future analgesic drug trials, we presented a novel method for assessing postoperative analgesic efficacy. The assessment method combines two inter-dependent analgesic outcomes, lowers the risk of mass significance and increases statistical power.

Dosage

As previously described, experimental studies in animals documented significant dose-dependent anti-nociceptive effects of melatonin in dosages ranging between 0.1 mg kg^{-1} - 300 mg kg^{-1} BW [26]. A recent randomized, placebo-controlled experimental study in humans administered up to 20 mg of sublingual melatonin, also demonstrating dose-dependent analgesic effects assessed by QST [51]. As opposed to the experimental studies, the general findings in clinical studies have been contradictory [98]. However, clinical studies documenting positive analgesic effects, administered oral melatonin dosages ranging between 3-10 mg [98]. Interestingly, a single dose of 10 mg kg^{-1} of intravenous melatonin administered during the intubation procedure produced long-term anti-nociceptive effects in pre-term infants [52]. In accordance to the positive findings in experimental animal studies and in pre-term infants, we chose to employ a high intravenous dose of melatonin to ensure sufficient doses/plasma levels, and optimal direct drug delivery. Furthermore, we administered an intermediate (and previously clinically applied) dose of 10 mg to assess possible dose-dependent analgesic effects [98]. Despite these considerations, we were unable to document any analgesic effects in the present experimental setup. In conclusion, the dosages of melatonin needed to induce anti-nociceptive/analgesic effects have varied extensively between studies. Studies, in both animals and in humans have documented dose-dependent effects, however, no final conclusions can be made at this moment concerning an optimal analgesic dose. Based on recent findings [51, 98], an oral or sublingual melatonin dose of 5-20 mg may be advocated.

Timing of administration

Another principal question relates to the timing of administration. In clinical studies, oral or sublingual melatonin was administered 30-60 minutes before surgery [98]. Interestingly, four clinical studies demonstrating positive analgesic effects, administered an additional dose of melatonin, the night before surgery [53-55, 58]. The exact mechanisms are unknown, but may relate to the sleep-regulating properties of melatonin indirectly affecting perioperative pain perception. Previous experimental- and population studies document significant correlations between sleep and pain [109, 110]. Similarly, the studies by Borazan et al. and Caumo et al. reported concomitant reduced pain intensity rating, reduced opioid consumption and improved postoperative sleep quality following melatonin administration [53, 54]. Correspondingly, the findings may also be explained by the documented anxiolytic properties of melatonin [98]. Experimental animal studies [111], and meta-analyses in humans have documented anxiolytic effects of exogenous melatonin [98]. Also, clinical studies have correlated preoperative anxiety and postoperative pain [112]. Finally, a possible correlation was demonstrated in six studies documenting both analgesic and anxiolytic effect of melatonin in the perioperative period [54-58, 63]. Conclusively, the results of our meta-analysis indicate that an administration 30-45 minutes before intended clinical effect can be recommend from a clinical point of view. However, substantial evidence implies that an administration, 12 hours before intended effect may also be advocated. Re-

peated pre- and postoperative administrations would seem rational, however, a superior clinical effect compared to standard preoperative administration still needs to be confirmed.

Administration route

Another consideration is the choice of optimal administration route. Melatonin is traditionally administered orally or sublingually in clinical perioperative trials, including those investigating analgesic effects [98]. Both routes of administration have been employed in human experimental- and clinical studies, documenting positive analgesic results [51, 98]. A study in pre-term infants applied intravenous administrations, also demonstrating significant anti-nociceptive and anti-oxidative effects [52]. Hence, it seems that the route of administration is not determinant of the clinical analgesic effects of melatonin. However, as presented in study III, the bioavailability of oral melatonin is only 3%. Hypothetically, these considerable differences in subsequent plasma concentrations between administration routes, may explain the variable clinical effects between studies, though no specific administration route have exhibited any clear advantage yet. Unfortunately, bioavailability (and other pharmacokinetic variables) of sublingual melatonin has not been established in humans, despite the widespread experimental- and clinical use.

From a clinical point of view, oral and sublingual formulations are simple to administer, and require a minimum of cooperation by the patient. Intravenous administration ensures optimal drug delivery, but requires intravenous access, limiting its use to a hospital setting. Furthermore, due to the limited solubility of melatonin in water, ethanol is normally required as solvent, making the manufacturing process of the melatonin solution more complicated. Finally, alternative administration routes, such as intranasal melatonin would be applicable in future analgesic drug trials. No studies have been performed yet, but intranasal formulations are commercially available, and would provide an easy administration procedure with a fast and direct drug delivery without significant first pass metabolism.

Experimental pain versus clinical pain in melatonin research

The introduction of new analgesics requires preclinical evaluations. Experimental studies, in animals or in humans may provide imperative information concerning the analgesic effects and safety of analgesic drugs [113]. Human experimental pain models typically apply electrical, mechanical or thermal stimulation to induce pain at different points of action, such as skin, bone, muscle and viscera [113]. We chose to employ a validated inflammatory pain model [76, 114, 115]. The human burn model has previously documented analgesic and anti-hyperalgesic effects of adenosine [77], non-steroidal anti-inflammatory drugs (NSAIDs) [78], lidocaine [79], ketamine [80] and opioids [81]. Importantly, Werner and colleagues documented a significant correlation between pain ratings during an experimental burn and postoperative pain scores in patients undergoing knee surgery [116]. Similarly, another study correlated preoperative heat pain response ratings to postoperative pain in laparoscopic tubal ligation surgery [117].

We were not able to document any significant analgesic, anti-hyperalgesic or anti-inflammatory effects of exogenous melatonin in our experimental setup. These findings are unexpected, since a recent experimental employing similar QST outcomes, demonstrated significant analgesic effects of sublingual melatonin compared to placebo [51]. However, as discussed in detail, our negative findings may relate to difference in dosage, or timing- or route of administration. Correspondingly, previous experimental studies investigating other well-established analgesics, such as local anaesthetics [102], NSAIDs [88] and glucocorticoids [89] have been unable to demonstrate positive results employing this model. It is possible that the limited tissue injury is insufficient to demonstrate significant analgesic difference between the groups. Hence, other experimental or clinical pain models, such as the incision model [118] or third molar extraction model [119] may be more ideal. Also, the limited psychophysiological test-paradigm of the burn injury model may not be optimal to demonstrate significant effects of exogenous melatonin. Therefore, the collective anxiolytic, sleep-regulating and analgesic effects may be instrumental to document a significant analgesic effect of melatonin [98].

The clinical pharmacokinetics of melatonin

A principal aim of this thesis was to estimate pharmacokinetic variables of exogenous melatonin. A limited number of low-volume studies in healthy subjects have previously demonstrated variable results [107]. T_{max} and $t_{1/2\ elimination}$ in humans are, however, well established [107]. Our data demonstrated a t_{max} of approximately 30-45 minutes, which is in accordance to previous trials [107]. From a pharmacokinetic point of view, the current timing of administration presented in our systematic review seems rational (30-45 minutes before surgical trauma), if maximal plasma concentrations coincide with maximal clinical effects. It is, however, important to emphasize that this correlation needs to be established further. We also demonstrated a $t_{1/2\ elimination}$ of approximately 45 minutes following both oral and intravenous administration of melatonin. These findings also correspond to previous trials [107]. $T_{1/2\ elimination}$ was characterized by first order elimination kinetics, and was similar between doses (10 mg *versus* 100 mg) and routes of administration (iv *versus* oral). In study III, oral bioavailability was estimated to 3%, as opposed to previous studies reporting higher values ranging from 9% to 33% [107]. Also, bioavailability varied considerably *between* subjects; in our data from 0.7% to 12.7%. It is widely accepted that the low oral bioavailability of melatonin is caused by significant first pass metabolism in the liver [120, 121], whereas the observed data variation between studies probably relates to methodological differences, such as applied methods of plasma analysis, pharmacokinetic analyses and number of included subjects. Another interesting observation in our data was the wide variation in C_{max} and $AUC_{0-\infty}$ values, which is also demonstrated in previous trials [107]. As an example, C_{max} ranged from 1,105 pg ml^{-1} to 58,900 pg ml^{-1} following oral administration of 10 mg melatonin. These variations may obviously relate to inter-individual differences in absorption, distribution, metabolism and elimination of the drug, however, more detailed knowledge is currently lacking. Finally, our data documenting *CL* and V_d values of approximately 0.025 l min^{-1} kg^{-1} and 1.5 l kg^{-1}, corroborate previous trials [107].

Several unanswered questions still remain with respect to the clinical pharmacokinetics of melatonin. *First*, the studies investigating plasma melatonin levels have employed several methods of analysis, including radioimmunoassay, enzyme-linked immunosorbent assay and mass spectrometry. Unfortunately, no consensus concerning the ideal method of analyses has been established yet [91]. These methodological differences may increase the risk of data variation between studies, and limit possible inter-study comparisons. *Second*, our data documented extremely variable plasma melatonin levels, even following administration of identical melatonin doses. Also, plasma melatonin levels varied extensively with the choice of administration route. At this moment only a single experimental study in humans has correlated actual

plasma levels and analgesic effects, indicating a positive dose-dependent correlation [51]. Future high-quality studies are, however, needed to further correlate plasma levels and analgesic effects, and to define clinically effective plasma levels; either by additional dose-response- or as more accurate pharmacokinetic-/pharmacodynamic studies. Finally, these data would also provide vital information concerning ideal administration route(s). *Third*, the metabolites of melatonin need to be examined further. Only, two preliminary low-volume studies have provided rough pharmacokinetic estimations of 6-hydroxymelatonin in human plasma [106, 120]. Future investigations are required in order to further characterize metabolism and elimination of both melatonin, and the subsequent metabolites following administration, preferably by different administration routes. Metabolites, such as N1-acetyl-5-methoxykynuramine (AMK) and N-acetyl-N-formyl-5-methoxykynuramine (AFMK) are becoming increasingly interesting, as recent studies indicate both physiological (and potentially clinically) activity [122]. Finally, the clinical use of melatonin requires further investigations in patients. At this moment only three clinical studies have estimated pharmacokinetic variables in critically ill patients [123, 124] and elderly suffering from insomnia [125], respectively. The available studies document reduced elimination and extended supra-physiological melatonin plasma levels [125-125]. These findings advocate for the need of more specific tailoring of dosing regimens in order to reduce the risk adverse effects, such as daytime sleepiness. Interestingly, no pharmacokinetic studies have been performed in surgical patients, however, a cohort study addressing this issue, is currently being performed by our research group (EUDRA-CT number: 2014-003789-25). Similarly, preliminary experimental studies indicate that external factors, such as fluvoxamine [126], oral contraceptives [106], caffeine-intake [105] and smoking [104] may impact the pharmacokinetics of melatonin. The possible clinical impact is, however, not established.

Conclusively, t_{max} and $t_{1/2\ elimination}$ of exogenous melatonin are well established. Also, it is recognized that oral bioavailability in humans is only 3%, whereas sublingual pharmacokinetic data are still completely lacking. Hence, several unanswered questions relate to the clinically effective plasma concentrations, and subsequent choice of proper administration route. Also, studies of melatonin metabolites are needed to characterize their biological functions, and the metabolism of these derivatives. Finally, pharmacokinetics in patients should also be investigated further in order to reduce the risk of potential adverse effects.

Safety and adverse effects

Exogenous melatonin has been available as drug treatment for more than five decades. During this period of time, melatonin has been widely applied in research, alternative- and conventional medicine. A large number experimental- [127, 128] and clinical studies in infants [52, 129-135], children [136-141] and adult patients [17, 22, 142] have assessed the adverse effects of melatonin. No serious adverse effects have been established, however, minor adverse effects such as sleepiness, dizziness, headache and nausea have been reported in levels corresponding to placebo treatments. In study IV, 10 and 100 mg of intravenous melatonin were administered, and pre-specified symptoms of nervousness, confusion, depressed mood, dizziness or headache were recorded. Also, volunteers were instructed to report any additional symptoms of adverse effect. High-dose intravenous melatonin did, however, not induce any symptoms of adverse effects in our experimental setup. A recent randomized study in surgical patients undergoing liver resection surgery administered a single dose of 3.5 g of oral melatonin following endotracheal intubation [143]. Similarly, no adverse effects were recorded. Also, general safety and adverse effects were evaluated in a recent systematic review of melatonin the perioperative period [144]. No serious adverse effects were reported, however, some studies indicated possible psychomotor impairment [63], sedation [53, 60, 61, 63] and loss of orientation [61] following melatonin treatment. When looking specifically at the risk of sedation, our data did not demonstrate any differences from placebo treatment. These findings corroborate previous experimental studies, documenting unaffected psychomotor function following melatonin treatment [145, 146].

Conclusively, exogenous melatonin can be considered as safe for short-term use e.g. in surgical patients, even in excessive intravenous doses. The safety of long-term melatonin treatment, however, still needs to be established further in high-quality randomized placebo-controlled studies assessing adverse effects as primary outcomes.

Exogenous melatonin in multimodal regimens

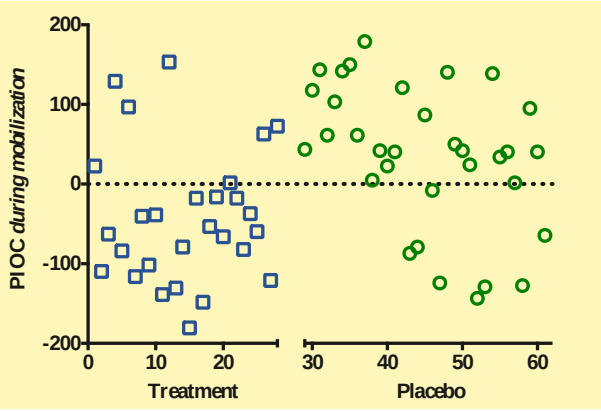
Postoperative pain remains a significant clinical problem [147]. Despite clinical advances, such as minimal invasive techniques and multimodal analgesic regimens, 82% of surgical patients still report pain in postoperative period [148]. Furthermore, commonly used analgesics, anxiolytics and hypnotics, such as NSAIDs, opioids and benzodiazepines may increase the risk of serious adverse effects and comorbidity [147, 149]. Recent studies document that NSAIDs increase the risk of cardiac morbidity [150] and stroke [151], in addition to well-known adverse effects, such as renal failure and GI-tract bleeding. Furthermore, Klein and colleagues documented an increased risk of anastomotic leakage in patients undergoing colorectal cancer surgery, if certain NSAIDs were administered for more than 2 days in relation to surgery [152]. Opioids increase the risk of adverse effects, such as sedation, dizziness, PONV, urine retention, itching and respiratory complications [153]. Similarly, benzodiazepines may induce sedation, dizziness and hypoventilation. As presented previously, these adverse effects are not present with melatonin treatment. Furthermore, as study I documents, exogenous melatonin may possess a number of beneficial effects in perioperative period [98]. *First*, high quality randomized studies indicate both analgesic- and anxiolytic effects of exogenous melatonin [54, 55]. Our meta-analysis confirmed these findings, though a substantial inter-study heterogeneity was present [98]. *Second*, studies imply that exogenous melatonin may improve perioperative sleep quality [53, 54, 62], in accordance with the positive hypnotic effects of melatonin in other patient groups [14]. *Third*, randomized studies in paediatric patients, indicate that emergence delirium is reduced following melatonin treatment compared to placebo and other sedatives/anxiolytics [154-156]. *Fourth*, melatonin reduces oxidative stress in experimental studies [157], and in clinical studies in pre-term infants [52, 132]. Interestingly, a recent randomized study investigating the anti-oxidative effects of high-dose intravenous melatonin in patients undergoing following abdominal aortic aneurism repair, documented reduced cardiac morbidity compared to placebo [158]. Finally, preliminary studies indicate a reduced need of anaesthetic requirements with the administration of melatonin [159, 160]. As a consequence, exogenous melatonin may be incorporated as part of several multimodal perioperative strategies. Despite the negative findings in our

experimental pain model, the results of a large number of experimental and clinical studies in humans advocate for further perioperative investigation in humans; among these, studies focusing on analgesic effects.

Future perspectives

In general, further high-quality randomized studies in surgical patients are needed to determine the clinical analgesic effects of melatonin. These investigations should include both minor and major surgical procedures. We propose investigating *three* different surgical procedures that would contribute individually with interesting and dissimilar aspects of the possible analgesic effects of melatonin. These include *third molar surgery*, *cosmetic breast surgery* and *laparoscopic gastric bypass surgery*, respectively. *First*, third molar surgery provides a clinical pain model, which has been used to assess the analgesic and anti-inflammatory effects of e.g. NSAIDs [119, 161]. The procedure is often performed bilaterally, and patients may, therefore, act as their own controls, minimizing possible inter-individual differences. Also, the model investigates a young study population without serious comorbidity, includes a standardized surgical procedure and produces a significant postoperative pain load [119]. Moreover, perioperative tissue biopsies would be easily be collected, potentially providing evidence of peripheral anti-inflammatory effects of melatonin in humans e.g. by measurements of inflammatory biomarkers or histopathological analysis. *Second*, cosmetic breast surgery would also provide a young and healthy patient population suffering from significant pain load during the postoperative period [162]. Interestingly, despite these obvious advantages, only a limited number of randomized analgesic drug trials studies have been performed so far [162]. *Finally*, patients undergoing laparoscopic gastric bypass surgery require specific anaesthesiological and surgical considerations due an inherent risk of hypoventilation and opioid-induced respiratory depression. Melatonin would provide a safe analgesic alternative in multimodal regimens, potentially reducing opioid consumption and subsequent adverse effects. These studies would employ the PIOC score, as presented in study V (See figure 7), providing both qualitative and statistical advantages compared to standard methods. Furthermore, additional perioperative effects, such as preoperative anxiety and postoperative sleep quality should be assessed in detail.

Figure 7:



An example of how PIOC scores may be distributed in a randomized analgesic drug trial. Each patient, represented by either squares or circles, situated above the zero line of the Y-axis, displayed an increased summed AUC-VAS and opioid consumption compared to the entire patient group (treatment + placebo). The X-axis documents the patient number (treatment (squares), n = 1-28 / placebo (circles), n = 29-61). Visually, it seems obvious that pain is reduced in the active treatment group. Correspondingly, the presented distribution corresponds to a significant difference between the active treatment- and the placebo group (P-value = 0.002). Pain intensity and opioid consumption, PIOC.

CONCLUSIONS

A large number of studies in animals have demonstrated significant dose-dependent anti-nociceptive effects of melatonin [26-28]. Similarly, a recent randomized trial in human volunteers documented increased pain thresholds following administration of sublingual melatonin [51]. In study I**,** we systematically reviewed all randomized studies investigating the clinical effects of perioperative melatonin [98]. Furthermore, we performed meta-analyses of the clinical outcomes, *preoperative anxiety* and *postoperative pain*. The analyses demonstrated significant analgesic and anxiolytic effects of exogenous melatonin. Conclusions were, however, limited by profound heterogeneity between the included studies. Furthermore, in study II, we aimed to investigate analgesic, anti-hyperalgesic and anti-inflammatory effects of exogenous melatonin in a validated human inflammatory pain model [74, 76]. The study was designed as a randomized, double-blind placebo-controlled crossover study. Primary outcomes were *pain during the burn injury* and *areas of secondary hyperalgesia*. No significant effects of exogenous melatonin were observed with respect to primary or secondary outcomes, compared to placebo. Two individual crossover studies, Study III and IV, respectively estimated the pharmacokinetic variables of exogenous melatonin. Oral melatonin demonstrated a t_{max} value of 41 minutes. However, bioavailability was only 3%. Eliminations $t_{1/2}$ were approximately 45 minutes following oral and intravenous administration, respectively. High-dose intravenous melatonin did not induce significant sedation, in terms of simple reaction times compared to placebo. Also, no other adverse effects were reported. Finally, in Study V, we aimed to re-analyse data obtained from a randomized analgesic drug trial. We employed a selection of standard statistical test, and, furthermore, presented an integrated assessment method of longitudinally measured pain intensity and opioid consumption. Our analyses documented that the choice of employed statistical methods may influence the statistical significance of postoperative analgesic outcomes in investigations of analgesic efficacy. Furthermore, the integrated assessment method may combine two interdependent outcomes, lower the risk of type 2 errors, increase the statistical power, and provide a more accurate description of postoperative analgesic efficacy.

Exogenous melatonin may offer an effective and safe analgesic drug. At this moment, however, the results of human studies have been contradictory [98]. High-quality randomized experimental and clinical studies are still needed to establish a "genuine" analgesic effect of the compound in humans. Furthermore, other perioperative effects of exogenous melatonin should be investigated, before melatonin can be introduced for clinical routine use in surgical patients. Despite promising experimental and clinical findings, several unanswered questions relate to optimal dosage, timing of administration and administration route of exogenous melatonin.

SUMMARY

The hormone, *melatonin* is produced with circadian rhythm by the pineal gland in humans. The melatonin rhythm provides an endogenous synchronizer, modulating e.g. blood pressure, body temperature, cortisol rhythm, sleep-awake-cycle, immune function and anti-oxidative defence. Interestingly, a number of experimental animal studies demonstrate significant dose-dependent

anti-nociceptive effects of exogenous melatonin. Similarly, recent experimental- and clinical studies in humans indicate significant analgesic effects. In study I, we systematically reviewed all randomized studies investigating clinical effects of perioperative melatonin. Meta-analyses demonstrated significant analgesic and anxiolytic effects of melatonin in surgical patients, equating reductions of 20 mm and 19 mm, respectively on a VAS, compared with placebo. Profound heterogeneity between the included studies was, however, present. In study II, we aimed to investigate the analgesic, anti-hyperalgesic and anti-inflammatory effects of exogenous melatonin in a validated human inflammatory pain model, *the human burn model*. The study was performed as a randomized, double blind placebo-controlled crossover study. Primary outcomes were *pain during the burn injury* and *areas of secondary hyperalgesia*. No significant effects of exogenous melatonin were observed with respect to primary or secondary outcomes, compared to placebo. Study III and IV estimated the pharmacokinetic variables of exogenous melatonin. Oral melatonin demonstrated a t_{max} value of 41 minutes. Bioavailability of oral melatonin was only 3%. Elimination $t_{1/2}$ were approximately 45 minutes following both oral and intravenous administration, respectively. High-dose intravenous melatonin was not associated with increased sedation, in terms of simple reaction times, compared to placebo. Similarly, no other adverse effects were reported. In Study V, we aimed to re-analyse data obtained from a randomized analgesic drug trial by a selection of standard statistical test. Furthermore, we presented an integrated assessment method of longitudinally measured pain intensity and opioid consumption. Our analyses documented that the employed statistical method impacted the statistical significance of postoperative analgesic outcomes. Furthermore, the novel integrated assessment method combines two interdependent outcomes, lowers the risk of type 2 errors, increases the statistical power, and provides a more accurate description of postoperative analgesic efficacy. Exogenous melatonin may offer an effective and safe analgesic drug. At this moment, however, the results of human studies have been contradictory. High-quality randomized experimental- and clinical studies are still needed to establish a "genuine" analgesic effect of the drug in humans. Other perioperative effects of exogenous melatonin should also be investigated, before melatonin can be introduced for clinical routine use in surgical patients. Despite promising experimental and clinical findings, several unanswered questions also relate to optimal dosage, timing of administration and administration route of exogenous melatonin.

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