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Effect of melatonin and melatonin agonists on postoperative sleep quality in adult patients: a protocol for systematic review and meta-analysis with trial sequential analysis

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ABSTRACT

Introduction The circadian rhythm of melatonin secretion is disturbed after general anaesthesia, leading to postoperative sleep disturbance. Small studies investigating the preventive effect of melatonin administration on postoperative sleep disturbance have not reached any conclusions. Therefore, we will conduct a systematic review and meta-analysis to obtain conclusive results.

Methods and analysis We prepared this protocol following the 2015 Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Protocols guidelines. We will conduct a search for randomised controlled trials that evaluated the effect of melatonin and melatonin agonists on postoperative sleep quality in adult patients undergoing general anaesthesia or regional anaesthesia with sedation. We will exclude patients undergoing regional anaesthesia without sedation. Relevant studies will be searched in the following eight databases: MEDLINE, the Cochrane Central Register of Controlled Trials, Embase, Web of Science and four preregistration sites from inception to 1 January 2021. No language restrictions will be applied. Two authors will independently scan and select eligible studies and perform data extraction and assessment of the risk of bias. The Visual Analogue Scale scores for sleep quality will be combined as the mean difference with a 95% CI using a random-effect model; we will use I^2 to assess heterogeneity. We will evaluate the quality of trials using the Cochrane methodology and assess the quality of evidence using the Grading of Recommendation Assessment, Development and Evaluation approach. If appropriate, trial sequential analysis will be performed.

Ethics and dissemination No ethical approval is required for this meta-analysis, as it does not include individual patient data. We will disseminate the results of this meta-analysis in a peer-reviewed journal.

PROSPERO registration number CRD42020180167.

Strengths and limitations of this study

- The protocol was prepared according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols) statement, and appropriate systematic review and meta-analytic methods will be used.
- We will conduct a comprehensive search of MEDLINE, the Cochrane Central Register of Controlled Trials, EMBASE, Web of Science and four preregistration sites.
- For the primary outcome, the trial sequential analysis will be performed to correct for random error and repetitive testing of accumulating and sparse data.
- We will grade the quality of evidence of the main outcomes using the Grading of Recommendations Assessment, Development and Evaluation approach.
- The limitation will be that the number of studies that can be synthesised may be small.

INTRODUCTION

Description of the condition

Postoperative sleep disturbance is common. Although pain is one of the most important contributing factors, patients with low postoperative pain levels also suffer from poor sleep quality.[1 2] Postoperative sleep disturbance is also associated with subsequent cognitive dysfunction,[3] and the importance of its prevention is drawing attention. In response, there is an expert consensus that postoperative sleep quality is one of the patient outcomes to be studied during the postoperative period.[4]

Description of the intervention and how the intervention may work

The intervention of interest is the postoperative administration of melatonin or melatonin agonists, compared with placebo or no intervention. Melatonin is a central circadian regulator, primarily produced by the pineal gland and a normal melatonin rhythm is partly instrumental in the regulation of the sleep-wake cycle.[5] Melatonin is also known

to have an excellent safety profile[6] and has no major adverse effects such as respiratory depression. The circadian rhythm of melatonin secretion is disturbed after surgery,[7 8] and this disturbance is thought to contribute to postoperative sleep disturbance.[9]

Why is it important to perform this review?

Small studies investigating the preventive effect of melatonin administration on postoperative sleep quality have not reached any conclusion.[10–12] Therefore, a systematic review and meta-analysis of these small studies could add value to our current knowledge of postoperative melatonin administration.

Zhang *et al* conducted a systematic review and meta-analysis of randomised controlled trials (RCTs) and suggested that melatonin intervention shows no significant influence on sleep quality compared with a control group.[13] However, since they limited the surgery to laparoscopic cholecystectomy and analysed only two RCTs for sleep quality, their conclusion may be underpowered. In this systematic review, we will include all types of surgery. Moreover, we will conduct a subgroup analysis to reveal the pivotal factors affecting the effects of melatonin and melatonin agonists on sleep quality.

Objectives

The primary purpose of this meta-analysis is to assess the Visual Analogue Scale (VAS) scores for sleep quality compared with no treatment or placebo. Secondary purposes are to assess sleep quality evaluated using other specific tools and adverse events. When significant heterogeneity is found, we will conduct subgroup analyses based on the following predefined factors:

1. Type of anaesthesia (regional anaesthesia, inhaled general anaesthesia and total intravenous general anaesthesia).
2. Surgery type.
3. Timing of surgery (daytime vs nighttime).
4. Drug type (melatonin vs melatonin agonists).
5. Dose of melatonin/melatonin agonists.
6. Age.
7. Type of control (placebo vs no treatment).

METHODS AND ANALYSIS

This protocol follows the guidelines according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA).[14] We will use the PRISMA guidelines[15] and the Cochrane Handbook for Systematic Reviews of Interventions.[16]

Eligibility criteria

Study designs

We will include all RCTs that tested the effect of melatonin or melatonin agonists on postoperative sleep quality in adult patients undergoing regional or general anaesthesia. We will exclude data from case reports, observational studies, reviews and animal studies.

Participants

We will include studies examining adults (≥18 years) undergoing general anaesthesia or regional anaesthesia with sedation for any surgery. We will exclude patients undergoing regional anaesthesia without sedation. Eligibility will not be restricted by language, type of surgery or type of anaesthesia.

Interventions

The intervention of interest will be the perioperative (7 days before and after the date of surgery) administration of melatonin or melatonin agonists. There will be no restrictions based on dosing, frequency, timing, route of administration or therapy duration.

Comparators

We will include no treatment or placebo as control interventions.

Information sources and search strategy

We will conduct a search in MEDLINE, the Cochrane Central Register of Controlled Trials, EMBASE and Web of Science. The reference lists of the relevant articles will be also searched. Further, we will conduct a search of ClinicalTrials.gov, WHO International Clinical Trials Registry Platform and the UMIN Clinical Trials Registry. The literature search will be limited to human subjects. The search strategy combining free text and Medical Subject Headings terms for PubMed is shown in table 1.

Study records

Data management and selection

Two authors (AT and TT) will independently scan the titles and abstracts of reports identified by the search strategies described above. We will use Mendeley[17] to remove duplicate titles and abstracts from our database search. We will export the remaining titles and abstracts to the Rayyan[18] application for screening titles and abstracts. If eligibility cannot be determined from the title or abstract, the full paper will be retrieved. Potentially relevant studies, chosen by at least one author, will be retrieved and evaluated in full-text versions. Articles that meet the inclusion criteria will be assessed independently by two authors, and any discrepancies will be resolved through discussion.

Data collection

Two authors (AT and TT) will extract data independently and in duplicate from each eligible study. To ensure consistency across reviewers, we will conduct calibration exercises before starting the review. Data abstracted will include demographic information, methodology, intervention details and all reported patient-important outcomes. Reviewers will resolve the disagreements by discussion. We will contact the study authors to resolve any uncertainties.

Data items

A data collection sheet will be created and will include data on participants (eg, age, sex, American Society of

Table 1 Search strategy for PubMed

Number	Search terms
#1	"melatonin"(MeSH Terms] OR "melatonin*"(Title/Abstract)OR "ramelteon"(Supplementary Concept)OR "ramelteon*"(Title/Abstract)OR "rozerem"(Title/Abstract)OR "tak 375"(Title/Abstract)OR "tasimelteon"(Supplementary Concept)OR "tasimelteon"(Title/Abstract)OR "valdoxan"(Title/Abstract)OR "S 20098"(Supplementary Concept)OR "S 20098"(Title/Abstract)OR "agomelatine"(Title/Abstract)OR "N-acetyl-5-methoxytryptamine"(Title/Abstract)OR "circadin"(Title/Abstract)OR "armonia"(Title/Abstract)OR "melamil"(Title/Abstract)OR "benedorm"(Title/Abstract)OR "sleepwell"(Title/Abstract)OR "BP2013"(Title/Abstract)OR "JL5DK93RCL"(Title/Abstract)
#2	"surgery"(MeSH Subheading] OR "surgery"(Title/Abstract)OR "surgical procedures, operative"(MeSH Terms] OR ("surgical"(Title/Abstract)AND "procedures"(Title/Abstract)AND "operative"(Title/Abstract)) OR "operative surgical procedures"(Title/Abstract)OR "general surgery"(MeSH Terms] OR ("general"(Title/Abstract)AND "surgery"(Title/Abstract)) OR "general surgery"(Title/Abstract) OR "surgery's"(Title/Abstract)OR "surgerys"(Title/Abstract)OR "surgeries"(Title/Abstract)
#3	"anesthesia"(MeSH Terms] OR "anaesthesia"(Title/Abstract)OR "anesthesia"(Title/Abstract)OR "anaesthetic*"(Title/Abstract)OR "anesthetic*"(Title/Abstract)
#4	("randomized controlled trial"(Publication Type] OR "controlled clinical trial"(Publication Type] OR "randomized"(Title/Abstract) OR "placebo"(Title/Abstract)OR "drug therapy"(MeSH Subheading] OR "randomly"(Title/Abstract)OR "trial"(Title/Abstract)OR "groups"(Title/Abstract)) NOT ("animals"(MeSH Terms] NOT "humans"(MeSH Terms))
#5	#1 AND (#2 OR #3) AND #4

Anesthesiologists-physical status), type of anaesthesia (regional anaesthesia, inhaled general anaesthesia and total intravenous general anaesthesia), type of surgery (including whether laparoscopic or open surgery), duration of surgery, drug treatment (type, timing, dose and duration of administration), type of control (placebo or no treatment), trial size, duration of follow-up, type and source of financial support and publication status from trial reports. If the actual numbers are provided, we will use them for the analysis. If the actual numbers are not provided, we will use the values originally provided as percentages and convert them back into actual numbers for analysis. If the data are reported only in graphs that indicate percentages or numbers of patients, we will measure the lengths of the graphs to obtain the percentages or numbers of patients. Two authors will extract the data independently from the included studies and then cross-check the data.

Outcomes and prioritisation

Primary outcome

The primary outcome will be the sleep quality measured with the VAS during the early postoperative period (ie, between the day after surgery and 3 days after surgery). If the outcomes are measured several times during the early postoperative period, we will use the mean value as the primary outcome.

The VAS consists of a line, which is often 10 cm long. The patient places a mark at a point on the line corresponding to their rating of sleep quality the previous night (0 mm=best conceivable sleep and 100 mm=worst conceivable sleep). When VAS measurements are shorter or longer than 10 cm, they will be scaled to a VAS of 10 cm in length. When the VAS score is defined as 0 mm as the best and 100 mm as the worst conceivable sleep, the scale will be reversed by 100 mm minus the reported VAS score so that 0 mm is the best conceivable sleep.

Secondary outcomes

The secondary outcomes will be as follows:

1. Sleep-onset latency (minutes).
2. Total sleep time (minutes).
3. Sleep interruption: number of awakenings and waking after sleep onset.
4. Sleep efficiency: percentage of time spent in bed asleep.
5. Sleep quality measured other than VAS during the early postoperative period. We anticipate that there will be studies using the total score of the Richards-Campbell Sleep Questionnaire (RCSQ).[19] Nevertheless, we did not limit our secondary outcomes to the RCSQ and included studies that assessed sleep quality using other methods. If there are studies using scores other than the RCSQ, we will synthesise these scores separately. When the outcomes are measured several times during the early postoperative period, we will adopt the first measured outcome for our primary outcome.
6. Sleep quality measured with VAS 1 week after surgery (postoperative days 4–10) and 1 month after surgery (following the period defined by each author).
7. Sleepiness during the daytime measured with validated questionnaires, such as the Karolinska Sleepiness Scale (KSS)[20] or Stanford Sleepiness Scale (SSS).[21] If there are studies using scores other than KSS or SSS, we will synthesise these scores separately.
8. Quality of recovery assessed by validated assessment tool scores such as QoR-40[22] and QoR-15.[23]
9. Opioid consumption: cumulative dose (milligram) of intravenous morphine or morphine equivalent.
10. Pain assessed by validated assessment tool scores such as VAS, Numerical Rating Scale and Verbal Categorical Rating Scale.
11. Any adverse events such as dizziness, desaturation event or delayed recovery

The RCSQ is a brief 5-item questionnaire used to evaluate perceived sleep depth, sleep latency (time to fall asleep), number of awakenings, efficiency (percentage of time awake) and sleep quality. Each RCSQ response was recorded on a 100 mm VAS, with higher scores representing better sleep and the mean score of these five items, known as the 'total score', representing the overall perception of sleep.

Sleep-onset latency, total sleep time, sleep interruption and sleep efficiency are noted in a sleep diary or measured by actigraphy. Actigraphy evaluates rest and activity by algorithmically processing gross motor activity data, usually collected by a non-invasive wristwatch-like accelerometer device.

Sleepiness during day time was measured using the following validated questionnaires: KSS[20] or SSS.[21] The KSS and the SSS are Likert scales to measure the subjective level of sleepiness at a particular time during the day. On this scale, subjects indicate which level best reflects the psychophysical state. The KSS is a 9-point scale from 'very alert' to 'very sleepy, fighting sleep, an effort to keep awake', 'the SSS is a seven-point scale from 'very alert' to 'completely exhausted, cannot function efficiently'.

Risk of bias of individual studies

We will assess the risk of bias using Cochrane's Risk of Bias tool (RoB 2) for RCTs.[24] The RoB 2.0 assessment for individually randomised trials (including cross-over trials) has five domains and one overall risk of bias domain, as follows:

1. Bias arising from the randomisation process.
2. Bias due to deviations from intended interventions.
3. Bias due to missing outcome data.
4. Bias in the measurement of outcome.
5. Bias for selection of the reported result.
6. Overall risk of bias.

The risk of bias will be assessed as 'low', 'some concern' or 'high' in each domain.

Data synthesis

Statistical analyses will be performed using R software (We will use the newest version at the time of analysis: R Development Core Team, Vienna, Austria) and RStudio (We will use the newest version at the time of analysis: RStudio, Boston, Massachusetts, USA). Continuous data will be summarised using the mean difference (MD) or the standardised mean difference (SMD) with a 95% CI. Dichotomous data will be summarised using the risk ratio with a 95% CI. If the 95% CI includes a value of 0 or 1 for continuous or dichotomous data, respectively, we will consider the difference not to be statistically significant. When there are missing data, we will attempt to contact the original authors of the study to obtain the relevant missing data. Heterogeneity will be quantified using the I^2 statistic and Cochran's Q statistic. We will consider that significant heterogeneity exists when the I^2 statistic exceeds 50%. We will conduct a subgroup analysis to explore the possible causes in cases of high heterogeneity. We will use either a random-effects model (DerSimonian and Laird methods[25]) or a fixed-effect model, considering clinical and methodological heterogeneity, to combine the results. Forest plots will be used to graphically represent and evaluate the effects of treatment.

We plan to conduct a subgroup analysis according to the following predefined factors when the I^2 statistic exceeds 50%: (1) type of anaesthesia (regional anaesthesia, inhaled general anaesthesia and total intravenous general anaesthesia), (2) type of surgery, (3) timing of surgery (daytime vs night-time), (4) drug type (melatonin vs melatonin agonists), (5) dose of melatonin/melatonin agonists, (6) age, (7) type of control (placebo vs no treatment) or (8) risk of bias (high risk of bias vs others). Subgroup analysis will not be performed if the number of studies is less than three.

For the primary outcome, trial sequential analysis (TSA) will be performed to correct for random error and repetitive testing of accumulating and sparse data using TSA viewer V.0.9.5.10 β (www.ctu.dk/tsa).[26] TSA monitoring boundaries (ie, monitoring boundaries for meta-analysis) and the required information size will be quantified, and the adjusted CIs will be calculated. The risk of type 1 error will be maintained at 5% with a power of 90%. A 10 mm reduction in MD is considered clinically meaningful. If the cumulative Z-curve does not cross the TSA monitoring boundaries, we will downgrade the quality of evidence owing to inaccuracies in the results.

Reporting bias and publication bias

To determine whether reporting bias is present, we will determine whether the RCT protocol was published before recruitment of patients for the study was initiated. For studies published after 1 July 2005, we will screen the Clinical Trial Register at the ClinicalTrials.gov (https://clinicaltrials.gov/), WHO International Clinical Trials Registry Platform (https://www.who.int/clinical-trials-registry-platform) and UMIN Clinical Trials Registry (https://www.umin.ac.jp/ctr/). We will evaluate whether selective reporting of outcomes is present (outcome reporting bias) by comparing the outcomes mentioned in the published study protocol or trial registry with the outcomes reported in the paper.

The small study effect will be assessed using a funnel plot and Egger's regression asymmetry test[27] and will be considered positive if $p<0.1$ in the regression asymmetry test.

Summary of evidence

We will grade the quality of evidence of the main outcomes using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach[28 29] with the GRADEpro guideline development tool(https://gradepro.org/). Judgements of the quality of evidence will be based on the presence or absence of the following variables: limitations in study design, inconsistency, indirectness, imprecision of the results and publication bias. The quality of evidence for the main outcomes will be graded as very low, low, moderate or high.

Limitation and implications

There are several tools for measuring postoperative sleep quality. The primary outcome will be the sleep quality measured by the VAS, which is considered to be the most

common assessment tool, but the number of studies that can be synthesised may be small. To compensate for this, we plan to synthesise other assessment tools as a secondary outcome. However, different measurement tools may not necessarily be assessing the same thing, and the conclusions reached, in that case, may be misleading.

The findings from this meta-analysis will indicate whether melatonin and melatonin agonists improve postoperative sleep quality in adult patients undergoing general anaesthesia. In addition, it may reveal the pivotal factors affecting the effects of melatonin and melatonin agonists on sleep quality. If the findings from this meta-analysis are inconclusive, they may help generate new hypotheses and contribute to the design of new RCTs.

Ethics and dissemination

The data used do not include individual patient data, and thus, there are no patient privacy concerns. This systematic review will be published in a peer-reviewed journal. Any significant changes to this protocol will be noted with a description of the change, the corresponding rationale and the date of the amendment. The results will also be presented at relevant conferences.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

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REFERENCES

1 Kain ZN, Caldwell-Andrews AA. Sleeping characteristics of adults undergoing outpatient elective surgery: a cohort study. *J Clin Anesth* 2003;15:505–9.
2 Cronin AJ, Keifer JC, Davies MF, *et al*. Postoperative sleep disturbance: influences of opioids and pain in humans. *Sleep* 2001;24:39–44.
3 Gögenur I, Middleton B, Burgdorf S, *et al*. Impact of sleep and circadian disturbances in urinary 6-sulphatoxymelatonin levels, on cognitive function after major surgery. *J Pineal Res* 2007;43:179–84.
4 Myles PS, Boney O, Botti M, *et al*. Systematic review and consensus definitions for the standardised endpoints in perioperative medicine (step) initiative: patient comfort. *Br J Anaesth* 2018;120:705–11.
5 Shochat T, Haimov I, Lavie P. Melatonin--the key to the gate of sleep. *Ann Med* 1998;30:109–14.
6 Yousaf F, Seet E, Venkatraghavan L, *et al*. Efficacy and safety of melatonin as an anxiolytic and analgesic in the perioperative period: a qualitative systematic review of randomized trials. *Anesthesiology* 2010;113:968–76.
7 Mihara T, Kikuchi T, Kamiya Y, *et al*. Day or night administration of ketamine and pentobarbital differentially affect circadian rhythms of pineal melatonin secretion and locomotor activity in rats. *Anesth Analg* 2012;115:805–13.
8 Cronin AJ, Keifer JC, Davies MF, *et al*. Melatonin secretion after surgery. *Lancet* 2000;356:1244–5.
9 Leardi S, Tavone E, Cianca G, *et al*. [The role of melatonin in the immediate postoperative period in elderly patients]. *Minerva Chir* 2000;55:745–50.
10 Hansen MV, Madsen MT, Andersen LT, *et al*. Effect of melatonin on cognitive function and sleep in relation to breast cancer surgery: a randomized, double-blind, placebo-controlled trial. *Int J Breast Cancer* 2014;2014:1–9.
11 Gögenur I, Kücükakin B, Bisgaard T, *et al*. The effect of melatonin on sleep quality after laparoscopic cholecystectomy: a randomized, placebo-controlled trial. *Anesth Analg* 2009;108:1152–6.
12 Borazan H, Tuncer S, Yalcin N, *et al*. Effects of preoperative oral melatonin medication on postoperative analgesia, sleep quality, and sedation in patients undergoing elective prostatectomy: a randomized clinical trial. *J Anesth* 2010;24:155–60.
13 Zhang J, Wang Y, Xu H, *et al*. The influence of melatonin on sleep quality after laparoscopic cholecystectomy: a meta-analysis of randomized controlled trials. *Surg Laparosc Endosc Percutan Tech* 2019;29:1–6.
14 Shamseer L, Moher D, Clarke M, *et al*. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015;350:g7647.
15 Moher D, Liberati A, Tetzlaff J, *et al*. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009;6:e1000097.
16 Higgins JPT, Thomas J, Chandler J. *Cochrane handbook for systematic reviews of interventions version 6.1*. Cochrane, 2020. www.training.cochrane.org/handbook
17 Elston DM. Mendeley. *J Am Acad Dermatol* 2019;81:1071.
18 Ouzzani M, Hammady H, Fedorowicz Z, *et al*. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* 2016;5:210.
19 Richards KC, O'Sullivan PS, Phillips RL. Measurement of sleep in critically ill patients. *J Nurs Meas* 2000;8:131–44.
20 Kaida K, Takahashi M, Akerstedt T, *et al*. Validation of the karolinska sleepiness scale against performance and EEG variables. *Clin Neurophysiol* 2006;117:1574–81.
21 Hoddes E, Zarcone V, Smythe H, *et al*. Quantification of sleepiness: a new approach. *Psychophysiology* 1973;10:431–6.
22 Myles PS, Weitkamp B, Jones K, *et al*. Validity and reliability of a postoperative quality of recovery score: the QoR-40. *Br J Anaesth* 2000;84:11–15.
23 Stark PA, Myles PS, Burke JA. Development and psychometric evaluation of a postoperative quality of recovery score: the QoR-15. *Anesthesiology* 2013;118:1332–40.
24 Sterne JAC, Savović J, Page MJ, *et al*. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 2019;366:l4898.
25 DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177–88.
26 Wetterslev J, Thorlund K, Brok J, *et al*. Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. *J Clin Epidemiol* 2008;61:64–75.
27 Egger M, Davey Smith G, Schneider M, *et al*. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629–34.
28 Atkins D, Best D, Briss PA, *et al*. Grading quality of evidence and strength of recommendations. *BMJ* 2004;328:1490.
29 Guyatt GH, Oxman AD, Vist GE, *et al*. Grade: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;336:924–6.