

Melatonin treatment in patients with burning mouth syndrome: a triple-blind, placebo-controlled, crossover, randomised clinical trial[†]

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Abstract

Aims: Melatonin (MLT) may improve the management of chronic pain. This study explored the efficacy of MLT in reducing burning mouth syndrome (BMS)-associated pain, a neuropathic pain for which there is no effective treatment. Sleep quality, anxiety, side effects, and serum and salivary MLT levels were also evaluated. **Methods:** In a triple-blind, randomised clinical trial, 20 BMS patients (mean age \pm standard deviation: 64.4 ± 11.5 years; range: 35–82 years) were enrolled to receive, in a crossover design, MLT (12 mg/day) or placebo (PLC) for 8 weeks. At baseline and after treatment, pain changes were ascertained by patient assessment and using a visual analogue scale. Secondary outcomes included evaluation of changes in sleep quality and anxiety. Data were subjected to analysis of variance (ANOVA, Fisher's exact test), paired t-test, Wilcoxon signed-rank test or χ^2 test, as appropriate. **Results:** MLT was not superior to PLC in reducing pain. MLT significantly improved anxiety scores, although without strong clinical relevance. Independently of the treatment, sleep quality did not significantly change during the trial, although MLT slightly increased the number of hours slept. After active treatment, the mean serum MLT level peaked at $1,520 \pm 646$ pg/mL. A generally safe pharmacological profile of MLT was observed and the PLC and MLT treatments resulted in similar adverse effects. **Conclusion:** Within the limitations of this study, MLT did not exhibit higher efficacy than PLC in relieving pain in BMS patients.

Introduction

Burning mouth syndrome (BMS) is a spontaneous, painful, burning sensation, recently categorised as a neuropathic pain, for which no dental or medical cause can be found ¹. Circadian variation of symptoms often occurs, as pain typically increases as the day progresses ². This intense pain significantly reduces patients' quality of life ³. The prevalence of BMS ranges from 0.7 to 7% of the general population, increasing to 12–18% in post-menopausal women ⁴. It is more frequent among anxious and depressed patients, suggesting that psychological factors play a role ^{5,6}.

The aetiology of BMS is unknown, although recent findings suggest alterations of the peripheral and central nervous systems^{4,7}. An imbalanced antioxidant status^{8,9} and an impaired inflammatory response¹⁰ have been reported, suggesting their influence on pathogenesis. One recent retrospective cohort study and three case-control studies have reported associations between sleep disorders and BMS¹¹⁻¹⁴. Compared with controls, BMS patients showed poorer sleep quality and an increased frequency of sleep disorders¹⁵; conversely, patients with sleep disorders showed a higher frequency of BMS¹⁵. These findings suggest that sleep disturbance is a risk factor for BMS, and a promising therapeutic target. The latter is, to date, largely empirical, due to the lack of strong scientific evidence¹⁶⁻¹⁸.

Many agents, from salivary substitutes to anxiolytics, antidepressants and anticonvulsants, have been proposed for coping with BMS and improving symptoms, but the results have been disappointing^{16,17,19}. Attention has focused on clonazepam, a benzodiazepine agonist of gamma-aminobutyric acid (GABA-A) receptors^{20,21}. This study investigated the efficacy of melatonin (MLT) for treating BMS. The rationale is related to the biological activities recently ascribed to this pleiotropic molecule, which possesses multiple mechanisms of action against chronic pain²². MLT is an indoleamine involved in the regulation of several chronobiological processes, such as the circadian and circannual rhythms, the sleep-wake cycle, and recovery from jet lag²³⁻²⁶. Moreover, it is an immunomodulating, antioxidant, anti-inflammatory and neuroprotective agent^{23,27,28}. MLT has, thus, been proposed to improve sleep and antioxidant status in patients affected by neurodegenerative disorders, including Parkinson's and Alzheimer's diseases²⁹, multiple sclerosis³⁰, and in post-menopausal women³¹. The effect of MLT in improving mood and anxiety and in promoting analgesia supports its potential for therapy of chronic pain, as shown for fibromyalgia³² and temporomandibular disorders^{22,33}. The anti-nociceptive effect of MLT involves activation of GABA-A-benzodiazepine receptors (similar to clonazepam) and increased endogenous β -endorphin release in the central nervous system³⁴.

Therefore, the aim of this clinical trial was to evaluate the efficacy of MLT in reducing BMS-related pain, compared with the placebo (PLC). Sleep quality, anxiety, side effects, and serum and salivary MLT levels were also evaluated.

Materials and Methods

Study design

A triple-blind (*i.e.* participants, care providers and researchers assessing outcomes were blinded, after assignment to interventions), cross-over, randomised, PLC-controlled, clinical trial was carried out at (.....), where the interventions were performed and data were collected and analysed.

Patient recruitment

Patients were enrolled at the Oral Medicine Service of the (.....), from October 2013 to July 2015, after approval by the Ethics Committee of the A.O. San Paolo (reference: BMS2013), in accordance with the ethical principles of the World Medical Association Declaration of Helsinki. Written informed consent was obtained from each patient. The trial was registered at www.clinicaltrials.gov (ref. number: NCT02580734), and the CONSORT statement for randomised clinical trials was applied (<http://www.consort-statement.org/consort-2010>). Consistent with Gremeau-Richard *et al.* ^{21,35}, the inclusion criteria were the presence of burning or stinging chronic oral pain, with or without xerostomia or dysgeusia, in patients with a normal oral mucosa upon clinical examination and without hyposalivation (salivary flow rate was assessed by the spitting method ³⁶). Pain should be present for more than 4 months, with no trigger paroxysm and not following a specific nerve trajectory. Any organic condition associated with an oral burning sensation was ruled out in all patients by laboratory tests (full blood cell count, and serum levels of iron, ferritin, folate, vitamin B12, glucose, and zinc)³⁵. Patients who were taking, or had previously taken, anxiolytics to induce sleep and/or antidepressants, anticonvulsants, other psychotropic drugs or who were receiving psychological therapy for anxiety and depression were included. The current study was designed to be as similar as possible to the clinical condition; *i.e.*, BMS patients are often anxious and/or depressed, for which they require drug treatment. The exclusion criteria were: previous or current therapy with MLT, serotonin or other analogues in the last month; previous or current therapy with phytotherapeutics or dietary supplements with MLT, serotonin and tryptophan in the last month; documented specific allergy or hypersensitivity to MLT; working at night; being treated with anticoagulants because of potential pharmacological interaction ²³; being pregnant or lactating; or being less than 18 years old.

Intervention

At baseline, the personal and clinical data of the participants were recorded and the inclusion/exclusion criteria were reviewed. Each patient performed two sequential 8-week treatments using MLT or PLC

compresses, separated by a washout period of 4 weeks. The clinical trial had a 20-week duration. Participants were randomly assigned to treatment sequence A (MLT → PLC) or B (PLC → MLT) (Fig. 1). During treatment and data analysis, the physicians, patients, and laboratory investigators were blind to the medication assignment. A simple randomisation method was applied using an online tool (<http://graphpad.com/quickcalcs/%20randomise1.cfm>). Allocation concealment was ensured because the person who generated the randomisation list and assigned the individuals to the two treatment sequences was not involved in evaluating patient eligibility for the study. Active compresses (Tranquillus[®], Functional Point s.r.l., Bergamo, Italy) contained 3 mg of N-acetyl-5-methoxytryptamine (MLT). MLT and PLC compresses had the same colour, shape and taste and were distributed in identical containers, without any labels. They had identical inert excipients; *i.e.*, microcrystalline cellulose, calcium phosphate, inulin, talc and magnesium stearate. Patients were instructed to take four compresses, for 8 weeks, at around 8:30 am, 12:30 am, 3:30 pm and 7:30 pm. The total dose of MLT was 12 mg/day, based on previous studies^{26,32,37,38}. Four experimental time points were recorded: T0, baseline visit before starting the first treatment; T1, visit at the end of the first 8 weeks of treatment; T2, baseline of the second treatment, after a washout period of 4 weeks; and T3, end of the second 8 weeks of treatment (Fig. 1). Each patient was examined at the same time of day at all four time points; in general, visits were scheduled between 8:30 am and 2:00 pm. At each time point, clinical data for the primary and secondary outcomes were recorded, and a clinical examination of oral mucosa was performed by the same physician who provided the compresses. Side effects were recorded and blood was drawn to measure serum and salivary MLT levels. Treatment compliance was self-reported using a questionnaire, and by counting the number of compresses left in the container at the end of each treatment.

Primary outcomes—pain evaluation

The primary endpoint was the self-reported perception of pain during the trial.

Patient global impression of pain change—Any change in pain intensity was recorded by the patients at T1 and T3. The patients were asked to express verbally their feelings about the treatment using the following five-point categorical scale: worse, no change, mild improvement, moderate improvement, and strong improvement (adapted from Farrar *et al.*³⁹).

Visual Analogue Scale—The visual analogue scale (VAS) consisted of a 10-cm horizontal line marked from 0 (no pain) to 10 (most severe pain experienced), on which the patient was requested to record the level of pain ^{40,41}. Changes in BMS symptoms were calculated as: $\Delta\text{VAS} = \text{baseline values} - \text{post-treatment values}$.

VAS for pain relief after treatment—The VAS consisted of a 10-cm horizontal line marked from 0 (no relief) to 10 (complete relief), on which the patient was requested to record the level of pain relief ^{40,41}.

Number of oral sites involved—The number of oral sites affected by the burning sensation was recorded and percentages calculated as the ratio of the number of sites measured to the total number of oral sites considered ($n = 15$) ⁴¹. Changes in oral sites were calculated as: $\Delta\% \text{ oral sites} = \text{baseline \% values} - \text{post-treatment \% values}$.

Secondary outcomes—sleep disturbances and anxiety

Quality of sleep—The Medical Outcomes Survey (MOS) sleep scale (baseline values – post-treatment values) questionnaire was administered. Items on the questionnaire were used to calculate various subscales according to the MOS Sleep Scale user manual (A Manual for Use and Scoring, version 1.0, November 2003). The MOS subscales included: raw sleep quantity (SLPQRAW), which refers to hours of sleep per night in the previous week; sleep disturbance (SLPD4), which assesses trouble falling asleep **and non-quiet sleep**, which assesses wakefulness during sleep time; snoring (SLPSNR1); shortness of breath (SLPSOB1), which evaluates waking up with shortness of breath or headache; sleep adequacy (SLPA2), which evaluates whether was sleep sufficient to feel rested upon waking in the morning; and daytime somnolence (SLPS3), which assesses drowsiness during the day, trouble in staying awake during the day or the need to take naps. By combining these items, the sleep problems index 2 (SLP9), which summarises sleep disturbances was calculated. SLP9 refers to sleep adequacy, respiratory impairment, somnolence, time to fall asleep, sleep quietness, and drowsiness during the day ¹³. Except for sleep quantity (SLPQRAW), for which lower scores indicate worse sleep, higher scores indicate more severe sleep problems for the other subscales and for SLP9.

Anxiety—The severity of anxiety symptoms was evaluated using the clinician-rated Hamilton Rating Scale for Anxiety (HAM-A) ^{6,12}: < 17, mild anxiety; 18–24, mild-to-moderate anxiety; and 24–30, moderate-to-severe anxiety ⁴². Changes in HAM-A score were calculated as follows:

$\Delta\text{HAM-A} = \text{baseline values} - \text{post-treatment values}$

Side effects

Diurnal sleepiness—Somnolence during the day was measured using the self-administered Epworth Sleepiness Scale (ESS). Respondents were asked to rate, on a 4-point scale (0–3), their typical probability of dozing or falling asleep while engaged in eight activities. The ESS score (the sum of the eight item scores, from 0, never, to 3, always) ranges from 0 to 24: the higher the ESS score, the higher the average sleep propensity of a person in daily life; *i.e.*, his/her ‘daytime sleepiness’⁴³. An ESS score of 10 has been proposed as a threshold for normality. The change in ESS score before and after each treatment was calculated as follows: $\Delta\text{ESS} = \text{baseline values} - \text{post-treatment values}$.

Other side effects—Any other side effect was recorded using a questionnaire about daily somnolence, dizziness, nausea and vomiting, impaired concentration, and appetite alteration.

Oral bioavailability—serum and salivary MLT levels

Serum and salivary samples were collected to measure MLT levels. Saliva was collected using the spitting method^{36,44}. Serum MLT levels were determined on blinded samples using a high-performance liquid chromatograph (Agilent 1290 Infinity Autosampler G4220B, Santa Clara, CA, USA) coupled with a triple quadrupole mass spectrometer (ABSciex QTrap 5500, Milan, Italy) (HPLC-MS/MS), using [2H₄]-N-acetyl-5-methoxytryptamine (98.3%) as the internal standard. After pre-analytical processing based on liquid-phase extraction, samples were injected in a Kinetex 2.6u XB-C18 100A column (Phenomenex, Torrance, CA, USA) (100 × 2.10 mm) at 30°C. The flow rate was 0.45 mL/min and the samples were eluted using the following gradient of mobile phase A (2 M ammonium formate and 0.1% formic acid) and B (acetonitrile): 90% A for 2 min, 30–70% A in 1 min, 70–15% A in 1 min, 90% B for 2 min, and 90–10% B in 2 min. The total run time was 10.5 min. The multiple reaction monitoring (MRM) technique was used to quantify MLT levels, with the optimised fragmentation *m/z* (Table 1). The limit of quantification was 5 pg/mL. Salivary MLT level was measured by enzyme-linked immunosorbent assay (ELISA) (BTB-E1013Hu, Human Melatonin, MT ELISA Kit, Li StarFISH S.r.l., Milan, Italy), following the manufacturer’s instructions.

Statistical analysis

The sample size ($n = 20$ patients) was calculated according to effect size and standard deviations derived from previous studies³⁶⁻³⁸, with a power of 80% and a type-I error of 0.05, considering a cross-over design and a 20% dropout rate. An intention-to-treat analysis (ITT) was performed to evaluate primary and secondary outcomes; the data of dropouts were included in the calculations. The last observation carried

forward (LOCF) approach, in which the last available data are assumed to be the same at all further time points, was used⁴⁸. The LOCF assumption was justified as the average unobserved outcomes, within each randomised group, did not change significantly over time^{33,49}. The means \pm standard errors of the mean (SEM) of variables were calculated, except for age, for which standard deviation was used. A Kolmogorov–Smirnov normality test was applied; at a level of 0.05, the data were considered to be from a normally distributed population. Normally distributed data were compared by one-way analysis of variance (ANOVA) (Fisher’s exact test), and pre- and post-treatment values were compared by paired t-test. A paired t-test was also used to compare Δ values; at the 0.05 level, results not considered to be from a normally distributed population were compared using the paired-sample Wilcoxon signed-rank test. Nonparametric variables were compared by χ^2 test. Spearman’s coefficient of correlation was used to identify linear correlations of VAS scores with serum and salivary MLT levels. Statistical significance was set at $p \leq 0.05$.

Results

A flowchart of the study design, including patient recruitment and dropouts, is shown in Fig. 1. During a 12-month period, 32 patients with a previous diagnosis of BMS were screened for participation. After application of the inclusion/exclusion criteria, 20 patients (16 females and 4 males; age: 64.4 ± 11.5 years) were enrolled in the study. The patients' sociodemographic data, clinical history, and current drug use are shown in Table 2.

During the first phase of the intervention, 8 of the 20 (40%) patients dropped out because of side effects ($n = 4$; self-reported heavy tremor, sexual disturbances, blurred vision, and severe heavy-headiness), lack of efficacy ($n = 2$), pain improvement ($n = 1$) or loss to follow-up ($n = 1$). They were equally distributed between the PLC ($n = 4$) and MLT ($n = 4$) groups, which allowed an LOCF approach for ITT analysis without reducing the statistical power. The patients who completed the study adhered to the protocol for more than 80% of the total therapy according to self-reporting, although some did not return the compresses to be checked.

Primary outcomes—pain evaluation

Patient impression of pain change—Most patients did not report significant changes in pain intensity during PLC and MLT treatments (Figs. 2A and B): half of the patients ($n = 10$, 50%) perceived no change in pain after PLC treatment, and 60% ($n = 10$) after MLT treatment. Interestingly, although not statistically significant, a moderate improvement in pain was reported after MLT treatment in 20% of cases ($n = 4$), slightly higher than that after PLC treatment ($n = 3$, 15%). Worsening of pain was recorded by 5% of patients ($n = 1$) after MLT treatment, and by 10% after PLC treatment ($n = 2$).

Pain intensity by VAS—The primary endpoint was improvement in BMS symptoms as measured by VAS. At all time points, VAS scores were > 3 ; *i.e.*, moderate-to-severe pain. In the PLC group, the baseline VAS score was 7.8 ± 0.3 , and decreased to 6.7 ± 0.4 post-treatment. In the MLT group, the VAS score at baseline was 7.6 ± 0.4 , and 7.0 ± 0.5 after treatment (Fig. 2C). VAS scores did not differ significantly among the four time points, but there was a significant difference in the PLC group between baseline and post-treatment ($p \leq 0.05$; Fig. 2C). The mean Δ VAS was 1.2 ± 0.4 for PLC and 0.6 ± 0.5 for MLT; these values did not differ significantly (Table 3). Patients consistently reported low pain relief scores; 2.0 ± 0.6 after PLC treatment and 1.9 ± 0.6 after MLT treatment (Fig. 2C).

Number of oral sites involved—Overall, no change in the number of oral sites affected by pain was recorded. At baseline, the mean percentage of involved oral sites was $42 \pm 6\%$ in the PLC group and $35 \pm 5\%$ in the MLT group; at the end of the treatment, the number of sites was unchanged after MLT treatment ($35 \pm 6\%$), and decreased slightly after PLC treatment ($37 \pm 7\%$), albeit not significantly. Δ values were similar: $5 \pm 4.2\%$ in the PLC group and $1 \pm 3.3\%$ in the MLT group (Table 3).

Secondary outcomes—sleep disturbances and anxiety

Sleep quality—The MOS subscale scores are shown in Table 4. At the time of enrolment (first baseline), the optimal sleep quantity (SLPQRAW), defined as 7–8 h, was recorded in five patients (25%); the remaining 75% ($n = 15$) reported a reduced sleeping time, with an average of 5.3 ± 1.5 h of sleep. MLT treatment slightly increased the number of hours slept during the night (SLPRAW), but PLC treatment did not (Table 4). In two patients, this increase corresponded to the optimal sleep quantity. In particular, the amount of sleep experienced by the first of these patients increased from 6 to 8 h per night after MLT treatment, but remained at 6 h after PLC treatment; however, this corresponded to a slight improvement in VAS score (Δ VAS: 0.2). The amount of sleep experienced by the second patient increased from 5 to 8 h per night after MLT treatment, but decreased from 5 to 4 h per night after PLC treatment; this patient showed a marked improvement in VAS score (Δ VAS: 4.6). MLT treatment also was correlated with a slight improvement in sleep disturbances (SLPD4) (Table 4). Sleep quality, in terms of SLP9 score, was 33.8 ± 3.8 at baseline in the PLC group and 32.2 ± 4.6 in the MLT group. The SLP9 score decreased after both treatments, albeit not significantly (Fig. 3A). This is consistent with the Δ SLP9 values (Table 3).

Anxiety—At the time of enrolment, the HAM-A score in BMS patients ranged from severe ($n = 4$, 20%) to moderate ($n = 3$, 15%) and mild ($n = 13$, 65%). After MLT treatment, a statistically significant decrease in comparison with baseline was recorded ($p \leq 0.05$; Fig. 3B). Interestingly, a patient in the MLT group showed a reduction in HAM-A score from 22 to 8. Δ HAM-A values were consistently higher in the MLT group than the PLC group (Table 3).

Oral bioavailability

MLT bioavailability was determined only in patients who completed the study ($n = 12$), for whom all serum and saliva samples were available. At baseline, serum MLT levels were less than 5 pg/mL in all patients; after treatment, the MLT concentration peaked at $1,520 \pm 646$ pg/mL, but remained at physiological levels

after PLC treatment (26 ± 16 pg/mL) ($p \leq 0.05$; Fig. 4A). No such difference was detected in saliva (Fig. 4B). The correlation between serum and salivary MLT levels was positive after MLT treatment (Spearman's coefficient: 0.1), but negative after PLC treatment (Spearman's coefficient: -0.5 ; $p < 0.05$). Serum and salivary MLT levels were correlated with the VAS score in each patient (Fig. 4C). Notably, after treatment, serum MLT concentrations increased markedly in around half of the patients ($n = 6$), but remained at physiological levels in the others (Fig. 4D). No strong correlation between VAS score and MLT concentration was found for either treatment, although values were negatively associated; *i.e.*, a higher MLT level corresponded to a lower VAS score (Spearman's coefficients: -0.2 for serum and -0.3 for saliva).

Side effects

Table 5 summarises the side effects recorded during the trial. The most frequent side effect was self-reported sleep impairment, which was slightly more common during MLT than PLC treatment (62.5%, $n = 10$ vs. 37.5%, $n = 6$, respectively), albeit without statistical significance. The ESS index was also used to evaluate diurnal somnolence. Overall, ESS values corresponded to normal daytime sleepiness, except in one patient in whom the values revealed moderately excessive daytime sleepiness at baseline and post-treatment (ESS score, 13). ESS scores increased after both treatments, supporting mild daytime sleepiness, with no significant difference between the MLT and PLC groups (Fig. 5; negative Δ ESS values, Table 3). Headache, dizziness, nausea and vomiting, and impaired concentration were reported slightly more frequently during PLC treatment, while appetite alteration was more marked during MLT treatment; however, these differences were not significant (Table 5). PLC also induced heart palpitations and severe tremor in two female patients, while MLT produced sexual disturbances and blurred vision in a male patient and severe **heavy-headiness** in a female patient; these side effects caused the patients to drop out of the trial.

Discussion

To the best of our knowledge, the present study is the first randomised, triple-blinded, PLC-controlled trial of 8 weeks of treatment with MLT in BMS patients, using pain intensity as the primary outcome.

The rationale of this study lies in the role of MLT in BMS treatment, and its mechanisms of action. Besides systemically regulating mood, the immune system, and circadian rhythms²², MLT may be useful for

treatment of oral diseases, including BMS, due to its neuro-protective, antioxidant and anti-inflammatory activities^{28,30,50-52}.

Our data show that MLT and PLC have comparable efficacy against BMS; no significant change in pain was detected. Patient-reported pain was largely unchanged in both treatments, with a slight improvement in Δ VAS post-treatment. This lack of difference can be attributed to the effect of PLC on BMS patients, as stated in a recent systematic review: “on average, treatment with placebos produced a response that was 72% as large as the response to active drugs”⁵³. The interpretation of this result must also consider incomplete compliance with the MLT therapy, as suggested by the physiological serum⁵⁴ MLT concentrations, despite self-reported satisfactory adherence to therapy.

We did not detect an association between MLT and improved sleep quality, which is consistent with a recent meta-analysis of MLT for sleep disorders in patients with neurodegenerative diseases²⁹. This is important, as sleep disturbances are associated with BMS¹¹⁻¹⁴. Anxiety and depression, which are independently associated with both sleep disturbances and BMS^{11,15}, may have confounded the results. Thus, the extant studies on this issue should be interpreted with caution because sleep assessment was based on self-reports, not polysomnographic analysis. This work found sleep deprivation in 75% of BMS patients who slept less than 7 h per day; MLT treatment improved sleep quantity slightly in these patients. Interestingly, a significant increase in sleep quantity was observed in two patients treated with MLT who reached the optimal number of hours slept, although only one of them showed a marked improvement in VAS score. Sleep quality was not significantly affected by either treatment. In the general population, the Medical Outcomes Study reported a mean SLP9 score of 29.2 ± 18.0 in 1988 and $25.8 \pm \text{xx}$ in 2005⁵⁵. In this trial, the mean values (around 30) were higher than these cut-offs, suggesting poor sleep quality in BMS patients. These findings were consistent with a previous report (SLP9 score of about 42)¹³.

The change in anxiety levels were also evaluated, as this is important for managing BMS in terms of coping with pain and improving quality of life. Overall, we found a moderate-to-severe anxiety level in 35% of cases, consistent with the 8–50% in previous reports⁹. Interestingly, MLT resulted in a significantly greater reduction in anxiety than PLC⁵, but this is likely clinically irrelevant. However, this finding is consistent with evidence supporting use of MLT as a premedication (orally or sublingually) to reduce preoperative anxiety in adults⁵⁶.

Despite its lack of efficacy, MLT exhibited a generally safe pharmacological profile, as reported previously^{23,38,56}. There was no difference in side effects or dropout rate between the MLT and PLC groups. The main side effect with both treatments was self-reported sleep impairment, suggesting that patients perceived that MLT (independently of the presence of the active ingredient) influenced their sleepiness. Indeed, no significant change in objective measures of sleep (SLP9 and EES) was observed. Other minor side effects, *e.g.*, dizziness, headache and nausea, were similar in the MLT and PLC groups. This suggests a nocebo effect, *i.e.*, negative, adverse reactions subsequent to intake of a PLC⁵⁸, as has been reported during treatment of BMS patients⁵⁷. The nocebo effect results in the patient experiencing side effects despite taking a PLC and can result in dropping out of trials, as occurred in one case in this study⁵⁹.

The limitations of this study must be considered when interpreting the findings; these include the small sample size, the high rate of dropouts, lack of body mass index measurements (which may have influenced pharmacokinetics), and questionable patient compliance. Despite the pragmatic approach to patient recruitment and the good safety profile of MLT, these limitations hamper the generalisability of the findings. In addition, the hospital setting and consequent involvement of oral medicine specialists in BMS further hinders direct translation of the findings to general practice.

Given the correlation between BMS and sleep quality, future studies should investigate sleep quality improvement as a treatment to alleviate BMS pain, as well as the ability of MLT to ameliorate sleep quality and/or quantity in BMS patients who are responsive to this molecule.

Conclusion

Although recent evidence suggests an association between sleep disorders and BMS, MLT was not superior to PLC in reducing BMS-related pain under our experimental conditions. Sleep quality did not change, while anxiety improved slightly after MLT treatment, although probably not to a clinically relevant degree. High-dose MLT showed a generally good safety profile, and the side effects were comparable to those in the PLC group.

Figure Legends

Figure 1. Study flowchart. This crossover, randomised clinical trial involved four clinic visits at the following time points: T0, baseline visit before starting the first treatment; T1, visit at the end of the first 8 weeks of treatment; T2, baseline of the second 8 weeks of treatment, after a washout period of 4 weeks; and T3, end of the second treatment. At all time points, primary and secondary outcomes were recorded, and blood and saliva samples were collected. Primary outcomes included change in pain scores, and secondary outcomes included sleep quality and anxiety. MLT, melatonin; PLC, placebo.

Figure 2. (A, B) Patient assessment of pain change. (A) Responses (n, %) PLC treatment; (B) responses (n, %) after MLT treatment. No differences were statistically significant (χ^2 test). **(C) Visual Analogue Scale (VAS) scores of pain intensity and pain relief.** Pain relief was evaluated only post-treatment. Asterisks indicate significant differences ($p \leq 0.05$, paired t-test). Error bars indicate standard errors of the mean (SEM). MLT, melatonin; PLC, placebo; BL, baseline; PT, post-treatment.

Figure 3. (A) Sleep quality before and after treatments, as evaluated using the Medical Outcomes Survey (MOS) sleep scale. No difference was significant (one-way ANOVA, paired-sample t-test). Error bars indicate standard errors of the mean (SEM). **(B) Anxiety before and after treatments,** as evaluated using the Hamilton Rating Scale for Anxiety (HAM-A) score. Asterisks indicate significant differences ($p \leq 0.05$, paired t-test). Error bars indicate standard errors of the mean (SEM). MLT, melatonin; PLC, placebo; BL, baseline; PT, post-treatment.

Figure 4. (A, B) Serum and salivary melatonin levels. (A) Serum (baseline values were below the LOQ, ≤ 5 pg/mL) and **(B) salivary melatonin levels.** Asterisks indicate statistically significant differences ($p \leq 0.05$, one-way ANOVA). Error bars indicate standard errors of the mean (SEM). **(C, D) Visual Analogue Scale (VAS) pain intensity scores and serum and salivary melatonin levels in individual patients.** Available data from compliers are shown ($n = 12$) for **(C) PLC** and **(D) MLT** treatment. MLT, melatonin; PLC, placebo; BL, baseline; PT, post-treatment.

Figure 5. Evaluation of diurnal somnolence using the Epworth Sleepiness Scale (ESS). No difference was significant (one-way ANOVA, paired-sample t-test). MLT, melatonin; PLC, placebo; BL, baseline; PT, post-treatment.

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