Cellular effects of melatonin on oral keratinocytes and human gingival fibroblasts

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BACKGROUND

- About 25% of children overall experience some type of sleep problem and poor-quality sleep is one of the most common complaints by parents to their pediatric practitioners.
- While there is no FDA-approved sleep medication for use in children, there is pediatric literature supporting the safety and efficacy of melatonin in its use as a sleep aid.
- Melatonin is a hormone produced by the pineal gland that has been determined to be a significant modulator of circadian rhythms and the diurnal day-night sleep cycle.
- Many studies have demonstrated the effect of melatonin supplementation on sleep duration and sleep quality, which can improve overall systemic health and disease prevention.
- However, the cellular effects of melatonin are not restricted to the central nervous system and have been demonstrated in various tissues, including both cardiac and reproductive systems.

STUDY OBJECTIVE

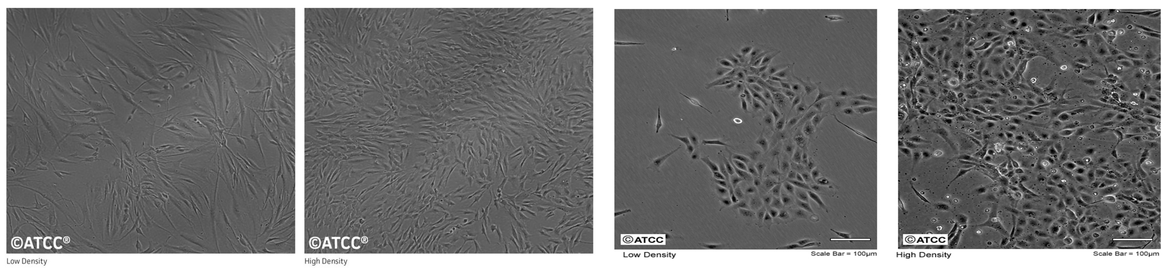
- More recently, melatonin has also been demonstrated to significant effects on oral health and tissues, including effects on periodontitis and periodontal inflammation.
- Despite the growing number of studies demonstrating the effects of melatonin on oral disease, no available studies have evaluated the effects of melatonin on normal oral tissues.
- Therefore, the primary objective of this study is to evaluate any potential effects of melatonin on normal oral cells and tissues within the physiologically relevant (supplementation) range.

RESEARCH QUESTION

Given the prevalence of over-the-counter melatonin, our research question is to determine “what are the cellular effects of melatonin in normal oral cells and tissues”?

METHODS

- No IRB approval was necessary for this in vitro, cell-based research study
- Normal oral keratinocytes (OKF4) and human gingival fibroblasts (HGF-1) were obtained and cultured for this study.
- Melatonin was administered in 96-well growth assays at supplement-equivalent physiologic concentrations at the low, mid and high range (1, 5 and 10 ug/uL) to determine any effects on cellular growth and proliferation.

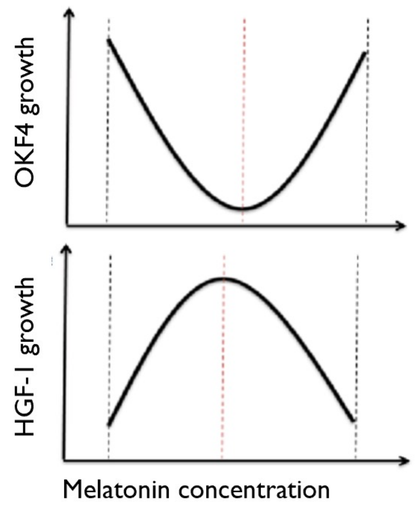


- Plates were fixed with formalin, stained with Gentian violet and read using a microplate reader at A630 nm absorbance.
- Absorbance data is measured on a scale (darker staining = more cells; lighter staining = fewer cells). These parametric data were analyzed using Microsoft Excel and two-tailed t-tests for statistical significance.
- In addition, RNA was extracted under each condition (control, low, mid and high melatonin) for subsequent qPCR screening for relevant mRNA expression.

RESULTS

Growth Assays

- 96-well growth and proliferation assays revealed a significant changes in OKF4 and HGF-1 growth under melatonin administration compared with controls (no treatment) - but no changes in cellular viability were observed.
- U-shaped dose responses were observed in OKF4cells under melatonin administration, ranging from +9.38% (low), to a maximum of -10.37% (mid) and +8.32% (high), p=0.022.
- U-shaped dose-responses among HGF-1 cells ranged from +9.4% (low), +15.5% (mid), and +10.4% (high), p=0.006.
- Interestingly, a diurnal pattern was also observed with effects of melatonin more strongly exhibited in early morning experiments compared with midday and afternoon.

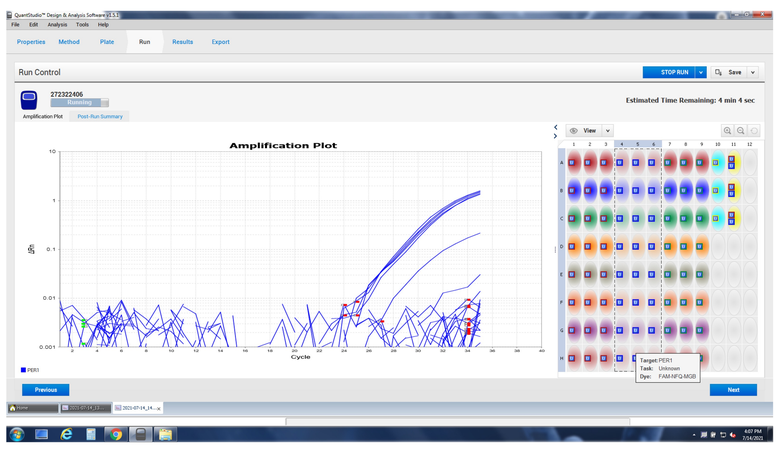


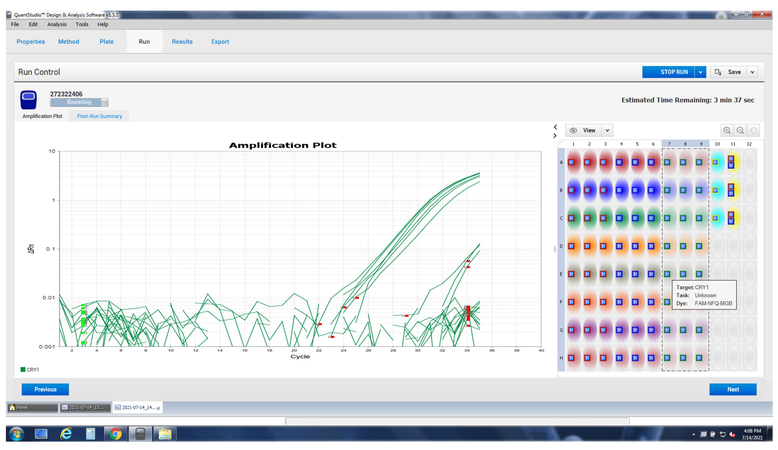
RNA Extraction/cDNA Synthesis

- RNA was successfully isolated from OKF4 and HGF-1 cells with and without melatonin treatment.
  - RNA concentrations ranged from 649 ng/uL to 4210 ng/uL.
- cDNA synthesis using the ThermoFisher high-capacity cDNA synthesis kit was successfully completed from the RNA.
  - cDNA concentrations ranging from 894 ng/uL to 4974 ng/uL.

qPCR Screening

- Preliminary qPCR screening for most of the melatonin-related pathway genes (BMAL,NPAS1, CRY1, CLOCK) has revealed no significant differences between the control and experimental groups with the exception of PER1 and CRY1, which were down-regulated by approximately two-fold in all treatment groups.
- Experiments are currently underway to determine if the diurnal (morning versus afternoon) cycle also affects these results





CONCLUSIONS

The goal of this study was to evaluate any potential effects of melatonin on normal oral cells and tissues within the physiologically relevant (supplementation) range.

- The results demonstrated that melatonin does significantly modulate growth and proliferation but not viability among these cell lines.
- In addition, the mRNA analysis has revealed that the major pro-apoptotic pathways controlled by BMAL and CLOCK are unaffected by melatonin in these cells, but the cell cycle (proliferation) genes controlled by PER1 and CRY1 may be differentially altered, which may explain these observations.
- These results suggest that melatonin may have some limited effects on oral tissues that may influence wound healing and repair but may not affect normal physiologic function or other cellular pathways.
- Future studies may explore the diurnal effects of melatonin on normal cells and tissues

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