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TITLE: Low Melatonin Production During Adulthood - Phase 2: Association with Levels of Hydroxyl Radical Scavenging and DNA Damage

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13. ABSTRACT (Maximum 200 Words)

The primary purpose of the proposed study is to develop cross-sectional evidence concerning whether or not lower melatonin production levels are associated with increased oxidative DNA guanine damage. If the results of this study are supportive, then confirmatory studies would be warranted, followed by prospective chemoprevention studies of melatonin supplementation. Adjuvant cancer treatment studies have not identified any serious melatonin toxicities.

High-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI MS/MS) will be used to simultaneously quantitate urinary levels of 8-hydrodeoxyguanosine (8-oxodG), 8-hydroxyguanosine (8-oxoGuo), and 8-hydroxyguanine (8-oxoGua). This assay appears superior to both the comet and the HPLC-ECD assays. Assays to estimate melatonin production have already been performed in Phase 1. The existing complete overnight urine samples have been properly processed and stored for the proposed study. Fifty-five (55) mother-daughter-father triples of urine samples along with detailed epidemiologic questionnaires are available.

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INTRODUCTION

The study is designed to develop preliminary data on whether oxidative DNA damage is increased in women with lower levels of melatonin production. The study uses the complete overnight urine samples collected under Phase 1 (funded by a DOD Concept Grant), with appropriate IRB and DOD approvals. The consent document allowed use of the specimens for the Phase 2 purpose. Specimens and data are identifiable only by code numbers, no longer linked to personal identifiers.

Breast cancer causes a reduction in melatonin production, making post-cancer measurements inappropriate for case-control studies. The objective of Phase 1 was to use a daughter's and her father's current melatonin production levels to estimate the mother's pre-cancer melatonin production level, making breast cancer case-control studies of low melatonin production appropriate. Cumulative overnight urinary 6-sulphatoxymelatonin (aMT6s) production, adjusted for urinary creatinine, was the measure of melatonin production.

Oxidative DNA damage can lead to malignant transformations and is considered an important factor in the development of breast cancer. Estradiol is a major contributor to the production of $HO^{\bullet}$, particularly in breast epithelial cells. $HO^{\bullet}$ has been shown to cause oxidative DNA damage, particularly to guanine. Melatonin appears to be a powerful scavenger of $HO^{\bullet}$. When guanine is oxidized, 8-hydrodeoxyguanosine (8-oxodG), 8-hydroxyguanosine (8-oxoGuo), and 8-hydroxyguanine (8-oxoGua) are excreted in urine. Several studies have found that melatonin inhibits mammary tumor development in animals prone to such tumors or exposed to a carcinogen. In animal studies, melatonin has demonstrated protective capabilities against natural oxidative DNA damage, as indicated by 8-oxodG levels.

BODY

The study had 3 primary tasks:

Task 1: To complete all preliminary work.
Task 2: To conduct the actual laboratory assays.
Task 3: To analyze the data and write the manuscript(s).

Task 1 has been completed, except the inclusion of 3-OHM in the assays proved not to be possible to accomplish in this study for technical reasons.

Task 2 has also been completed. High-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI MS/MS) has been used to simultaneously quantitate urinary levels of 8-oxodG, 8-oxoGuo, and 8-oxoGua. This assay appears superior to both the comet and the HPLC-ECD assays (Weimann et al, 2002). Assays to estimate melatonin production were already performed in Phase 1.

8-oxodG, 8-oxoGuo and 8-oxoGua analyses have been completed on 118 women in the

original sample, with 67 being daughters and 51 mothers.

The data have been edited and combined with the Phase 1 melatonin metabolite and epidemiologic data. Analyses have just begun.

KEY ACCOMPLISHMENTS

The key accomplishments to date have been the completion of the HPLC-ESI MS/MS assays.

REPORTABLE OUTCOMES

The analyses have not progressed sufficiently to provide any reportable outcomes.

CONCLUSIONS

Conclusions will not be available until the data have been analyzed.

REFERENCES

Weimann A, Belling D, Poulsen HE. Quantification of 8-oxo-guanine and guanine as the nucleobase, nucleoside and deoxynucleoside forms in human urine by high-performance liquid chromatography-electrospray tandem mass spectrometry. Nucleic Acids Res 2002;30:E7.

APPENDICES

None.