POSTER # P20

GENETIC TOXICITY EVALUATION OF MELATONIN IN THE BACTERIAL REVERSE MUTATION ASSAY

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TAKEAWAY MESSAGE

Melatonin exhibited no evidence of mutagenic activity under a Bacterial Reverse Mutation Test, supporting its safety profile at the tested dose range. These findings contribute to the non-clinical safety assessment of melatonin as part of the IGC-AD1 development program.

BACKGROUND

IGC Pharma has an ongoing Phase II Clinical Trial (NCT05543681) that explores the impact of the dual-API oral formulation IGC-AD1, an investigational drug combining low-concentration Delta-9-THC and melatonin that targets agitation in Alzheimer's disease.

Both are well-known compounds, but Melatonin is not yet considered a pharmaceutical substance. It is a neurohormone commonly used as a supplement that aids sleep-related disorders, as it regulates circadian rhythms. It also exhibits neuroprotective effects in Alzheimer's Disease ("AD"), as it has been proven to have the ability to reduce oxidative stress, preserve cognitive abilities, and neuronal survival[1,2]. Also, melatonin has anti-inflammatory properties related to the inhibition of TNF-α[3,4].

While melatonin is Generally Recognized As Safe ("GRAS"), its non-clinical safety profile remains inadequately characterized due to the lack of formal FDA approval. To test the safety profile of melatonin, it is necessary to evaluate its genetic toxicology.

A Bacterial Reverse Mutation Test ("Ames test") is part of the genetic toxicology battery that evaluates a compound's ability to induce reverse mutations in several bacterial strains of *Salmonella typhimurium* and *Escherichia coli*, both in the presence and absence of an exogenous mammalian metabolic activation system containing microsomal enzymes (rat S9).

This study evaluates the mutagenic potential of melatonin using the Bacterial Reverse Mutation Test, an established assay for assessing genetic toxicity[5].

METHODS

The assay employed multiple histidine- or tryptophan-dependent bacterial strains, including *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and the *Escherichia coli* strain WP2 trp uvrA, which are exposed to melatonin at a range of concentrations up to the standard limit dose of 5000 µg/plate, including 1.6, 5, 16, 50, 160, 500, 1600, 5000 µg/plate using dimethyl sulfoxide ("DMSO") as the vehicle (observed in **Figure 1**).

As recommended by the regulatory guideline[5]; Sodium Azide ("SAZ"), 9-Aminoacridine Hemihydrate ("9AC"), 2-Nitrofluorene ("2NF"), 4-Nitroquinoline N-oxide ("NQO"), 2-Aminoanthracene ("2AA") and Benzo[a]pyrene (B[a]P") are the mutagens used as positive controls.

The test is conducted under Good Laboratory Practices ("GLP"), in triplicate per treatment to count revertant colonies using the Plate Incorporation Method in the presence or absence of an exogenous metabolic activation system (rat S9).

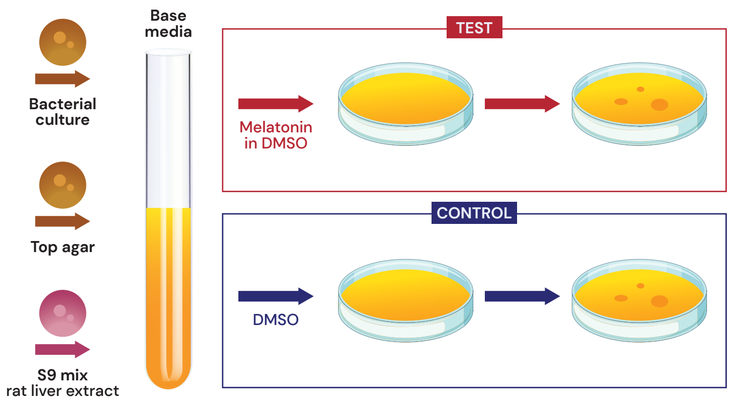

Figure 1. *Bacterial Reverse Mutation Test Methodology with S9 Mix Metabolic Activation System.*

RESULTS

Across all tested doses and bacterial strains, the Fold Response values remained consistently below 2, both with (+S9) and without (-S9) metabolic activation as observed in **Figures 2 and 3**. According to Ames test acceptance criteria[6], these results indicate no evidence of mutagenic activity at any concentration evaluated.

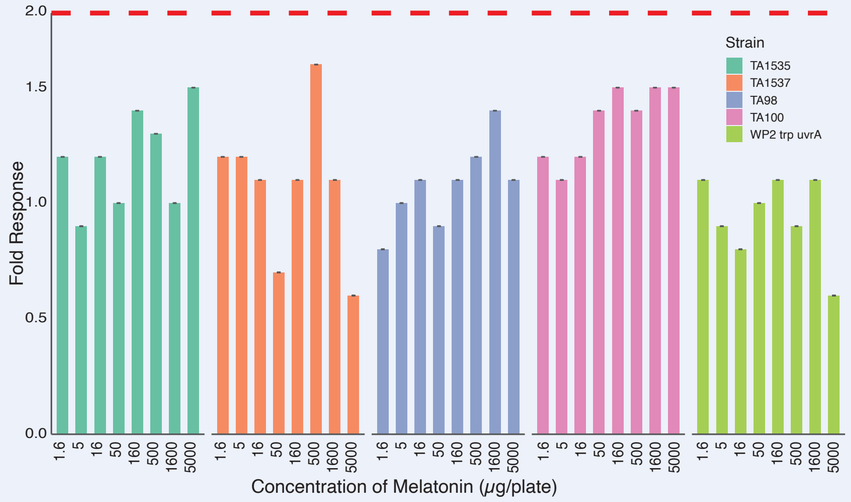

Figure 2. *Fold Response values for melatonin across all tested strains in the absence of metabolic activation (-S9).*

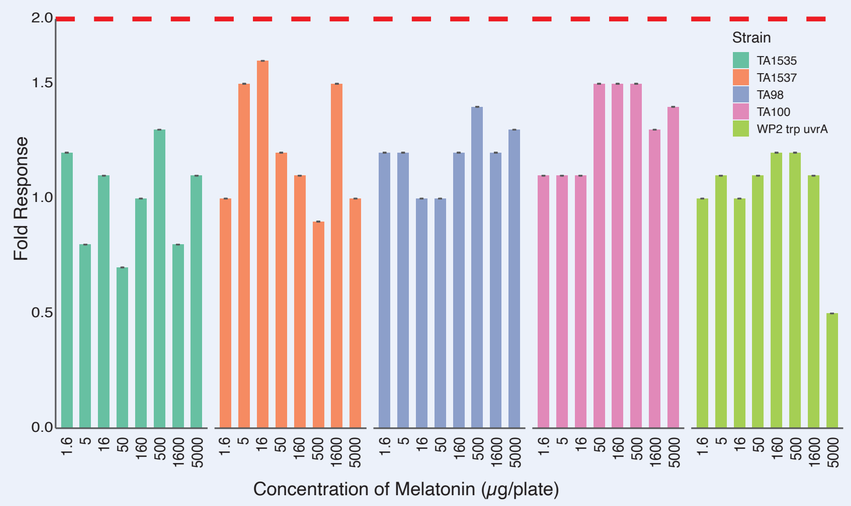

Figure 3. *Fold Response values for melatonin across all tested strains in the presence of metabolic activation (+S9).*

An analysis of the number of revertant colonies was also performed, as part of the evaluation of mutagenic potential. The data corresponding to this analysis are shown in **Figures 4 and 5**. Melatonin did not induce a significant or dose-dependent increase in the number of revertant colonies in any of the strains tested under either metabolic condition. Positive and negative controls met the acceptance criteria, confirming assay validity.

Statistical analysis using Dunnett's test[7] revealed that, for all tested doses of melatonin, p-values were greater than 0.05 when compared to the negative control across all strains and metabolic conditions.

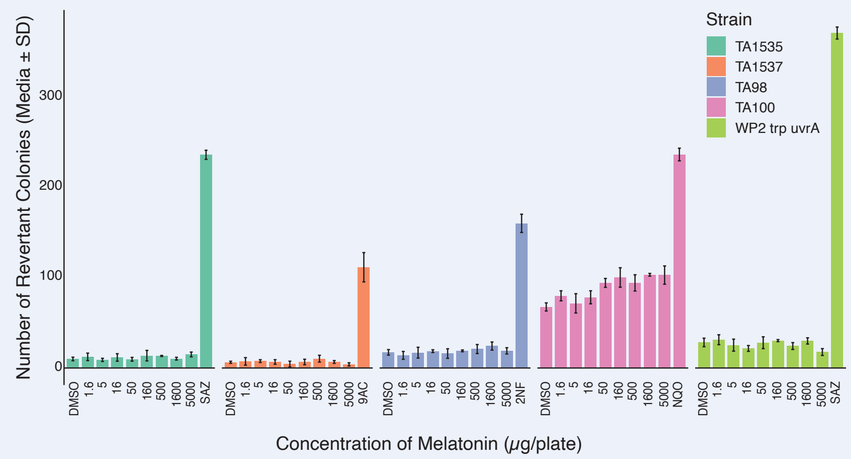

Figure 4. *Number of revertant colonies in the absence of metabolic activation (–S9) following treatment with different doses of melatonin compared to the vehicle and positive controls. Data represent mean ± standard deviation of three independent experiments.*

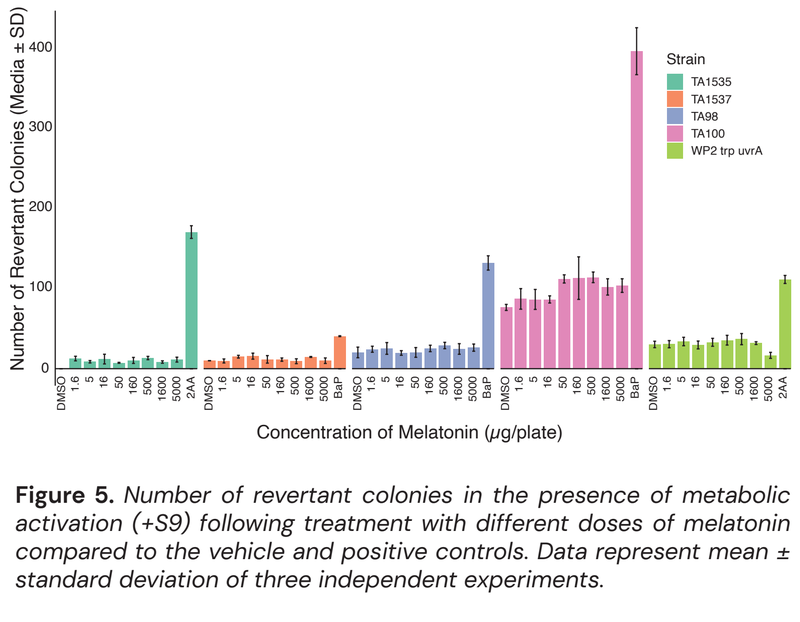

Figure 5. *Number of revertant colonies in the presence of metabolic activation (+S9) following treatment with different doses of melatonin compared to the vehicle and positive controls. Data represent mean ± standard deviation of three independent experiments.*

DISCUSSION AND CONCLUSIONS

Melatonin exhibited a safe genetic toxicity profile at the standard limit doses tested in *Salmonella typhimurium* strains (TA1535, TA1537, TA98, and TA100) and the *Escherichia coli* strain WP2 trp uvrA, as per OECD Guideline 471[5] and ICH S2 (R1)[8].

The consistently low fold response values, remaining below the mutagenicity threshold[6] across all strains and conditions, confirm that melatonin does not induce genetic mutations under the tested conditions, reinforcing its non-mutagenic profile in this assay.

Dunnett's test showed that all p-values exceeded 0.05. This, together with the consistent maintenance of revertant counts within the negative control range and substantially lower than those of the positive controls, supports the conclusion that melatonin does not induce mutagenic responses under the experimental conditions evaluated.[7]

Results support melatonin's safety profile at the tested dose range. These findings contribute to the non-clinical safety assessment of the compound as one of IGC-AD1's APIs.

FUNDING

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The test was performed at ITR Laboratories Canada Inc.

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