

## FINAL PROGRESS REPORT

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### Layman's Report

Skin depigmentation in vitiligo is the result of melanocyte destruction via mechanisms that are still unclear. However, individuals with pre-existing vitiligo are sensitive to the depigmenting potency of substituted phenolic substrates of melanin synthesizing enzyme tyrosinase. Clinical and experimental data support the capability of the substituted phenols to selectively cause toxicity in melanocytes, explaining the loss of functional melanocytes in depigmented lesional skin of patients with vitiligo.

The mechanism(s) by which substituted phenols, 4-tertiary butylphenol (4-TBP) in particular, destroy melanocytes has been an area of active investigation. It has been established that exposure to 4-TBP results in melanocyte destruction by the process of programmed cell death (apoptosis). It has been suggested that metabolites of 4-TBP most likely are responsible for melanocyte specific toxicity. Preliminary evidence in our laboratory supported a role for cellular glutathione conjugation detoxification pathway in the conversion of 4-TBP to a metabolite with potential to cause toxicity in melanocytes.

In the project proposal, the hypothesis to be tested was that  $\gamma$ -glutamyltranspeptidase (GGT) enzyme, a key enzyme involved in the maintenance of cellular glutathione (GSH), may be responsible for the conversion of 4-tertiary butylphenol (4-TBP) to a toxic metabolite capable of destroying melanocytes in occupational vitiligo. Immortalized cultures of human melanocytes PIG1 and PIG3V established from a normal and vitiligo patient respectively were used as an experimental model to accomplish the goals described in the proposal. Experiments were designed and implemented to determine (a) the baseline levels of cellular glutathione (GSH), a cellular antioxidant molecule that reacts with 4-TBP to make it non-toxic, and (b) characterize endogenous activity of glutathione S-transferase (GST), the enzyme that facilitates the addition of GSH to 4-TBP to make the 4-TBP-GSH (non-toxic) conjugate.

The project has resulted in several key observations that are critical for the development of new strategies for treatment of vitiligo. Experimental evidence demonstrates an inherent defect in major factors that regulate intracellular levels of glutathione in the melanocytes. Specifically, PIG-3V vitiligo melanocytes have significantly lower glutathione levels, decreases in the baseline activities of glutathione S-transferase and  $\gamma$ -glutamyl transpeptidase enzymes. In the event of GSH loss from the PIG-3V vitiligo melanocytes, their ability to recover from the lost GSH is much slower compared to the normal PIG-1 melanocytes. These results demonstrate that the PIG-3V vitiligo melanocytes are inherently deficient in GSH, the most important and abundant anti-oxidant molecule in the cell capable of dealing with oxidative stress. In addition, results demonstrate that PIG-3V vitiligo melanocytes have decreased ability to synthesize GSH and are unable to detoxify chemicals such as 4-TBP following external exposure. Thus, results from this research suggest that future studies must explore ways to increase the ability of vitiligo melanocytes to directly/indirectly increase cellular GSH levels as a potential treatment to prevent progressive loss of melanocytes in lesional skin. This would greatly enhance our ability to devise clinically relevant treatment strategies for patients with vitiligo.

## Scientific Report

Cultures of PIG1 and PIG3V cells from a normal and vitiligo patient, respectively, were used to test the hypothesis that  $\gamma$ -glutamyltranspeptidase (GGT), the enzyme essential for maintenance of cellular GSH, is responsible for conversion of 4-tertiary butylphenol to a melanocytotoxic metabolite.

Results demonstrate that total GSH content (per mg total cellular protein) in PIG3V cells was 30% lower compared to PIG1 cells. Exposure to 100 $\mu$ M and 250 $\mu$ M 4-TBP increased (250 $\mu$ M > 100 $\mu$ M) total GSH in both PIG1 and PIG3V cells. However, the increase in PIG3V cells was lower than that observed in PIG1 cells even at 24 hours.

Exposure to 10mM acivicin, a specific inhibitor of GGT, did not affect GSH content in PIG1 cells, but caused a 15-18% reduction in PIG3V cells. Treatment with 10mM buthionine sulfoximine, an irreversible and specific inhibitor of  $\gamma$ -glutamyl cysteine synthase (GCS), resulted in decreasing cellular GSH levels in both PIG1 and PIG3V cells. However, extent of GSH depletion was greater and 24 hours recovery in cellular GSH content was significantly lower in PIG3V cells compared to PIG1 cells. Treatment with ACV and BSO provided significant protection against 4-TBP toxicity in PIG-3V cells compared to PIG-1 cells supporting our initial hypothesis that GSH conjugation and related biochemical pathway is most likely responsible for the converting 4-TBP to a toxic metabolite. We are currently in the process of determining the GGT activities in both cell lines. The first two experiments have provided data which demonstrate that PIG-3V cells have very minimal GGT activity at the plasma membrane compared to PIG-1 cells.

The overall conclusion is that PIG-3V cells are inherently deficient in their ability to maintain adequate GSH levels in the cells contributing to their overall inability to combat oxidative stress. Results also provide the basis for the role of GSH in the bioactivation of 4-TBP to a metabolite that is specifically toxic to the melanocytes.