

ADDITIVE BENEFITS OF THE COSMETIC INGREDIENTS NIACINAMIDE AND N-ACETYL GLUCOSAMINE TOWARDS THE *IN VITRO* REGULATION OF MELANIN PRODUCTION

C Millikin, L Laughlin, D Bushnell, M Robinson, T Reichling, G Hurley, and E Jewell-Motz
P&G Beauty, Cincinnati, Ohio, USA

INTRODUCTION

In vitro studies have shown that niacinamide, which inhibits melanosome transfer between the melanocyte and the keratinocyte and N-acetyl glucosamine (NAG), an inhibitor of tyrosinase glycosylation, independently inhibit melanin synthesis. Here we confirm the ability of niacinamide and NAG to decrease melanin production *in vitro* and investigate the differential gene expression of cultures treated with niacinamide alone and in combination with NAG.

METHODS

Skin-Equivalent Cultures

- Melanocyte- and keratinocyte- containing cultures, reconstituted from human epidermis, were purchased from SkinEthic (Nice, France).
- Niacinamide and niacinamide + NAG treatments were applied topically to the cultures for 2 days (n=3).
- Water was applied topically to the control cultures daily for 2 days (n=4).
- Cultures were harvested after the second day of treatment
- RNA was isolated from the harvested cultures and preserved using RNeasy (Qiagen, Austin, Texas).

RESULTS

Statistical analysis of pigmentation-relevant genes revealed two interesting expression profiles:

Genes specifically up-regulated by the NAG + niacinamide combination

(Figure 1):

- **Syntaxin-8 (stx8)**: responsible for small molecule transport, which could increase melanin turnover.
- **Vesicle transport through interaction with t-SNAREs homolog (vti1)**: lysosomal transport protein, which could increase lysosomal melanin degradation.
- **Super oxide dismutase 1 (SOD1)**: an enzyme with strong anti-oxidant activity

Genes specifically down-regulated by the NAG + niacinamide combination (Figure 1):

- **Dopachrome tautomerase (DCT)**: also known as tyrosinase-related protein-2 (TRP2), regulates the expression of eumelanin (dark pigment) versus pheo-melanin (light pigment). Down-regulation of DCT would favor pheomelanin production.
- **Prostaglandin-endoperoxide synthase (PTGS2)**: also known as cyclooxygenase 2 (COX2), responsible for prostaglandin-mediated pro-inflammatory responses. Inhibition of PTGS2 would minimize inflammatory-mediated pigmentation.
- **Golgi SNAP receptor complex member 1 (GOSR1)**: a cellular transport protein that could reduce melanosome transport

Total Melanin Synthesis: (Figure 2)

- Mouse melanoma F1 cells treated with niacinamide alone produced 74% of the melanin produced by cells receiving no active treatment.
- Mouse melanoma F1 cells treated with both niacinamide and NAG produced 61% of the melanin produced by cells receiving no active treatment.

Figure 2. Relative melanin synthesis from mouse melanoma F1 cells treated with niacinamide and NAG (◆) vs. niacinamide alone (▲).

OBJECTIVE

To explore the additive benefits of niacinamide and NAG in reducing melanin production and to investigate additional mechanisms of action.

Genomic Analysis

- RNA isolated and GeneChip targets generated using the Enzo BioArray™ HighYield™ labeling kit and standard procedures.
- Affymetrix HG-U133 Plus 2.0 GeneChips were hybridized, washed, stained, and scanned using standard parameters.
- Summary statistics were calculated using standard methods

Total Melanin Analysis

- Mouse melanoma F1 cells were cultured to a confluency of 20 - 30 % and treated with media containing test compounds.
- Two days post treatment, a small amount of media was removed to determine cell viability using a ToxiLight viability assay kit.
- A solution containing 0.75% sodium hydroxide was then added to the remaining culture to lyse the cells and neutralize pH.
- Melanin was detected colorimetrically at 405nm using a spectrophotometer.

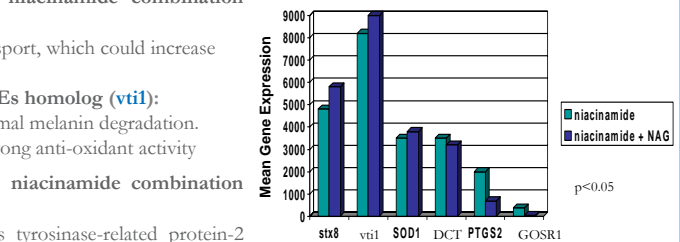
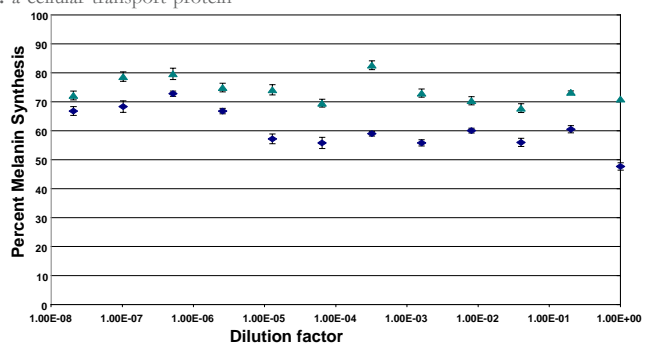


Figure 1. stx8, vti1, and SOD1 were up-regulated according to the profile: niacinamide + NAG > niacinamide while DCT, PTGS2, and GOSR1 were down-regulated according to the profile: niacinamide + NAG < niacinamide



CONCLUSIONS

- Genomics analysis suggests that the observed decrease in melanin formation is the result of genes that regulate both melanin synthesis and melanin degradation.
- The combination of niacinamide and NAG provides an additional benefit for the *in vitro* inhibition of melanin synthesis as compared to treatment with niacinamide alone.

This work was funded by P&G Beauty.

REFERENCES:

1. Bissett DL, McPhail SJ, Farmer TL, et al. Pig Cell Res. 2006; 19:373.
2. Bissett DL, Robinson LR, Li JX, et al. Pig Cell Res. 2006; 19:376.
3. Bissett D, Kimball A, Robinson L. J Am Acad Dermatol. 2006; 54:AB43.
4. Bissett D, Robinson L, Li J, et al. J Am Acad Dermatol. 2006; 54:AB43.
5. Bissett DL, Robinson LR, Raleigh PS, et al., J. Cosme Derm., 2007; 6:20.

P&Gbeauty