**STUDY #1:INVESTIGATION OF ERYTHEMA SUPPRESSION PROPERTIES ON UV INDUCED ERYTHEMA IN HUMAN SUBJECTS**

AMA Laboratories, Inc June, 2006

In a clinical trial, AMA Laboratories (New York) applied LIFT + FIX on a group of volunteers. They then exposed the panel to UV-A and UV-B radiation. The study results were summarized by AMA as follows:

 ***Study Results: AMA Lab No.: K-8371 when tested on 5 panelists as described herein, compared to untreated skin and integrating a lotion control, CPPs demonstrated a remarkable 95.3% (Table 1, Chart 1) reduction of induced erythema distributed over a 72 hour period when applied topically 15 minutes prior to exposure. Moreover, even in a relatively small panel, this data is considered to be statistically significant for evaluations conducted 24 and 48 hours post irradiation across most of the UV dose levels investigated.***

***It is also notable that a 42.0% suppression of erythema (also compared to untreated skin and integrating a lotion control) could be observed when the test product is applied immediately after exposure but not before.***

**Study #2: EFFECT OF CELL PROTECTION PROTEINS ON PROTEIN EXPRESSION** **in Skin Cells**

BioInnovation Laboratories (Lakewood, Colorado Study Numbers 05-0061 and 06-0012 May 31, 2006), analyzed the effect of CPPs on skin cells and found that almost all genes associated with skin protection and regeneration increased the amount of protein produced. The study results were summarized as follows:

***Discussion:Normal human keratinocytes were treated with 0.01% Cell Protection Proteins (CPPs) for 24 hours. At the end of the treatment period, RNA was isolated from the keratinocytes and analyzed for changes in gene expression versus untreated control keratinocytes. With respect to the genes of interest, CPPs appear to induce highly similar responses with respect to changes in gene expression, increasing the amount of protein produced.***

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| --- | --- |
| Protein Expression in Control Cells: | 100% |
| WHEN CPP IS ADDED: |
| **Heat Shock and Chaperonin Genes** |
| **Protein Expression:** | **191%** |
| **Replication Genes** |
| **Protein Expression:** | **176%** |
| **Transcription Related Genes** |
| **Protein Expression:** | **159%** |
| **Translation Related Genes** |
| **Protein Expression:** | **201%** |
| **Epidermal Differentiation Genes** |
| **Protein Expression:** | **196%** |
| **Antioxidant Genes** |
| **Protein Expression:** | **167%** |

**Further Comments from Dr. Holtz of BioInnovation:**It looks like the treatment really increased RNA transcription.  We recovered more RNA from the treated cells than the untreated cells, and the DNA microarray shows an upregulation of the RNA polymerases.  In addition, it looks like there was an dramatic increase in RNA translation.  The array shows an increase of a lot of ribosomal genes.  This suggests that the cells were really making a lot of protein.  There was also an increase in the HSPs and chaperonins (also helpful in thermal shock),probably to assist in making sure the proteins being made were in the proper confirmation.  The interesting question is what are they making?  Looking at the array data it appears that there was a lot of proliferation going on, suggesting that the material may be beneficial in promoting a skin rejuvenation effect. Expression of the cyclins was increased, as was the expression of the DNA polymerases.  In addition, there was an upregulation of several genes involved in epidermal differentiation including: KeratinV,ATP2C1,BPAG1,CLSPEVLP,FABP5,EGFR,GJB2,,LAMA3,NICE-4,MAPK1,

Interestingly there was also an increase in some of the keratin isoforms involved in hair production (KRTHA3A, KRTHB1 and KRTHB4).  There was also an increase in a few of the antioxidant genes (GPX1, PRDX2, AOP2and PON2).The changes in keratin may translate to benefits for hair care products,
while the changes in the antioxidant genes may translate into protection
of the skin from oxidative insults.

**STUDY #3: OSMOTIC STRESS PROTECTION (BIOINNOVATION LABORATORIES)**

Study Number 06-0014 April, 2006

**Purpose**

This assay procedure was used to screen materials for their ability to protect against osmotic stress using cultured human cells as the testing model. This assay used changes in cell viability as its endpoint.

**Discusion and Result**

**The purpose of this study was to test the ability of CPPs to protect against osmotic stress.** Cultured keratinocytes were pretreated with CPPs, sorbitol or left untreated and then an osmotic stress (hypertonic) was then induced by the addition NaCl. Although the addition of 50 or 75 mM NaCl was not observed to affect cell viability, the addition of 100, 125 or 150 mM of NaCl to the untreated groups of cells was observed to induce a dose dependent decrease in cell viability.

If the keratinocytes were pretreated with low concentrations of sorbitol, which induced a mild osmotic (hypertonic) stress, then the adverse affects of the higher NaCl concentrations on cell viability were partly blunted. This is evidenced by mean viability scores for the cells pretreated with either 12.5 or 25 mM sorbitol and exposed to 100 or 125 mM NaCl being significantly greater than viability scores for untreated cells exposed to similar levels of NaCl. However, the pretreatment with sorbitol was not able to confer any protection when the concentration of NaCl was increased to 150 mM. At this NaCl concentration, the mean viability scores for the untreated cells and the cells pretreated with sorbitol were not significantly different.

Pretreatment with CPP was also able to reduce the adverse effects of NaCl on cell viability, although the **protective effects of CPP were significantly greater than the effects of sorbitol.** While pretreatment with sorbitol was only effective against the addition of 100 and 125 mM NaCl, pretreatment with CPP provided protection at all three concentrations of NaCl observed to negatively impact cell viability. **Mean viabilities of keratinocytes pretreated with all three concentrations of CPP were significantly greater than untreated cells when the cells were exposed to 100, 125 and 150 mM NaCl. In addition, mean viabilities of keratinocytes pretreated with all three concentrations of CPP were also significantly greater than the mean viabilities of keratinocytes pretreated with both concentrations of sorbitol when the cells were exposed to 125 and 150 mM NaCl.**