tion of the final dose of cis-UCA as indicators of alterations in immune function: thymic and splenic organ weights, total cellularity and leukocyte cellularity of the thymus and spleen, splenic macrophage phagocytosis, and splenic leukocyte production of hydrogen peroxide (chemiluminescence assay). Significant decreases in thymic leukocyte cellularity were seen in both strains of mice in the four-week dosing regime, supporting previous observations of thymic effects in mice exposed subacutely to cis-UCA. (Supported by NIH RO1-ES 09642-01.)

725 ROLE OF METABOLISM IN ARSENIC-INDUCED CYTOKINE PRODUCTION IN MURINE KERATINOCYTES.

E. Corsini, M. Marinovich and C. L. Galli. *University of Milan, Milan, Italy.*

Inorganic arsenicals are skin carcinogens both in humans and rodents. It has recently been demonstrated that arsenic induces overexpression of keratinocyte-derived growth factors, which are likely to have a significant role in arsenic-induced skin hyperkeratoses and cancer. The mechanism(s) involved in this induction are, however, still elusive as the role of arsenic valence. The purposes of this study were to investigate the early intracellular events that follow *in vitro* treatment with sodium arsenate in a murine keratinocyte cell line (HEL30), which leads to interleukin-1α (IL-1α) production, and second, to characterize the role of the cell metabolism in arsenic toxicity. It has been known for some years that arsenic affects mitochondrial enzymes and impairs tissue respiration. We demonstrated by electron microscopy analysis that arseniate induced a dramatic alteration in keratinocyte mitochondria, that could be prevented by rotenone pretreatment, suggesting the possible involvement of mitochondria-derived reactive oxygen species (ROS). The first intracellular event following the binding of As to mitochondria is reactive oxygen species generation, followed by activation of transcription factors (NF-κB and AP-1), production of IL-1α and proliferation of keratinocytes. In mammals, the biotransformation of arsenic is based on several steps: arsenate species must be reduced to arsenite, through glutathione consumption. Arsenite is sequentially methylated, first to form monomethylarsonic acid and subsequently dimethylarsinic acid (DMA), which are less reactive and are rapidly excreted in urine. In our cells, DMA up to 1mM did not induce IL-1α production, confirming that the methylation of inorganic arsenic is indeed a detoxification mechanism. Regarding the effect of As valency on IL-1α production, we found that As(III) is five times more potent then As(V). However, this is due to a difference in cell uptake between As(III) and As(V), in particular, to a competition of phosphate anions with As(V) uptake. In medium without phosphates, As(V) shows the same potency of As(III). On the contrary the phosphates content does not influence the effect of As(III) on IL-1α production. Glutathione deprivation exacerbates both As(III) and As(V)-induced IL-1α production. Thus, it still remains to be elucitated the role of intracellular reduction of As(V), in order to identify the species responsible for IL-1α neosynthesis. (Acknowledgement: this study was partially supported by a grant from UNIPRO.)

726 QUENCHING OF CITRAL SENSITIZATION DEMONSTRATED IN A HUMAN REPEATED INSULT PATCH TEST.

A. M. Api and D. Isola. *Research Institute for Fragrance Materials, Inc., Hackensack, NJ.*

Quenching is the term used to describe the phenomenon of inhibition of sensitization. Citral is a fragrance ingredient that has a powerful lemon scent. It has been demonstrated that although citral produces sensitization reactions when applied alone, it does not produce any reactions in a human maximization test when combined with d-limonene (Opdyke, 1976). In this study, a solution of 4% citral and 1% d-limonene in diethyl phthalate was tested in a repeated insult patch test with a total of 118 human subjects. The test method was an adaptation of the Draize Patch Test that included an induction phase consisting of nine repetitive 24-hour occluded applications on the same skin site for three weeks. This was followed by a two-week rest period. The challenge phase consisted of a 24-hour occluded application to a naive site. No sensitization reactions were observed. In summary, quenching has been clearly demonstrated for citral with d-limonene in two different human sensitization tests (a human maximization test and a human repeated insult patch test).

727 SKIN PENETRATION BY THE NATURAL RUBBER LATEX PROTEINS, HEVEIN, AND RUBBER ELONGATION FACTOR.

M. D. Howell[1], B. B. Hayes[1], H. Alenius[2] and B. J. Meade[1]. *[1]National Institute for Occupational Safety and Health, West Virginia University, Morgantown, WV, and [2]National Institute for Occupational Safety and Health, Helsinki, Finland.*

Differences in IgE antibody patterns have been demonstrated in latex allergic individuals who have undergone multiple surgeries and those with limited surgical exposure to natural rubber latex (NRL). Adult health care workers show a higher prevalence of antibody towards hevein (Hev b 6.02) whereas children requiring multiple surgeries have shown a higher prevalence of recognition of Rubber Elongation Factor (REF, Hev b 1). The mechanisms underlying the differences in protein recognition by these risk groups is presently not known. These studies were designed to examine the extent of dermal penetration of hevein and REF using hairless guinea pig skin in an *in vitro* flow through dermal penetration model. Proteins were radiolabeled with 125-I using a modified chloramine-T method and applied to dermatomed skin sections (250 μm) that were unaltered or abraded via tape stripping to remove the stratum corneum. In comparing the penetration of hevein and REF into intact and abraded skin samples (n=6), no significant differences were observed between the two proteins. For intact skin, 0.34% and 0.57% of the total applied radiolabeled proteins were recovered for hevein and REF, respectively. Dermal abrasion increased the penetration into the skin with 5.22% of hevein and 3.32% of REF being retained. For both hevein and REF, less than 2% of the proteins penetrated through the intact skin while greater than 20% was able to penetrate through abraded skin. Mass balance for all penetration studies was greater than 94%. Immunohistochemistry of skin sections revealed localization of proteins in the stratum corneum of intact samples with more intense staining in the viable layers of the epidermis in abraded samples. Based on the results of these studies, the differences in sera recognition of hevein and REF between HCW and multiple surgery patients does not appear to be related to the ability of the proteins at equal concentrations to penetrate the skin. (These studies were supported in part by the NIOSH/NIEHS interagency agreement #Y02ES10189.)

728 DERMAL AND SYSTEMIC TOLERABILITY OF TOPICALLY APPLIED ISIS 2105, A PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDE (PS ODN), IN SPRAGUE-DAWLEY RATS.

R. E. Morgan, K. Lemonidis, A. Chappell and M. V. Templin. *Isis Pharmaceuticals, Inc., Carlsbad, CA.*

A topical formulation has been developed for dermal delivery of antisense ODNs. Local and systemic tolerability has been characterized using ISIS 2105, a representative 20-base P=S ODN, at concentrations of 0.2%, 0.5%, 2.0% and 10% (w/w). Vehicle absent ODN or formulated with ISIS 2105 was applied to the dorsal region of rats and an occlusion barrier placed over application sites for 6 hours per day. Local tolerability of ISIS 2105 was assessed following daily treatments for 14 days. Dermal tolerability was dose (conc)-dependent with moderate erythema and mild ulcerations at 10%; mild erythema and minimal ulcerations at 2.0%; minimal erythema at 0.5%; and no apparent effects at either 0.2% or in vehicle controls. Evidence of immune stimulation, e.g. a mononuclear cell infiltrate at the epidermal/dermal junction, was found at 10% and to a lesser extent at 2.0%. Immunohistochemical localization illustrated deposition of ODN in both dermis and epidermis. Quantitation of ODN in treated skin revealed non-linear, dose-dependent uptake; 5.8, 20.3, 51.1, and 362.0 μg/g in skin treated with 0.2%, 0.5%, 2.0% and 10%, respectively. Metabolism in skin was minimal, with 40% to 90% of total ODN measured as parent compound. Systemic exposure was low and dose-dependent. There was no evidence (hematology, serum chemistry, histology) of systemic toxicity. Most importantly, these studies verify epidermal and dermal uptake of ODN in intact skin. Dermal alterations were dose-dependent and correlated to significant local exposure. Systemic exposure was nominal, consistent with minimal toxicity. This suggests therapeutic dose regimens may be developed for safe/effective topical ODN delivery.

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