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**DOSE-RESPONSE STUDY OF D. PTERONYSSINUS, CAT DANDER AND GRASS POLLEN ALLERGENS FOR PATCH TESTING IN ATOPIC ECZEMA** U. Darsow<sup>1</sup>, D. Vieluf<sup>1</sup>, J. Berger<sup>1</sup>, J. Ring<sup>1</sup> for the APT study group, <sup>1</sup>Dept. of Dermatology and Allergology Biederstein, Technical University Munich, <sup>2</sup>University Hospital Eppendorf, Hamburg, Germany

Patch testing with aeroallergens can elicit eczematous reactions in atopic eczema patients (atopy patch test, APT). In a randomized, blinded multicenter trial, 253 patients (15-63 yrs) were epicutaneously tested with lyophilized allergen preparations (house dust mite D. pter., cat dander and grass pollen in petrolatum) on untreated unbraded back skin. The dose-response study was performed with 3000, 5000, 7000 and 10,000 PNU/g concentrations. Reactions were evaluated after 48 and 72 hrs according to patch test guidelines. The ratio of clear-cut positive/negative APT vs. questionable (only erythema) reactions or irritation was compared stepwise using the McNemar test.

The raw percentages of patients with at least one clear-cut positive APT reaction were 34% to D. pter., 12% to cat dander and 18% to grass pollen, the frequency of positive reactions increased with allergen concentration. Dose-dependency analysis showed optimal results for D. pter. at 7000, for cat at 5000/7000 and for grass pollen at 5000 PNU/g. APT results were associated with skin prick test, specific IgE ( $p < 0.0001$ ) and a predictive history of aeroallergen-induced eczema flares ( $p < 0.001$ ). Patients with a positive APT reaction to cat dander were significantly more likely to have atopic eczema lesions predominantly in air-exposed skin areas ( $p < 0.05$ ). No severe side effects were seen. These data may contribute to the concurrent standardization of the APT for clinical routine and experimental atopic eczema models. An allergen concentration of 7000 PNU/g is proposed to obtain optimal results using APT with the most frequent aeroallergens on unbraded skin.

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**CELLULAR BASIS FOR THE SPECIFIC ANTIMELANOMA ACTION OF N-PROPIONYL-4-S-CYSTEAMINYL PHENOL.** Panakkezhum D. Thomas, Shradha Singh, and Kowichi Jimbow, Division of Dermatology, University of Alberta, Edmonton, CANADA.

Earlier studies from our laboratory have shown that cysteaminy phenol and its derivative, N-acetyl-4-S- cysteaminy phenol (NACCAP), are good antimelanoma agents when tested in cultured cells and in B16 melanoma-bearing mice. Recently, we synthesized N-propionyl-4-S-cysteaminy phenol (NPrCAP) which was found to be more effective than NACCAP. However, the mechanism of the action of the drug leading to specificity is poorly understood.

Specific uptake of the drug by melanoma cells is one of the possibilities to explain this tissue-specific action. In order to verify this, we synthesized [<sup>14</sup>C]NACCAP and [<sup>14</sup>C]NPrCAP and measured the uptake by different cell lines using standard uptake assay (incubation of harvested cultured cells in a buffer with labelled compounds followed by filtration on glass fibre filters). Both the drugs are taken up by SK-MEL-23, a human melanotic melanoma cell line in a time- and dose-dependent manner. The addition of 1 mM (cold) NACCAP inhibited the uptake > 90%, thereby suggesting that the uptake is specific. Amelanotic melanoma cell lines and non-melanocytic cell lines failed to show any uptake, thereby suggesting that specific uptake could be the mechanism responsible for the specific action on melanoma. This is also reflected in irreversible damage to DNA synthesis machinery. These studies suggest that cysteaminy derivatives are promising candidates for developing melanoma therapy.

## 1239

**BIOCONVERSION OF TOCOPHERYL ACETATE TO TOCOPHEROL IN HUMAN SKIN: USE OF HUMAN SKIN ORGAN CULTURE MODELS.** Zeenat Nabi, Amir Tavakkol, Nadia Soliman and Thomas G. Polejka, Personal Care Products, Colgate Palmolive Co., Piscataway, NJ, USA.

There is substantial interest in delivering the antioxidant benefits of vitamin E (tocopherol) to the skin. Since free tocopherol is inherently unstable, it is usually delivered to the skin in the form of tocopheryl acetate. The premise behind this approach is that the skin's enzymes (esterases/lipases) would bioconvert the pro-vitamin E to the active vitamin. However, recent clinical studies by Alberts, et al. (*Nutr. Cancer*, 1996, 26: 193) suggest that human skin lacks the enzyme(s) necessary for this bioconversion. Thus, the objective of this study was to use human skin explants to examine the bioconversion of tocopheryl acetate to tocopherol. For comparison, two organotypic models, Living Skin Equivalent (LSE, Skin2™ ZK130f), Advanced Tissue Sciences, CA) and the EpiDerm™ Skin Model (EPI-100, MatTek Corp., MA) were also used in these studies.

Samples of viable human skin obtained from breast reduction surgery were sliced into 0.5 cm<sup>2</sup> pieces and maintained at the air-liquid interface in RPMI medium supplemented with 5% fetal bovine serum. The organotypic models were used according to manufacturer's recommendation. All organ cultures were treated topically (4 mg/cm<sup>2</sup>) with a lotion (oil in water emulsion) ± 1% tocopheryl acetate and incubated at 37°C in a 5% CO<sub>2</sub> environment. Skin samples were extracted with ethanol at various time points to recover tocopheryl acetate and tocopherol, which were then quantified by HPLC. Bioconversion of tocopheryl acetate to tocopherol was observed by the appearance of a new peak in the HPLC chromatogram. This bioconversion reached maximum levels at 6 hr in LSE and 10 hr in the skin explants. Additionally, in the EpiDerm model, pre-treatment with tocopheryl acetate reduced the peroxide-induced cell damage as determined by MTT assay.

The results of these studies show that 1) human skin and LSE possess the enzymes necessary to bioconvert tocopheryl acetate to the active tocopherol from topical formulation and 2) the skin explant can serve as a useful model for studying the skin's metabolic activity.

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**TOPICAL PHOTODYNAMIC THERAPY WITH 5-AMINOLEVULINIC ACID FOR ACTINIC KERATOSES USING THE SCLEROLASER® AT 585 NM** S. Karrer, C. Abels, W. Bäuml, M. Landthaler, R.M. Szymieski, Dept. of Dermatology, Univ. of Regensburg, Regensburg, Germany

Photodynamic therapy (PDT) after application of 5-aminolevulinic acid (ALA) and irradiation with incoherent or coherent light sources is effective for the treatment of actinic keratoses. However, this therapy causes severe burning pain during light treatment. With the aim to reduce this main side effect, in-vitro and in-vivo efficacy of PDT using the sclerolaser® (flashlamp-pumped dye laser with a of pulse length of 1.5 ms, 585 nm, Candela Corp., USA) was investigated.

HaCaT human keratinocytes were incubated with ALA (3 mmol/l) for 24 h and subsequently irradiated with different light doses (0 - 50 J/cm<sup>2</sup>) using the sclerolaser at 585 nm, 595 nm or 600 nm vs. an incoherent light source (580-740 nm, 40 mW/cm<sup>2</sup>). Cytotoxic effects were determined using the MTT-assay 24 h following irradiation. Controls (cells irradiated only without ALA) showed no response to laser or light treatment. Maximal cytotoxic effects could be achieved using either the sclerolaser at 585 nm or the incoherent light source, whereas the sclerolaser at 595 nm and 600 nm yielded a significantly lower cytotoxicity.

After proving the efficacy of PDT using the sclerolaser at 585 nm in-vitro, 11 patients with 65 actinic keratoses on the head received ALA-PDT once after application of a 20% ALA ointment for 6 h and irradiation with an incoherent light source (160 mW/cm<sup>2</sup>, 160 J/cm<sup>2</sup>) versus the sclerolaser® (585 nm, 16 J/cm<sup>2</sup>). Therapeutic efficacy was judged using an established score (0-8) evaluating infiltration (0-4) and keratosis (0-4) of AKs. Pain was monitored by a pain scale (0-10). In AKs with initially lower score (score < 4) the use of both light sources resulted in complete remission of all lesions within 28 days after PDT. AKs with higher score (≥ 4) responded better to PDT using the incoherent light source (incoherent laser: CR 88%, sclerolaser: CR: 71%), although the difference was not statistically significant. Control lesions (sclerolaser® treatment alone without ALA) did not show a significant score reduction. However, patient's acceptance was far better for PDT using the sclerolaser®, because the short exposure time (1.5 ms per lesion) caused significantly less pain as compared to a 16 min-irradiation using the incoherent lamp.

These results show the effectivity of topical ALA-PDT using both light sources. However, tolerability of PDT could be significantly improved by using the sclerolaser.

## 1241

**CULTURED SKIN SUBSTITUTES INCREASE UTILIZATION OF DONOR SITES FOR TREATMENT OF FULL-THICKNESS WOUNDS** Steven Boyce, Richard Kagan, Kevin Yakuboff, David Greenhalgh, and Glenn Warden Shriners Burns Institute & Dept of Surgery Univ of Cincinnati, Cincinnati, OH

Prompt and permanent closure of full-thickness wounds remains a limiting factor in recovery from burns and cutaneous ulcers. Cultured skin substitutes (CSS) may provide greater availability of grafts for permanent closure of excised, full-thickness wounds. CSS consist of autologous keratinocytes and fibroblasts attached to collagen/chondroitin-sulfate substrates incubated at the air-liquid interface for two weeks to stimulate formation of epidermal barrier. CSS were prepared for treatment of seven patients with large total body surface area full-thickness burns (range 58-91%) as an adjunctive therapy to conventional 1:4 meshed split-thickness skin grafts. Areas of healed wounds were determined by planimetry, and divided by areas of skin biopsies to calculate donor site utilization.

CSS covered excised burns at healed:donor area ratios ranging from 19 - 197 (mean ± SEM, 88 ± 29). Healed:donor ratios for CSS at day 28 were significantly greater ( $p < 0.05$ ) than conventional 1:4 meshed autografts. Therefore, utilization of donor skin with CSS was greater than with conventional grafts. These results suggest that:

1) acute-phase recovery of patients with large burns is facilitated by adjunctive use of CSS together with conventional skin grafting, and 2) autologous CSS may reduce morbidity from donor sites in treatment of cutaneous ulcers.

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**THE EFFECT OF TOPICAL RU58841 (ANDROGEN RECEPTOR BLOCKER) COMBINED WITH MINOXIDIL ON HAIR GROWTH IN MACAQUE ANDROGENETIC ALOPECIA.** K. Imamura, A. Bonfils, A. Diani, and H. Uno, Wisconsin Primate Research Center, Univ. Wisconsin, Madison, WI, USA, Hoechst Marion Roussel, Romainville, France, and Pharmacia-Upjohn, Kalamazoo, MI, USA

Our previous studies demonstrated that the treatment with either RU58841 (RU) or minoxidil (M) alone induced significant hair regrowth in the bald stumptailed macaques. However, the rate of hair growth has not significantly progressed after 6 months. This fact implies that M stimulates follicular cell proliferation but is unable to halt androgenetic follicular regression. On the contrary, RU prevents androgenetic follicular regression but appears to have no direct stimulating effect on follicular cell growth.

The present study was aimed to evaluate the effect of combined RU 5% (RU-5) with M 2% (M-2) or M 5% (M-5) solution on hair regrowth by both photographic recording and micromorphometrical analysis of follicular growth (folliculogram). The vehicle, mixture of propylene glycol, alcohol, and water, was used for both compounds and was applied to the placebo group. Each group has 3 monkeys. The data of hair growth for RU-5, M-2, and M-5 groups were used the results from our previous studies. The results revealed that the initial hair regrowth was noticed as early as 1 month after treatment in RU-5 + M-5 and 2 months in RU-5 + M-2 group. The similar degree of hair growth noticed at 3 months after treatment with RU-5 and M-5 alone. In M-2 group, the hairness maintained but showed no noticeable regrowth. At 3 months, regrowth of long terminal hairs appeared in both RU-5 + M-2 and RU-5 + M-5 groups and these hairs maintained for 6 to 12 months. The overall rate of anagen conversion and follicular enlargement were higher in combined groups compared to RU-5 and M-5 alone and revealed no significant difference between RU-5 + M-2 and RU-5 + M-5. The follicular growth was not found in M-2 group. Progressive effect of hair growth by combined treatment of antiandrogen with hypertrichotic agent showed most remarkably in early stage, but within one year the overall effects of hair regrowth revealed no significant difference compared to the rate in RU-5 or M-5 alone. Long-term observation will be necessary to find the synergetic effects on the follicular growth.