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LASER DOPPLER FLOWMETRY COMPARISON OF PHARMACODYNAMIC EFFECTS OF CETIRIZINE AND LORATADINE ON HISTAMINE INDUCED SKIN RESPONSE. Van Neste D.(1), de Brouwer B.(1), Valentin B.(2) and Coulie P.(2), (1) Skin Study Center, Skinterface, Tournai and (2) UCB International, Braine l'Alleud (Belgium)

In previous studies, reproducibility of the skin response to histamine administered by intradermal pricking (HP) was established using laser Doppler flowmetry (LDF). Hence the method appeared acceptable for performing *in vivo* evaluation of anti-H1 activity in clinically relevant terms to the dermatologist i.e. at the skin level.

In this study, LDF monitoring of the skin response (expressed as perfusion units above baseline; PU) to histamine (100mg/ml) was performed at 4 cardinal points located at 1 cm from the HP sites on the volar aspect of the forearm of 9 non atopic adults. All subjects gave their written informed consent to participate in a randomized, placebo controlled, double blind study comparing the anti-H1 activity of cetirizine (10mg) or loratadine (10mg). Skin responses to HP were evaluated 1h and 5h after a single oral intake of test compounds with a washout period of 8 days between test sessions. Statistical analysis was by analysis of variance; a profile of treatment related variation was evaluated for significance with Scheffe F-test ($p<0.05$).

As compared with skin responses observed after placebo, LDF readings did not show significant variations 1h after drug intake. However, 5h after drug administration, we recorded the expected change, i.e. a reduction of LDF signal at 1cm from HP sites with a significant difference between cetirizine (87.59± 118.97 PU) and loratadine (463.34± 350.06 PU). As indicated by the 95% confidence limits (from -3.8 to 179 and 194 to 732 PU for cetirizine and loratadine respectively) there was no overlap between skin PU recorded 5h after intake of drugs with significantly lower values after cetirizine ($p<0.05$). Both drugs clearly show a different activity profile at their skin targets.

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STUDY OF CHLORIDE CHANNEL REGULATION IN CUTANEOUS CELLS USING CELL VOLUME IMAGE ANALYSIS OF A SINGLE DISSOCIATED CELL. *M. Ohtsugawa, T. Toyomoto,*M. Morohashi, F. Sato, and K. Sato, Departments of Dermatology, University of Iowa, College of Medicine, Iowa city, Iowa, USA and *Toyama Medical and Pharmaceutical University, Faculty of Medicine, Toyama, Japan.

Chloride (Cl) channels are ubiquitous and involved in homeostasis and membrane transport in all living cells. Unfortunately, the study of ionic transport, especially that of ion channels, has been neglected in cutaneous cells such as keratinocyte, fibroblasts, and appendageal cells including the sweat gland cells, hair cells, and sebaceous cells. We hypothesize that ion channels in cutaneous cells are dynamically regulated in both normal and pathological conditions (e.g., spongiotic keratinocyte, effects of cytokines on cutaneous cell membrane, differentiation vs. membrane transport, effect of growth factors, cancerous cells). Although membrane channels can be studied using the patch clamping technique, we propose that a simple cell image analysis of cell volume regulation is equally suitable for the study of Cl channels. Freshly dissociated rhesus sweat clear cells and human keratinocytes were used as model systems. The method is based on the fact that if the cell membrane is made permeable to exogenous K channels with valinomycin, then activation of endogenous Cl channels by agents (Ca, ATP, cAMP, PKC activation, cytokine and growth factors, etc.) should cause cell shrinkage due to outflux of KCl from the cell. In contrast, if the cell membrane is made permeable to gramicidin-derived Na channels, then activation of endogenous Cl channels should cause cell swelling due to an influx of extracellular NaCl. In dissociated human keratinocytes, gramicidin-treated cells swell by 10% in response to 1mM ATP-gamma-S, indicating that ATP is one of the regulators of Cl channels in keratinocytes. In the valinomycin system, we also observed a small delayed decrease in the cell volume in response to 1mM CT-cAMP, suggesting that CFTR (cystic fibrosis tranport regulator)-encoded Cl channels are also present. We therefore conclude that the cell volume analysis is a promising and simple methodology applicable to the study of a wide variety of cutaneous cells in health and disease.

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IN VITRO EVALUATION OF GENETIC PREDISPOSITION TO TOXIC EPIDERMAL NECROLYSIS Pierre Wolkenstein, Dominique Charue, Jean-Claude Roujeau, Jean Revuz, and Martine Bagot, Department of Dermatology, Université Paris XII, Créteil, France.

The pathogenesis of hypersensitivity reactions has been hypothesized to be dependent on genetic predisposition involving cell defense mechanism. The aim of the present study was to identify genetic detoxication defects involved in severe cutaneous drug reactions. Lymphocytes of 26 patients (including 17 with Lyell or Stevens-Johnson syndromes) were tested for their susceptibility to reactive metabolites generated from drugs by a microsomal oxidation system.The culprit drugs were sulfonamides or anticonvulsants (respectively 13 and 13 patients). Toxicity of culprit drug reactive metabolites (CDRMs) toward patients lymphocytes (9.5% ± 2.2%) was higher than toward controls (3.5% ± 2.2 %) ($p<0.05$). First relatives of 4 patients with Lyell (3 to sulfonamides, 1 to phenobarbital) were also tested. In each family a relative was more susceptible to CDRMs than controls. In order to precise the detoxication defect involved in sulfonamide and anticonvulsivant reactions, we challenged lymphocytes from 11 patients (7 with hypersensitivity to sulfonamides, and 4 to anticonvulsants) to menadione, formaldehyde, and trichloropropene oxide (TCPO). Menadione induces toxicity by oxigen species. Formaldehyde is detoxified by aldehyde deshydrogenase, oxidase and reductase. TCPO is a potent inhibitor of epoxide hydrolase. After a 2 h incubation with one of these three chemicals, no difference of toxicity was found between patients and controls lymphocytes. In conclusion severe cutaneous reactions, especially Lyell or Stevens-Johnson syndromes to sulfonamides and anticonvulsants may be linked to a constitutional and inherited highly specific defect in the detoxication of CDRMs. Our results show that this genetic defect does not involve epoxide hydrolase, oxigen free radicals and / or aldehyde detoxication pathways.

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THE OCCLUSIVE EFFECTS OF PROTECTIVE GLOVES ON THE BARRIER PROPERTIES OF THE STRATUM CORNEUM. C.J. Graves, C. Edwards, R. Marks. Department of Dermatology, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, U.K.

The aim of this study has been to characterise the effects of occlusion by gloves on the stratum corneum in terms of its physical and functional properties.

A series of volunteer trials have been carried out, looking at the effect of occlusion by patches of PVC glove material on the stratum corneum. Percorneal permeability was assessed using time to onset of nicotinate induced hyperaemia by measuring laser Doppler blood flow. Impairment of barrier function was assessed by measuring trans-epidermal water loss. Surface roughness was measured using silicone rubber skin surface replicas and a stylus profilometer. Compliance of the stratum corneum was assessed by measuring the change in skin surface profile after application of a linear extension device. Hydration was assessed by measuring skin conductance using a skin conductance meter and the water sorption-desorption test.

We found there is at least a short term increase in percorneal permeability, measured as a 38% reduction in time to onset of nicotinate induced hyperaemia; and a temporary impairment of barrier function, measured as an increase in trans-epidermal water loss of 2.2 $gm^{-2}h^{-1}$. We found a temporary reduction in stratum corneum surface roughness and skin compliance, and a temporary increase in basic hydration as measured by skin conductance. However even after baseline conductance measurements have returned to normal levels, we found that peak conductivity after water sorption is still significantly elevated. Peak conductance after water sorption has been related to the ability of the stratum corneum to take up water. This effect was observed up to 3 hours after patch removal.

On the basis of these results we carried out a volunteer study looking at the effects of wearing PVC gloves on stratum corneum barrier properties on the dorsum of the hand. The regimen length was two days. Our results show that there are the beginnings of a cumulative effect. Measurements of TEWL remained elevated by 1.5 $gm^{-2}h^{-1}$ the day after removal of an occlusive glove.

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EFFECT OF GINSENG SAPONINS ON THE GROWTH OF CULTURED HAMAN KERATINOCYTE AND MELANOCYTE. Nack-in Kim, Tae-jin Yoon, Jae-kyung Park, Choong-rim Haw, Department of Dermatology, College of Medicine, Kyung Hee University, Seoul, Korea

The antiproliferative effect on the cultured Keratinocyte (KC) and Melanocyte (MC) with panax ginseng saponins from red ginseng were investigated. Purified ginseng saponins, such as total saponin (TS), panaxadiol saponin : ginsenoside Rb1, panaxatriol saponin : ginsenoside Rg1, and ginseng water extract were provided by Korea Ginseng and Tobacco Research Institute.

Epidermal KC and MC were isolated from the neonatal foreskin and cultured using MCDB 153 and modified TIC media, respectively. And also we try to make the Living Skin Equivalent (LSE) using human fibroblast and bovine collagen. After then various concentrations of ginseng saponins and ginseng water extract were added to each culture system and LSE.

The effects on the cell proliferation were evaluated by inverted microscope and survival cells were calculated using hemocytometer. Ginseng saponins treated LSE were stained with hematoxylin-eosin and Masson-trichrome. Epidermal thickness was observed under the light microscope.

The results were as follows : First, Ginseng saponins and ginseng water extract inhibited proliferation of the cultured KC in a dose-dependent manner. Second, there were no effects on the proliferation or melanization process of the melanocyte. Third, epidermal thickness of the LSE revealed that ginseng saponin treated groups were reduced than control subject. Fourth, apparent cytotoxic effects on the KC were observed in the panaxatriol with concentration of 100 μg/ml or above dosage. This study suggested that ginseng saponins from red ginseng may play a role in the treatment of hyperproliferative skin disease.

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THE EFFECT OF TERBINAFIN IN SOME EXTENSIVE DERMATOMYCOSES. A.L.Mashkilleyson, M.A.Gomberg, Department of Dermatology, Semashko Moscow Medical School, Moscow, Russia

Dermatomycoses (onichomycoses, **Tinea pedis, corporis, cruris**) are among the most common infections in humans. We have studied the effect of terbinafin (Lamisil) in patients with some extensive dermatomycoses including those with acquired immunodeficiency. **Trichophyton rubrum** was found to be the main ethilogic agent isolated from the lesions of our patients. Terbinafin was administered orally in a dose of 250 mg (1 tablet) daily. Bacterioscopy and bacteriologic tests were screened every 2-4 weeks. Patients with **Tinea corporis, pedis** and **cruris** were bacteriologically cured in 2 weeks of the therapy; the average times of bacteriologic cure in onichomycoses of the finger and toe nails were 4 and 8 weeks, respectively, including patients with immunodeficiency. The rate of growth of healthy nails was markedly increased if terbinafin was combined with pentoxifylline (Trental) - the drug enhancing microcirculation. Terbinafin is highly effective in dermatomycoses even in immunodeficiency.