

Vitiligo and Skincare Physicians Meeting

September 15-16, 2016 Berlin, Germany

Posters



Vitiligo and Skincare 2016

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September 15-16, 2016 Berlin, Germany

5-HT_{1A/1B} receptors as targets for optimizing Pigmentary responses in C57BL/6 mouse skin to stress

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Stress has been reported to induce alterations of skin Pigmentary response. Acute stress is associated with increased turnover of serotonin (5-hydroxytryptamine; 5-HT) whereas chronic stress causes a decrease. 5-HT receptors have been detected in pigment cells, indicating their role in skin pigmentation. To ascertain the precise role of 5-HT in stress-induced pigmentary responses, C57BL/6 mice were subjected to chronic restraint stress and chronic unpredictable mild stress (CRS and CUMS, two models of chronic stress) for 21 days, finally resulting in abnormal pigmentary responses. Subsequently, stressed mice were characterized by the absence of a black pigment in dorsal coat. The down-regulation of tyrosinase (TYR) and tyrosinase-related proteins (TRP1 and TRP2) expression in stressed skin was accompanied by reduced levels of 5-HT and decreased expression of 5-HT receptor (5-HTR) system. In both murine B16F10 melanoma cells and normal human melanocytes (NHMCs), 5-HT had a stimulatory effect on melanin production, dendricity and migration. When treated with 5-HT in cultured hair follicles (HFs), the increased expression of melanogenesis-related genes and the activation of 5-HT_{1A}, 1B and 7 receptors also occurred. The serum obtained from stressed mice showed significantly decreased tyrosinase activity in NHMCs compared to that from non-stressed mice. The decrease in tyrosinase activity was further augmented in the presence of 5-HTR_{1A}, 1B and 7 antagonists, WAY100635, SB216641 and SB269970. *In vivo*, stressed mice received 5-HT precursor 5-hydroxy-L-tryptophan (5-HTP), a member of the class of selective serotonin reuptake inhibitors (fluoxetine; FX) and 5-HTR_{1A/1B} agonists (8-OH-DPAT/CP94253), finally contributing to the normalization of pigmentary responses. Taken together, these data strongly suggest that the serotonergic system plays an important role in the regulation of stress-induced depigmentation, which can be mediated by 5-HT_{1A/1B} receptors. 5-HT and 5-HTR_{1A/1B} may constitute novel targets for therapy of skin hypopigmentation disorders, especially those worsened with stress.

Biography

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Vitiligo and Skincare Physicians Meeting

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The exploration of therapeutic target of vitiligo based on butin

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Vitiligo is a kind of skin depigmentation disorder which pathological mechanism is still unclear. There was less progress in the drug treatment of vitiligo within decades due to the unidentified pathological mechanism and therapeutic target. We found that butin, a new kind of small molecule compound derived from herb treated vitiligo, which could regulate the pigmentation process in melanoma cells and organ-cultured human skin. For further study the targets of the butin, we synthesized a novel fluorescent butin-BODIPY, which could improve melanogenesis in B16F10 cell line by selectively binding to its endoplasmic reticulum; we employed the proteomic approach of isobaric tags for relative and absolute quantification (iTRAQ), followed by LC-MS/MS, using the organ-cultured human skin with or without butin as a model. In total, 4550 unique proteins were identified. Of those, 60 were up-regulated and 242 down-regulated after 48 hours treatment with butin (10 μ M). The study aims at discovering therapeutic target of vitiligo and focus on the new mechanism of butin.

Biography

Jinpeng Lv is currently pursuing Doctorate in Pharmacology in China Pharmaceutical University, China. His main research is therapeutic target of vitiligo and proteomic.

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Vitiligo and Skincare Physicians Meeting

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Halobetasol propionate loaded nanostructured lipid carriers for topical delivery

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Vitiligo is a skin disorder with estimated prevalence of 1% of worldwide population and is characterized by depigmented macules due to the loss of functionality or destruction of melanocytes. The etiology is unknown, but one hypothesis is an autoimmune pathogenesis. Several treatments have been tested for this skin disorder, including psoralens, UVA therapy and steroids. Halobetasol propionate (Hb) is a potent corticosteroid commonly used for skin diseases such as eczema, rash and vitiligo, as initial treatment because of the severe side effects that may occur. To improve this characteristic, it is necessary to develop a controlled release system for this type of drugs. Nanostructured lipid carriers (NLC) constitute an alternative to improve drug delivery and decrease secondary effects. The purpose of this study was to design and optimize a formulation of NLC for topical delivery of HB based on lipid nanoparticles. This NLC were composed of Precirol, LAS and Tween 80 and the hot high pressure homogenization method was used. A factorial design based on three variables was used to plan the experiments, namely, amount of lipid, HB concentration and stabilizer concentration. The results showed that increasing lipid amount was followed by an enhanced tendency to produce larger particles with small polydispersity index (PI). The selected formulation showed size <200 nm; PI <0.230; Zp ~15 mV and Encapsulation efficiency (EE) >90%. These results are acceptable parameters for a formulation for topical delivery and are an alternative for controlled release of Hb.

Biography

Paulina Andrea Carvajal Vidal is a Pharmacist from Chile. She has completed her Master's degree of "Investigation, Development and Control of Medicines" and currently pursuing PhD at the Department of Pharmacy, Pharmaceutical Technology and Physical Chemistry at Barcelona University. Her studies are focused on the field of lipid nanoparticles for topical delivery, the utilization of corticosteroids and skin diseases.

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Vitiligo and Skincare Physicians Meeting

September 15-16, 2016 Berlin, Germany

The utility of performing routine blood investigations for associated autoimmune disease in patients with non-segmental vitiligo in South Africa

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The exact prevalence of vitiligo in South Africa is not known, but what is well known is the severe impact on quality of life, particularly in dark-skinned individuals. Uncertainty prevails on the importance and cost-effectiveness of autoimmune screening blood investigations in asymptomatic patients newly diagnosed with non-segmental vitiligo (NSV). The aim of this study was to ascertain whether the investigations routinely requested in all patients with newly diagnosed NSV yield any clinically relevant abnormalities. Over a one-year period we performed full-blood count, iron, ferritin, vitamin B12, thyroid stimulating hormone (with additional thyroxin T4 if abnormal), antinuclear antibodies, thyroid antibodies (including thyroid antimicrosomal antibody also known as antithyroid peroxidase antibodies and thyroglobulin antibodies) and a random blood glucose level on 110 patients (mean age 34.0 years, 74 females). 41 (37.3%) had significant abnormalities. In total, 35 patients (31.8%) were diagnosed with clinically significant related diseases at the time of presentation or within 12 months follow up. The diagnoses included iron-deficiency anemia (n=16), autoimmune thyroid diseases (n=11) and type 1 diabetes mellitus (n=3). After one year, 4 patients with antimicrosomal antibodies and 2 patients with antinuclear antibodies remained free of any systemic involvement. In conclusion, almost a third of patients with NSV had an associated clinically significant systemic disease, suggesting that “routine” screening remains relevant.

Biography

Susanna M H Kannenberg is a graduate of Stellenbosch University, South Africa. She has qualified Medical doctor degree in 1999 and worked in various hospitals and community health centers in South Africa and the United Kingdom. She has received her MMed in Dermatology cum laude from Stellenbosch University in 2012. She is a Dermatology Consultant at Tygerberg Academic Hospital in Cape Town, South Africa with limited private practice. She is also involved with the dermatology training of pre and postgraduate students. She has a special interest in vitiligo, atopic dermatitis and pediatric dermatology.

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Accepted Abstracts



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Patients with vitiligo are immune against skin malignancies and infections

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Autoimmune diseases like pemphigus and lupus erythematosus are protective against skin tumors and infections although they are on prolonged drug induced immunosuppression and this is in contrast with kidney transplant patients, who are highly liable for skin malignancies like basal and squamous cell carcinomas and infections like bacterial, viral and fungal. This new hypothesis has encouraged us to go further and test this hypothesis on other autoimmune diseases like vitiligo. And from our daily clinical practice; we observed that patients with universal vitiligo have no freckles and nevi and no skin cancers, melanoma or Kaposi's sarcoma. These observations have supported by many studies that have carried out in our department. It has been found that P53 is high in vitiliginous areas when compared with normal adjacent skin and no increase in skin cancers. While in another studies, there are low frequency of infections, photodermatoses and skin tumors in patients with vitiligo compared with patients with kidney transplants. Also patients with severe vitiligo especially universal type have lower frequency of skin tumors and infections when compared with patients with mild vitiligo and control with apparently healthy individuals. The etiology behind these finding is difficult to elucidate but we can speculate that the high P53 in vitiligo skin and the possibility of more effective DNA repair of solar damage by vitiligo skin are responsible for this protection. While the importance of depletion of melanin stores in vitiligo patients remain query and need explanation. In conclusion, patients with vitiligo like any other autoimmune diseases have protection against skin tumors, photodermatoses and infections.

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Tumorigenicity assessment of cultured melanocytes *in vitro* and *in vivo*

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Vitiligo is a multi-factorial polygenic disorder with an incidence rate of 0.1-2.0% worldwide, characterized by patchy loss of pigment in the skin due to abnormal melanocyte function. Autologous cultured melanocyte transplantation is one of the most effective and safe therapeutic methods for treating vitiligo. Utilization of appropriate culture media and growth factors provide safe and efficacious system for culture of melanocytes. The aim of this study was to find the best culturing media and assess the cultured melanocytes *in vitro* and *in vivo*. The melanocytes were isolated from skin samples of 8 vitiligo patients and then cultured in MGM-M2. The best condition media was selected according to proliferation and MTT assessments. We characterized cells using immunocytochemistry with their specific antibodies. Karyotype, real time PCR and gene sequencing for detection of chromosomal instability, gene expression and mutation in important genes of melanoma in different passages (0, 1, 3, 5 & 7) were performed. The cells of different passages were injected to nude mice's skin for tumorigenicity assessments. A375, D10 and NA8 melanoma cell lines were cultured and characterized as control group. ICC results confirmed cultured cells are melanocytes, cytogenetic analysis and real time-PCR did not show any chromosomal instability and changes gene expression. Mutation in famous genes was not observed. No detectable tumors formed. Histopathology confirmed the presence of cultured melanocyte cells were not tumorigenic. Our data show cultured melanocytes of different passages were not associated with any tumor formation in nude mice as well as *in vitro* results support the safety of cells for transplantation.

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Fabrication and characterization of anti-vitiligo cream containing 5% hydroalcoholic extract of *Psoralea corylifolia*

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Aims & Background: Vitiligo is a common disorder of human skin pigmentation leading to devastation and disfiguring the normal color of skin. Although multifactors have been suggested in etiology and different management options but still the medical science is in the search for definitive cause and ultimate cure of vitiligo. Plants have been reported to possess the anti-vitiligo spectrum particularly *Psoralea corylifolia* (PC) but there is a scientific exploration of this plant. This study was designed to formulate skin friendly emulsion (cream) from containing PC extract.

Materials & Methods: Hydroalcoholic extract of grinded seed of PC was screened for the presence of phytochemistry. Antioxidant potential was measured by various radical scavenging assays (DPPH, nitric oxide, superoxide and hydroxyl). Total phenolic contents were calculated spectrophotometrically. Thirteen formulations (F1-F13) were attempted by variable concentration of emulsifying agent in accordance with response surface methodology (RSM). Three of the formulations containing 5% extract of PC were tested for stability at different temperature and humidity to optimize *in vitro*, the most suitable formulation. Rheology studies were performed to determine the viscosity and shear rate.

Results: It was found that hydro alcoholic extracts of PC contained tannins, flavonoids, saponins, triterpenoids, steroids, glycosides and anthraquinones. Hydroalcoholic extract of PC possess significant radical scavenging potential (77.27%, 77.72%, 82.50 and 89.84% with increasing concentrations) and phenolic contents. An emulsion formulation containing 5% hydroalcoholic extract of PC was found stable at 0 oC, 8 oC, 25 oC and 30 oC.

Conclusion: An emulsion containing 5% hydroalcoholic extract of PC can be used to explore bioactive natural products that may serve as leads in the development of new pharmaceuticals for vitiligo. The *in vivo* study of this finding will support the traditional use of PC for vitiligo.

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Diagnostic of onychomycosis

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Onychomycosis is one of the most commonly occurring dermatological conditions. This study aimed to determine the aetiology of these onychomycosis in Tlemcen city in Algeria. A cross-analytic study was conducted from September 2015 to Mars 2016 in University Hospital Center of Tlemcen. All patients of onychomycosis diagnosed clinically were studied for clinical forms. Onychomycosis were proved in 73% of patients. Toenails were involved in 46.57%. Plantar reached (63.64%) and intertrigo interorteil (57.14%) were the two skin lesions most common in the feet. On fingernails, *C. albicans/dubliniensis* was isolated in 55.56% of cases. On toenails, *T. rubrum* was responsible for 81.82% of onychomycosis. In our study, yeasts were the main agents isolated on fingernails and dermatophytes on toenails.

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Stable vitiligo can be treated by various surgical procedures

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Background & Aim: Stable vitiligo can be treated by various surgical procedures. Non-cultured melanocyte grafting techniques were developed to overcome time consuming process of culture while at the same time providing acceptable results. All the techniques using non-cultured melanocyte transfer involve trypsinization as an integral step. Jodhpur technique used by the author is autologous, non-cultured, non-trypsinized, melanocyte and keratinocyte grafting. This is the only technique that does not require split thickness graft.

Methods: At the donor site, dermabrasion was done with the help of micromotor dermabrader at slow rpm till pin point bleeding was seen. Dermabrasion was then continued till the upper dermis and then stopped. The paste like material obtained by this procedure containing melanocytes and keratinocytes was harvested with spatula and was subsequently spread over the recipient area. Recipient site was prepared in the same manner as donor site except no harvesting of epidermal particles was done. After 10 days dressing at both sites was removed taking utmost care at the recipient site as there was theoretical risk of dislodging epidermal cells.

Results: In a study of 437 vitiligo patches, more than 75% re-pigmentation (excellent improvement) was seen in 41% of the patches. Lesions on thigh (100%), face (75%) and trunk (50%) showed maximal excellent improvement while patches on joints and acral areas did not show much improvement.

Conclusions: This technique is a cheaper, less time consuming alternative to other techniques which involve trypsinization of melanocytes and at the same time provides satisfactory uniform pigmentation.

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Evaluation of synergistic effect of red clover extract with Bimatoprost in the treatment of eyelash discoloration in patients with vitiligo

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Vitiligo is an autoimmune disease in which the body makes antibody against its own melanocytes and the skin loses its pigment and color in patches. Also, the amount of hydrogen peroxide (H₂O₂) is very high compared to the normal individual range. Increased tumor necrosis factor alpha (TNF alpha) and interleukin-1 levels directly cause apoptosis and activate cell death pathway in eyelash follicle melanocytes. As a result, eyelashes turn white. Treatment methods such as psoralen and ultraviolet A irradiation (PUVA) or the use of steroids are effective in treating skin discoloration. But these methods are not usually effective in the case of eyelashes. When bimatoprost is used in Cerita eyelash enhancer solution, it increases melanin synthesis via induction of tyrosinase activity. Therefore, result indicated that bimatoprost increased the melanogenesis process. Also, red clover extracts is used in this solution. Red clover is a rich source of isoflavones and A, C, E vitamins (The maximum scale was used in the solution combination). Consequently, these compounds effectively reduce catalase activity and the amount of H₂O₂. Moreover, bimatoprost and red clover have a synergistic effect on each other. This study was performed on 50 infected patients. 25 people used Cerita eyelash enhancer solution and 25 people used a concurrent placebo control daily for a month. The result of observation in the scanning electron microscope confirmed that the color of eyelashes changed and gets darkened eyelashes in the first group 60% compared with the control group.

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Effect of UV-B radiations on the antibacterial potential of filamentous cyanobacterium *Nostoc muscorum* on skin pathogens

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Chronic skin conditions are not curable and can be managed using drugs and by paying close attention to your lifestyle. Applications of algae in cosmetics and pharmaceutical industries are rapidly growing because of increasing demands from customers; the reason being the safety and efficacy of novel bioactive substance from natural sources than artificial substances. Cyanobacteria are regarded as a potential candidate for this as antibacterial, antiviral, antifungal, algicide and cytotoxic activities have been reported in these organisms. Radiations in the UV-B range have deleterious effects on humans as they generate free radicals and active oxygen species. These deleterious compounds are inactivated by antioxidants. Among them are the carotenoids and phycocyanin which protect against photodynamic action in different ways. Stress plays an important role in the production of bioactive metabolites from organisms. *Nostoc muscorum* was studied for antibacterial activity against various pathogenic bacteria resistant to a number of available antibiotics after being exposed to UV-B radiation. The antibacterial activity of *Nostoc muscorum* was studied on five potent skin pathogens. The highest antibacterial activity was seen the methanol extracts of 24 h UV-B exposed cultures of *N. muscorum*. It can be concluded that there was potent antibacterial activity. Results showed stress, solvent and dose dependent activity. This antibacterial activity might be due to the enhanced synthesis of carotenoids and phycocyanin under UV-B stress. The purpose of the present study was to relate the inhibitory effects of the cyanobacterial compounds specifically on skin pathogens with exposure to UV-B radiation as UV protecting compounds are already reported in these organisms.

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Statistical analysis of skin texture and color for classification and disease diagnosis of human skin

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Texture refers to visual patterns or spatial arrangement of pixels. Skin texture analysis plays a vital role in assessing the skin health and to diagnosis of skin disorders. Texture analysis is carried out by Structural, statistical, model based, transform based techniques. Statistical Texture analysis depend mainly on feature extraction which may be done using GLCM (Gray level Co-occurrence Matrix) and WDM (wavelength division multiplexing) techniques. The extracted features are used to classify texture. Skin texture can be analyzed using pixel intensity matrix parameters and GLCM. The research carried out by our team shows that Pixel intensity matrix often performs better than GLCM for analyzing skin texture. The following are the research outputs of our "Texture Analysis and disease diagnosis" research team. The pilot study carried out by our texture analysis team of Bio-Medical dept. ACSCE, Bangalore. In my lecture I would flash the output of our research on skin texture analysis to classify the skin of different parts of the body. The pixel intensity matrix parameters perform better than GLCM in identifying the skin texture GLCM. Region of interest is then selected; the selected region of interest is an RGB image which is then converted to gray image. The ROI must have at least 400 to 600 pixels to get reliable results. For this work a set of normal mole and another set of abnormal moles (three images for each set) are taken for experimentation. The covariance, standard deviation, means, f_v (maximum intensity pixel) are found for every image. From the values obtained a decision rule is framed to test the abnormality of the image of interest. There is a strong relation between the mole skin color and its pathological status i.e whether it is healthy or cancerous. When the mole is cancerous one its color highly differs from that of the one which is healthy that is why in the golden rule (A, B, C, D, and E) parameter diagnosis method used in skin cancer diagnosis the color plays a vital role.

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Fabrication of anti-vitiligo ointment containing *Psoralia corylifolia*: In vitro and in vivo characterization

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Vitiligo vulgaris is an ailment that has profound impact on pigmentation of the human skin. Different therapeutic regimens are used to treat vitiligo but none of them alone is too successful to be called as vitiligo cure. Recently, a change has been observed with the development of anti-vitiligo ointment containing hydroalcoholic extract (distilled water and methanol in 1:1 ratio) of ground seed of *Psoralia corylifolia* (P.C). The study aimed to explore the clinical outcome of ointment formulation containing hydroalcoholic extract of *Psoralia corylifolia*. 20 patients (aged 25-65 years) were included in this study. Formulation was applied on effected white patches of patients and some effected portion of the same patient was regarded as control (self control study design). The formulation that passed the required tests (pH, the temperature stability tests in 8 ± 0.1 °C, 25 ± 0.1 °C, 40 ± 0.1 °C and the physical properties like color, bleeding and rheology) was selected. FTIR studies of hydroalcoholic extract of P.C seeds and formulation were performed. The pigmentation of white spots of vitiligo was photographically evaluated before, during and after 12 weeks of treatment. A significant ($p\leq 0.05$) reduction in the pigmentation of skin was determined in the volunteers used herbal extract. Pre and post treatment difference in the levels of pigmentation was statistically significant ($p=0.05$). Hydrophilic ointment prepared from herbal extract was found effective in vitiligo as compared to self control. Ointment formulation containing hydroalcoholic extract of P.C could be an effective monotherapy for vitiligo.

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1, 3-Bis (3, 5-dichlorophenyl) urea compound 'COH-SR4' inhibits proliferation and activates apoptosis in melanoma

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The current clinical interventions in malignant melanomas are met with poor response to therapy due to dynamic regulation of multiple melanoma signaling pathways following administration of single target agents. In this context of limited response to single target agents, novel candidate molecules capable of effectively inducing tumor inhibition along with targeting multiple critical nodes of melanoma signaling assume translational significance. In this regard, we investigated the anti-cancer effects of a novel dichlorophenyl urea compound called COH-SR4 in melanoma. The SR4 treatment decreased the survival and inhibited the clonogenic potential of melanomas along with inducing apoptosis in the in vitro cultures. SR4 treatments lead to inhibition of GST activity along with causing G2/M phase cell cycle arrest. Oral administration of 4 mg/kg SR4 leads to effective inhibition of tumor burdens in both syngeneic and nude mouse models of melanoma. The SR4 treatment was well tolerated and no overt toxicity was observed. The histopathological examination of resected tumor sections revealed decreased blood vessels, decrease in the levels of angiogenesis marker, CD31 and proliferation marker Ki67, along with an increase in pAMPK levels. Western blot analyses of resected tumor lysates revealed increased PARP cleavage, Bim, pAMPK along with decreased pAkt, vimentin, fibronectin, CDK4 and cyclin B1. Thus, SR4 represents a novel candidate for the further development of mono and combinatorial therapies to effectively target aggressive and therapeutically refractory melanomas.

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Quick direct transplant of melanocytes in patients with vitiligo using needling micrografting, dermabrasion techniques and intralesional injection of melanocytes by spade like needle

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In refractory cases of vitiligo where medical therapy fails, surgical procedures are highly indicated where melanocytes are transplanted into vitiliginous area. There are many surgical procedures like punch mini-graft, suction blister epidermal grafting, split thickness grafting, cultured and non-cultured melanocytes transplant and others. These maneuvers are usually time consuming, need sophisticated labs and well trained personals and very costly. Recently we invented procedures by quick direct transplant of melanocytes from normal donor area into recipient vitiliginous skin. These methods are needling micrografting, dermabrasion techniques and intralesional injection of melanocytes by spade like needle. These new techniques are quick, take very short time, give rapid re-pigmentation, highly effective and without complications during and following operations. Also these new methods are simple, not need for sophisticated equipment, well trained personals and not costly. These new maneuvers will be discussed.

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The study of release of alarmin HMGB1 in keratinocytes Kuanhou Mou¹, Pan Li² and Wei Liu¹

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Vitiligo, a common idiopathic acquired depigmentation disorder, occurs mostly in young people who are typically very concerned about their appearance. Although the causes of vitiligo are still unknown, so, it is still not easy to treat with. In the past, scholar always focused on abnormal of melanocytes (MC), but recently we found not only MC but also keratinocytes had malfunctions. For example, HMGB1 could be passively released by apoptosis and necrosis keratinocytes or actively released in vitiligo. In order to investigate the release of HMGB1 by HaCaT cell and keratinocytes of tissue from vitiligo patients, HaCaT cells were treated with UV light or with apoptosis drugs or with cytokines and freeze-thaw process. The supernatant was condensed and subjected to SDS-PAGE to detect the release of HMGB1. Measure the HMGB1 and cleaved caspase-3 expression in skin biopsies of normal control subjects and vitiligo patients by immunofluorescence. HMGB1 was detected in the supernatant of HaCaT after treated and control supernatant was not detected. Cleaved caspase-3 was found in the stratum corneum of vitiligo patients and control patient was not found. HMGB1 was expressed in the nucleus of keratinocytes of control patients and transferred from nucleus to cytoplasm in vitiligo patients.

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Discoidin domain receptor-1 as a player in impairment of melanocytes adhesion process in vitiligo

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Aim: To study immune histochemical expression of DDR1 in lesional and non-lesional skin of vitiligo patients in comparison to controls, to explore its possible implication vitiligo pathogenesis.

Methods: 20 patients of non-segmental vitiligo (NSV) were subjected to punch biopsy from lesional and non-lesional vitiligo skin, in addition to punch biopsy from 10 healthy subjects. All specimens were examined by H&E staining and by immunohistochemistry for DDR1 expression.

Results: Significantly decreased expression of DDR1 in lesional vitiligo skin in comparison to non-lesional skin was observed. In addition, decreased lesional and non-lesional DDR1 expression in vitiligo skin in comparison to controls was found.

Conclusion: Reduced DDR1 expression may be implicated in impaired melanocyte adhesion process involved in vitiligo pathogenesis.

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Systemic effects of photo and photochemotherapy

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Phototherapy (UVA1)/photochemotherapy (PUVA) is a well established treatment of several dermatoses, such as cutaneous lymphoma, psoriasis and vitiligo among others. Under this treatment, patients develop generalized hyperpigmentation due to increased skin melanization and melanocyte proliferation, which is the rationale for its use in vitiligo and other hypopigmentary skin disorders. There is clinical evidence that hyperpigmentation not only affects exposed but also unexposed skin, albeit to a lesser extent, denoting that an endocrine effect results from release of melanocyte mitogens. To evaluate this possible systemic (endocrine) effect, plasma was collected from patients (n=16) treated with UVA1 and PUVA before and 1 and 2 h following irradiation at day 0 and day 14 of therapy. Plasma endothelins (1-3), α -MSH, basic-FGF, HGF, SCF and GM-CSF were assessed by ELISA. Results revealed significant elevation of both endothelins (potent melanocyte mitogens) and α -MSH (a melanocyte mitogen and immunomodulator) 2 h following irradiation when compared with values before irradiation session on day 0 ($p < 0.05$). This significant difference, however, was not observed on day 14 of treatment. Nevertheless, both endothelins and α -MSH values at day 14 remained higher than their corresponding time points (before and 2 h following irradiation session) at day 0 ($p < 0.01$ and < 0.05 , respectively). Interestingly, endothelin values were almost doubled under PUVA treatment than under UVA1 therapy, whereas α -MSH levels showed no clear difference between UVA1 and PUVA therapy. On the other hand, no significant changes in basic-FGF, HGF, SCF and GM-CSF plasma levels were detected after photo and photochemotherapy. Generalized tanning (hyperpigmentation) was clinically obvious in all treated patients. These findings indicate that UV light irradiation, whether alone or in combination with psoralene has a systemic (endocrine) and sustained effect through release of endothelins and α -MSH in the circulation. This may provide an explanation not only for the generalized tanning effect but also for the local and systemic anti-inflammatory effects of UV light irradiation. Furthermore, total body irradiation may be more beneficial than localized irradiation in inflammatory and hypopigmentary skin disorders.

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Emerging trends in the management vitiligo

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Vitiligo, histologically is characterized by loss of melanocytes with diminished or absent activity of melanocyte tyrosinase on the melanin pigment precursor dihydroxy phenyl alanine (DOPA). Worldwide, prevalence of vitiligo is of 0.5-1.0%; 1 in 136 or 0.74% or 2 million people in USA. In India, incidence of vitiligo is around 0.5-2.5%. The ratio of males and females is 1:1 and the age at onset is 20 or 30 years and 50% of the disease population develops their full clinical picture before attaining their early adult life. A disorder consisting of areas of macular depigmentation, commonly on extensor aspects of extremities, on the face or neck and in skin folds involving unilateral, bilateral, symmetrical distribution. Emotional distress and discrimination is also usually associated with vitiligo. Therefore, it is important to develop immune modulators to control the aggravation of the disease, melanogenic drugs to induce repigmentation at the same time to overcome the resistance phase.

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Novel method for generating fractional epidermal micro-grafts

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Epidermal suction blister grafting is an effective treatment for wounds or vitiligo but tedious and limited to small areas. We developed two novel strategies to create "fractional" epidermal grafts and compared them. Epidermal blisters were raised from fresh human skin ex vivo at 38-40 oC, with suction of 380-510 mm Hg. In Strategy-1, a 1 cm blister was micro-meshed into ~500 pieces, transferred to elastic adhesive dressing, then pneumatically expanded to ~9x the original blister area. Strategy-2: a 25 cm² array of 100 small blisters was raised, simultaneously harvested and captured directly onto an adhesive dressing. The pneumatic expansion limit, release of micro-blisters upon hydration of the dressing adhesive, light microscopy, epidermal cell viability and DOPA positive melanocyte presence in blisters were measured. Both strategies yielded viable fractional epidermal micro-blisters, carried on a dressing for transfer to graft-recipient sites. The micro-blisters were gradually released upon hydration of the dressing adhesive. Strategy-2 has major advantages; only small blisters are made at the donor site, skillful dissection and physical expansion are not required and the strategy can be scaled to create large area grafts. Strategy-2 is practical for fractional epidermal micro-grafting and has recently been commercialized.

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Vitiligo and Skincare Physicians Meeting

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Vitiligo and coeliac disease related or coincidental

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In coeliac disease gluten triggers an autoimmune reaction, which affects not only the gastrointestinal tract with inflammation, malabsorption and villous atrophy but can also be associated with other autoimmune diseases as diabetes mellitus I, thyroid disease and skin disease like dermatitis herpetiformis, linear IgA bullous dermatosis, urticaria, psoriasis and vitiligo. However there is an ongoing controversial discussion, whether vitiligo is related to coeliac disease. The prevalence of vitiligo in the general population is reported to be 0.5-2%. In cohorts of celiac patients higher prevalence of 3.8-9.1% were observed. In studies of vitiligo patients for autoimmune antibodies for celiac disease, the results are conflicting. In some studies a statistical correlation was found, in others no correlation could be determined. A convincing argument for a relation between vitiligo and coeliac disease would be the amelioration of vitiligo in patients with coeliac disease after introduction of a gluten free diet. The largest study for the amelioration of symptoms in coeliac patients after introduction of gluten free diet was performed by Norstrom et al in Sweden. Here in 1031 adult patients with coeliac disease of different ages the results of a gluten free diet on a wide range of symptoms was assessed. Among these patients 3.8% (n=39) had vitiligo. After introduction of a gluten free diet, 34 patients showed an amelioration of vitiligo ($p < 0.01$). In children with coeliac disease vitiligo was reported at a prevalence of 2% similar to the general population. In one child with coeliac disease re-pigmentation of vitiligo was reported after the introduction of gluten free diet. Vitiligo and coeliac disease can occur together. Adult patients with coeliac disease and vitiligo can benefit from a gluten free diet. In children with coeliac disease and vitiligo only singular reports are exist for the improvement of skin re-pigmentation after a gluten free diet. The large Swedish study by Norstrom et al., suggests a relationship between the two diseases. Therefore patients with vitiligo should be screened for transglutaminase IgA antibodies including the determination of total serum IgA in order to assess underlying coeliac disease.

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Efficacy of shwitravyoga (polyherbal powder) in vitiligo

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This is a single blind randomized clinical study on patients of vitiligo, a disease rather difficult for cure. Aetiology is multifactorial, it may be hereditary, autoimmune, hormonal imbalance, dietary, stress, secondary to other systemic diseases like diabetes mellitus and hypothyroidism etc. This study is carried out on 200 outdoor patients suffering with vitiligo. Patients from both sexes from age group 5-70 years complaining mainly as white patches are studied. Patients are treated with ayurvedic polyherbal powder mixture containing *Psoralea corylifolia* (Bakuchi) as main ingredient supplemented with local application and phototherapy (natural). Duration of treatment is of one month to one year depending upon response and requirement for the treatment. Follow up is biweekly to monthly. The response to treatment is observed in terms of reduction in area of depigmentation after treatment. Complete cure is noted in 12% patients. Good result is noted in 18% cases, moderate in 45% cases and mild result in 25% cases noted. The result is statistically highly significant at 0.1% level. No major side effects of the treatment given observed. The treatment is effective in all types of vitiligo. The results of internal medications and local treatment are aggravated after panchakarma.

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A comparative study of split thickness skin grafting followed by topical corticosteroid versus split thickness skin grafting followed by PUVA-sol therapy in stable vitiligo

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Background: Achromic fissures and perigraft halo are well known complication of split thickness skin grafting in stable vitiligo. PUVA and PUVA-sol are established therapies for these unwanted side effects but no comparative study till now has been done for topical steroid in the treatment of perigraft hallow.

Objectives: The aim of this study was to evaluate the efficacy of topical corticosteroid in perigraft hallow and achromic fissures and to compare it with perigraft pigmentation after PUVA in patients with stable vitiligo.

Methods: 50 patients with stable vitiligo of various clinical variants were subjected to split thickness skin grafting. They were randomized into two groups: One group received post grafting PUVA (group I) and the other group post grafting topical application of fluticasone propionate cream, 0.05% (group II). During the next 6 months follow-up, 4 patients were lost to follow-up and two patients were excluded from the study; 40 patients were evaluated for pigment spread and side effects.

Results: In group I, the average pigment spread was 7.44 mm, whereas in group II, it was 7.94 mm, showing a slightly higher pigment spread in group II, which was statistically not significant. 19 cases (76%) of group I compare to 21 cases (84%) of group II showed >90% pigmentation on vitiligo area severity index. There was no difference in response to therapy in patients having segmental vitiligo as compared with non-segmental vitiligo. Infection, milia, hypertrophy of graft, hyperpigmentation and hypopigmentation of graft were few side effects seen in some patients in both groups.

Conclusion: The study shows that the pigment spread with topical corticosteroid is comparable to that with PUVA. However, long-term follow-up are required to establish this. The advantages of topical corticosteroid are that its use is easy, less cumbersome, cheaper and more cost effective than PUVA.

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The use of 308-nm excimer laser for treatment of vitiligo

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2²¹ patches of stable vitiligo from 97 patients of different ethnic backgrounds were treated with this modality of treatment. 54 were males and 43 were females. Mean age was 33.5 years. 30 lesions were left untreated as controls. Patients were treated for 30 sessions or 75% re-pigmentation or more, whichever comes first. Initial dose was 100 mj/cm² which is increased by 1/2 MED (50 mj/cm²) until erythema developed. Treatment was given twice per week. 50.6% of treated lesions showed 75% re-pigmentation or more. 64.3% of lesions showed 50% re-pigmentation or more. Lesions on the face showed the best response with 77.6% of them achieved 75% re-pigmentation or more and 81.3% had 50% re-pigmentation or more. Patients with skin phototype III-VI had the best results. None of the control patches developed pigmentation. No significant side effects were encountered and none of the responding patients lost their pigment during the two year follow up.

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Pediatric vitiligo: A study to delineate the clinical profile of a morbid disease

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Introduction: Approximately 50% of vitiligo patients are less than 20 years, while 25% of children experience vitiligo before the age of 10 and its onset is on the rise since two decades.

Aim: To study clinical pattern in childhood vitiligo.

Material & Methods: A total of 120 patients with vitiligo were included. The diagnosis of vitiligo was made on clinical grounds by two different dermatologists independently. The inclusion criteria were unequivocal clinical diagnosis of vitiligo and age of onset up to 18 years. The cases whose clinical diagnosis was in doubt, which did not consent to take part in the study, were excluded. Relevant differential diagnoses were ruled out on basis of assessment and appropriate investigation. A detailed history of the patient and his/her relatives was taken and clinical examination was done.

Results: Out of 120 patients in study, 75 (62.5%) were females and 45 (37.5%) were males. The mean age of onset was 8.4 years. A positive family history was seen in 27 (22.5 %). Clinically, 45 (37.5%) patients had vitiligo vulgaris, 36 (30%) had segmental distribution, local variant in 24 (20%) while 15 (20%) showed acral distribution. The most common site was trunk (33.9%) followed by head and neck (32.14%), extremities (23.4%) and palms/soles (10.7%). 6 (5%) patients had additional mucosal involvement. 18 (15%) of the patients relatives had autoimmune disorder, most common being hypothyroidism. Leucotrichia was seen in 42 (35%) of the patients, premature graying in 12.5% of patients. Koebnerization was seen in 15 (12.5%) while nail involvement was seen in 57 (47.5%) of patients with the most common finding being striate leuconychia (32.5%) followed by pitting (15%).

Conclusion: In recent times, vitiligo is affecting younger generation with an increasing incidence. The occurrence of vitiligo in young may offer a more clear understanding of its pathogenesis. Its short duration and limited history can point to the triggering event more clearly. Further childhood vitiligo requires special consideration both in terms of pharmacological and psychological management.

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Radiotherapy-induced vitiligo in a patient with carcinoma buccal mucosa

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Vitiligo significantly affects self-esteem and deteriorates the quality of life of affected persons. Radiotherapy has several early and late effects but it is not known to induce vitiligo. This is a case report of a patient suffering from carcinoma buccal mucosa that had developed vitiligo in the radiotherapy portal. To the best of my knowledge this is one of the first case reports of its kind as this patient had no history of vitiligo but developed it soon after radiotherapy. Since radiotherapy is an essential component of cancer management, the radiation oncologist must be aware of this toxicity of radiotherapy. Choosing the high energy of photon beam may reduce the risk of such toxicities.

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