stead, a novel finding was that IL-4-positive mast cells were highly correlated with the wheal size ($p<0.003$). Furthermore, tryptase-, chymase- and IL-4-positive mast cells correlated significantly ($p<0.05$) with the levels of total IgE in serum but not with cow-specific IgE. These findings point to the clinical relevance of cutaneous mast cells and the significance of IL-4 though further studies are needed to elucidate its functions.

Prick tests were performed on the forearm skin of 51 atopic subjects sensitive to cows with a crude cow dander extract to investigate the effect of intracutaneously injected mepyramine, nordihydroguaiaretic acid (NDGA) and indomethacin (each at 10 and 100 μg/ml) on the subsequent wheal size. Mepyramine decreased the wheal by 33% ($p<0.001$) whereas NDGA showed no effect and indomethacin increased it by 27% ($p<0.02$). Fourteen subjects of 51 did not respond to mepyramine pretreatment with a diminished wheal size. Similarly, the leukotriene antagonist, zafirlukast (40 mg), was inefficient and indomethacin (100 mg) increased the wheal by 16.6% ($p=0.035$) when administered perorally to 5 other randomly chosen atopic subjects before prick-testing.

It is unhelpful to argue that one particular mast cell mediator is important in the allergic wheal reaction as histamine, LTC_4, PGD_2, chymase, tryptase and IL-4 are probably all important and present study give a little new information of them.

List of original publications

1. Saarinen JV, Harvima RJ, Naukkarinen A, Horsmanheimo M and Harvima IT. Release of histamine, leukotriene C_4 in immediate allergic wheal reaction as measured with the microdialysis technique. Arch Dermatol Res 2000; 292: 333–340.
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3. Saarinen JV, Harvima RJ, Naukkarinen A, Horsmanheimo M, Harvima IT. The release of histamine is associated with the inactivation of mast cell chymase during immediate allergic wheal reaction in the skin. Clin Exp Allergy 2001; 31: 593–601.
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Long-wave Ultraviolet Radiation (UVA1) and Visible Light. Therapeutic and Adverse Effects on Human Skin

Desiree Wiegleb Edström
Department of Dermatology, Karolinska Hospital, Karolinska Intitutet, Stockholm, SE-171 76 Karolinska Hospital, Sweden.
E-mail: desiree.edstrom@ks.se

The effects on human skin of repeated UVA1 irradiation and visible light

Sun exposure is widely accepted as the major risk factor for developing skin cancer. Ultraviolet B radiation (290-320 nm) is considered the causative agent. However, it has been shown that long-wave ultraviolet A (UVA1: 340-400 nm) also induces non-melanoma skin cancer development in hairless mice.

The *p53* is a tumour suppressor gene, a gene that contributes to the development of cancer when it is inactivated. *P53* gene mutations are induced by ultraviolet radiation and found in squamous cell carcinoma, basal cell carcinoma and in actinic keratosis. We studied the effects of repetitive suberythemal fluences of long-wave ultraviolet UVA1 radiation and visible light in normal sun-shielded skin, using immunohistochemical staining for p53 and the downstream mediator p21WAF-1, a cycline-kinase inhibitor, bcl-2 an apoptosis inhibitor, Ki67 and cyclin A, proliferation markers. An increased expression of Ki67 after UVA1 and visible light were observed as a sign of increased proliferation. By comparison to untreated skin, increased expression of p53 protein, but not

p21WAF-1 in epidermis after UVA1, were seen. After visible light only a slightly increased expression was observed. These results suggested that suberythemal doses of UVA1 – and even visible light – may cause DNA damage.

To investigate the *p53* status of the scattered immunoreactive cells resulting from UVA1 exposure, genetic analysis of the *p53* gene in single cells was performed. Single *p53* positive cells from UVA1-irradiated, earlier sun-shielded, skin were therefore microdissected and thereafter PCR-amplified (polymerase chain reaction) with p53 sequence analysis. This showed three mutations, all CA transversion (Cytosine to Adenine). One of the mutations was found in codon 231 of exon 7 (coding DNA) and the other two in the intron (non-coding DNA). The average mutation was 1 per 8,700 bases or 1 per 12 cells, correlating well with the earlier findings of ultraviolet A signature mutations in normal sun-exposed human skin. There are strong indications that even relatively low doses of UVA1 can give rise to *p53* mutations.

The effects of dye laser in treating portwine stains

The wavelength at which the dye laser operates is in the visible range commonly used in treating portwine stains (PWS). The treatment result depends on the wavelength, pulse duration and fluence of the laser system. Twenty-two patients with PWS were treated with flashlamp-pulsed dye laser (FPDL) using the wavelengths of 585 and 600 nm. There was significantly less lightening with 600 nm than with 585 nm when equal fluences were used. When 1.5 and 2 times the 585 nm fluence were applied with 600 nm, the lightening was equal to that of 585 nm. However, in individual cases (11 of 22), 600 nm showed superior lightening of at least 20% compared to 585 nm.

We compared the FPDL at 0.45 msec pulse duration and spot size 5 mm with an argon-pumped dye laser with a robotized scanning laser handpiece (Hexascan) at 70–190 msec pulse duration and spot size 1 mm. Both were tuned to 585 nm. Thirty patients with PWS were treated on test areas using both laser systems. Twelve weeks later the degree of lightening was evaluated and biopsies were taken. The skin sections were immunohistochemically stained with the marker of endothelial cells, CD34, to count the vessels. The clinical result showed a significantly better lightening using the flashlamp-pulsed dye laser. The histological result showed significantly fewer vessels of a diameter larger than 20 μm in treated PWS than in untreated, but no difference between the two laser types. However, there was a tendency towards more small vessels (diameter < 10 mm) after one treatment with the FPDL compared to untreated. This might reflect angiogenesis.

The effect of photodynamic therapy in mycosis fungoides

Mycosis fungoides is a cutaneous T-cell lymphoma commonly treated with psoralen and ultraviolet A (PUVA). In photodynamic therapy (PDT), porphyrin-based photosensitizers are used which absorb light energy resulting in cellular damage. We used the prodrug 5-aminolevulinic acid topically on mycosis fungoides lesions and thereafter exposed the lesions to red visible light. Skin biopsies were taken before treatment, after clinical improvement and after clinical remission. The expression of CD3 (pan T-cells), CD4 (helper T-cells), CD7 (pan T-cells), CD8 (suppressor T-cells), CD1a (Langerhans' cells), CD34 (endothelial cells), CD68 (macrophages), CD71 (transferrin receptor), Ki67, bcl-2, and p53 was studied immunohistochemically. There was complete clinical clearance in 7 of 9 plaque lesions but not of two tumour lesions. The biopsies confirmed a regress of the infiltrate after treatment. In the sparse remain-

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ing infiltrate a few CD4+ and CD8+ cells were found, most showing normal bcl-2. There were also fewer proliferating cells, illustrated by a decrease of Ki67 and CD71. The result showed that PDT has good clinical and histological effects in treating local plaque mucosis fungoides lesions.

List of original publications

The thesis was based on the following papers:

I Wiegleb Edström D, Porwit A, Ros A-M: The effects on human skin of repetitive ultraviolet-A1 (UVA1) irradiation and visible light. Photodermatol Photoimmunol Photomed 2001; 17 :66–70.

II Persson ÅE, Wiegleb Edström D, Bäckvall H, Lundeberg J, Pontén F, Ros A-M, Williams C: The mutagenic effects of UVA1 in human skin – studied by analysing the p53 gene in single cells.(Submitted for publication)

III Wiegleb Edström D, Ros A-M: The treatment of port-wine stains with the pulsed dye laser at 600 nm. Br J Dermatol 1997; 136: 360–363.

IV Wiegleb Edström D, Hedblad M-A, Ros A-M: Flashlamp-pulsed dye laser and argon-pumped dye laser in the treatment of port-wine stains. A clinical and histological comparison. Br J Dermatol 2002;146: 285–289.

V Wiegleb Edström D, Porwit A, Ros A-M: Photodynamic therapy with topical 5-aminolevulinic acid for mycosis fungoides: clinical and histological response. Acta Derm Venereol 2001; 81: 184–188.

The Role of Mast Cell Proteinases in the Formation of Skin Blisters

Renata Kaminska

Department of Dermatology, University of Kuopio, FIN-70210 Kuopio, Finland. renata.kaminska@kpshp.fi

Tryptase and chymase are serine proteinases and are the major proteins residing in the secretory granules of cutaneous mast cells. The role of tryptase and chymase in skin biology and pathology is obscure, but these enzymes may degrade the extracellular matrix of the skin. Hence, the purpose of this work was to elucidate the role of mast cell tryptase and chymase in skin blister formation in different experimental settings.

In the histochemical part of the work, the number of mast cells with tryptase and chymase activity and the percentage of mast cells showing immunoreactivity for α1-proteinase inhibitor (α1-PI) and α1-antichymotrypsin (α1-AC) were studied in skin specimens obtained from different phases (healthy-looking, erythematous and blistering skin) of intraepidermal (herpes zoster and pemphigus) and subepidermal (pemphigoid, dermatitis herpetiformis, linear IgA dermatosis, erythema multiforme and infective bullous eruption) blistering diseases. Significant changes were noted in mast cells during the development of the skin diseases, but these changes were essentially the same in the different blistering diseases. The number of mast cells with chymase activity, and the ratio of mast cells with chymase activity to those with tryptase activity showed a steady decrease in parallel with the progress of the blistering diseases. This indicates that there is inactivation of chymase since concurrently also the percentage of α1-PI- and/or α1-AC-positive mast cells increased. Moreover, in the inflamed skin from herpes zoster patients, the double-staining method revealed the appearance of cells showing chymase immunoreactivity but not chymase activity indicating that chymase had been inactivated. The number of mast cells with tryptase activity was largely unchanged in the erythematous skin but decreased in the vesicular skin specimens, suggesting solubilization of tryptase. Nevertheless, mast cells were found only occasionally in apparent contact with the basement membrane (BM).

In the first experimental study, normal skin specimens were incubated with purified skin tryptase or compound 48/80 (a mast cell degranulator) for up to 24 h and changes in dermis-epidermis (DE) separation and BM components were studied. Tryptase induced focal DE separation above intact collagen IV and laminin already after an 8-h incubation. The separation did not, however, progress to its full extent by 24 h. The appearance of focal DE separation was delayed by including 1,10-phenanthroline (a metalloproteinase inhibitor) in the incubation mixture with tryptase suggesting involvement also of metalloproteinases. Immunostaining of the extra domain A (EDA) of cellular fibronectin (cFn), but not that of the cell-binding region of Fn,