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

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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,

in the BM zone, blood vessels and dermal connective tissue was decreased by tryptase, even in the presence of 1,10-phenanthroline. Compound 48/80 induced almost complete DE separation above intact collagen IV and laminin by 24 h, a separation which could be attributed to the activities of endogenous tryptase and metalloproteinases. In addition, focal disappearance in EDA-cFn immunostaining in the BM by compound 48/80 was noted, possibly due to the action of tryptase. The involvement of chymase or cathepsin G in the DE separation by compound 48/80 does not seem to occur under the experimental conditions used since an inhibitor of chymotryptic serine proteinases (TPCK), could not prevent these changes. Tryptase was also found to cleave Fn in vitro into 173, 161 and 28 kDa fragments. The extent of this cleavage process was associated with decreased adherence and spread of cultured keratinocytes on plastic surfaces coated with tryptase-treated Fn.

Renata Kaminska (*right*), Department of Dermatology, University of Kuopio defended her thesis on December 7, 2001. Professor Maija Horsmanheimo (*middle*), was Chairman, Department of Dermatology, Kuopio University Hospital, and Faculty Opponent was Professor Aarne Oikarinen(*left*), Department of Dermatology, University of Oulu.

In the second experimental part of the study, the suction blister method and freezing of the skin with liquid nitrogen were used to induce blisters on the normal skin. Tryptic activity, but not chymotryptic activity, was detected in all samples of suction blister fluid, also after pretreatment of blister-induction sites with intracutaneous injections of 100 μg/ml compound 48/80 for 2–4 days and topical application of 0.1% capsaicin cream for 7–10 days. However, these pretreatments did not affect the rate and size of suction blister formation. Chymotryptic activity was not detected even when compound 48/80 was injected immediately before suction blistering, although this injection resulted in accelerated suction blister formation. In freezing blister fluids, significant tryptic activity was detected at 1 and 2 days after freezing, whereas chymotryptic activity was weak or undetectable. In blister fluids obtained from some different blistering diseases (e.g., pemphigoid and herpes zoster patients) both tryptic and chymotryptic activity were detectable, indicative of the presence of both tryptase and chymase activity in more chronic inflammatory conditions. Tryptic activity, present in freezing and suction blister fluids, reflects the good stability of tryptase. In contrast, chymase seems to be rapidly inactivated and/or it can only poorly diffuse through the extracellular matrix. In general, these blister fluid results are in line with the results obtained after the incubation of skin specimens with compound 48/80. Moreover, the results may explain why an urticarial wheal and mast cell degranulation do not lead to blister formation despite the presence of potent tryptase and chymase in the