Adverse effects of ultraviolet irradiation in atopic dermatitis

Lay-out: Optima BV Rotterdam

Cover: triple stringer longboard close up, istockphoto.

Print: Optima BV Rotterdam

ISBN: 978-90-8559-105-4

Adverse effects of ultraviolet irradiation in atopic dermatitis

Ongunstige effecten van ultraviolette straling bij constitutioneel eczeem

(met samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 7 december 2010 des ochtends te 10.30 uur

door

Onno ten Berge

geboren op 26 maart 1978 te Utrecht.

Promotor

Prof. dr. C.A.F.M. Bruijnzeel-Koomen

Co-promotoren

Dr. E.F. Knol

Dr. V. Sigurdsson

Publication of this thesis was financially supported by:

Abbot, Alk-Abelló, Astellas Pharma, Bauerfind, Beiersdorf, Dermasilk - Cara C'air, Pfizer, Fagron, Farmadomo Homecare, Galderma, Janssen, J.E. Jurriaanse stichting, La Roche-Posay, Leo Pharma, Louis Widmer, Novartis, Phadia, Raadgevers-Kuijkhoven, Stiefel - a GSK company, Vereniging van Mensen met Constitutioneel eczeem, Vision - All Day Sun Protection - Nycomed, Waldmann.

The board is in there, you just have to find it…
-Big 'Z'

Contents

General introduction

1 2 3 4 5 6 7

Atopic Dermatitis

Atopic Dermatitis (AD) is a highly pruritic chronic inflammatory skin disease that commonly presents during early infancy and childhood, but can persist or start in adulthood. [1,2] The worldwide prevalence of AD in children is estimated to be around 3-10% and 1-3% in adults. [2-5] The first known description of a possible case of AD was given by the historian Suetonius: The Roman emperor Augustus showed skin and respiratory traits corresponding with atopy.[6] Atopy is derived from the Greek "ατοπία" originally meaning "strangeness". This term was offhandedly introduced into the medical world by E.D. Perry in 1923, a (non medical) professor of Greek during a dinner conversation with Drs. A.F. Coca and R.A. Cook who subsequently published the term for the first time in "On the classification of the phenomena of hypersensitiveness". [7] Currently atopy is regarded as a tendency to produce IgE to low dose allergens, usually proteins, potentially accompanied by the typical clinical symptoms involving eczema (AD), allergic conjunctivitis, allergic rhinitis and allergic asthma.[8] Some, mainly paediatricians and dermatologists, also consider ''atopy'' a constitutional trait. They find ''atopy'' to be a clinically useful term since IgE-mediated allergy is common in children and young adults, and often runs in families.[9]

Not every patient with AD is however atopic. And the name constitutional eczema with or without atopy should be considered instead of AD.[9] The debate on nomenclature is however not the focus of this thesis. The current diagnosis of AD is based on its clinical presentation. Consensus criteria for the main clinical features of AD based on the diagnostic criteria for AD by Hanifin and Rajka have led to a short list of reliable and valid discriminators that are used worldwide (table 1). [1,10,11]

Pathogenesis

The pathomechanism of AD is still largely unknown and different key mechanisms are pointed out to be calling the tune. Consequently AD is thought to be a complex sum of genetic susceptibility, determining defects in the skin (barrier) function, systemic and local immunologic responses, and the host's environment.

Immunological characteristics

Acute inflammation in AD skin is histologically characterized by spongiosis and an infiltration of eosinophills, macrophages and T cells.[2,12,13] The $CD4^+$ T cells that

Table 1. Criteria for the diagnosis of atopic dermatitis

The diagnosis requires evidence of itchy skin (or parental report of scratching or rubbing) plus three or more of the following:
History of involvement of the skin creases (e.g. fronts of elbows, back of knees, front of ankles and areas around the neck or eyes)
History of asthma or hay fever (or history of atopic disease in a first-degree relative if the child is under 4 years of age)
History of generally dry skin in the past year
Onset in a child under two years of age (criterion not used if the child is under four years of age)
Visible flexural dermatitis (including dermatitis affecting the cheeks or forehead and outer aspects of limbs in children under four years of age)

are found in acute AD lesions are characterized by a Th2 phenotype shifting to a dynamically balanced Th1 phenotype in chronic lesions.[12,13] A similar switch from Th2-type to a more Th1-type CD4+ phenotype is found in the first 48 hours after an atopy patch test.[90,103] Non-lesional and lesional AD skin harbours IgE bearing Langerhans cells and dendritic cells.[14]

Genetically

AD is strongly heritable, suggesting an important role for genetic involvement.[15] A number of replicable disease loci have been discovered, and a small number of positional, theory based, or both candidate genes have been identified.[15] One gene in particular was reported; the filaggrin gene, which was very recently pointed out to predispose to ichthyosis vulgaris and associated with AD.[16-19] A filaggrin gene code mutation is found in 20% of AD patients vs. 10% in healthy controls [19] and is considered a filament-associated protein which bind to keratin fibers in epithelial cells. Filaggrin is a key protein in the formation of the cornified envelope and is critical for an effective skin barrier. [16-19] It has been hypothesized that a decreased skin barrier may facilitate sensitization to environmental allergens.[14,20],

The role of allergy

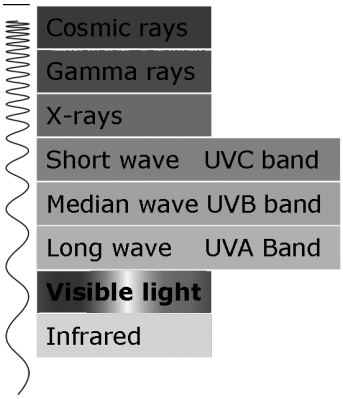
As stated above, AD is strongly associated with allergic asthma and rhinitis. Around 70% of AD patients is sensitised for aeroallergens such as house dust mite (HDM), grass pollen and animal dander, and 50% of these patients has a positive atopy patch test for aeroallergens.[90-91] Provocation with HDM via inhalation is known to worsen AD.[92] However allergen avoidance studies with HDM did not show a significant clinical effect.[93] The avoidance measures consisted of applying HDM-impermeable encasings for mattresses, pillows, and duvets at home, suggesting that reduction of allergens in other environments (work, school, and outdoors) might be equally important in improving symptoms of AD [93], which seems an infeasible goal.

Ultraviolet radiation and the (AD) skin

Spectrum

The Ultraviolet (UV) radiation spectrum is divided in UVA (315-400nm), UVB (280-315) nm and UVC (100-280 nm) based on physiological characteristics of the wavelengths range, divided (figure.1). UV is responsible for variety of effects in the skin: erythema, pigmentation, vitamin D3 conversion, skin aging, immunosuppression, induce or aggravate dermatoses, and cause skin cancer.[22-33] UVB accounts for just 0.2% of the total radiation energy of the sun but is responsible for most of these effects in the skin, because many important

Figure 1. The electromagnetic (UV) spectrum.



components of skin cells absorb UVB. UV-A can also cause these effects, but need much higher radiation doses to do so.[23, 25, 34-36] UVC does not reach the earth's surface naturally due to its absorption by the ozone layer.
In reaction to UV-exposure healthy human skin shows clear changes in epidermal and dermal regions: damage of keratinocytes, the so called sunburn cells,[31] immigration of neutrophils and monocytes into the skin,[32,37,38] mast cell degranulation [39,40] and emigration of dendritic cells to the draining lymph nodes.[41] It was recently shown that infiltrating neutrophils produce IL-4 and IL-10, suggesting that they can have an immunomodulatory function after UV irradiation.[94,95]

Photosensitivity

Human skin may also show a pathological response to UV. This is diagnosed as a photosensitivity disorder or a photodermatosis. The photosensitivity disorders can be classified into 4 groups: 1) idiopathic, such as polymorphic light eruption (PLE), solar urticaria or chronic actinic dermatitis, 2) secondary to metabolic pathway disorders; the porphyrias, 3) photoexacerbated dermatoses, such as AD, lupus erythematodes or psoriasis, and 4) secondary to exogenous agents (e.g. plants, coal tar, topical and systemic drugs). PLE is the most common photodermatosis with a mean European prevalence of up to 18% of the general population.[42]
In earlier studies on the pathogenesis of PLE, a decreased number of skin infiltrating neutrophils after UVB irradiation (3x the minimal erythemal dosis (MED), Philips TL-12) is suggested to attribute to the mechanism of photosensitivity, probably via the reduced expression of TNF-alpha, IL-4, and IL-10.[43,44] As a consequence of reduced TNA-alpha expression, decreased emigration of Langerhans cells following UVB exposure (6x MED, Philips TL-12) was demonstrated.[45]

Photosensitivity in AD

Paradoxically, UV irradiation can both improve and worsen the course of AD. The majority of AD patients benefit from sunlight or ultraviolet radiation (UVR) therapy (UVB/UVA1/PUVA) due to its immunosuppressive effects.[46] On the other hand, an estimated 10 % of patients with AD experience an adverse reaction upon exposure to sunlight or artificial UVR.[47,48]
The terminology to describe photosensitivity in AD is variable namely photo-aggravation of AD,[47, 49-61] photosensitive eczema,[62-64] light exacerbated eczema,[65,66]

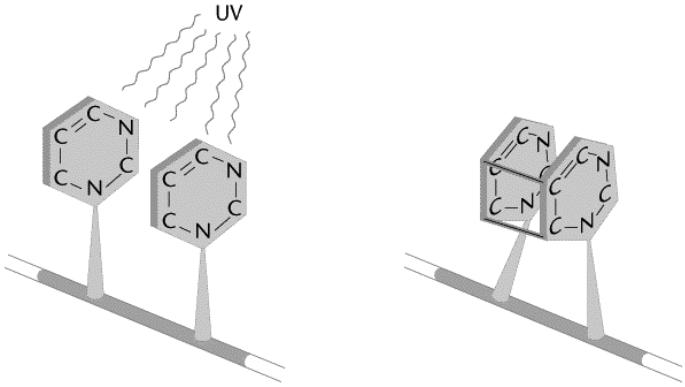
photo-exacerbated AD,[67] or even chronic actinic dermatitis (CAD).[68-71] There are also reports of the co-existence of polymorphic light eruption (PLE) with AD. [46, 72-74] CAD and PLE have clinical different characteristics. This suggests that photosensitivity in AD is heterogeneous both from a clinical and aetiological point of view. There are no data available on the cellular aspects of the different photosensitive reaction patterns in patients with AD at present time.

Ultraviolet radiation and skin cancer

Photocarcinogenesis is considered to be the predominant cause of skin cancer, accounting for 90% of skin cancers.[96] Risk factors for skin cancers, other than UV exposure, are; age, genetic susceptibility (suh as mutations in the sonic hedgehog pathway e.g. PTCH1 and SMO gene mutations), exposure to carcinogens (arsenic, ionizing radiation) and the use of systemic immunosuppressive drugs, particularly calcineurin inhibitors (CI).[96-101]

In the past decades skin cancer incidence rates are rising, possibly because of changing sunbathing habits.[75] Especially UVB radiation is highly mutagenic, and extensive epidemiological evidence has indicated that UVB radiation is responsible for the majority of non melanoma skin cancers (NMSC).[76-77] UVB is directly absorbed by DNA and induces dimers between adjacent pyrimidine derivatives the so-called DNA-photoproducts. Thymine cyclobutane pyrimidine dimers (TT-CPDs) are the major photoproduct upon UVB exposure (figure 2).[78]

Figure 2. Forming of thymine dimers by UV visualised Adapted with permission from S.M. Carr, © 2002 Griffiths et al. Modern Genetic Analysis, 2nd ed. Prentice-Hall.



Photoproducts are primarily repaired by nucleotide excision repair (NER). In NER the damaged stretch is excised, leaving a single strand gap of 25-30 nucleotides. Subsequently, new residues are inserted at the site of damage by DNA polymerase. To prevent replication of the cell before DNA photo damage is repaired and tumor suppressor genes like p53 are upregulated, which induce a cell cycle arrest at the G1-phase.
Deficiencies in, or ineffectiveness of DNA repair, apoptotic mechanisms or suppressor genes can lead to the formation of mutations over time, and increases the risk of developing skin cancer.[26, 29]

Immunosuppressive drugs and non-melanoma skin cancer

Topical corticosteroids and emollients are the mainstay of AD treatment. Due to the chronicity of the disease maintenance therapy is necessary. Long term use of corticosteroids can lead to local side effects e.g., atrophy, acne, striae, telangiectasia, and due to systemic absorption, adrenal gland suppression.[79]
In the last decade a new breed of topical anti-inflammatory drugs based on calcineurin inhibition entered the therapeutic field. The two available drugs at present time are tacrolimus and pimecrolimus. Inhibition of calcineurin leads to diminished translocalization of the transcription factor Nuclear Factor of Activated T-cells (NFAT), and blocks the expression of important genes, such as cytokine genes, resulting in an inhibition of T cell activation.[80]
The increased incidence of skin cancers on sun exposed sites in the setting of post transplant immunosuppression has been linked to the use of systemic administration of calcineurin inhibitors (CI), such as cyclosporine or tacrolimus. In renal transplantation patients, for example, the standardized incidence rates for invasive NMSC (33-fold increase) and *in situ* carcinoma of the skin (65-fold increase) are significantly increased.[101] Moreover, the risk for invasive squamous cell carcinoma (SCC) was increased 82-fold compared with a non-transplanted population.[101]
Different mechanisms have been described how CI may promote carcinogenesis. Apart from an indirect immunosuppressive effect CI may also directly promote carcinogenesis.[83-89] Previous in vitro studies showed a decrease in DNA repair rate and apoptosis in CI treated cultured human keratinocytes and peripheral blood mononuclear cells after UVB irradiation.[82-84]

The relation between systemic use of CI and skin cancers has raised questions about the potency of topical use of these compounds to promote UV-mediated carcinogenesis.[81, 82] So far studies on the topical use of CI to promote carcinogenesis are contradictory. UV irradiation of topically CI (tacrolimus vs. pimecrolimus) treated mice showed a decrease in induction of DNA damage after UV irradiation but failed to show a decrease in DNA damage repair rate.[85, 86] Enhanced photocarcinogenesis was demonstrated by Niwa et al. in mice treated with topical CI in combination with UV irradiation, but this could not be confirmed by Lerche et al.[85, 87] Two in vivo studies in healthy volunteers treated with pimecrolimus showed no effect on induction or repair of UV induced DNA damage or on P53 (oncogene) expression within the first 24 hours after irradiation.[81, 102] There are no in vivo study data on the effect of topical tacrolimus on DNA damage repair.

Outline of this thesis

As described above UV irradiation plays an important but bivalent role in AD: beneficial via its immunosuppressive potency or deteriorating due to the induction of photosensitivity. In addition, UV irradiations may complicate AD treatment by its interaction with CI resulting in increased carcinogenesis of the skin.
This thesis will focus on the two major adverse effects of UV in AD: 1) The clinical (in adults and children) and immunohistochemical (only in adults) aspects of photosensitivity and 2) UV-induced DNA damage and repair in lesional AD skin after treatment with topical tacrolimus.

1) In AD patients with photosensitivity it is important to determine the type of clinical response: is this AD with co-existing PLE or a photo-induction of eczema. Therefore, we aimed to define the clinical characteristics and underlying pathomechanism of photosensitivity in AD. Two retrospective studies were carried out to determine the characteristics of photosensitivity in adults and children with AD (**chapter 2 & 3**). To learn more about the aetiology of photosensitivity in AD, we examined the inflammatory cell composition in UV irradiated skin of photosensitive and non-photosensitive AD patients (**chapter 4**).

2) The effect of topical tacrolimus on UVB induced DNA damage and its subsequent repair in patients with AD was evaluated with an improved method to quantify

UVB induced DNA damage and repair in the epidermis (**chapter 5**). Lesional AD skin was treated with topical tacrolimus and subsequently irradiated with UVB. UV induced DNA damage and repair in tacrolimus treated lesional AD skin was compared to that in topical triamcinolone acetonide treated lesional AD skin (**chapter 6**).

Reference List

1 Hanifin JM. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Suppl.) (Stockholm) 1980;90:44-47

2 Leung DY. Atopic dermatitis. Lancet 2003; 362(9352):151-60.

3 Hanifin JM. Epidemiology of atopic dermatitis. Monogr Allergy 1987;21:116-131.

4 Schultz LF. The epidemiology of atopic dermatitis. Monogr Allergy 1993;31:9-28.

5 Williams H. Worldwide variations in the prevalence of symptoms of atopic eczema in the International Study of Asthma and Allergies in Childhood. J.Allergy Clin.Immunol. 1999;103:125-38.

6 Ring J. [1st description of an "atopic family anamnesis" in the Julio-Claudian imperial house: Augustus, Claudius, Britannicus]. Hautarzt 1985;36(8):470-1.

7 Beltrani VS. "Atopy" What Does It Really Mean? Pediatric Asthma, allergy and immunology 2007; 20(1):52-3.

8 Spergel JM. Atopic dermatitis and the atopic march. J.Allergy Clin.Immunol. 2003;112(6 suppl):118-27.

9 Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001;56(9):813-24.

10 Williams HC. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. Br.J.Dermatol. 1994;131(3):406-16.

11 Williams HC. Clinical practice. Atopic dermatitis. N.Engl.J.Med. 2005;352(22):2314-24.

12 Fiset PO. Immunopathology of atopic dermatitis. J.Allergy Clin.Immunol. 2006;118(1):287-290.

13 Grewe M. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol.Today 1998;19(8):359-61.

14 Bieber T, Bruijnzeel-Koomen C. [Langerhans cells in the physiopathology of atopic dermatitis]. Ann Dermatol Venereol 1990;117(3):185-93.

15 Morar N. The genetics of atopic dermatitis. J.Allergy Clin.Immunol. 2006;118(1):24-34.

16 Palmer CN. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat.Genet. 2006;38(4):441-6.

17 Weidinger S. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J.Allergy Clin.Immunol. 2006;118(1):214-9.

18 Weidinger S. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J.Invest Dermatol. 2007;127(3):724-6.

19 Weidinger S. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J.Allergy Clin.Immunol. 2008;121(5):1203-9.

20 Cork MJ. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. J.Allergy Clin.Immunol. 2006;118(1):3-21.

21 Zutavern A. Atopic dermatitis, extrinsic atopic dermatitis and the hygiene hypothesis: results from a cross-sectional study. Clin.Exp.Allergy 2005;35(10):1301-8.

22 Backvall H. Similar UV responses are seen in a skin organ culture as in human skin in vivo. Exp.Dermatol. 2002;11(4):349-56.

23 de Gruijl FR, Sterenborg HJ, Forbes PD, Davies RE, Cole C, Kelfkens G, et al. Wavelength dependence of skin cancer induction by ultraviolet irradiation of albino hairless mice. Cancer Res 1993 Jan 1;53(1):53-60.

24 de Gruijl FR. [Adverse effects of sunlight on the skin]. Ned.Tijdschr.Geneeskd. 1998;142(12):620-5.

25 de Gruijl FR. In situ molecular dosimetry and tumor risk: UV-induced DNA damage and tumor latency time. Photochem.Photobiol. 1998;68(4):555-60.

26 de Gruijl FR. Skin cancer and solar UV radiation. Eur.J.Cancer 1999;35(14):2003-9.

27 Duthie MS. The effects of ultraviolet radiation on the human immune system. Br.J.Dermatol. 1999;140(6):995-1009.

28 Ledo E. Photodermatosis. Part I: Photobiology, photoimmunology, and idiopathic photodermatoses. Int.J.Dermatol 1993;32(6):387-96.

29 Matsumura Y. Short-term and long-term cellular and molecular events following UV irradiation of skin: implications for molecular medicine. Expert.Rev.Mol.Med. 2002;1-22.

30 Matsumura Y. Toxic effects of ultraviolet radiation on the skin. Toxicol.Appl.Pharmacol. 2004;195(3):298-308.

31 Norval M. Effects of solar radiation on the human immune system. J.Photochem.Photobiol.B 2001;63(1-3):28-40.

32 Soter NA. Acute effects of ultraviolet radiation on the skin. Semin.Dermatol. 1990;9(1):11-15.

33 Urbach F. Welcome and introduction: evidence and epidemiology of ultraviolet-induced cancers in man. Natl. Cancer Inst.Monogr 1978;50:5-10.

34 Kelfkens G, van WH, de Gruijl FR, van der Leun JC. The influence of dose rate on ultraviolet tumorigenesis. J Photochem Photobiol B 1991 Jul;10(1-2):41-50.

35 van Weelden H, de Gruijl FR, van der Putte SC, Toonstra J, van der Leun JC. The carcinogenic risks of modern tanning equipment: is UV-A safer than UV-B? Arch Dermatol Res 1988;280(5):300-7.

36 van Weelden H, van der Putte SC, Toonstra J, van der Leun JC. UVA-induced tumours in pigmented hairless mice and the carcinogenic risks of tanning with UVA. Arch Dermatol Res 1990;282(5):289-94.

37 Hawk JL. The presence of neutrophils in human cutaneous ultraviolet-B inflammation. Dermatology 1988;118(1):27-0.

38 Lee PL. Neutrophil infiltration in normal human skin after exposure to different ultraviolet radiation sources. Photochem.Photobiol. 2008;84(6):1528-34.

39 Kim MS. Acute exposure of human skin to ultraviolet or infrared radiation or heat stimuli increases mast cell numbers and tryptase expression in human skin in vivo. Br.J.Dermatol. 2009;160(2):393-402.

40 Walsh LJ. Ultraviolet B irradiation of skin induces mast cell degranulation and release of tumour necrosis factor-alpha. Immunol.Cell Biol. 1995;73(3):226-33.

41 Kolgen W. Epidermal langerhans cell depletion after artificial ultraviolet B irradiation of human skin in vivo: apoptosis versus migration. J.Invest Dermatol. 2002;118(5):812-7.

42 Rhodes LE. Polymorphic light eruption occurs in 18% of europeans and does not show higher prevalence with increasing latitude: multicenter survey of 6,895 individuals residing from the Mediterranean to Scandinavia. J.Invest Dermatol. 2010;130():626-8.

43 Kolgen W. Differential expression of cytokines in UV-B-exposed skin of patients with polymorphous light eruption: correlation with Langerhans cell migration and immunosuppression. Arch.Dermatol. 2004;140(3):295-302.

44 Schornagel IJ. Decreased neutrophil skin infiltration after UVB exposure in patients with polymorphous light eruption. J.Invest Dermatol. 2004;123(1):202-6.

45 Kolgen W. CD11b+ cells and ultraviolet-B-resistant CD1a+ cells in skin of patients with polymorphous light eruption. J.Invest Dermatol. 1999;113(1):4-10.

46 Majoie IM. Narrowband ultraviolet B and medium-dose ultraviolet A1 are equally effective in the treatment of moderate to severe atopic dermatitis. J.Am.Acad.Dermatol. 2009;60(1):77-84.

47 Rajka G. clinical aspects; other essential features; photosensitvity. Essential Aspects of Atopic Dermatitis 2006;chapter 2.6.2:27.

48 Vieluf D. Complications and Diseases associated with Atopic Eczema. Handbook of Atopic Eczema 1991;p.54-79.

49 Photoaggravated dermatoses; Atopic dermatitis. In: Frain-Bell W, editor. Cutaneous photobiology. Oxford university press; 1985;p.60-1.

50 Photoaggravated dermatoses, including lupus erythematosus; atopic dermatitis. In: Harber, Bickers, editors. Photosensitivity diseases: principles and treatment. 2 ed. B.C.Decker; 1989; p.299.

51 Crouch RB, Foley PA, Baker CS. Analysis of patients with suspected photosensitivity referred for investigation to an Australian photodermatology clinic. J Am Acad Dermatol 2003 May;48(5):714-20.

52 Deguchi H, Umemoto N, Sugiura H, Danno K, Uehara M. Ultraviolet light is an environmental factor aggravating facial lesions of adult atopic dermatitis. Dermatol Online J 1998 Oct;4(1):10.

53 Keong CH, Kurumaji Y, Miyamoto C, Fukuro S, Kondo S, Nishioka K. Photosensitivity in atopic dermatitis: demonstration of abnormal response to UVB. J Dermatol 1992 Jun;19(6):342-7.

54 Kerker BJ, Morison WL. The photoaggravated dermatoses. Semin Dermatol 1990 Mar;9(1):70-7.

55 Murphy GM. Diseases associated with photosensitivity. J Photochem Photobiol B 2001 Nov 15;64(2-3):93-8.

56 Murphy GM. Investigation of photosensitive disorders. Photodermatol Photoimmunol Photomed 2004 Dec;20(6):305-11.

57 Pigatto PD, Altomare G, Polenghi MM, Roveroni S, Menni S, Docchio F. Atopy and photosensitivity in children. Photodermatol 1986 Oct;3(5):303.

58 Tajima T, Ibe M, Matsushita T, Kamide R. A variety of skin responses to ultraviolet irradiation in patients with atopic dermatitis. J Dermatol Sci 1998 Jun;17(2):101-7.

59 V.A.deLeo. other photoaggravated diseases. In: van Vloten WA, editor. photosensitivity: topics in clinical dermatology.Tokyo: Igaku-Shoin; 1992. p. 155-7.

60 photomedicine; abnormal responses to UV radiation: idiopathic and photoexacerbated. In: I.M.Freedberg et AL., editor. Fitz-Patrick's Dermatology in General Medicine I. Mc-graw-Hill; 2004. p. 1294.

61 Amblard P, Leccia MT. [Skin diseases with photosensitivity]. Rev Prat 1992 Jun 1;42(11):1365-8.

62 Other photodermatoses; Photosensitive eczema. In: Magnus, editor. Dermatological photobiology: clinical and experimental aspects. Blackwell; 1976. p.194-5.

63 Ramsay CA, Black AK. Photosensitive eczema. Trans St Johns Hosp Dermatol Soc 1973;59(2):152-8.

64 Yokel BK, Hood AF, Morison WL. Management of chronic photosensitive eczema. Arch Dermatol 1990 Oct;126(10):1283-5.

65 Hawk, Magnus. Diseases cause by environmental exposure or trauma; Chronic actinic dermatitis; Photosensitive eczema. In: Braun, Falco, Plewig, Wolff, editors. Dermatology. 2 ed. Springer; 1996. p. 559.

66 Harber LC, Whitman GB. Photosensitivity. Classification. Dermatol Clin 1986 Apr;4(2):167-9.

67 Morison WL. Clinical practice. Photosensitivity. N Engl J Med 2004 Mar 11;350(11):1111-7.

68 Russell SC, Dawe RS, Collins P, Man I, Ferguson J. The photosensitivity dermatitis and actinic reticuloid syndrome (chronic actinic dermatitis) occurring in seven young atopic dermatitis patients. Br J Dermatol 1998 Mar;138(3):496-501.

69 Yones SS, Palmer RA, Hextall JM, Hawk JL. Exacerbation of presumed chronic actinic dermatitis by cockpit visible light in an airline pilot with atopic eczema. Photodermatol Photoimmunol Photomed 2005 Jun;21(3):152-3.

70 Ogboli MI, Rhodes LE. Chronic actinic dermatitis in young atopic dermatitis sufferers. Br J Dermatol 2000 Apr;142(4):845.

71 Creamer D, McGregor JM, Hawk JL. Chronic actinic dermatitis occurring in young patients with atopic dermatitis. Br J Dermatol 1998 Dec;139(6):1112-3.

72 Pearson IC, Holden CA. Atopic dermatitis - a simple entity? Clin Exp Dermatol 2002 Jan;27(1):88-9.

73 Tajima T, Ibe M, Matsushita T, Kamide R. A variety of skin responses to ultraviolet irradiation in patients with atopic dermatitis. J Dermatol Sci 1998 Jun;17(2):101-7.

74 D.Vieluf, T.Ruzicka. photosensitivity. In: T.Ruzicka, J.Ring, B.Przybilla, editors. Handbook of atopic eczema. first ed. Springer-Verlag; 1991. p. 68.

75 Fatima Birch-Johansen et al. Trends in the incidence of nonmelanoma skin cancer in Denmark 1978–2007: rapid incidence increase among young Danish women, Int. J. Cancer 2010.

76 Katiyar SK. Kinetics of UV light-induced cyclobutane pyrimidine dimers in human skin in vivo: an immunohistochemical analysis of both epidermis and dermis. 2000;72(6):788-93.

77 Urbach F. Welcome and introduction: evidence and epidemiology of ultraviolet-induced cancers in man. 1978;50:5-10.

78 Douki T. Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. 2001;40(*):2496-2501.

79 de Bruin-Weller MS. [Topical immunomodulators, such as tacrolimus and pimecrolimus, in the treatment of atopic dermatitis]. 2005;149(20):1096-1100.

80 Allen BR. Tacrolimus ointment: its place in the therapy of atopic dermatitis. J.Allergy Clin.Immunol. 2002;109(3):401-3.

81 Doelker L. Production and clearance of cyclobutane dipyrimidine dimers in UV-irradiated skin pretreated with 1% pimecrolimus or 0.1% triamcinolone acetonide creams in normal and atopic patients. 2006;15(5):341-6.

82 Yarosh DB. Calcineurin inhibitors decrease DNA repair and apoptosis in human keratinocytes following ultraviolet B irradiation. J.Invest Dermatol 2005;125(5):1020-5.

83 Canning MT, Nay SL, Pena AV, Yarosh DB. Calcineurin inhibitors reduce nuclear localization of transcription factor NFAT in UV-irradiated keratinocytes and reduce DNA repair. J Mol Histol 2006 Sep;37(5-7):285-91.

84 Herman M, Weinstein T, Korzets A, Chagnac A, Ori Y, Zevin D, et al. Effect of cyclosporin A on DNA repair and cancer incidence in kidney transplant recipients. J Lab Clin Med 2001 Jan;137(1):14-20.

85 Niwa Y, Terashima T, Sumi H. Topical application of the immunosuppressant tacrolimus accelerates carcinogenesis in mouse skin. Br J Dermatol 2003 Nov;149(5):960-7.

86 Tran C, Lubbe J, Sorg O, Doelker L, Carraux P, Antille C, et al. Topical calcineurin inhibitors decrease the production of UVB-induced thymine dimers from hairless mouse epidermis. Dermatology 2005;211(4):341-7.

87 Lerche CM, Philipsen PA, Poulsen T, Wulf HC. Topical pimecrolimus and tacrolimus do not accelerate photocarcinogenesis in hairless mice after UVA or simulated solar radiation. Exp Dermatol 2009 Mar;18(3):246-51.

88 Wu X, Nguyen BC, Dziunycz P, Chang S, Brooks Y, Lefort K, et al. Opposing roles for calcineurin and ATF3 in squamous skin cancer. Nature 2010 May 20;465(7296):368-72.

89 Norman KG, Canter JA, Shi M, Milne GL, Morrow JD, Sligh JE. Cyclosporine A suppresses keratinocyte cell death through MPTP inhibition in a model for skin cancer in organ transplant recipients. Mitochondrion 2010 Mar;10(2):94-101.

90 Langeveld-Wildschut EG, Thepen T, Bihari IC et al. Evaluation of the atopy patch test and the cutaneous late-phase reaction as relevant models for the study of allergic inflammation in patients with atopic eczema. J Allergy Clin Immunol. 1996;98:1019-1027.

91 Bruijnzeel PL, Kuijper PH, Kapp A, Warringa RA, Betz S, Bruijnzeel-Koomen CA. The involvement of eosinophils in the patch test reaction to aeroallergens in atopic dermatitis: its relevance for the pathogenesis of atopic dermatitis. Clin Exp Allergy. 1993;23:97-109.

92 Tupker RA, de Monchy JG, Coenraads PJ, Homan A, van der Meer JB. Induction of atopic dermatitis by inhalation of house dust mite. J Allergy Clin Immunol. 1996;97:1064-1070.

93 Oosting AJ, de Bruin-Weller MS, Terreehorst I et al. Effect of mattress encasings on atopic dermatitis outcome measures in a double-blind, placebo-controlled study: the Dutch mite avoidance study. J Allergy Clin Immunol. 2002;110:500-506.

94 Piskin G, Bos JD, Teunissen MB. Neutrophils infiltrating ultraviolet B-irradiated normal human skin display high IL-10 expression. Arch Dermatol Res 2005 Jan;296(7):339-42.

95 Teunissen MB, Piskin G, di NS, Sylva-Steenland RM, de Rie MA, Bos JD. Ultraviolet B radiation induces a transient appearance of IL-4+ neutrophils, which support the development of Th2 responses. J Immunol 2002 Apr 15;168(8):3732-9.

96 Murphy GM. Ultraviolet radiation and immunosuppression. Br J Dermatol. 2009;161 Suppl 3:90-95.

97 Begnini A, Tessari G, Turco A et al. PTCH1 gene haplotype association with basal cell carcinoma after transplantation. Br J Dermatol. 2010;163:364-370.

98 Iwasaki JK, Srivastava D, Moy RL, Lin HJ, Kouba DJ. The molecular genetics underlying basal cell carcinoma pathogenesis and links to targeted therapeutics. J Am Acad Dermatol. 2010.

99 Silverstein TD, Johnson RE, Jain R, Prakash L, Prakash S, Aggarwal AK. Structural basis for the suppression of skin cancers by DNA polymerase eta. Nature. 2010;465:1039-1043.

100 Tamura D, DiGiovanna JJ, Kraemer KH. Founder mutations in xeroderma pigmentosum. J Invest Dermatol. 2010;130:1491-1493.

101 Moloney FJ, Comber H, O'Lorcain P, O'Kelly P, Conlon PJ, Murphy GM. A population-based study of skin cancer incidence and prevalence in renal transplant recipients. Br J Dermatol. 2006;154:498-504.

102 Gambichler T, Schlaffke A, Tomi NS, Othlinghaus N, Altmeyer P, Kreuter A. Tacrolimus ointment neither blocks ultraviolet B nor affects expression of thymine dimers and p53 in human skin. J Dermatol Sci 2008 May;50(2):115-22.

103 Thepen T, Langeveld-Wildschut EG, Bihari IC et al. Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial TH2 response to a TH1 response in situ: an immunocytochemical study. J Allergy Clin Immunol. 1996;97:828-837.

Throwing a light on photosensitivity in atopic dermatitis

A Retrospective Study

Onno ten Berge, Huib van Weelden, Carla A.F.M. Bruijnzeel-Koomen, Marjolein S. de Bruin-Weller and Vigfus Sigurdsson

Department of Dermatology and Allergology, University Medical Centre Utrecht, the Netherlands

American Journal of Clinical Dermatology 2009;10:119-23

Abstract

Background

Photosensitivity in atopic dermatitis (AD) is a well known but ill-defined phenomenon.

Objectives

To determine the prevalence of photosensitivity in patients with AD, define its clinical characteristics, and analyze the photo provocation test (phototest) results.

Methods

A retrospective study of patients with AD who were phototested because of suspected photosensitivity at our department during the period 1994–2004.

Results

The total number of patients with AD seen in our department between 1994 and 2004 was 3804, of whom 145 patients (45 men and 100 women) were phototested. Photosensitivity was confirmed in 108 (74%) of these 145 patients (33 men and 75 women). The minimal erythema dose (MED) for UVB was decreased in eight of these 108 patients (7%) and the MED for UVA in five patients (5%). Two major clinical reaction patterns were observed: a polymorphic light eruption-type reaction in 51 patients (47%) and an eczematous reaction in 44 patients (41%). Seventy-two of the 108 patients (67%) had a pathologic reaction to UVA and UVB, 18 patients (17%) were only UVB sensitive, and 18 patients (17%) were only UVA sensitive. Photopatch tests were performed in 125 patients (86%). Twenty-nine patients (23%) had a positive photocontact reaction to one or more substances.

Conclusion

Photosensitivity is found in approximately 3% of patients with AD and the majority is female. Photosensitivity in patients with AD consists of two clinical reaction patterns distinguishable by phototesting. Patients were diagnosed with either AD and co-existing polymorphic light eruption or photosensitive AD.

Background

Atopic dermatitis (AD) is a common inflammatory skin disease. the majority of AD patients benefit from sunlight or UV radiation therapy (UVB/UVA1/psoralen plus UVA). Unfortunately, a small group of patients experience an adverse reaction to sunlight or artificial UV radiation. An estimated 10% of patients with AD are reported to deteriorate after solar exposure.[1,2] Although sunlight may improve or exacerbate AD, the effect of UV light on AD in clinical practice is often unclear, since the symptoms may also be explained by a host of unrelated circumstantial factors such as humidity, scratching, pollen exposure, psychological factors, skin care, and co-morbidities such as polymorphous light eruption (PLE).[3] In the literature, photosensitivity in AD is reported as Photoaggravation of AD,[1,4-16] photosensitive eczema,[17-19] light exacerbated eczema,[20,21] photoexacerbated AD,[22] and even chronic actinic dermatitis (CAD). [23-26] There are also reports of co-existence of PLE with AD.[1,3,13,27]

Although photosensitivity in AD has often been mentioned in the literature, a thorough description of this phenomenon is lacking. The description of the clinical picture is minimal and there are no phototest criteria. The aim of this retrospective study was to determine the prevalence of photosensitivity in patients with AD, define its clinical characteristics, and describe the outcome of phototests in this patient group.

Methods

All patients with AD who were phototested in the Department of Dermatology and Allergology, University Medical Centre Utrecht, during the period 1994–2004 were included in this study. Patients were tested because of a clinical suspicion of photosensitivity (patient's history and/or sun exposed skin distribution of the lesions). The diagnosis of AD was made according to the criteria of Hannifin and Rajka.[28] Diagnostic phototesting consisted of determination of the minimal erythema dose (MED) for UVB and UVA, provocation with these wavelengths, and photopatch tests.

Radiation Sources

The radiation sources used have been described previously by Boonstra et al.[29] Briefly, the UVA source (Mutzhas UVASUN 3000S) consists of a high-pressure mercury arc with special filtering to filter out all UVB and short-wave UVA (UVA2), creating a UVA1 source (resulting in a 340–450-nm emission spectrum). The UVB source consists of four Philips TL20W/12 tubes with a continuous emission spectrum (280–360 nm) with a maximum of approximately 305nm. These light sources for UVA and UVB were used for both MED and phototesting.
Doses of light were measured in J/cm2 using a Waldmann UV detector device (Waldmann, Schwenningen, Germany). Because the Waldmann meter does not fully correspond with the emission of the Mutzhas lamp, it was calibrated with the spectro-radiometer, model 752 (Optronic Laboratories Inc., Orlando, FL, USA).

Phototesting Procedure

The MEDs for UVB and UVA were determined using a specially designed apparatus that simultaneously exposes a series of nine areas of skin of 3 x 10mm to increasing doses. These doses are geometrically progressive with increments of 41% between successive skin fields. Irradiation was given in the morning and assessment was performed after 8 and 24 hours by an experienced observer.
The MED is defined as the dose of UVB/A necessary to induce a just perceptible erythema with no marked borders. The MED was considered decreased if the skin was at least four times more sensitive than normal. The normal MED for UVB is about 50–70 mJ/cm2 (Philips 20W/TL12). The lower limit of a normal MED for UVA is about 25 J/cm2 (Mutzhas UVASUN 3000S). The upper limit of this UVA test is 40 J/cm2. Photo provocation is a useful method for reproducing photosensitive skin lesions. In this test, non-lesional skin is exposed on a daily basis to increasing doses of UV light. This is done separately for UVB and UVA on skin areas of 6 x 10cm, starting with twice the MED to limit irritation of the skin and then increasing daily by 20-40%, depending on the reaction of the skin. Exposure is stopped as soon as a pathologic reaction occurs or after six exposures. The photo provocation sites used in this study were the upper arms, lower arms, and the back. UVA and UVB provocation sites were always symmetrically distributed over the body. Observations were made by an experienced observer. Tests were carried out in a special temperature-controlled phototesting area at our photodermatology unit. Photopatch tests were carried out in the standard fashion.[30] Depending on the

patient's history, personal skin care products and sunscreen products used by the patient were added to the fixed series used in the standard photopatch test.

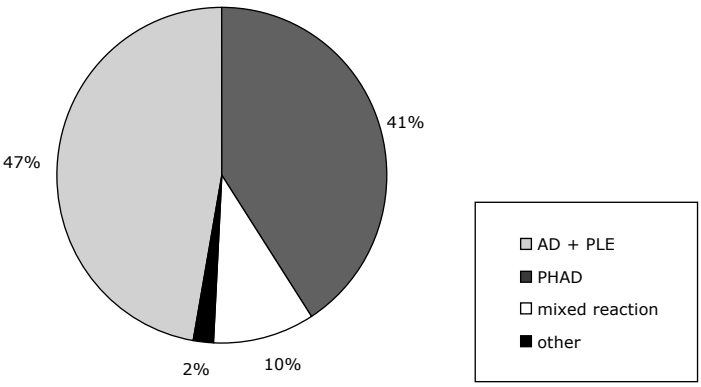
Results

Between 1994 and 2004 our department tested 145 patients (45 men and 100 women) with AD and a clinical suspicion of photosensitivity. The average age of onset of complaints of photosensitivity was 37 years. The age of the patients at testing ranged from 4 to 65 years. The majority of the patients had Fitzpatrick skin types II or III. Only a few patients had Fitzpatrick skin type I. During the same period, 3804 patients were diagnosed with AD in our department.

Phototest Results

Pathologic photo-provoked reactions were observed in 108 (74%) of the 145 patients (33 men and 75 women). Thus, the prevalence of photosensitivity in our population of patients with AD was 3% (108/3804). The MED for UVB was decreased in eight patients (7%). The geometric mean MED for UVB was 56 mJ/cm2. A decreased MED for UVA was observed in five patients (5%). The photo provocation tests identified two main clinical reaction patterns: an erythematous, papulous, and pruritic reaction in 51 patients (47%) and an eczematous reaction

Figure 1. Morphologic spectrum after photo provocation tests in patients with atopic dermatitis (AD).



PhAD= photosensitive AD; PLE = polymorphous light eruption.

in 44 patients (41%), (Figure 1). Eleven patients (10%) manifested a combination of these two reactions. One patient (1%) showed a clinical picture of CAD, with a lowered MED for UVA and UVB and one patient (1%) had a lupus erythematosus-like provocation reaction. Histopathologic examination confirmed the diagnosis of lupus erythematosus in this patient.

The erythematous, papulous, and pruritic reaction resembled the reaction seen in PLE provocations and was therefore called 'PLE type' (Figure 2). This reaction developed within hours (0.5–48 hours) and resolved within days without scarring. These patients were diagnosed with AD and coexisting PLE.

The provoked reaction was termed 'eczematous' when it displayed development of a pruritic erythematous and papulo-vesicular reaction within hours or days (0.5 hours to 5 days), subsequently resulting in a polymorphic confluent clinical picture with erythema, papules, scales, vesiculation, and excoriations (Figure 3).

Figure 2. Polymorphous light eruption-type reaction following 3 days of photo provocation testing in a patient with atopic dermatitis.

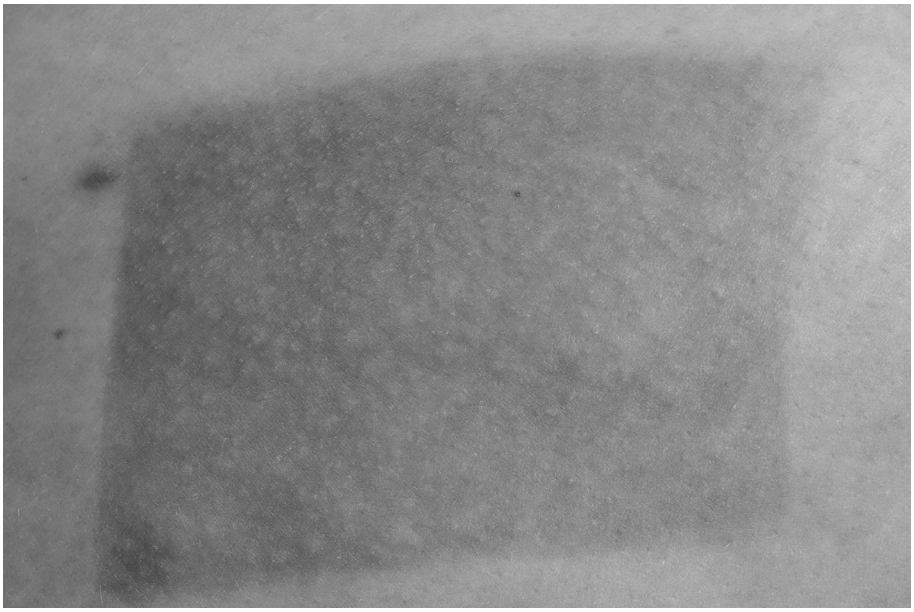
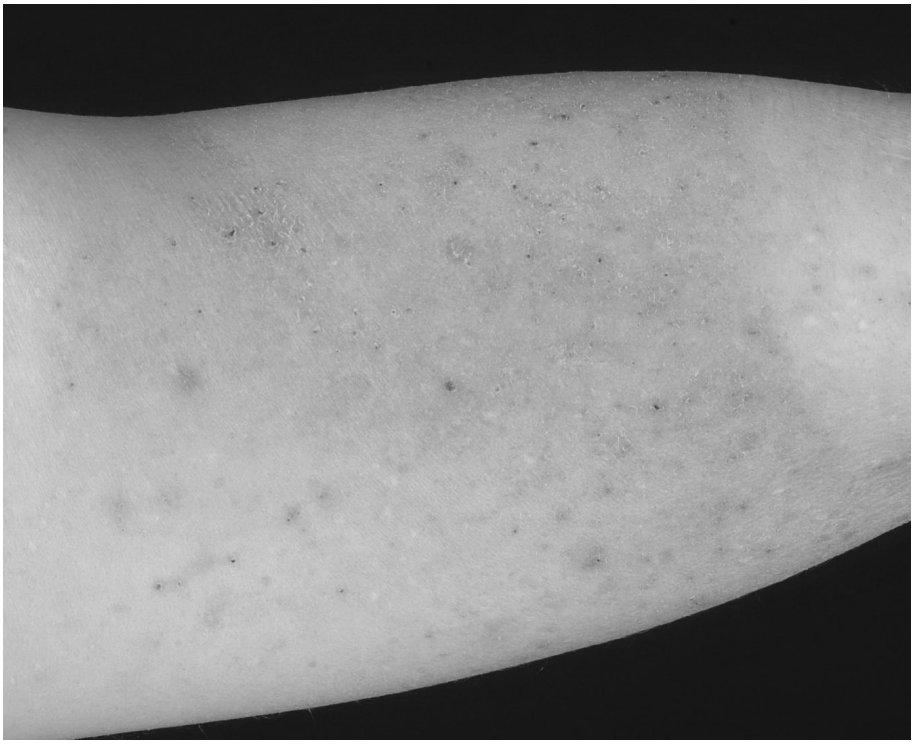


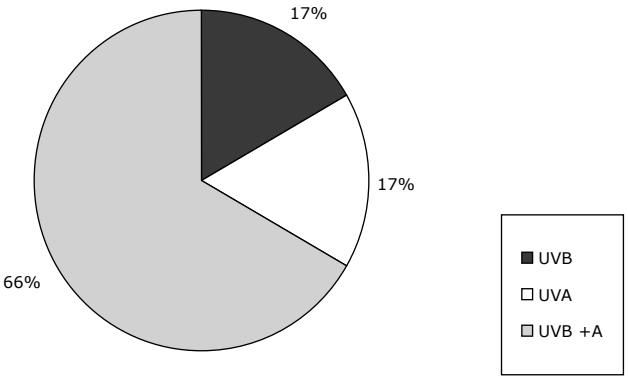
Figure 3. Photosensitive atopic dermatitis-type reaction following 5 days of photo provocation testing in a patient with atopic dermatitis.



The eczematous reaction, which was diagnosed as photosensitive AD (PhAD), took longer (days) to develop and the lesions had a more persistent character.

The age of onset of complaints in the PLE-type group was 35 years (standard deviation (SD) 19.1) compared with 39 years (SD 17.8) in the eczematous group. In a smaller group of patients the reaction initially resembled the PLE-type reaction but eventually became a more 'chronic eczematous' reaction. This was termed a 'mixed reaction pattern.' Sensitivity to both UVA and UVB was detected in 72 (67%) of the 108 patients, 18 patients (17%) showed only UVB sensitivity, and 18 patients (17%) showed only UVA sensitivity (figure 4). No difference in UV wavelength sensitivity could be detected between the PLE-type and eczematous reaction groups. There were no significant differences between men and women. Three times as many women as men with AD were tested for photosensitivity and

Figure 4. UV spectrum sensitivity after photo provocation tests in patients with atopic dermatitis.



three times as many women as men demonstrated photosensitivity. No correlations between sex or age and specific phototest results could be established in our analysis. No biopsies were taken from these photo provocation sites.

Photopatch test

Photopatch tests were performed on 125 of 145 patients (86%). Positive reactions were predominantly elicited by sunscreen substances (48%), skincare products (16%), and fragrance mix (15%). Positive photocontact tests to one or more substances were found in 29 patients (23%). The sunscreen substances eliciting a positive (photo)contact reaction are shown in table I. We found no differences between the PLE-type and the eczematous groups with regard to the photopatch test results.

Treatment

Treatment depended on the patient's history and the results of the phototests. Patients with severe photosensitivity, based on phototest results, and patients with unsatisfactory relief of complaints despite medical advice regarding sun exposure, use of sunscreen, and treatment with topical corticosteroids for symptomatic relief were treated with UVB hardening therapy.[29] Patients were classified as having severe photosensitivity on the basis of a number of factors, that is, lowered MEDs, the number of provocations required to induce abnormal reactions, and persistence of these reactions. Twenty-six patients (24%) were treated with UVB hardening

therapy (11 patients with PhAD, 11 patients with AD and PLE, and 4 patients with a mixed reaction pattern). Repeated photosensitivity tests to evaluate the effect of hardening therapy were not performed. UVB hardening therapy was clinically successful in 20 patients (77%) in terms of time spent outdoors.
Treatment with UVB hardening was unsuccessful in six patients with severe AD and severe photosensitivity, all of whom were patients with PhAD. In these patients, the effect of UVB hardening was hindered by aggravation of existing AD lesions.

Table I. Sunscreen substances eliciting a positive (photo)contact reaction (N=125)

		No. of reactions (%)	
		Photocontact	contact
4-tert-butyl-4methoxy-dibenzoylmethane	(Parsol 1789, Eusolex 9020)	7 (21%)	0 (0%)
Contralume ultra ®		5 (15%)	10 (43%)
Vichy spf 60 ®		5 (15%)	4 (17%)
2-hydroxy-4-methoxy benzophenone	(Oxybenzone, Benzophenone 3)	4 (11%)	1 (4%)
Roc minesol spf 40 bébé ®		4 (11%)	1 (4%)
2 Hydroxy 4 methoxybenzophenone	(Benzophenone 4, MS-40)	3 (9%)	4 (17%)
Vision spf 28 ®		2 (6%)	3 (13%)
2-ethyl-p-methoxycinemate	(Parsol MCX, Eusolex 2292)	2 (6%)	0 (%)
2-ethylhexyl-p-dimethyl-aminobenzoate	(Escalol 507, Eusolex 6007)	2 (6%)	0 (%)
Total No. of positive (photo)contact reactions of these substances		34	23

Discussion

AD has a complex aetiology and many different factors influence the course of this disease. One of these factors is sunlight, in particular the UV spectrum, which can both improve and worsen the course of AD. Approximately 3% of our population of patients with AD was photosensitive. Furthermore, the actual percentage might have been slightly higher because photosensitivity can easily be missed. Patients can easily overlook the correlation between complaints and (sun)light exposure, especially if they are sensitive to UVA only. In such cases, induction of skin lesions may occur even on cloudy days.[23] Nevertheless, we believe that the prevalence

of 3% identified in our study is the most precise estimate of photosensitivity in patients with AD to date and nearer to the true prevalence than earlier reports in the literature.[1,2] This is because, first, our prevalence number was based on phototest results, and secondly, estimates in the literature include not only true photosensitivity patients but also patients with exacerbation of eczema due to heat intolerance, drug-induced photosensitivity, and sunburn.[1,2] However, it is also important to note that our group of patients was a selected secondary care population and may therefore not reflect the true prevalence of photosensitivity in AD in a general population. Photosensitivity in patients with AD consists mainly of two clinical reaction patterns distinguishable by phototesting.

Patients were diagnosed either with PhAD or AD and co-existing PLE. Differences in histology or histochemical analysis between these two clinical patterns could not be affirmed because no biopsies were obtained. Nevertheless, the two clinical patterns may represent different pathologic mechanisms and these mechanisms should be investigated more intensively. These two reaction patterns were first suggested 'in children with AD' by Frain-Bell.[31] Tajima et al.[13] also described patients with normal MEDs and abnormal reactions to UV irradiation and considered these patients as having PLE co-existent with AD or having a true exacerbation of their AD due to UV irradiation. The described co-existence of PLE and AD in our group of patients is in keeping with reports in previous publications. [1,18,23,27] A mixed reaction pattern was seen in a small number of patients. In these patients, an initial PLE-type reaction progressed to an eczematous reaction. We believe the eventual eczematous reaction is a 'Koebner-like' phenomenon following the initial PLE reaction. Only one patient in this analysis qualified for the diagnosis of CAD. Patients with CAD have a chronic and infiltrated dermatitis with severe photosensitivity to UVB, UVA, and often visible light. Patients with this condition have reduced MEDs and (photo)contact allergies are often noted.[32,33] We speculate that PhAD might be a preliminary stage of CAD that eventually results in this fierce persistent eczematous eruption. However, this possibility does not seem to exclude the existence of CAD at a young age. [23,25,26,34]

In our experience, advice and protection combined with local therapy is sufficient to treat most patients' complaints. UV hardening appears to be effective in most patients with AD and photosensitivity who experience persistent problems despite advice, protection, and local treatment.

Conclusion

Identifying photosensitivity, which we found in approximately 3% of our population of patients with AD, can be difficult in this patient group. Clinically, there are two reaction patterns: patients with PLE coexisting with their AD and patients with PhAD. So long as patients with PhAD and AD with co-existent PLE are given similar treatment, differentiating between the two patterns will not be necessary. However, phototesting of patients with AD should be considered if the history is not convincing, if there is a suspicion of photocontact allergy, or when patients have severe or persistent complaints despite photo protection and local treatment.

References

1 Rajka G. Clinical aspects; other essential features; photosensitivity. In: Rajka G, editor. Essential aspects of atopic dermatitis. Berlin: Springer-Verlag, 2006: 27

2 Vieluf D, Ring J. Complications and diseases associated with atopic eczema. In: Ruzicka T, Ring J, Przybilla B, editors. Handbook of atopic eczema. Berlin: Springer, 1991: 54-79

3 Vieluf D, Ruzicka T. Photosensitivity. In: Ruzicka T, Ring J, Przybilla B,
editors. Handbook of atopic eczema. 1st ed. Berlin: Springer-Verlag, 1991: 68

4 Frain-Bell W. Photoaggravated dermatoses; atopic dermatitis. In: Frain-Bell W, editor. Cutaneous photobiology. Oxford: Oxford University Press; 1985: 60-1

5 Photoaggravated dermatoses, including lupus erythematosus; atopic dermatitis. In: Harber LC, Bickers DR, editors. Photosensitivity diseases: principles and treatment. 2nd ed. Toronto (ON): B.C. Decker, 1989: 299

6 Crouch RB, Foley PA, Baker CS. Analysis of patients with suspected photosensitivity referred for investigation to an Australian photodermatology
clinic. J Am Acad Dermatol 2003 May; 48 (5): 714-20

7 Deguchi H, Danno K, Sugiura H, et al. Sun exposure is an aggravating factor responsible for the recalcitrant facial erythema in adult patients with atopic dermatitis. Dermatology 2002; 204 (1): 23-8

8 Keong CH,KurumajiY,Miyamoto C, et al. Photosensitivity in atopic dermatitis: demonstration of abnormal response toUVB. JDermatol 1992 Jun; 19 (6): 342-7

9 Kerker BJ, Morison WL. The photoaggravated dermatoses. Semin Dermatol 1990 Mar; 9 (1): 70-7

10 Murphy GM. Diseases associated with photosensitivity. J Photochem Photobiol B 2001 Nov 15; 64 (2-3): 93-8

11 MurphyGM. Investigation of photosensitive disorders. Photodermatol Photoimmunol Photomed 2004 Dec; 20 (6): 305-11

12 Pigatto PD, Altomare G, Polenghi MM, et al. Atopy and photosensitivity in children. Photodermatol 1986 Oct; 3 (5): 303

13 Tajima T, Ibe M, Matsushita T, et al. A variety of skin responses to ultraviolet irradiation in patientswith atopic dermatitis. JDermatol Sci 1998 Jun; 17 (2): 101-7

14 de Leo VA. Other photoaggravated diseases. In: van VlotenWA, editor. Photosensitivity: topics in clinical dermatology. Tokyo: Igaku-Shoin, 1992: 155-7

15 Hawk JLM, Norris PG, Ho¨ nigsmann H. Photomedicine; abnormal responses to UV radiation: idiopathic and photoexacerbated. In: Freedberg IM, Eisen AZ, Wolff K, et al., editors. Fitzpatrick's dermatology in general medicine: I. Columbus (OH): McGraw-Hill, 2004: 1294

16 Amblard P, Leccia MT. Skin diseases with photosensitivity. Rev Prat 1992 Jun 1; 42 (11): 1365-8

17 Other photodermatoses; photosensitive eczema. In:Magnus IA, editor.Dermatological photobiology: clinical and experimental aspects.Oxford: Blackwell, 1976:194-5

18 Ramsay CA, Black AK. Photosensitive eczema. Trans St Johns Hosp Dermatol Soc 1973; 59 (2): 152-8

19 Yokel BK, Hood AF, Morison WL. Management of chronic photosensitive eczema. Arch Dermatol 1990 Oct; 126 (10): 1283-5

20 Hawk JLM,Magnus IA. Diseases caused by environmental exposure or trauma; chronic actinic dermatitis; photosensitive eczema. In: Braun-Falco O, Plewig G, Wolff HH, et al., editors. Dermatology. 2nd ed. Berlin: Springer, 1996: 559

21 Harber LC, Whitman GB. Photosensitivity: classification. Dermatol Clin 1986 Apr; 4 (2): 167-9

22 Morison WL. Clinical practice: photosensitivity. N Engl J Med 2004 Mar 11; 350 (11): 1111-7

23 Russell SC, Dawe RS, Collins P, et al. The photosensitivity dermatitis and actinic reticuloid syndrome (chronic actinic dermatitis) occurring in seven young atopic dermatitis patients. Br J Dermatol 1998 Mar; 138 (3): 496-501

24 Yones SS, Palmer RA, Hextall JM, et al. Exacerbation of presumed chronic actinic dermatitis by cockpit visible light in an airline pilot with atopic eczema. Photodermatol Photoimmunol Photomed 2005 Jun; 21 (3): 152-3

25 Ogboli MI, Rhodes LE. Chronic actinic dermatitis in young atopic dermatitis sufferers [letter]. Br J Dermatol 2000 Apr; 142 (4): 845

26 Creamer D, McGregor JM, Hawk JL. Chronic actinic dermatitis occurring in young patients with atopic dermatitis. Br J Dermatol 1998 Dec; 139 (6): 1112-3

27 Pearson IC, Holden CA. Atopic dermatitis: a simple entity? Clin Exp Dermatol 2002 Jan; 27 (1): 88-9

28 Hannifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol Stockh 1980; Suppl. 92: 44-7

29 Boonstra HE, van Weelden H, Toonstra J, et al. Polymorphous light eruption: a clinical, photobiologic, and follow-up study of 110 patients. J Am Acad Dermatol 2000 Feb; 42 (2 Pt 1): 199-207

30 Bryden AM, Moseley H, Ibbotson SH, et al. Photopatch testing of 1155

patients: results of the U.K. Multicentre Photopatch Study Group. Br J
Dermatol 2006; 155 (4): 737-47
31 Frain-Bell W. The photosensitive child. Trans St Johns Hosp Dermatol Soc 1973; 59 (2): 159-65
32 Roelandts R. Chronic actinic dermatitis. J Am Acad Dermatol 1993 Feb;
28 (2 Pt 1): 240-9
33 Lim HW, Morison WL, Kamide R, et al. Chronic actinic dermatitis: an analysis of 51 patients evaluated in the United States and Japan. Arch Dermatol 1994 Oct 1; 130 (10): 1284-9
34 Kurumaji Y, Kondo S, Fukuro S, et al. Chronic actinic dermatitis in a young patient with atopic dermatitis. J Am Acad Dermatol 1994 Oct; 31 (4): 667-9

Photosensitivity testing in children

Onno ten Berge, MD [a], Vigfus Sigurdsson, MD, PhD [a], Carla A. F. M. Bruijnzeel-Koomen, MD, PhD [a], Huib van Weelden, MSc [a], and Suzanne G. M. A. Pasmans, MD, PhD [b]

From the Department of Dermatology/Allergology/Venereology [a] and Wilhelmina Children's Hospital [b] University Medical Center Utrecht.

Journal of the American Academy of Dermatology 2010, in press.

Abstract

Background

Phototesting is an important diagnostic tool to objectify light-related symptoms. Data on phototesting procedures in children are scarce. Objective: The aim of this study was to evaluate phototest results in photosensitivity disorders in children.

Methods

The phototest procedures are described. All children phototested in our department between 1995 and 2007 were included in this retrospective study. Children given the diagnosis of polymorphic light eruption (PLE) were selected for follow-up.

Results

A total of 92 children (39 boys and 53 girls, age range 4-16 years) were successfully phototested. A photosensitivity disorder was confirmed in 56 children (61%, 24 boys and 32 girls). PLE was diagnosed in 39%, photosensitivity associated with atopic dermatitis in 23%, and erythropoietic protoporphyria in 23%. Other diagnoses were less common. Ten children with PLE were followed up for at least 5 years. Seven reported their photosensitivity had not changed over time, in two cases it had diminished, and in one patient the photosensitivity had disappeared.

Limitations

Retrospective study design is a limitation.

Conclusion

Phototesting in children is feasible when performed in a case- and child-dependent manner. PLE was the most prevalent diagnosis in our series followed by photosensitivity in atopic dermatitis.

Introduction

Photosensitivity disorders in children can be classified into 4 groups: (1) idiopathic, (2) secondary to endogenous agents, (3) Photoexacerbated dermatoses, and (4) secondary to exogenous agents (Table I).[1] Polymorphic light eruption (PLE) is the most common photodermatosis.[2,3] Early onset of photosensitivity is often the clue to identifying a rare genetic or metabolic disease.[4] A diagnosis of a photosensitivity disorder can usually be made based on the history and clinical examination. Phototesting is necessary in only a minority of cases.[5] It is indicated if there is clinical uncertainty or if a child has persistent symptoms despite adequate sun protection and topical treatment. It is performed to objectify photosensitivity and/or to clarify the diagnosis. Phototests can also determine the specific eliciting wavelengths for the photodermatosis,[6-9] establish the extent of photosensitivity, evaluate the effects of treatment, and monitor the photosensitivity over time.[10-12] Descriptions of phototesting procedures in children are scarce and limited.[3,10]

Methods

All children younger than 16 years who underwent diagnostic phototests in our department between 1995 and 2007 were included in the study. Their diagnoses and accompanying phototests were analyzed. Children given the diagnosis of PLE with a minimum 5-year follow-up period were contacted about their current disease status.

The study was approved by the medical ethics committee of our university medical center.

Phototesting procedures

Phototesting in children was performed with ultraviolet (UV)B, UVA, and visible light (VL). The wavelengths included in the test were chosen based on the patient's history and clinical features. Radiation sources and their emission spectrum, filters, the UV intensity measuring, and calibration have been described previously.[6-9,13,14] The final diagnosis of a photosensitivity disorder was based on both the clinical features and the phototest results.

Table I. Classification of photosensitivity disorders in children

Idiopathic
Polymorphic light eruption
Actinic prurigo
Hydroa vacciniforme
Juvenile spring eruption
Solar urticaria
Chronic actinic dermatitis
Secondary to endogenous agents
Porphyrias
Porphyria cutanea tarda
Erythropoietic protoporphyria
Photoexacerbated dermatoses
Auto-immune diseases
Lupus erythematosus
Dermatomyositis
Genodermatoses
Bloom syndrome
Rothmund- Thomson syndrome
Kindler syndrome
Xeroderma pigmentosum
Cockayne syndrome
Trichothiodystrophy
Hartnup disease
Smith-Lemli-Opitz syndrome
Infectious disease
HIV
Nutritional deficiencies
Pellagra
Pyridoxine deficiency
Primary dermatologic diseases
Atopic dermatitis (AD)
Psoriasis
Secondary to exogenous agents
Photo allergy, contact and systemic
Photo toxicity, contact and systemic

Minimal erythema dose

The minimal erythema dose (MED) is defined as the dose of UVB, UVA, or VL necessary to induce a just perceptible erythema with no marked borders. The MED for UVB and UVA was determined with a specially designed apparatus that simultaneously exposes a series of 9 areas of skin each measuring 3 x 10 mm to increasing UV doses (geometrically progressive with increments of 41% between successive skin fields).[6] The MED for VL was determined by sequential exposure of different skin fields to increasing doses of white light with a factor two: 5,10, 20, 40, and 80 J cm^2.[6] Irradiation was administered in the morning and the assessment was performed after 8 and 24 hours. The normal MED for UVB is about 50 to 70 mJ cm2 (Philips 20W/TL12). The normal MED for UVA has a lower limit of about 25 J cm^2 and an upper limit of 40 J cm^2 (Mutzhas UVASUN 3000S). The normal MED for VL is greater than 100 J cm^2.[6] The normal MEDs for UVB, UVA, and VL are based on a series of healthy volunteers with Fitzpatrick sun-reactive skin type II and III.[15] The MED was considered decreased if the skin was at least 4 times more sensitive than normal.

Photoprovocation

Photoprovocation by repeated exposures is a way to reproduce photosensitive skin lesions (Figs 1 and 2). Non-lesional skin was exposed daily to an increasing dose of UVB, UVA, or VL. Repeated irradiations were administered on skin areas

Fig 1. Measurement of ultraviolet (UV)B intensity preceding photoprovocation with UVB.

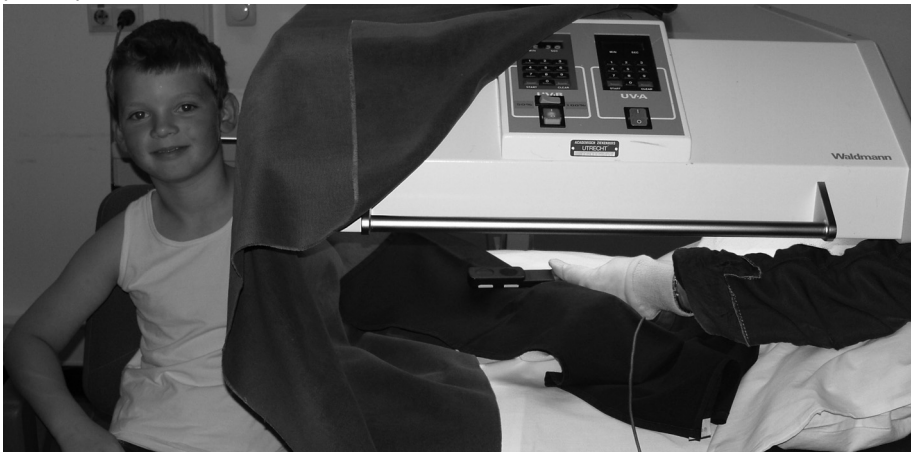
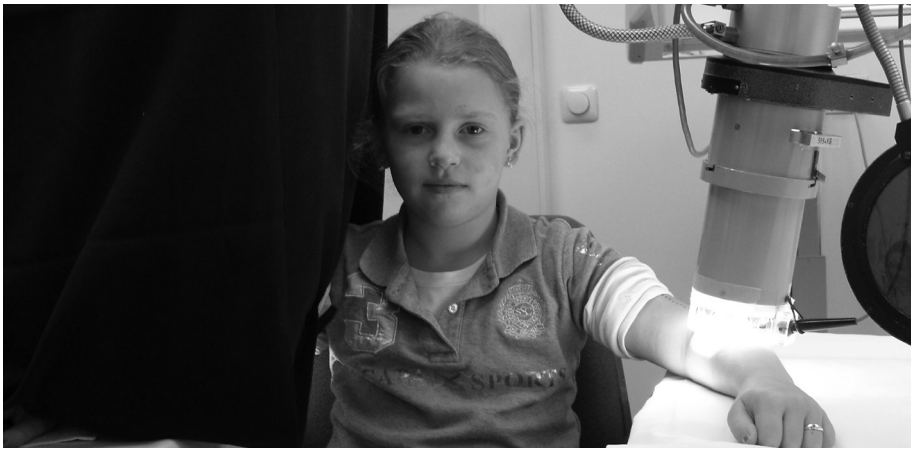


Fig 2. Simultaneous photoprovocation with ultraviolet A (right arm) and visible light (left arm).



measuring 60 cm^2, starting with 2 x MED to limit irritation and increasing daily by 20% to 40% depending on the skin reaction. If marked erythema, edema, or both developed, the same dose was given as on the previous day. If the previous exposure resulted in slight erythema, the next dose was increased by 40%. If there was hardly any visible reaction, the dose was doubled. Repeated exposures were mostly administered a maximum of 4 times. The maximum summation dose with VL was 120 J cm^2. If an abnormal reaction was observed, exposure was stopped. Because of the high intensity of the light source in VL testing, the skin temperature of the VL test area was monitored to prevent thermic influences (Ellab a/s, DU-3, Copenhagen).

As UVB and UVA are the main eliciting wavelengths in PLE, lupus erythematosus, actinic prurigo (AP), or photosensitivity in atopic dermatitis (AD), testing with UVA and UVB specific wavelengths was performed when one of these photodermatoses was suspected. Solar urticaria can be elicited by all sorts of light or combinations of UV and VL. Erythema, swelling, and itching develop within minutes to half an hour and disappear within 1 or 2 hours. The skin was therefore directly observed for 60 minutes after single exposures with UVB, UVA, or VL. To measure the extent of the photosensitivity, patients with erythropoietic protoporphyria (EPP) were sometimes phototested. The inciting wavelengths to produce a photodynamic reaction with the accumulated photosensitizing porphyrins in the skin in EPP are mainly purple (405

nm) and a minor degree of green (546 nm) light and there is no need for repeated exposures.[16] Therefore; MED for purple and sometimes green light was performed in these children. The normal MED for purple and green light is greater than 100 J cm^2.[16]
Observations were made by an experienced observer and all tests were carried out in a special temperature-controlled phototesting room.

Contraindications
Phototests in children should not be performed on inflamed skin or if the patient is on systemic immunosuppressive therapy. Topical treatment with corticosteroids or with potentially phototoxic medications or sunscreens should be stopped (if possible) before testing. For younger children it was necessary to confer with the parents about the ability of the child to cooperate. Phototesting was deferred in cases of photosensitivity presenting before 4 years of age.

Photopatch tests

When there was a suspicion of a (photo)contact reaction photopatch tests were performed. These tests were carried out in a standard fashion: the test series consisted of 11 established and candidate organic sunscreen agents, a few possible commercial sunscreens for future protection (as is), and white soft paraffin as a control. If a patient suspected that one or more of their skin-care products caused a reaction these products were tested as is.[17]

Results

A total of 92 children, 39 boys (42%) and 53 girls (58%) (aged 4-16 years) were phototested. A photosensitivity disorder was confirmed in 56 children (61%). This group consisted of 24 boys (43%) and 32 girls (57%). In 36 children (39%) no photosensitivity was demonstrated with Phototesting despite a history of sunlight-related symptoms. The majority of the children had Fitzpatrick skin types II or III. Nearly all children were referred to our department by a dermatologist. No problems were encountered with any of the children undergoing the test procedures.

Diagnosis

The diagnoses made in these 92 children are presented in Table II.

Table II. Phototest outcome of 92 phototested children

Photosensitive disorders	Number of children	Percentage
Polymorphic light eruption (PLE)	22	39 %
Photosensitivity in Atopic dermatitis	13	23 %
– Photosensitive atopic dermatitis (PhAD)	*8*	*14 %*
– Atopic dermatitis with coexistent PLE (AD+PLE)	*5*	*9 %*
Erythropoietic protoporphyria	13	23 %
Solar urticaria	3	5 %
Lupus erythematosus	1	2 %
Actinic prurigo	1	2 %
Rothmund-Thomson	1	2 %
Xeroderma pigmentosum	1	2 %
Undefined genodermatosis	1	2 %
Total	56 (61 %)	100 %
Non photosensitive disorders		
Atopic dermatitis	16	44,5 %
Contact dermatitis	4	11 %
No diagnosis / No photosensitivity	16	44,5 %
Total	36 (39 %)	100 %

Phototest results

The phototest results are described separately for each encountered diagnosis.

Polymorphic light eruption

Children with PLE showed a transient pruritic erythematous and papular reaction after provocation. Vesicles or edema were observed in some cases. The reaction disappeared within days without scarring. A total of 22 children (37%) were given the diagnosis of PLE (8 boys and 14 girls). The average age was 11 years. A lowered MED for UVB was found in two children and for UVA in two children. Photosensitivity for UVB was found in 32%, for UVA in 28%, and for UVB and

UVA in 40% of the children. Two boys and two girls demonstrated severe PLE photosensitivity showing strong skin reactions within two provocations for UVB and UVA. One of the two girls had a lowered MED for UVB. No difference between boys and girls concerning age and photospectrum sensitivity was found.

Photosensitivity in children with AD

Thirteen children with AD (23%; average age 12 years) experienced photosensitivity (5 boys and 8 girls). Eight were given the diagnosis of photosensitive AD (PhAD), and 5 the diagnosis of AD and coexistent PLE. The MED for UVA and UVB was measured in all these children. The MED for VL was measured in 9. One child showed a lowered MED for UVA and UVB, and one showed a lowered MED for UVB. Photosensitivity for UVA and UVB was found in 7 children (54%), two (15%) were photosensitive for only UVB, two (15%) showed sensitivity for UVA and VL, one (8%) showed sensitivity for only UVA, and one (8%) showed sensitivity for UVA, UVB, and VL. The reaction of the skin to VL was similar to the reactions of UVB and UVA. Two girls with PhAD and one girl with AD and coexistent PLE demonstrated severe photosensitivity. They showed strong pathologic skin reactions within two irradiations for both UVB and UVA. Their MEDs for UVB and UVA were in the normal range. No difference in UV wavelength sensitivity, sex ratio, or age could be demonstrated between the PhAD and the AD and coexistent PLE group. The two children with lowered MEDs were given the diagnosis of PhAD.

Erythropoietic protoporphyria

Provocation reactions of patients with EPP consisted mainly of burning sensations during testing and erythema and swelling afterward. In some cases, vesicles, bullae, and purpura were noted. A small percentage of patients showed fast transitory urticarial reactions. Thirteen children (23%) with EPP were phototested (7 boys and 6 girls). The average age was 10 years. All but one were given the diagnosis of EPP before phototesting, based on red blood cell porphyrin analysis. In the exception, the diagnosis was suggested by the history and the outcome of the phototest, and was confirmed by laboratory testing. 18 In children with EPP, phototesting was performed to measure the extent of photosensitivity. Patients showed lowered MEDs for VL; the average MED for green light was 83 J cm^2 (N = 11) and the average MED for purple light was 27 J cm^2 (N = 13). Two boys showed severe EPP photosensitivity with an MED for purple light less than 5 J cm^2.

Solar urticaria

Solar urticaria was diagnosed in 3 children (5%; two boys and one girl) with an average age of 12 years. All 3 children showed normal MEDs for UVA, UVB, and VL. Provocation with UVA and VL showed sensitivity with urticarial reactions for VL in two children and for UVA and VL in one boy.

Lupus erythematosus

A 4-year-old girl with lupus erythematosus was phototested to measure the extent of her photosensitivity. She showed normal MEDs for UVA, UVB, and VL. The erythematous reaction after provocation with UVA and UVB slowly progressed to persistent erythema with papules within days.

Actinic prurigo

A 9-year-old girl was given the diagnosis of AP. The clinical course and photoprovocation result resembled PLE but was coupled with seasonal independence, severe persistent pruritus, and excoriations typical for AP.[19] A normal MED for UVA, UVB, and VL was found. Provocation with UVA gave an erythematous and papular reaction with itching.

Rothmund-Thomson syndrome

This autosomal recessive disorder is typified by photosensitivity and poikiloderma. A 13-year-old girl, previously given the diagnosis of Rothmund-Thomson syndrome, was phototested to determine the extent of photosensitivity. She showed normal MEDs for UVB and UVA. An erythematous papular reaction was seen after provocation with UVB.

Xeroderma Pigmentosum

A 4-year-old boy was suspected of having Xeroderma Pigmentosum. He presented with photosensitivity symptoms, lentigines on the face, and hypopigmentation and hyperpigmentation on the extensor surfaces of the arms and legs. Limited phototests were performed while awaiting DNA analysis. MEDs for UVB and UVA were normal. One single dose (2x3 MED) of UVB showed persistent erythema and hyperpigmentation. The diagnosis of Xeroderma Pigmentosum was thereafter confirmed by DNA analysis, which showed nucleotide excision repair defects corresponding with this disease.

Photopatch tests

These tests were performed in 49 (53%) of the 92 children. In 9 cases (16%, all with a photosensitivity disorder) a (photo)contact reaction was demonstrated. One showed photocontact reactions, two showed both photocontact and contact reactions (for different substances), and 6 had positive contact reactions. The main substances eliciting photocontact and contact reactions were sunscreens (90%). The most prevalent sunscreen UV filters eliciting a positive reaction were 4-tert-butyl-4methoxy-dibenzoylmethane (Parsol 1789, Eusolex 9020) and 2-hydroxy-4-methoxy benzophenone (oxybenzone, benzophenone 3). There was no difference between boys and girls and there was no age correlation.

Therapy

All children were advised to use an adequate sunscreen, varying from sun protection factor 15 to 50, depending on the severity of their symptoms, and to avoid exposure to intense sunlight. In the case of idiopathic photodermatoses and photoexacerbated dermatoses, children and parents were told about the benefit of gradually increasing exposure to sunlight to achieve tolerance.[9,20,21] Topical steroids were used in some cases to provide symptomatic relief. Ten children with severe photosensitivity based on phototest results who had unsatisfactory relief of symptoms despite sunlight protection and use of topical steroids were treated with UVB hardening therapy.[6,7] This included 5 children with PLE, 3 children with PhAD, one child with an ambiguous photosensitivity (PLE and PhAD), and one child with AP. Because of the retrospective nature of our study it was not possible to evaluate the effect of this therapy. No repeated phototests were performed.

Follow-up of PLE

Based on our inclusion criterion of a minimum 5-year follow-up period after phototesting, 15 of the 22 children given the diagnosis of PLE were identified for assessment. Ten were successfully recalled (4 boys and 6 girls). The average age at testing was 11 years (range 4-16 years). The mean age of these patients at follow-up was 18 years (range 10-28 years). Patients were contacted an average of 7 years after phototesting. The results are presented in Table III.

Table III. Results of a minimum 5-year follow-up of children given diagnosis of polymorphic light eruption.

PLE patient	Age at diagnosis	Age at follow-up	Male/ Female	General photosensitivity since diagnosis	Current protection	Photosensitivity complaints after advice following testing	Treatment of complaints	Photosensitivity under control
A	9	14	M	Stayed the Same	Sunscreen	Decreased	Topical corticosteroid	Yes
B	3	12	F	Decreased	Sunscreen	Decreased	Skincare product	Yes
C	14	23	F	Stayed the same	Natural hardening	Decreased	Nothing; sun avoidance	Yes
D	7	16	F	Stayed the same	Avoiding solar exposure	Stayed the Same	Nothing; sun avoidance	No
E	12	17	M	Decreased	Sunscreen Protective clothing	Decreased	Nothing; sun avoidance	Yes
F	16	28	F	Decreased	Sunscreen and Natural hardening	Decreased	Skincare products	Yes
G	4	10	M	Stayed the same	Sunscreen and avoiding solar exposure	Decreased	Nothing; sun avoidance	Yes
H	16	26	F	Stayed the same	Sunscreen	Stayed the Same	Nothing; sun avoidance	No
I	13	20	F	Stayed the same	Sunscreen	Stayed the Same	Cooling (shower), Prednisone	No
J	10	15	M	Stayed the same	Sunscreen	Decreased	Antihistamines sun voidance	Yes

Discussion

Children require a different approach to conducting photomedical testing when compared with adults. The phototest procedures are not technically different but they need to be carried out in a case and child-dependent manner. Most important is a clear explanation of the test procedures to the child and the parents. Ferguson[10] was first to point out the diagnostic potential and value of determining wavelength dependencies to guide preventive measures and recommendations in photosensitive children. The data analysis of photosensitivity testing in children at our photodermatology department during a 12-year period illustrates the phototesting properties and the characteristics of photosensitivity.
The diagnostic potential of phototesting is well demonstrated by the number of children in whom photosensitivity was not verified upon testing (39%), despite sun-related symptoms.

As in earlier series and in adults, PLE was the most prevalent photodermatosis in our patients.[2,3,10,22] More girls than boys were affected, in keeping with previous reports.[1,23] Comparable with earlier analysis, although variable in ranking (with the exception of PLE), PLE, photosensitivity in AD, and EPP were the most prevalent photosensitivity disorders of childhood.[2,3,10] Photosensitivity in AD and solar urticaria were diagnosed more often in our series, whereas AP and juvenile spring eruption were diagnosed less frequently.[2,3,10] Our study also demonstrated a greater variety of photosensitivity disorders than previous reports.[2,3,10]

The association of photosensitivity and atopy in childhood was reported earlier.[24] We found that childhood photosensitivity in AD, as in adults, consisted mainly of two clinical reaction patterns distinguishable by phototests: PhAD and AD with coexisting PLE.[8] These two reaction patterns were first suggested in children with AD by Frain-Bell.[23] Tajima et al[25] also described this phenomenon in adults with normal MEDs and abnormal reactions to UV irradiation; they considered the patients to have PLE with coexistent AD or a true exacerbation of their AD caused by UV irradiation. The described coexistence of PLE and AD is in keeping with previous publications.[26-29]

The purpose of our follow-up of the patients with PLE was to gain more information on the natural course of early-onset PLE. A decrease in symptoms and, in some

cases, in the extent of photosensitivity was observed on reaching adolescence in most children, suggesting a relatively favourable short-term clinical course. This observation cannot be used to draw conclusions about long-term prognosis, which would necessitate follow-up of a larger group of patients over a longer period.

The main weaknesses of this study are the retrospective design and the fact that it describes phototest results at a tertiary referral center. Furthermore, as photosensitivity disorders in children can be diagnosed clinically in most cases, not all children referred to our photodermatology department with suspected photosensitivity were phototested.[10] Consequently; this study does not provide data on the prevalence of photosensitive disorders in children. Nevertheless, it represents one of the largest series of phototesting in children to date.

References

1 Lim HW, Epstein J. Photosensitivity diseases. J Am Acad Dermatol 1997;36:84-90.

2 Horkay I, Emri G, Varga V, Simics E, Remenyik E. Photosensitivity skin disorders in childhood. Photodermatol Photoimmunol Photomed 2008;24:56-60.

3 Jansen CT. Photosensitivity in childhood. Acta Derm Venereol Suppl (Stockh) 1981;95:54-7.

4 Garzon MC, DeLeo VA. Photosensitivity disorders in childhood. Adv Dermatol 1997;13:307-52.

5 Poh-Fitzpatrick MB, Ramsay CA, Frain-Bell W, Tamayo L, Kahn TA, Bickers DR. Photodermatosis in infants and children.Pediatr Dermatol 1988;5:189-200.

6 Boonstra HE, van Weelden H, Toonstra J, van Vloten WA. Polymorphous light eruption: a clinical, photobiologic, and follow-up study of 110 patients. J Am Acad Dermatol 2000;42:199-207.

7 Sanders CJ, Lam HY, Bruijnzeel-Koomen CA, Sigurdsson V, van Weelden H. UV hardening therapy: a novel intervention in patients with photosensitive cutaneous lupus erythematosus. J Am Acad Dermatol 2006;54:479-86.

8 ten Berge O, van Weelden H, Bruijnzeel-Koomen CA, de Bruin-Weller MS, Sigurdsson V. Throwing a light on photosensitivity in atopic dermatitis: a retrospective study. Am J Clin Dermatol 2009;10:119-23.

9 van Weelden H. [Lichtonderzoek en therapie bij lichtdermatosen]. Mod Med 1985;739-46.

10 Ferguson J. Investigation of the photosensitive child. Pediatr Dermatol 1992;9:348-50.

11 Murphy GM. Diseases associated with photosensitivity. J Photochem Photobiol B 2001;64:93-8.

12 Schornagel IJ, Knol EF, van Weelden H, Guikers CL, Bruijnzeel-Koomen CA, Sigurdsson V. Diagnostic phototesting in polymorphous light eruption: the optimal number of irradiations. Br J Dermatol 2005;153:1234-6.

13 Sigurdsson V, Knulst AC, van Weelden H. Phototherapy of acne vulgaris with visible light. Dermatology 1997;194: 256-60.

14 Toonstra J, Henquet CJ, van Weelden H, van der Putte SC, van Vloten WA. Actinic reticuloid: a clinical photobiologic, histopathologic, and follow-up study of 16 patients. J Am Acad Dermatol 1989;21:205-14.

15 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988;124:869-71.

16 Bijlmer-Iest JC, Baart de la FH, van Asbeck BS, van HJ, van Weelden H, Marx JJ, et al. Protoporphyrin photosensitivity cannot be attenuated by oral N-acetylcysteine. Photodermatol Photoimmunol Photomed 1992;9:245-9.

17 Bryden AM, Moseley H, Ibbotson SH, Chowdhury MMU, Beck MH, Bourke J, et al. Photopatch testing of 1155 patients: results of the UK multicenter photopatch study group. Br J Dermatol 2006;155:737-47.

18 Lecluse AL, Kuck-Koot VC, van Weelden H, Sigurdsson V, Russel IM, Frank J, et al. Erythropoietic protoporphyria without skin symptomseyou do not always see what they feel. Eur J Pediatr 2008;167:703-6.

19 Man I. Actinic prurigo. In: Ferguson J, Dover JS, editors. Photodermatology. 1st ed London: Manson Publishing; 2006.pp. 51-3.

20 Hensley DR, Hebert AA. Pediatric photosensitivity disorders.Dermatol Clin 1998;16:571-8.

21 Moriwaki S, Kraemer KH. Xeroderma Pigmentosum; bridging a gap between clinic and laboratory. Photodermatol Photoimmunol Photomed 2001;17:47-54.

22 Millard TP, Fryer AA, McGregor JM. A protective effect of glutathione-S-transferase GSTP1*Val(105) against polymorphic light eruption. J Invest Dermatol 2008;128:1901-5.

23 Frain-Bell W. The photosensitive child. Trans St Johns Hosp Soc 1973;59:159-65.

24 Frain-Bell W, Scatchard M. The association of photosensitivity and atopy in the child. Br J Dermatol 1971;85:105-10.

25 Tajima T, Ibe M, Matsushita T, Kamide R. A variety of skin responses to ultraviolet irradiation in patients with atopic dermatitis. J Dermatol Sci 1998;17:101-7.

26 Pearson IC, Holden CA. Atopic dermatitisea simple entity? ClinExp Dermatol 2002;27:88-9.

27 Rajka G. Clinical aspects; other essential features; photosensitivity. In: Rajka G, editor. Essential aspects of atopic dermatitis. Berlin: Springer-Verlag; 2006. p. 27.

28 Ramsay CA, Black AK. Photosensitive eczema. Trans St Johns Hosp Soc 1973;59:152-8.

29 Russell SC, Dawe RS, Collins P, Man I, Ferguson J. The photosensitivity dermatitis and actinic reticuloid syndrome (chronic actinic dermatitis) occurring in seven young atopic dermatitis patients. Br J Dermatol 1998;138:496-501.

UVB responses in atopic dermatitis patients with photosensitivity

Onno ten Berge, Edward F. Knol, Huib van Weelden, Marjolein S. de Bruin-Weller, Carla A. F. M. Bruijnzeel-Koomen

University Medical Centre Utrecht, Department of Dermatology & Allergology, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands

Submitted 2010

Abstract

Background

Two types of photosensitivity in atopic dermatitis (AD) are clinically recognized: Photosensitive AD and AD with coexistent polymorphic light eruption (PLE). In patients with PLE without AD a decreased influx of neutrophils after UVB exposure was demonstrated.

Objectives

To compare the infiltration of inflammatory cells after a single UVB exposure in AD patients with and without photosensitivity.

Patients and methods

Eight non-photosensitive AD patients, eight with Photosensitive AD and eight with AD and coexisting polymorphic light eruption were included in this analysis. Non-lesional buttock skin was exposed to 3 times the minimal erythemal dose (MED) UVB and skin biopsies were taken at t=0, 6 and 24 hours. Skin sections were immunohistochemically analysed for specific UV-induced changes.

Results

In the first 24 hours following UV exposure there was an influx of neutrophils, a decrease in the number of mast cells and epidermal dendritic cells, and an increase of dermal T cell numbers in the skin. Hardly any eosinophils were present. There was no difference in these cell numbers between the three patient groups. Furthermore, there was no difference in filaggrin protein expression nor was this influenced by UV during the first 24 hours.

Conclusion

In contrast to PLE there was no association between photosensitivity and the number of neutrophils infiltrating the skin upon UVB irradiation. A single UVB irradiation induces mast cell degranulation in atopic dermatitis patients with or without photosensitivity without a clinical noticeable skin reaction. The analysis of a single irradiation did not unravel the pathomechanism of photosensitivity in AD.

Introduction

UV radiation is capable of inducing both inflammation and immunosuppression in human skin.[1-3] Consequently, patients with atopic dermatitis (AD) can benefit from this immunosuppressive effect, e.g. through UV-therapy [4], but can also experience deterioration.[5;6] Approximately 3-10% of patients with AD experience an adverse effect after exposure to UV-radiation.[5;6]
By means of photoprovocation tests two types of photosensitivity are clinically recognized in AD: Photosensitive AD (PhAD) and AD with co-existent polymorphic light eruption (AD+PLE). [5] Until now the mechanism of photosensitivity in AD patients has not been studied.

UV-exposure of the skin of healthy controls induces changes such as damage and apoptosis of keratinocytes, visible as sunburn cells, immigration of neutrophils and monocytes into the skin, mast cell degranulation and emigration of dendritic cells from the epidermis into the draining lymph nodes.[7-12]

In patients with PLE a decreased number of skin infiltrating neutrophils after a single UVB irradiation (TL12, 3x MED) was noted.[13] Since neutrophils express immunoregulatory cytokines TNF-alpha, IL-4, and IL-10, [13, 14] it was hypothesized that a decrease in the number of infiltrating neutrophils upon UVB exposure could be responsible for diminished immunoregulation.

Another study of patients with PLE demonstrated an increased number of CD1 positive cells in the epidermis after UVB irradiation (TL12, 6x MED), suggesting a decrease in dendritic cell emigration from the epidermis.[15]
An acute eczematous reaction in AD patients is histologically characterized by spongiosis and infiltration of eosinophils, dendritic cells and T cells in the dermis.[16-18] Neutrophils are hardly observed.[19] Since neutrophils are supposed to have an immunoregulatory function following UV exposure, we hypothesized that a lack or decrease of infiltrating neutrophils would be responsible for photosensitivity in patients with AD.

About 40 % of the AD patients have a loss of function mutation of the filaggrin gene.[20-25] Since filaggrin protein is degraded to urocanic acid which is involved in UV tolerance we also measured the filaggrin protein expression in the epidermis.

Materials and methods

Subjects

Eight patients with AD (not photosensitive), 8 with photosensitive AD (PhAD) and 8 with AD with coexistent polymorphic light eruption (AD+PLE) were age matched and included in this study. Patients with AD + PLE show an erythematous, papulous, and pruritic phototest reaction resembling the reaction seen after UVB provocation in patients with PLE; developing within hours and resolving within days without scarring. Patients with PhAD show an eczematous reaction with erythema, papules, scales, vesiculation, and excoriations upon provocation. This reaction takes longer (days) to develop and the lesions have a more persistent character.[5]

This study was approved by the medical ethical committee of the University Medical Centre Utrecht and the participants gave written informed consent. Photosensitivity was determined by means of Phototesting. The protocol and patient disease definition was reported earlier. [5]

Exclusion criteria were the use of systemic immunosuppressive drugs, UV-hardening, other forms of phototherapy or exposure to direct sunlight or tanning bed exposure at the location of the biopsy site within two months prior to this study, co-existence of possible light exacerbating disease other than AD or an immunologic disorder. The test region had to be clear of topical immunosuppressive drugs for a minimal period of 2 weeks before inclusion. The overall AD severity was assessed using the Investigator's Global Assessment (IGA) classification: 0 = clear; 1= almost clear; 2 = mild disease; 3 = moderate disease; 4 = severe disease; 5 = very severe disease.[26]

Minimal erythemal dose (MED), irradiation and biopsies

The MED is defined as the dose of UV necessary to induce a just perceptible erythema with no marked borders. Determination of MED and UV irradiation was done with 4 Philips TL12W/12 tubes with a continuous emission spectrum (280-360 nm) with a maximum around 305 nm. They were used at an intensity of 1.5m W/ cm^2.

The MED for UVB was determined with a specially designed apparatus that simultaneously exposes a series of 9 areas of skin of 3 x 10 mm to increasing doses. These doses are geometrically progressive with increments of 41% between

successive skin fields. Irradiation was given in the morning and assessment was done after 8 and 24 hours by an experienced observer. The normal MED for UVB is about 50 -70 mJ cm-2 (Philips 20W/TL12), typical for Fitzpatrick skin type II-III.

In all patients a circular area with a diameter of 5 cm of non-lesional buttock skin was irradiated with 3 times the MED for UVB (TL012). Subsequently, 4 mm punch biopsies were taken from this area at t= 0 (directly following exposure), 6 and 24 hours (using 2% xylocaïne with adrenalin as local anaesthetic). One control biopsy was taken from non-irradiated, non-lesional skin (next to the irradiated area, t=C). Immediately after the biopsy was taken the material was snap frozen in liquid nitrogen, embedded in OCT compound (Tissue-Tek; Sakura, Zoeterwoude, the Netherlands), snap frozen again and stored at -80°C until use. None of the patients developed a pathological skin reaction after a single exposure with three times the MED for UVB. Observations were made by an experienced observer and all tests were carried out in a special temperature-controlled phototesting room.

Reagents and antibodies

For immunohistochemical staining, monoclonal antibodies against elastase (clone NP57, DAKO A/S, Glostrup, Denmark, diluted 1:40), CD1a (DAKO A/S, Glostrup, Denmark, diluted 1:50), AA-1 (DAKO A/S, Glostrup, Denmark, diluted 1:100), CD3 (DAKO A/S, Glostrup, Denmark, diluted 1:50) and EG-2 (Pharmacia & Upjohn Diagnostic AB, Uppsala, Sweden, diluted 1:25) were used. For the staining of filaggrin a polyclonal antibody was used (Rabbit polyclonal IgG antibody, ab24584, Abcam, Cambridge, UK, diluted 1:500). Biotinylated horse anti-mouse immunoglobulin (Vector, Burlingame, California, diluted 1:800) or biotinylated goat anti-rabbit (Vector, Burlingame, California, diluted 1:300) were used as a secondary antibody. Levamisol, naphthol AS-BI/AS-MX phosphate and 3-amino-ethyl-carbazole (AEC) were all purchased from Sigma (St Louis, Missouri).

Immunohistochemistry

Frozen sections (6 micrometer) of the skin biopsies on 3-amino-propyltriethoxysilane-couted glass slides were used for immunohistochemical staining. The slides were fixed for 10 minutes in dry acetone at room temperature and incubated for 20 minutes in a 10% blocking reagent (PBS containing 10% normal human serum and 10% normal horse or goat serum) to prevent non-specific binding. The skin sections were incubated at room temperature for 1 hour with

the primary antibody, diluted in 1% blocking reagent (PBS containing 1% normal human serum and 1% normal horse or goat serum). The glass slides were washed with PBS containing 0.05% Tween 20. Subsequently, skin sections were incubated for 45 minutes with a biotinylated secondary antibody followed by incubation with alkaline phosphate-labelled streptavidine for 45 minutes.
Antibody binding was visualized by horseradish peroxidase-conjugated avidin–biotin complex (DAKO A/S, diluted 1:50) and stained with AEC. The skin sections were counterstained with Mayer's Haematoxylin.

Quantification of staining

The skin sections were evaluated using a light microscope at x 400 magnification with a standard eye-piece. Neutrophils, T cells, mast cells and eosinophils were quantified by counting the absolute number of cells per mm2 in the dermis. Epidermal dendritic cells and filaggrin were quantified by semi-quantitative analysis in skin sections. Presence of CD1+ epidermal dendritic cells was graded as: no or hardly any positive cells present (-), presence of scattered positive cells (±), clear abundant presence of cells (+), close maze of positive cells (++).15 The intensity of the filaggrin immunostaining was graded on a scale from 0 to 5, with 0 indicating no staining and 5 the most intense staining.[20]

Statistical analysis

Data were analysed and illustrated using GraphPad Prism v.5 (GraphPad Software Inc., San Diego, CA). Data were expressed as mean ± SEM. Differences between the three groups were assessed with the one-way ANOVA analysis with Kruskal-Wallis post test. $P < 0.05$ was considered significant.

Results

Patients

Patient characteristics are shown in Table I. No significant differences concerning sex, age, disease activity or MED between the groups were demonstrated.

MED and single exposure of UVB

The mean MED for UVB for the 8 patients with AD was 77 mJ cm-2, for PhAD 80 mJ cm-2 and for AD + PLE 85 mJ cm-2, normal for AD.5 None of the patients showed a diminished MED.

Table 1. Patient characteristics.

Diagnosis	n	age	male/female	MED (mJ $cm^{-2)}$
AD	8	46	4/4	77
PhAD	8	47	5/3	80
AD + PLE	8	48	4/4	85

Immunohistochemical results

No neutrophils, visualized by their elastase content, were present in un-irradiated, non-lesional buttock skin (t=C) in any patient group. UVB irradiation induced a significant dermal neutrophil infiltration of the skin in all three groups at t=6 and t=24 hours. There were no significant differences in neutrophil influx numbers between PhAD, AD+PLE or AD patients without photosensitivity within 24 hours after UVB exposure (P>0.05, figure 1). A strong variation of dermal neutrophil infiltration numbers was noted at t=24 in all three groups.

UVB irradiation caused a decrease in the epidermal CD1a+ dendritic cells after 24 hours in all groups (figure 2). There was no significant difference in numbers between the three groups at either t=C or t=24.
No eosinophils were present in non-lesional skin before UV exposure (t=C). Only a few eosinophils infiltrated the skin at 24 hours after UV exposure (figure 3). Furthermore, a small increase in existing CD3+ T cell numbers was observed in all patient groups at t= 6 and t=24 hours (figure 4).

The average number of mast cells at t=C was normal in all three groups compared to healthy controls.27 There was a significant decrease in AA1+ mast cells directly following UV exposure in all three groups at t=0 and at t=6 (figure 5).

Filaggrin staining intensity at t=C did not differ between the AD groups and was in the same range as in healthy controls. Furthermore filaggrin staining intensity did not change following UV exposure (figures 6 and 7).

Figure 1. Significant increase in the number of elastase positive neutrophils in the dermis in time after 3x MED UVB. Mean number of cells per mm^2 ± SEM. No significant statistical difference between the three patient groups.

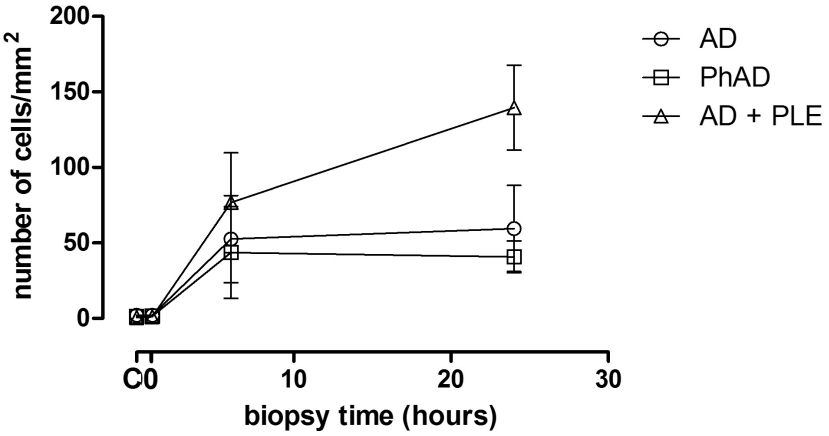


Figure 2. Presence of CD1+ epidermal dendritic cells at t=C and t= 24 hours after irradiation with 3x MED UVB, graded as: no or hardly any positive cells present (-), presence of scattered positive cells (±), clear abundant presence of cells (+), close maze of positive cells (++).

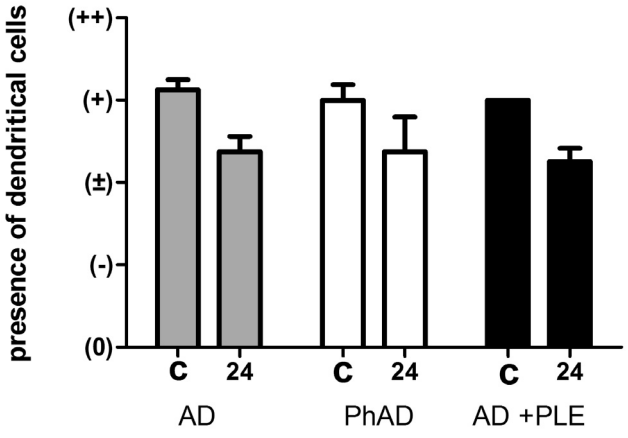


Figure 3. EG-2 positive eosinophil numbers in time following 3x MED UVB. Mean number of cells per mm^2 ± SEM.

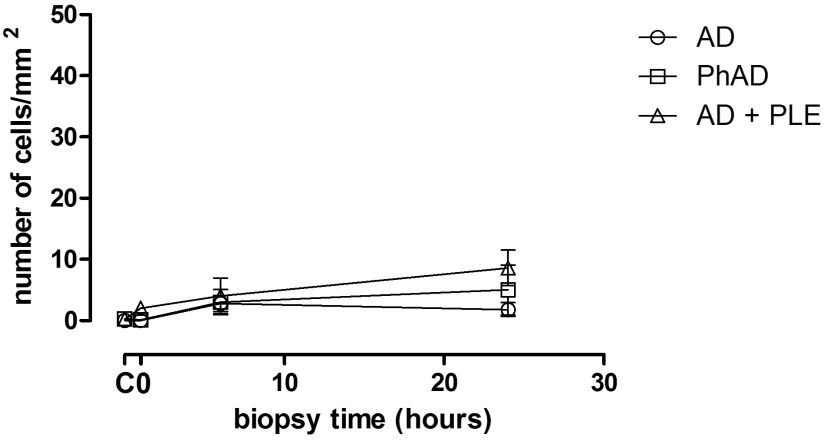


Figure 4. Moderate increase of CD3 positive T-cell numbers in time after 3x MED UVB. Mean number of cells per mm^2 ± SEM.

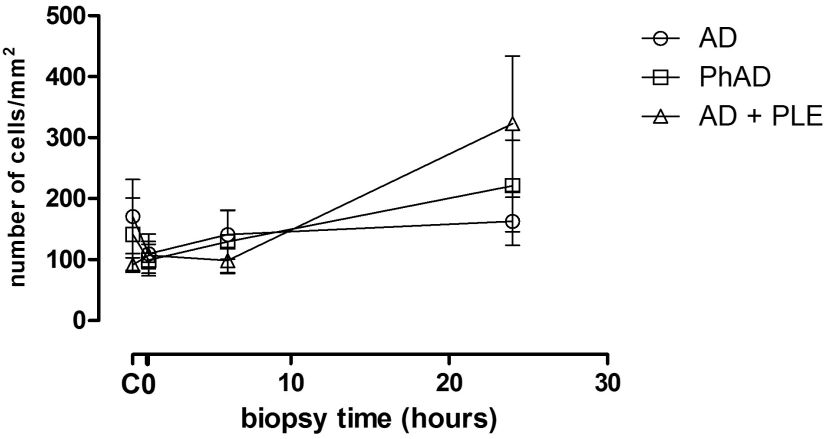


Figure 5. Significant decrease in the number of AA-1 positive mast cells in the dermis after 3x MED UVB. Mean number of cells per mm^2 ± SEM. No significant statistical difference between the three patient groups.

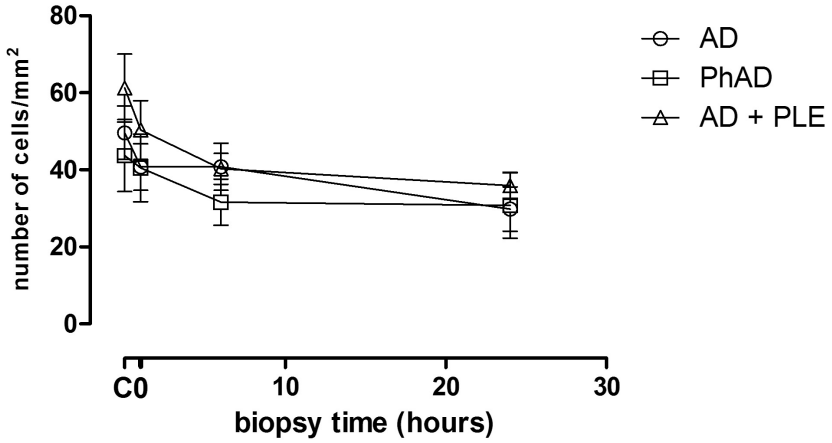


Figure 6. Filaggrin immunostaining intensity: graded on a scale from 0 to 5, with 0 indicating no staining and 5 the most intense staining, ± SEM.

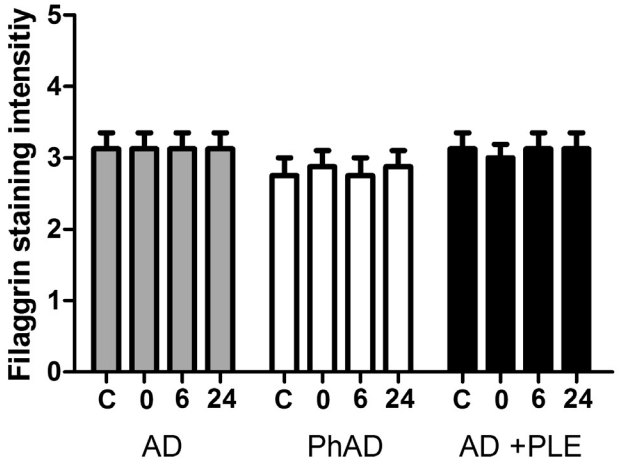
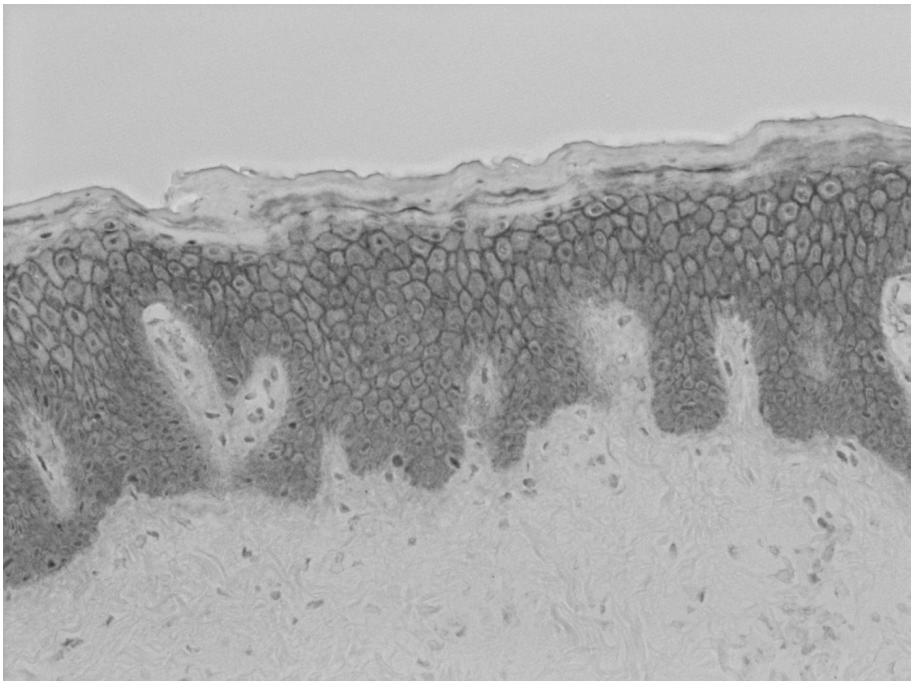


Figure 7. Filaggrin immunostaining intensity example of AD skin.



Discussion

Irradiating non-lesional skin of AD patients with and without photosensitivity with 3x MED of UVB (TL12) demonstrated an influx of neutrophils in the dermis and a decrease of dendritical cells in the epidermis 24 hours after exposure. Hardly any eosinophils were observed. There was a rapid decrease in mast cell numbers. There was no mutual divergence between patients with AD, PhAD or AD +PLE.

Although previous research did show a decreased number of neutrophils infiltrating the dermis after UVB exposure patients with PLE,[13] there was no negative correlation between photosensitivity and neutrophil numbers in the AD patients with photosensitivity in our study. All the AD patients (with and without photosensitivity) showed an infiltration of neutrophils, which was in the same range as the numbers observed in healthy controls.[10,13, 28] Moreover, AD + PLE patients even showed a tendency toward even higher number of neutrophils 24 hours after irradiation

compared to patients with either AD or PhAD. This shows that photosensitivity in AD is not due to a decreased number of neutrophils infiltrating the skin and furthermore suggests there is no decreased neutrophil immunosuppression.[13, 29;30]

Kölgen demonstrated a deviant dendritical cell behavior after UVB irradiation (6x MED TL12) in PLE patients in whom there was no decrease in numbers of dendritic cells 24 hours after exposure, as was noted in healthy controls.[15] It was suggested that a defect in this emigration might allow inadvertent immune reactions to develop after ultraviolet (over)exposure. We could not demonstrate a decrease in dendritic cell emigration upon UVB exposure in AD patients with photosensitivity, However we used a 3x MED UVB irradiation, which could have resulted in a milder local effect.

The significant decrease in mast cell numbers directly following irradiation in all three patient groups (fig. 5) is most likely due to the degranulation of mast cells. Degranulation of mast cells in vivo directly following UV(B) exposure has been demonstrated in earlier studies.[12] The degranulation of mast cells in our study was not followed by an influx of eosinophils or by a skin whealing. This in contrast to solar urticaria where degranulation of mast cells is associated with a visible or UVA induced swift dermal eosinophilic infiltration,[31;32] Mast cell degranulation induced by a skin prick test with allergen is also followed by an influx of neutrophils and eosinophils after 6-24 hours.[19, 33] This suggests that UVB induced mast cell degranulation differs from degranulation induced by other light wavelengths or allergens. Interestingly, in recent studies, mast cells were found to possess anti-inflammatory or immunosuppressive potential, producing e.g. IL-10, after UVB exposure.34 IL-10 may limit UVB induced pro-inflammatory signals, and therefore result in mast cell degranulation without clinically noticeable effects after a single UVB irradiation.

In AD an impaired barrier function of the skin is suggested to contribute to its pathogenesis.[20-25] Filaggrin is a key protein in the formation of the cornified cell envelope and is critical for an effective skin barrier.[20-25] Common loss-of-function variants of filaggrin is a predisposing factor for AD.[35] Trans-urocanic acid (trans-UCA) is formed from filaggrin protein breakdown products in the corneal layer. Urocanic acid is a major UV absorbing component of the skin. Upon UV irradiation photo-isomerization from trans- to cis-UCA is induced. We hypothesized that a

decrease in the production of UCA because of filaggrin loss of function mutations might alter UV induced skin dynamics.[36] However there was no difference in filaggrin protein expression between photosensitive and non-photosensitive AD patients at t=C.
Expression does not equal function and is not representative for the amount of UCA present in the skin and unfortunately we did not genotype our patients. It would be interesting to do so and measure UCA values in time after UV irradiation in a filaggrin loss of function mutation AD population compared to healthy controls.

We studied only a single exposure to UVB, which may not have been sufficient to unravel differences in cellular inflammatory reaction patterns in our patient groups. Unfortunately an in vivo UV-summation model with more time point analyses is hard to standardize, since the number of irradiations needed for each individual patient widely varies, making it difficult to compare individual patients.

In conclusion: After a single 3x MED UVB irradiation we could not detect differences in cell kinetics between AD patients with and without photosensitivity. In contrast to PLE there was no correlation between neutrophil skin infiltration and photosensitivity. Future studies regarding photosensitivity (in AD) should not only be focussed on cell types but also on their functionality.

References

1 Millard TP, Hawk JL. Photosensitivity disorders: cause, effect and management. Am J Clin.Dermatol 2002; 3: 239-46.

2 Duthie MS, Kimber I, Norval M. The effects of ultraviolet radiation on the human immune system. Br J Dermatol. 1999; 140: 995-1009.

3 Schornagel IJ. The polymorphous light eruption-severity assessment score does not reliably predict the results of phototesting. J Eur Acad Dermatol Venereol. 2008; 22: 675-80.

4 Majoie IM, Oldhoff JM, van Weelden H et al. Narrowband ultraviolet B and medium-dose ultraviolet A1 are equally effective in the treatment of moderate to severe atopic dermatitis. J.Am.Acad.Dermatol. 2009; 60: 77-84.

5 ten Berge O, van Weelden H, Bruijnzeel-Koomen CA et al. Throwing a light on photosensitivity in atopic dermatitis: a retrospective study. Am.J.Clin.Dermatol. 2009; 10: 119-23.

6 Rajka G. clinical aspects; other essential features; photosensitvity. Essential Aspects of Atopic Dermatitis 2006: 27.

7 Hawk JL. The presence of neutrophils in human cutaneous ultraviolet-B inflammation. Br J Dermatol.1988; 118: 27-30.

8 Kim MS. Acute exposure of human skin to ultraviolet or infrared radiation or heat stimuli increases mast cell numbers and tryptase expression in human skin in vivo. Br J Dermatol. 2009 160: 393-402.

9 Kolgen W. Epidermal langerhans cell depletion after artificial ultraviolet B irradiation of human skin in vivo: apoptosis versus migration. J Invest Dermatol. 2002; 118: 812-7.

10 Lee PL. Neutrophil infiltration in normal human skin after exposure to different ultraviolet radiation sources. Photochem.Photobiol. 2008; 84 :1528-34 .

11 Soter NA. Acute effects of ultraviolet radiation on the skin. Semin Dermatol.1990; 9: 11-5.

12 Walsh LJ. Ultraviolet B irradiation of skin induces mast cell degranulation and release of tumour necrosis factor-alpha. Immunol.Cell Biol. 1995; 73: 226-33

13 Schornagel IJ. Decreased neutrophil skin infiltration after UVB exposure in patients with polymorphous light eruption. J Invest Dermatol. 2004. 123; 202-6.

14 Kolgen W, van MM, Jongsma M et al. Differential expression of cytokines in UV-B-exposed skin of patients with polymorphous light eruption: correlation with Langerhans cell migration and immunosuppression. Arch.Dermatol. 2004; 140: 295-302.

15 Kolgen W. CD11b+ cells and ultraviolet-B-resistant CD1a+ cells in skin of patients with polymorphous light eruption. J Invest Dermatol. 1999; 113: 4-10

16 Fiset PO, Leung DY, Hamid,Q. Immunopathology of atopic dermatitis. J Allergy Clin Immunol. 2006; 118: 287-90.

17 Grewe M. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today.1998; 19: 359-61.

18 Leung DY. Atopic dermatitis. Lancet. 2003; 361: 151-60.

19 Langeveld-Wildschut EG, Thepen T, Bihari IC et al. Evaluation of the atopy patch test and the cutaneous late-phase reaction as relevant models for the study of allergic inflammation in patients with atopic eczema. J Allergy Clin Immunol. 1996; 98: 1019-27.

20 Howell MD. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol. 2007; 120: 150-5 .

21 Irvine AD, McLean WH. Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. J.Invest Dermatol. 2006; 126: 1200-2.

22 Palmer CN. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006; 38: 441-6.

23 Weidinger S. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol. 2006; 118: 214-9.

24 Weidinger S. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J Invest Dermatol. 2007; 127: 724-6.

25 Weidinger S. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol. 2008; 121: 1203-9.

26 Meads DM, McKenna SP, Kahler K. The quality of life of parents of children with atopic dermatitis: interpretation of PIQoL-AD scores. Qual.Life Res. 2005; 14: 2235-45.

27 Janssens AS, Heide R, den Hollander JC et al. Mast cell distribution in normal adult skin. J.Clin.Pathol. 2005; 58: 285-9.

28 Rijken F, Kiekens RC, van den WE et al. Pathophysiology of photoaging of human skin: focus on neutrophils. Photochem.Photobiol.Sci. 2006; 5: 184-9.

29 Terui T, Tagami H. Mediators of inflammation involved in UVB erythema. J.Dermatol.Sci. 2000; 23 Suppl 1: S1-S5.

30 Teunissen MB, Piskin G, di NS et al. Ultraviolet B radiation induces a transient appearance of IL-4+ neutrophils, which support the development of Th2 responses. J.Immunol. 2002; 168: 3732-9.

31 Botto NC, Warshaw EM. Solar urticaria. J.Am.Acad.Dermatol. 2008; 59: 909-20.

32 Norris PG, Murphy GM, Hawk JL et al. A histological study of the evolution of solar urticaria. Arch.Dermatol. 1988; 124: 80-3.

33 Langeveld-Wildschut EG, Bruijnzeel PL, Mudde GC et al. Clinical and immunologic variables in skin of patients with atopic eczema and either positive or negative atopy patch test reactions. J.Allergy Clin. Immunol. 2000; 105: 1008-16.

34 Grimbaldeston MA, Nakae S, Kalesnikoff J et al. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. Nat.Immunol. 2007; 8: 1095-104.

35 Marenholz I, Nickel R, Ruschendorf F et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J.Allergy Clin.Immunol. 2006; 118: 866-71.

36 Kezic S, Kammeyer A, Calkoen F et al. Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. Br.J.Dermatol. 2009; 161: 1098-104.

Assessment of cyclobutane pyrimidine dimers by digital photography in human skin

Onno ten Berge, Sara G.A. van Velsen, Barbara Giovannone, Carla A. F. M. Bruijnzeel-Koomen, Edward F. Knol, Kees Guikers, Huib van Weelden

University Medical Centre Utrecht, Department of Dermatology & Allergology, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands

Poster Nederlandse vereniging voor experimentele dermatologie 2009.
Submitted 2010

Abstract

UV-mediated DNA damage and repair are important mechanisms in research on UV-induced carcinogenesis. UV-induced DNA-damage and repair can be determined by immunohistochemical staining of photoproduct positive nuclei of keratinocytes in the epidermis. We developed a new method of analysing and quantifying thymine dimer (TT-CPD) positive cells in the epidermis.
Normal skin of healthy controls was exposed to UVB *ex vivo* and *in vivo*. Skin samples were immunohistochemically stained for TT-CPDs. Digital images of the epidermis were quantified for TT-CPDs both visually and digitally.
There was a UVB-dose dependent induction of TT-CPDs present in the ex vivo UVB-irradiated skin samples. The linear measurement range of the digital quantification was increased compared to the manual counting. The average 24-hour repair rate of the initiated TT-CPDs elicited by the UVB irradiation at T=0 of the 8 HCs showed a 34 % decrease of TT-CPD photoproducts by the manual counting method and a 51 % decrease determined by digital counting.
The digital quantification method improves immunohistochemical quantification of DNA photo damage. It is more sensitive in measuring the extent of DNA-damage per nucleus.

Introduction

Ultraviolet irradiation (UV) is a major factor in the development of skin cancer. Some ultraviolet radiations are directly absorbed by DNA and induce dimers between adjacent pyrimidine derivatives; DNA-photoproducts. UVB-induced bipyrimidine photoproducts include: cyclobutane pyrimidine dimers (CPDs) and pyrimidine 6-4 photoproducts (6-4PP's) and their Dewar valence isomers.(de Gruijl, 1998;Douki, 2001;Matsumura, 2004) CPDs and 6-4 PPs are induced by UV in a dose dependent manner.(Berg, 1995;Katiyar, 2000;Mouret, 2006) CPD is the predominantly induced photoproduct.(Yoon, 2000; Cooke,2003) Bipyrimidine photoproducts are preferentially produced at thymine-thymine (TT) and thymine-cytosine (TC) sites whereas lower amounts are formed at CT and CC sites.(Mouret, 2008a) Thymine dimers (TT-CPDs) are the major photoproduct upon UVB exposure. (Douki,2001)

Quantitative assessment of photoproducts in irradiated epidermis at different points in time reflects both the amount of absorbed radiations and the rate of dimer removal as a result of DNA repair, apoptosis, cell cycle delay or other biological mechanisms.(Bykov, 1999) Deficiencies in, or ineffectiveness of the DNA repair mechanisms can lead to the formation of mutations over time and increase the risk of developing skin cancer.(de Gruijl, 1999;Matsumura, 2002) The quantification of UV induced DNA damage and its repair is therefore important in research on carcinogenesis.

Other methods to quantify photoproducts in human skin are DNA extraction analysis and immunohistochemical (IHC) analysis.
There are some advantages to using IHC quantification compared to DNA extraction. Firstly, histology shows in which skin compartment DNA damage has occurred, to what depth cells are affected and which subpopulations of cells are damaged. (Chadwick, 1995) This allows measurement of the DNA damage specifically per compartment or even per nucleus. Secondly, in contrast to DNA extraction analysis, IHC can analyse different targets within the same material not only proteins but also mRNA.

Manual counting of photoproduct positive IHC-stained nuclei in the epidermis is a frequently used method for quantification of DNA repair.(Al, 2002;Caproni, 2007;de,

2004;Ling, 2001;Nijhof, 2007;van Praag, 1993;Yamada, 2006) However, manual quantification is semi-quantitative, relatively time consuming and at risk of inter-observer variation.(Lejeune, 2008) The specific and precise quantification of stained cells is, in manual assessment, subject to evaluation by the human eye, which has difficulties in distinguishing subtle differences in staining intensity, especially at the extremes of the continuous colour scale.(Yamada, 2006) Moreover, differentiation in the number of photoproducts in a single positive nucleus cannot be made since the intensity of the staining per cell cannot be quantified. This implicates that the amount of photoproducts in one nucleus can be large directly after UV radiation and decreased after DNA repair, but the cell will still be considered positive in both states when counted visually.
We developed a new method of analysing and quantifying photoproduct positive cells in the epidermis.

Materials and methods

Ex vivo human skin

Two human skin sections (healthy females, 23 and 35 years old, Fitzpatrick skin type II) were obtained directly (<10 min) following elective reduction surgery (abdominal and mammary reduction skin). 7 skin fields were exposed to UVB radiation with gradually increasing doses of respectively 3, 6, 12, 25, 50, 100 and 200 mJ/cm^2 at 37°C. 4 mm punch biopsies were taken from each field directly after irradiation. Biopsies were snap frozen in liquid nitrogen and then stored at -80 ºC until further analysis. Biopsies were taken from an unexposed site as a control.

Healthy controls

In 8 healthy volunteers (HVs) the minimal erythema dose (MED) for UVB was determined in un-tanned skin from the buttock region. An area of 3x5 cm was exposed to 2 x the MED of broadband UVB (Philips 20W/TL12).

Directly following UV irradiation (<10 min, T=0), and 24 hours after irradiation (T=24) a 4 mm punch skin biopsy was taken from the irradiated skin field after local anaesthesia with lidocaine-epinephrine. A reference biopsy (un-irradiated skin) was taken next to the site of exposure. Biopsies were snap frozen in liquid nitrogen and stored at -80 ºC until further analysis.

Biopsy time points directly following exposure and 24 hours later were chosen according to prior observations, in which a maximum of photoproducts directly following exposure and after 24 hours a reduction of up to 98% of *in vivo* CPDs in the epidermis was demonstrated.(D'Ambrosio, 1981a)

This study was approved by the medical ethical committee of the University Medical Centre Utrecht and the participants gave written informed consent.

MED procedure

The MED for UVB in the HVs was determined with a specially designed apparatus that simultaneously exposes a series of 9 areas of skin of 3 x 10 mm to increasing UV doses. These doses are geometrically progressive with increments of 41% between successive skin fields. Irradiation was given in the morning and assessment was done after 8 and 24 hours by an experienced observer. The MED is defined as the UV dose necessary to induce a just perceptible erythema with no marked borders. The average normal MED for UVB is about 50-70 mJ cm^{-2} (based on a series of healthy volunteers with Fitzpatrick sun reactive skin types II and III (Philips 20W/TL12)(Fitzpatrick, 1988;ten Berge et al., 2009).

Radiation sources

The UVB source used consists of 4 Philips TL20W/12 tubes with a continuous emission spectrum (280-360 nm) with a maximum around 305 nm.
This light source was used for both determination of the MED and exposure of the skin. Dosimetry was carried out at a flow rate of 0.5 mW cm^{-2}. Doses of light were measured in mW cm^{-2} with a Waldmann UV detector device (Waldmann, Schwenningen, Germany). It was calibrated with the spectro-radiometer, model 752, Optronic laboratories Inc., Orlando USA. The light source also emits wavelengths below 280 and above 315 nm. However 98 % of the effective irradiation lies within the UVB range.

Immuno-staining for thymine dimers

To detect UV-induced DNA damage, IHC-staining of the most prevalent UVB photoproduct, TT-CPD, was performed. 7 µm-thick frozen skin sections were thawed and fixed in acetone for 10 minutes and air dried for two minutes. Slides were then kept in freshly prepared 70mM NaOH in 70% ethanol for 2 minutes to denature

DNA. Slides were washed three times in phosphate-buffered saline (PBS) with 0.05 % Tween for three minutes each.

After washing, the slides were incubated for 10 minutes in 10% rabbit serum in PBS to prevent non-specific antibody binding. Sections were subsequently incubated with thymine dimer-specific monoclonal antibody (Kamiya Biomedical Company Seattle, WA; clone KTM53, IgG, diluted 1:4000) in PBS containing 1% rabbit serum for 1 hour and subsequently washed three times in PBS with 0.05 % tween for three minutes each.

Bound anti-thymine dimer antibody was detected by incubation for one hour with alkaline phosphatase conjugated rabbit anti-mouse IgG1 with (Dako A/S; diluted 1:50) and washed three times in PBS with 0.05 % tween for five minutes each. Slides were developed with fuchsine (fuchsine substrate-chromogen K0624, DAKO A/S) as a substrate for 10 minutes. All sections were then rinsed with water. Slides were mounted using Imsol® and covered with a cover glass using Entellan®. Optimal staining was defined as a clear signal without background staining. No counter stain was used.
All specimens were stained in one batch in order to prevent possible variations between staining sessions.

Assessment of cyclobutane pyrimidine dimers by digital photography

Digital bright field images were obtained using a Zeiss Axioskop 40 light microscope with a 1.4 mega pixel Axiocam ICc 1 camera (Zeiss, Goettingen Germany). Images were made at a 400x magnification (Carl Zeiss W-PI 10x/23 + Zeiss EC plan - Neofluor 40x/0.75 - ∞/0.17). After allowing the lamp to stabilize and adjusting the illumination intensity to a level where the histogram is not saturated, a control image of a blank field was captured. The blank field image was used to correct for unequal illumination of the stained specimens. This correction was achieved by dividing the stained image by the blank field image and multiplying the resulting image with the mean density of the blank field image. The final image has a homogenous illumination and a neutral background. No further processing was performed before image analysis. This illumination intensity range of the microscope was fixed for the complete series of images. Images were captured with Zeiss Axiovision software (release 4.6.3) at basic adjustments. Digitized images

have a resolution of 1392x1038 pixels with 24 bit RGB colour format and were saved in uncompressed tagged image file format (TIFF).

The complete epidermis was photographed at three different sites of the biopsy per time point (>30 µm apart). The mean integrated red-intensity of the positive nuclei was measured. Since the extent of antibody binding corresponds to the mean UV-induced- DNA-damage, the plug-in was designed to measure the mean integrated red-intensity of (positive) nuclei. The mean integrated red-intensity was measured in a selection of the epidermis and expressed as the mean integrated redness per unit area.

The automatic thresholding algorithms, incorporated in most imaging software programs, are not suitable for the separation of red coloured areas from background staining, due to interference by melanocytes, keratin and other structures. Therefore the captured 24-bit bright field images were composed of three 8-bit monochromatic channels (red, green and blue (RGB)). A color in the RGB color model is described by indicating how much of each of the red, green, and blue is included in a pixel. In computing, the component values are often stored as integer numbers in the range 0 to 255, the range that a single 8-bit byte can offer. Red areas in the skin sections are characterized by a significant drop in the green channel values and a much less pronounced or even virtually absent decrease of the red channel values (Fig. 1).
By this approach we are able to ignore reddish-appearing melanocytes (Fig. 1). The ratio of the decline in the red and green channel values per pixel (Q) is defined as

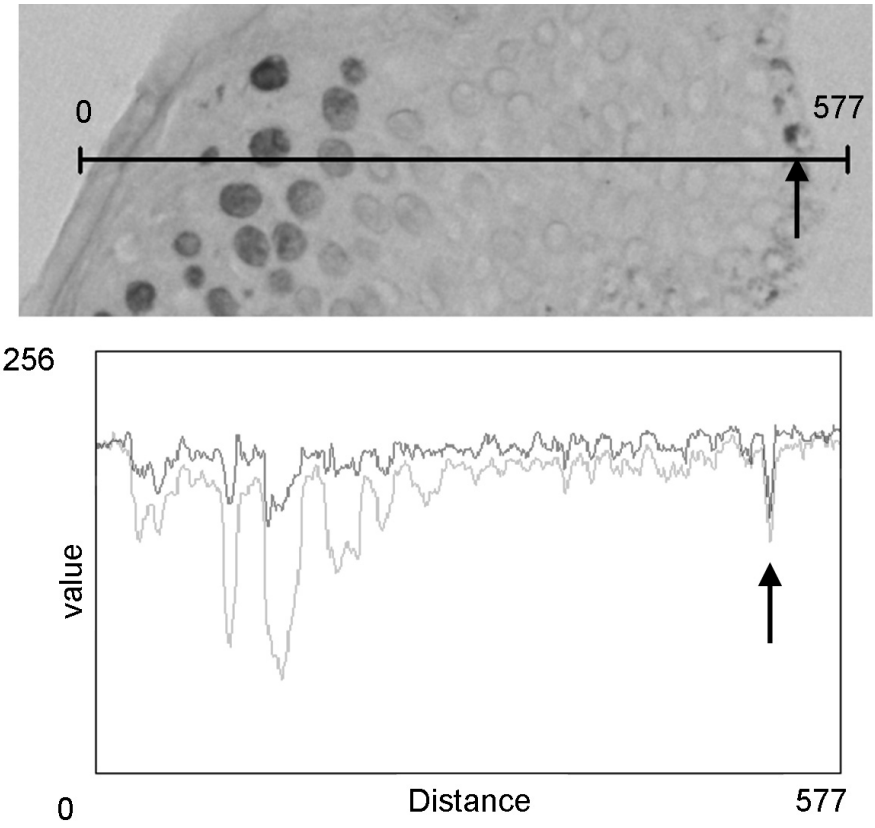
$$Q = \frac{255 - green}{255 - red}$$

The red intensity for each pixel (R) is defined as

$$R = (255 - green) - (255 - red) = red - green$$

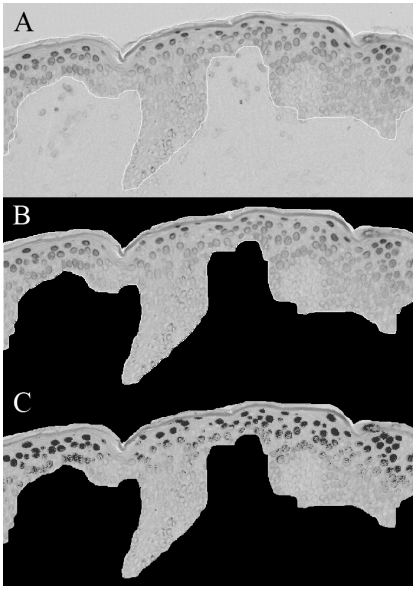
A threshold (Th) for Q is chosen interactively and every pixel for which Q > Th is set to monochromatic blue in a duplicate picture. By comparing the original and duplicate pictures an optimal separation between red pixels (relevant pixels) and background staining can be achieved by adjusting the value of Q. Once this

Figure 1. Close up of a section of an image of the UVB irradiated epidermis (above) measured across the black line (0-577) with a Red and Green value profiler in ImageJ. Red areas in the skin sections are characterized by a significant drop in the green channel values and a much less pronounced or even virtually absent lowering of the red channel values (diagram below). Note the decrease in both red and green values when measuring the melanin layer of the skin (indicated by ↑).



optimal separation has been achieved, the red intensity (R) is integrated over all relevant pixels in the selected area and this sum is divided by the total number of pixels in the selection, giving the redness per unit area. The stepped procedure is shown in Figure 2.

Figure 2. Digital quantification process of TT-CPDs in UVB irradiated skin (T=0L): selection of the region of interest (A), clearing the outside (B) and measuring the red intensity is integrated over all relevant pixels in the selected area (C); this sum is divided by the total number of pixels in the selection, giving the redness per unit area.



The plug-in which automates these procedures was written in Java and compiled in ImageJ, version 1.37c, an image analysis program freely downloadable from the website of the National institutes of health of the U.S. Department of health and Human Services. (http://www.nih.gov/)

Manual quantification of TT-CPDs

The number of TT-CPD positive nuclei was counted in the epidermis in the same images used for digital analysis. Positive nuclei were tagged and counted using the particle analysis cell counter available in the imageJ software. The surface area of the epidermis was measured by area selection and the measure function in imageJ and converted to square millimetre. In this manner thymine dimers were quantified by counting the absolute number of thymine dimer positive nuclei per unit area (in the epidermis).

Inter- and intra- observer variation

A random selection of 60 images was analysed by three different independent observers both manually and digitally to assess the inter- and intra- observer bias between these two methods.

Statistical analysis

Linearity of the UVB dose dependence induction of TT-CPDs was determined by linear regression analysis with a 95% confidence interval. Differences in 24-hour repair rates were analysed using a paired two tailed Wilcoxon signed rank test. Differences in inter and intra observer quantification between the two methods was analysed by comparing the coefficients of variation with a 95% confidence interval after bootstrapping (x2000). Statistics were calculated with SPSS 15.0 and illustrated using GraphPad Prism v.5 (GraphPad Software Inc., San Diego, CA).

Results

Measurement of UVB Dose dependent DNA damage

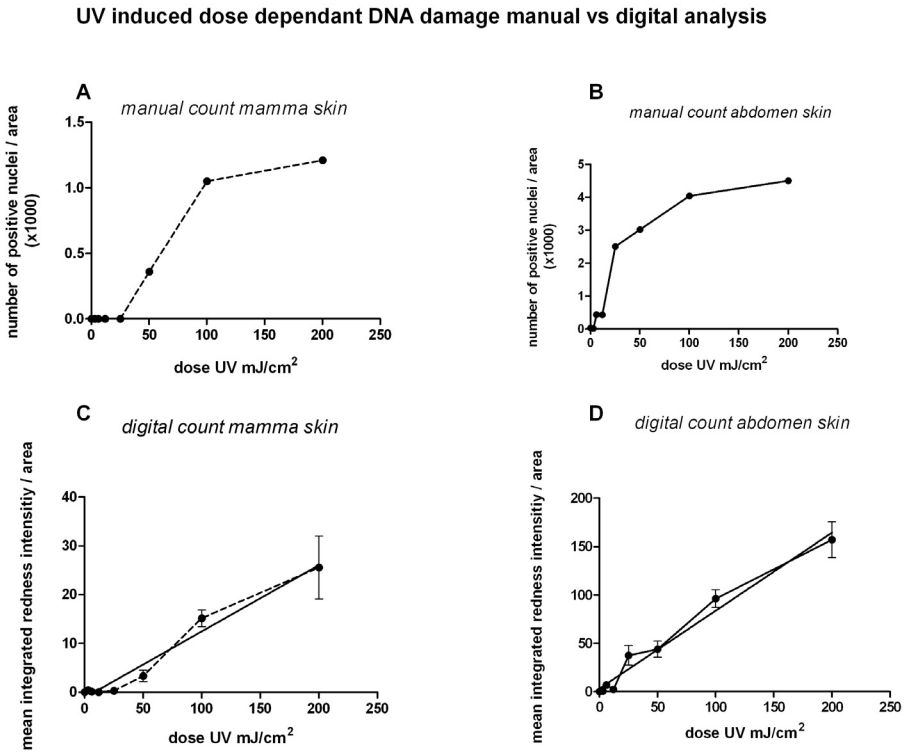
UVB exposure caused a dose-dependent induction of TT-CPD in *ex vivo* irradiated human skin (Fig. 3). The manual counting of TT-CPD positive nuclei (Fig. 3a and b) resulted in a dose dependent increase until the upper limit of the number of countable positive nuclei reached about 50-100 mJ cm^{-2} (also demonstrating inter-individual differences) . The digital quantification assessment (Fig. 3c and d) showed a linear regression (r^2 =0.96 and 0.97) up to 200 mJ cm^{-2}.

The major difference is that the manual analysis did saturate, indicating that the human eye has its limitations that can be overcome by machines, demonstrating an increased linear measurement range of the digital quantification.
The complete epidermis was photographed and analysed at three different sites on the biopsy per time point.

UVB-induced DNA damage repair measurement

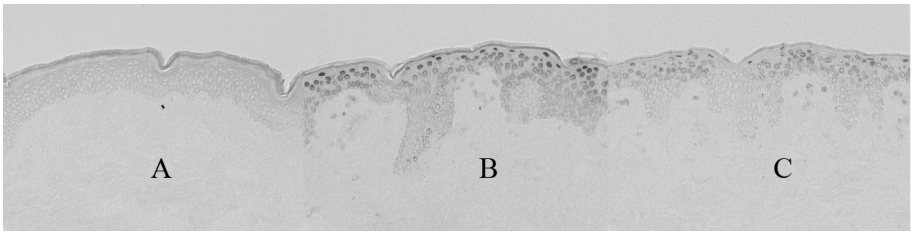
Eight HVs were included in this study (7 male and 1 female, average age 41 years, range 23-62 years) with Fitzpatrick skin types II-III. The average MED was 85 mJ cm^{-2} (range 51-145 mJ cm^{-2}; buttock skin).

Figure 3. Dose-dependence of TT-CPD induction by UVB in *ex vivo* skin: manual count (A and B) and digital quantification assessment (C and D) in breast and abdomen skin. The digital quantification assessment demonstrated a linear regression in both skins (Mamma skin r^2 =0.96 / abdomen skin r^2 =0.97).



An average of 19 images per time point after UVB exposure was analysed. As an example the damage and repair images of the fuchsine-stained skin sections are shown in Fig. 4. No TT-CPDs were detected in non-UVB-exposed skin sites (Fig. 4a). A single dose of 2 MED UVB resulted in a strong positive nuclear staining indicating the presence of TT-CPDs (Fig. 4b). After 24 hours part of the initial damage had been excised and repaired; part of it was still present (Fig. 4c).
The average 24-hour repair rate of the initiated TT-CPDs elicited by the UVB irradiation at T=0 of the 8 HVs showed a 34.8% (±SEM 6.4) decrease in the number of TT-CPD positive nuclei determined by manual counting and 51.0% (±SEM 6.1)

Figure 4. Visualization of TT-CPDs in human skin by Immunohistochemical microscopy without UVB irradiation (A) directly following 2 MED UVB (B) and 24 hours after exposure (C).



decrease in mean integrated red-intensity determined by digital counting. The digital quantification method resulted in a significantly higher 24-hour repair rate compared to manual quantification ($p < 0.0156$, Wilcoxon rank test). Results are shown in Fig. 5, Individual characteristics of the HVs are shown in table 1.

No significant differences in inter- or intra-observer bias between these two methods could be demonstrated (coefficient of variations test with bootstrapping (2000x), $p > 0.1$). Variations in outcome were mainly caused by the material itself rather than by the observers (mean square 0.692 vs. 10.698, ANOVA test).

Based on these 8 HVs no correlation with the initial amount of damage at T=0 or the extent of repair could be found (data not shown). In this small series we were unable to demonstrate any age-repair correlation, either for the initial amount of TT-CPDs induced or for their 24-hour repair rate.

Discussion

This study introduces a digital quantification IHC method, specifically designed for the analysis of UV-induced DNA damage and repair dynamics in human skin.
The digital quantification method improves IHC quantification of DNA photo damage because it increases the linear measurement range compared to manual quantification. Furthermore, the digital quantification method has proven to be less time consuming. The software was designed in such a way as to select the stained signal and neglect false positively stained textures such as melanin-containing cells.

Figure 5. 24 hour repair rates: manual counting of UVB induced TT-CPD positive nuclei/area (A) vs. quantitative digital analysis of mean integrated redness intensity/area (B). There is a significant difference in remaining TT-CPDs at 24 hours (*); Wilcoxon rank test $P < 0.0156$. The results are expressed in percentage of residual TT-CPDs and are the mean ± SEM of the 8 different donors.

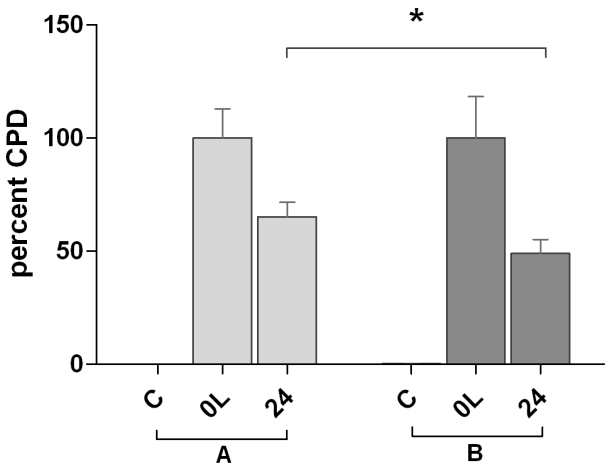


Table 1. Individual HC characteristics: age, MED and percentage TT-CPD repair 24 hours after UVB irradiation.

HCs	Age (years)	MED (mJ cm^{-2})	TT-CPD repair (% manual)	TT-CPD repair (% digital)
1	23	51	28	46
2	31	51	2	21
3	35	51	52	79
4	38	73	43	61
5	40	102	47	58
6	46	145	15	48
7	48	73	45	57
8	62	102	47	37
Mean	40	81	35	51

Dose-dependent induction of TT-CPDs in human skin is a well demonstrated phenomenon.(Cooke, 2003;Potten, 1993) The digital quantification method showed a linear correlation to the point where all TT-CPDs are positively stained in the epidermis. This results in an increased linear measurement range. Manual counting also shows a linear correlation, but the linear measurement range of TT-CPDs is restricted to a maximum point at which all keratinocytes are stained positive.

The TT-CPD repair rates in human skin 24 hours after UVB irradiation have been described to range from no repair at all to 98%.(D'Ambrosio, 1981b;Doelker, 2006;Mouret, 2008b;Segerback, 2008;Xu, 2000) The explanation of this strong variation lies in the fact that different UV sources, different skin types, different staining methods and different quantification methods have been applied.
In our study the average 24-hour repair rate of the 8 HVs was 34 % by the manual counting method and 51 % by the digital counting method. The significant difference in repair rate in this analysis is clearly due to the increased linear range of the digital analysis method.
Interpersonal variation in DNA repair rates is reported in earlier literature. and may be explained by individual predisposition to form and repair TT-CPDs. (Sutherland, 2002) This may explain the differences found within our HVs.

Although previously suggested in 12 healthy controls,(Yamada,200) we could not observe an age dependency in the DNA repair rates in this series of patients. The lack of age dependency was also shown in a larger series of patients by Xu et al.(2000) (n=30). Previously, Potten et al.(1993) reported a method for the digital analysis of UVB-induced DNA damage on paraffin–embedded human skin sections. Both studies quantified DNA damage based on the integrated optical density. We however decided to avoid staining enhancement by an avidin-biotin complex but rather to quantify TT-CPDs more directly.

Solar radiation is recognized as the most important environmental factor involved in the pathogenesis of skin cancers by producing DNA photoproducts. It is therefore important to evaluate determinants interfering of DNA damage and interference in its repair mechanisms.

Digital quantification offers an efficient and sensitive method to measure IHC stained DNA damage of the epidermis. This method is applicable for the analyses of

disorders that are associated with anomalous UV mediated DNA induction or repair and for the analysis of treatment interfering with DNA repair of the skin.

References

1 Al M M, Yaar M, Phillips T J, Bhawan J, Gilchrest B A. Effect of sunscreen application on UV-induced thymine dimers. Arch Dermatol 2002: 138: 1480-1485.

2 Berg R J, de Bueger S C, Guikers,K, van Weelden H, van Vloten W A, van der Leun J C, de Gruijl F R. Induction and disappearance of thymine dimers in human skin exposed to UVB radiation: flow cytometric measurements in replicating and nonreplicating epidermal cells. Photochem Photobiol 1995: 62: 970-975.

3 Bykov V J, Sheehan J M, Hemminki K, Young A R. In situ repair of cyclobutane pyrimidine dimers and 6-4 photoproducts in human skin exposed to solar simulating radiation. J Invest Dermatol 1999: 112 : 326-331.

4 Caproni M, Torchia D, Antiga E, Terranova M, Volpi,W, Del,Bianco E, D'Agata A, Fabbri P. The comparative effects of tacrolimus and hydrocortisone in adult atopic dermatitis: an immunohistochemical study. Br J Dermatol 2007: 156: 312-319.

5 Chadwick C A Potten C S, Nikaido O, Matsunaga T, Proby C, Young A R, The detection of cyclobutane thymine dimers, (6-4) photolesions and the Dewar photoisomers in sections of UV-irradiated human skin using specific antibodies, and the demonstration of depth penetration effects. J Photochem Photobiol B 1995: 28: 163-170Cooke M S, Podmore I D, Mistry N, Evans M D, Herbert K E, Griffiths H R, Lunec J. Immunochemical detection of UV-induced DNA damage and repair. 2003: J Immunol Methods 280: 125-133.

6 D'Ambrosio S M, Slazinski L, Whetstone J W, Lowney E. Excision repair of UV-induced pyrimidine dimers in human skin in vivo. J Invest Dermatol 1981a: 77: 311-313.

7 D'Ambrosio S M, Whetstone J W, Slazinski L, Lowney E. Photorepair of pyrimidine dimers in human skin in vivo. Photochem Photobiol 1981b: 34: 461-464.

8 de Gruijl F R, Berg R J. In situ molecular dosimetry and tumor risk: UV-induced DNA damage and tumor latency time. Eur J Cancer 1998: 68: 555-560.

9 de Gruijl F R. Skin cancer and solar UV radiation. Eur J Cancer 1999: 35: 2003-2009.

10 de Vries E, Louwman M, Bastiaens M, de Gruijl F, Coebergh J W. Rapid and continuous increases in incidence rates of basal cell carcinoma in the southeast Netherlands since 1973. J Invest Dermatol. 2004: 123: 634-638.

11 Doelker L, Tran C, Gkomouzas A, Grand D, Sorg O, Saurat J H, Lubbe J. Production and clearance of cyclobutane dipyrimidine dimers in UV-irradiated skin pretreated with 1% pimecrolimus or 0.1% triamcinolone acetonide creams in normal and atopic patients. Exp Dermatol 2006: 15: 342-346.

12 Douki T, Cadet J. Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. Biochemistry 2001: 40: 2495-2501.

13 Fitzpatrick T B. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988: 124: 869-871.

14 Katiyar S K, Matsui M S, Mukhtar H. Kinetics of UV light-induced cyclobutane pyrimidine dimers in human skin in vivo: an immunohistochemical analysis of both epidermis and dermis. Photochem Photobiol 2000: 72: 788-793.

15 Lejeune M, Jaen J, Pons L, et al. Quantification of diverse subcellular immunohistochemical markers with clinicobiological relevancies: validation of a new computer-assisted image analysis procedure. J Anat 2008: 212: 868-878.

16 Ling G, Chadwick C A, Berne B, Potten C S, Ponten J, Ponten F. Epidermal p53 response and repair of thymine dimers in human skin after a single dose of ultraviolet radiation: effects of photoprotection. Acta Derm Venereol 2001: 81 : 81-86.

17 Matsumura Y, Ananthaswamy H N. Short-term and long-term cellular and molecular events following UV irradiation of skin: implications for molecular medicine. Expert Rev Mol Med 2002: 1-22.

18 Matsumura Y, Ananthaswamy H N. Toxic effects of ultraviolet radiation on the skin. Toxicol Appl Farmacol 2004: 195: 298-308.

19 Mouret S, Baudouin C, Charveron M, Favier,A, Cadet J, Douki T. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. Proc Natl Acad Sci USA 2006: 103 : 13765-13770.

20 Mouret S, Charveron M, Favier A, Cadet J, Douki T. Differential repair of UVB-induced cyclobutane pyrimidine dimers in cultured human skin cells and whole human skin. DNA Repair 2008: 7 : 704-712.

21 Nijhof J G, van Pelt C, Mulder A A, Mitchell D L, Mullenders L H, de Gruijl F R.. Epidermal stem and progenitor cells in murine epidermis accumulate UV damage despite NER proficiency. Carcinogenesis 2007: 28: 792-800.

22 Potten C S, Chadwick C A, Cohen A J, Nikaido O, Matsunaga T, Schipper N W, Young A R. DNA damage in UV-irradiated human skin in vivo: automated direct measurement by image analysis (thymine dimers) compared with indirect measurement (unscheduled DNA synthesis) and protection by 5-methoxypsoralen. Int J Radiat Biol 1993: 63: 313-324.

23 Segerback D, Strozyk M, Snellman E, Hemminki K. Repair of UV dimers in skin DNA of patients with basal cell carcinoma. Cancer Epidemiol Biomarkers Prev 2008: 17: 2388-2392.

24 Sutherland B M, Hacham H, Bennett P, Sutherland J C, Moran M, Gange R W. Repair of cyclobutyl pyrimidine dimers in human skin: variability among normal humans in nucleotide excision and in photorepair. Photodermatol Photoimmunol Photomed 2002: 18: 109-116.

25 ten Berge O, van Weelden H, Bruijnzeel-Koomen C A, de Bruin-Weller M S, Sigurdsson V. Throwing a light on photosensitivity in atopic dermatitis: a retrospective study. Am J Clin Dermatol 2009: 10: 119-123.

26 van Praag M C, Roza L, Boom B W, Out-Luijting C, Henegouwen J B, Vermeer B J, Mommaas A M. Determination of the photoprotective efficacy of a topical sunscreen against UVB-induced DNA damage in human epidermis. J Photochem Photobiol B 1993: 19 : 129-134.

27 Xu G, Snellman E, Bykov V J, Jansen C T, Hemminki K. Effect of age on the formation and repair of UV photoproducts in human skin in situ. Mutat Res 2000: 459: 195-202.

28 Yamada M, Udono M U, Hori M, Hirose R, Sato S, Mori T, Nikaido O. Aged human skin removes UVB-induced pyrimidine dimers from the epidermis more slowly than younger adult skin in vivo. Arch Dermatol Res. 2006: 297: 294-302.

29 Yoon J H, Lee C S, O'Connor T R, Yasui A, Pfeifer G P. The DNA damage spectrum produced by simulated sunlight. J Mol Biol 2000: 299: 681-693.

Topical tacrolimus in atopic dermatitis: no effect on UV-induced DNA damage repair

Onno ten Berge, Barbara Giovannone, Marjolein de Bruin-Weller, Kees Guikers, Huib van Weelden, Carla Bruijnzeel-Koomen, Edward Knol

University Medical Centre Utrecht, Department of Dermatology & Allergology, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands

Submitted 2010

Summary

Background:
The increased incidence of skin cancers on sun-exposed sites during post-transplant immunosuppression with systemic drugs has raised questions about the potency of topical calcineurin inhibitors to promote UV-mediated carcinogenesis.

Objective:
The current study was performed to evaluate the in vivo effect of topical tacrolimus compared to topical triamcinolone acetonide on the repair of UV-induced Thymine-cyclobutane pyrimidine dimers (TT-CPDs) in atopic dermatitis (AD) skin.

Method:
Eight AD patients were treated with tacrolimus 0.1% ointment (TACR) and eight AD patients were treated with triamcinolone acetonide 0.1% ointment (TCA).
In each patient a treated and a non-treated skin site was exposed to two times the minimal erythema dose of broadband UVB irradiation. Skin biopsies were taken directly after irradiation and at t= 24 hours. Skin sections were immunohistochemically analysed and quantified for TT-CPDs using a digital quantification method.

Result:
There were neither differences in the amount of TT-CPD's directly after UVB irradiation nor in their repair rate 24 hours after UVB irradiation between non-treated and treated skin sites in both patient groups.

Conclusion:
Our *in vivo* data do not show an effect of topical tacrolimus treatment on UVB induced DNA damage repair in the skin of patients with AD.

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disease. Topical corticosteroids are the mainstay of AD treatment. Due to the chronicity of the disease long term use of topical corticosteroids is often necessary. However, long term and extensive use of topical corticosteroids, may lead to local side effects e.g. atrophy, acne, striae telangiectasia and occasionally, pituititary-adrenal axis suppression.
In the last decade a new breed of topical anti-inflammatory drugs entered the therapeutic field, the topical calcineurin inhibitors (CI).The lack of cutaneous atrophy is the main advantage compared to the use of topical corticosteroids. This makes the topical CI's a treatment of choice for atrophy sensitive regions such as the face.
Calcineurin inhibition leads to a reduced translocation of the transcription factor called Nuclear Factor of Activated T-cells (NFAT). This blocks the expression of important genes, such as cytokine genes, resulting in the inhibition of T cell activation.[1]

The increased incidence of non-melanoma skin cancers (NMSC) on sun-exposed sites during post-transplant immunosuppression with systemic CI has raised questions about the photocarcinogenetic potency of topical use.[2] In 2006 the US Food and Drug Administration issued a black box warning on topical CI concerning an increased long-term malignancy risk, especially in combination with UV exposure.
In photocarcinogenesis, apoptosis, differentiation, cell-cycle regulation and DNA repair are the four important cellular events. In particular, ultraviolet- B (UVB) irradiation is highly genotoxic and the thymine-cyclobutane pyrimidine dimer (TT-CPDs) is the major DNA photoproduct upon skin exposure with UVB.[3] These photoproducts are primarily repaired by Nucleotide Excision Repair (NER). Deficiencies in DNA repair mechanisms as well as a delay in repair, can lead to accumulation of cellular DNA damage over time, thereby increasing the risk of developing NMSC.[4-6]

Cutaneous photocarcinogenesis in humans is a stepwise process that, in contrast to mice,[11, 12] progresses over decades before eventually resulting in clinically visible

skin cancer. One way to circumvent this obstacle in carcinogenesis research is to study UV induced DNA damage and its repair.
Previous *in vitro* studies showed a decrease in DNA repair rate and apoptosis in CI treated cultured human keratinocytes and peripheral blood mononuclear cells after UVB irradiation.[7-9] More recently, these data could not be confirmed by others.[10] Animal studies showed that pimecrolimus in combination with simulated solar irradiation and both pimecrolimus and tacrolimus in combination with UVA did not accelerate photocarcinogenesis in hairless mice.[11, 12]
Two *in vivo* studies, one in healthy volunteers and AD patients treated with pimecrolimus, using solar simulated irradiation, the other in tacrolimus-treated healthy volunteers using UVB, could not find an effect on induction or repair of UV induced DNA damage or on P53 expression within the first 24 hours after irradiation.[13, 14] However, differences in radiation sources, CI compounds and study protocols make it difficult to compare these results. Furthermore, topical CI are used on lesional skin, which has different absorption kinetics compared to healthy skin.[15] The current study was therefore performed to evaluate the effect of topical tacrolimus on the repair of TT-CPD's after UVB irradiation in AD skin.

Materials and methods

Patients

Sixteen patients with AD and Fitzpatrick skin type II-III [16, 17] were included in this study. Exclusion criteria were the use of systemic immunosuppressive drugs, UV exposure of the biopsy skin sites within two months prior to this study or known co-morbidity for photodermatoses. The test region had to be clear of topical immunosuppressive drugs for a minimal period of 2 weeks before inclusion. Patients were randomly enrolled in the study protocol. This study was approved by the medical ethical committee of the University Medical Centre Utrecht and the participants gave written informed consent.

Treatment

The AD patients were observer blinded simple randomized included into the study protocol and were either treated with tacrolimus 0.1% ointment (TACR) (Protopic ®, Astellas, Staines, United Kingdom) or with triamcinolone acetonide 0.1% ointment (TCA) (Pharmachemie B.V., Haarlem, the Netherlands). The treatment

was twice a day during two weeks in both groups on a marked lower back or buttock skin region with lesional AD.[18-20] The patients of the TACR group consisted of 4 males and 4 females, with a mean age of 37 years. The AD patients of the TCA group consisted of 6 males and 2 females, with a mean age of 47 years.

UVB irradiation

The minimal erythema dose (MED, described below) for UVB was determined on lesional skin at the end of the two week treatment period. Consecutively, the either TCA or TACR treated skin was exposed to two times the MED of broadband UVB irradiation (20W/TL12, Philips, Eindhoven, the Netherlands) on an area of 3x5 cm. The MED for UVB was also determined on a non-treated non-lesional skin region symmetrically opposing the TCA or TACR treated skin area. This skin region was also exposed to 2 MED UVB as a control.

Directly following UV irradiation (<10 min, t=0), and twenty-four hours after irradiation (t=24) 4 mm punch skin biopsies were taken from the treated and untreated irradiated skin fields after local anesthesia with lidocaine-epinephrine (2% / 1:80.000). A non-irradiated reference biopsy (C) from a treated and non-treated skin area was taken adjacent to the area of UVB exposure. Biopsies were snap frozen in liquid nitrogen and stored at -80 ºC until further analysis. Biopsy time points directly following exposure and 24 hours later were chosen according to prior observations.[14]
The overall AD severity was assessed using the Investigator's Global Assessment (IGA) classification: 0 = clear; 1= almost clear; 2 = mild disease; 3 = moderate disease; 4 = severe disease; 5 = very severe disease. [21]

Irradiation source and minimal erythema dose (MED)

The UVB source used consists of 4 Philips TL20W/12 tubes (Philips, Eindhoven, the Netherlands) with a continuous emission spectrum (280-360 nm) with a maximum around 305 nm. Dosimetry was carried out at a flow rate of 0.5 mW/cm2. Doses of light were measured in mW/cm2 with a Waldmann UV detector device (Waldmann, Schwenningen, Germany). It was calibrated with the spectro-radiometer (model 752, Optronic laboratories Inc., Orlando USA). This light source also emits wavelengths below 280 and above 315 nm; however 98 % of the effective irradiation lies within the UVB range.

The MED is defined as the UV dose necessary to induce a just perceptible erythema with no marked borders. The MED procedure was described earlier.[22]The normal MED for UVB is about 50-70 mJ cm-2 (based on a series of healthy volunteers with Fitzpatrick sun reactive skin type II and III, 20W/TL12)

Immuno-staining for thymine dimers

To detect UV-induced DNA damage, immunohistochemical staining of the most prevalent UVB photoproduct, the thymine cyclobutane pyrimidine dimers (TT-CPD), was performed. 7 µm-thick frozen skin sections were thawed and fixed in acetone for 10 minutes and air dried for two minutes. Slides were then kept in freshly prepared 70mM NaOH in 70% ethanol for 2 minutes to denature the DNA. Slides were washed three times in phosphate-buffered saline (PBS) with 0.05 % Tween for three minutes each.

After washing, the slides were incubated for 10 minutes in 10% rabbit serum in PBS. Sections were subsequently incubated with thymine dimer-specific monoclonal antibody (Kamiya Biomedical Company Seattle, WA; clone KTM53, IgG, diluted 1:4000) in PBS containing 1% rabbit serum for 1 hour and subsequently washed three times in PBS with 0.05 % Tween for three minutes each.

Bound anti-thymine dimer antibody was detected by incubation for one hour with alkaline phosphatase-conjugated rabbit anti-mouse IgG1 (Dako A/S; diluted 1:50) and washed three times in PBS with 0.05 % Tween for five minutes each. Slides were developed with fuchsine (fuchsine substrate-chromogen K0624, DAKO A/S) as a substrate for 10 minutes. All sections were then rinsed with water. Slides were mounted using Imsol® and covered with a cover glass using Entellan®. Optimal staining was defined as a clear signal without background staining. No counter stain was used. All specimens were stained in one batch in order to prevent possible variations between staining sessions.

Digital assessment of cyclobutane pyrimidine dimmers

The digital quantification assessment has been described previously (Chapter 5) In brief, digital bright field images were obtained using a Zeiss Axioskop 40 light microscope with a 1.4 mega pixel Axiocam ICc 1 camera (Zeiss, Goettingen Germany). Images were made at a 400x magnification (Carl Zeiss W-PI 10x/23 + Zeiss EC plan - Neofluor 40x/0.75 - ∞/0.17). Images were captured with Zeiss

Axiovision software (release 4.6.3) at basic adjustments. Digitized images have a resolution of 1392x1038 pixels with 24 bit RGB color format and were saved in uncompressed tagged image file format (TIFF). The complete epidermis was photographed at three different sites of the biopsy per time point. Measurements were made from at least three sections per specimen with each section separated by >30 µm apart.
It was assumed that the extent of antibody binding equals mean UV-mediated DNA-damage. The mean integrated red-intensity of the positive nuclei was measured using a ImageJ based plug-in.[23] The mean integrated red-intensity was measured in a selection of the epidermis and expressed as the mean integrated redness per unit area. The skin slides were coded and analyzed in a blinded manner.

Histometric analysis

For estimation of epidermal thickness, care was taken to cut the sections perpendicular to the surface. Epidermal thickness was determined at regular intervals on the digital images of each sample of each patient of treated and non-treated skin sections at t=C (2x30 observations per patient) with the Zeiss Axiovision software, using the scaling option (the stratum corneum was excluded).

Statistical analysis

Grouped column statistics were calculated with two tailed Wilcoxon signed rank tests. Statistics were calculated and illustrated using GraphPad Prism v.5 (GraphPad Software Inc., San Diego, CA).

Results

Patients

No significant differences concerning age or disease activity between the groups was demonstrated; both scored an average around 3 on the IGA scale (moderate disease). Topical treatment with both TCA and TACR resulted in a complete clinical remission of AD activity at the treated skin sites.

MED and single exposure of UVB

The mean MED for UVB in the patients within the TCA group was for non-treated, non-lesional skin 65.6 mJ/cm2 ±SEM 4.9 and for treated lesional skin 91.9 mJ/cm2

±SEM 17.5 mJ/cm2. The MED for UVB in the patients within the TACR group was for non-treated, non-lesional skin 75.2 mJ/cm2 ±SEM 12.2 and for treated lesional skin and 94.3 mJ/cm2 ±SEM 19.4. The treated skin showed higher, but not significantly different MED's in both groups. None of the patients showed a diminished MED (< 4 times decreased compared to healthy subjects).[24]

TT-CPD induction directly after 2 MED UVB (t=0)

A single dose of 2 MED UVB resulted in a strong positive nuclear staining indicating the presence of TT-CPD's in the biopsies from both the treated and non-treated skin sites. (Figure 1) The TCA treated skin sites expressed a significantly higher amount of TT-CPD's at t=0 compared to non-treated skin (expressed as the mean integrated redness per area, p = 0.0019, Wilcoxon signed rank test). This was not demonstrated in the TACR treated skin (p = 0.3). (Figure 2).

Figure 1. Visualization of TT-CPD's in human skin by Immunohistochemical microscopy 200x. Without UVB irradiation (C) directly following 2 MED UVB (t=0) and 24 hours after exposure (t=24), in non-treated skin (control), triamcinolone acetonide 0,1 % cream treated skin (TCA) and tacrolimus 0,1% cream treated skin (TACR).

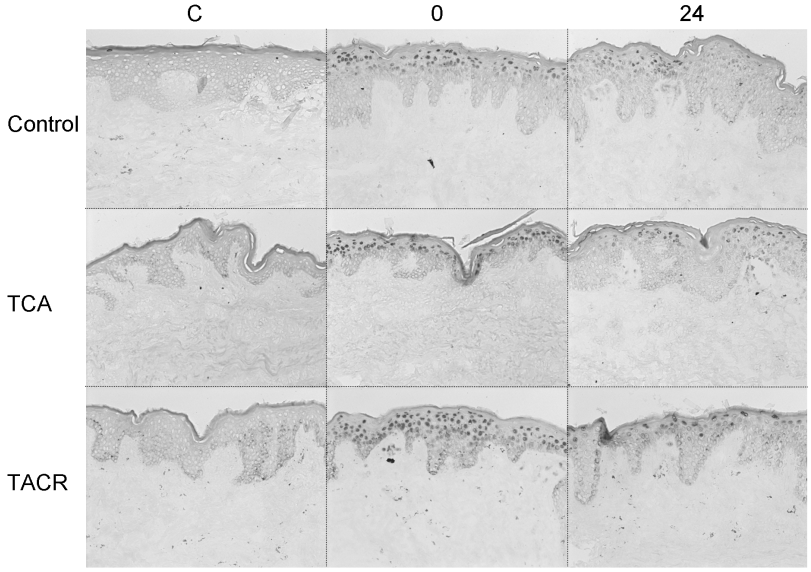
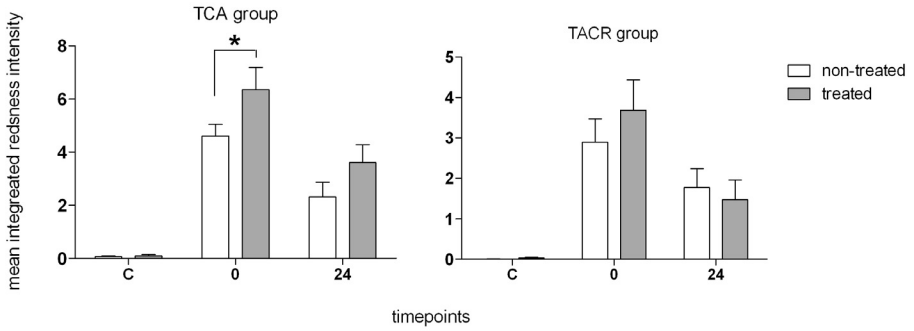


Figure 2. TCA and TACR treated and non-treated skin sites: mean integrated redness intensity/area at t=C, 0 and 24 hours. Mean ± SEM. TT-CPD induction proved to be significantly higher at t=0 in TCA treated skin compared to non-treated skin (* = significant, *p* = 0.0016, Wilcoxon signed rank test).



Repair 24 hours after 2 MED (t=24)

The amount of UV induced TT-CPD's at t=24 was significantly decreased in all groups compared to t=0 ($p < 0.01$). (Figure 1). There was neither a significant difference in DNA damage repair rate between treated and non-treated skin in the TCA patients group (untreated repair rate 53.5%, relative SEM ±23.6 vs. treated 46.1%, relative SEM ±18.7: $p > 0.7$) nor between treated an non-treated skin in the TACR patients group (untreated 47.6%, relative SEM ±26.3 vs. treated 65.1%, relative SEM ±32.: $p > 0.2$). In addition, there was no significant difference in DNA damage repair rate between TCA and TACR treated skin sites (46.1% relative SEM ±18.7 and 65.1 relative SEM ±32.8, $p > 0.08$). (Figure 3).

Skin thickness

Mean epidermal skin thickness in non treated, non lesional, non irradiated AD skin proved to be 84.2 µm (SEM ±9.2, N=16). Two weeks of treatment with TCA resulted in a significant decrease in epidermal thickness with a mean skin thickness of 52.6 µm (SEM ±4.9 vs. non-treated 88.8 µm ±SEM 9.9, $p = 0.007$, Mann-Whitney test) This was not observed in the TACR group (non-treated 79.9 µm ±SEM 5.8 vs. treated 74.8 µm ±SEM, $p = 0.4$). (Figure 4).

Figure. 3. TCA and TACR treated and non-treated skin sites: significant 24 hour DNA damage repair rates: The results are expressed as the percentage repaired TT-CPD's. Mean ± relative SEM (p < 0.01 for all groups, Wilcoxon signed rank test).

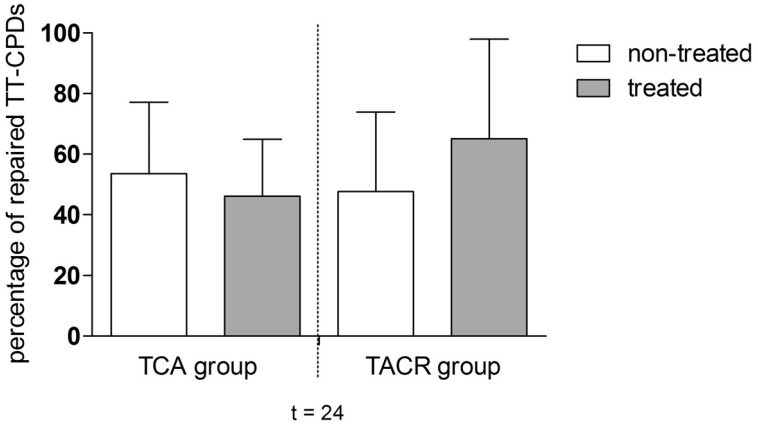
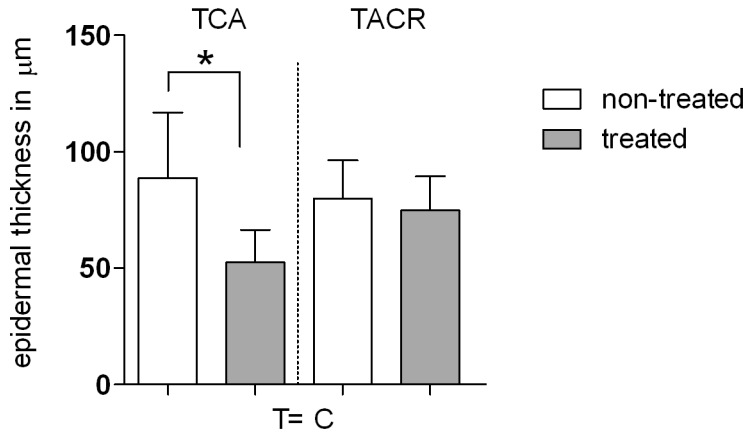


Figure. 4. Epidermal thickness of TCA and TACR treated skin vs. non treated skin.
Epidermal diameter was measured in µm, mean ± SEM. A significant difference in epidermal thickness was demonstrated in the TCA treated group (* = significant, p = 0.0078, Wilcoxon signed rank test).



Discussion

Non-melanotic skin cancer (NMSC) is a major complication of treatment with systemic calcineurin inhibitors in organ recipients. Their predilection for sun-exposed sites suggests an important role for UV. This raised questions about the long term safety of topical use of these compounds. We performed a small scale randomized controlled and observer blinded pilot study on UV induced DNA damage repair after a two week topical TACR treatment regime of lesional AD skin. Furthermore, we compared the effects on DNA damage repair with TCA treated skin.

At t=0 a significantly higher amount of TT-CPD's was induced in TCA treated skin compared to non-treated skin. This coincided with a significant decrease of epidermal thickness of TCA treated skin after two weeks. Both aspects could not be observed in the TACR group.
At t=24 the TT-CPD repair rates in either TCA or TACR treated skin did not differ from those in non-treated, non-lesional skin. This suggests that both topical treatment with TACR and TCA do not cause an increased residual DNA damage at 24 hours post UVB exposure in the epidermis. A limitation of our study is the small number of patients making detection of minute differences in repair rates not possible.
Doelker et al.[13] also demonstrated that the topical CI pimecrolimus and TCA did not inhibit TT-CPD repair rates, 24 hours after exposure. This was found both on skin of healthy controls and non-lesional skin of AD patients even after application on tape stripped skin preceding occlusion for a period of 3 days.

Although our observations suggest that UVB exposure on topical steroid treated skin induces more DNA damage, the repair rates showed that DNA damage may be restored in an equally effective manner. Furthermore these data suggest that in contrast to TCA the anti-inflammatory effect of TACR does not influence the MED for UVB. This fits with the recent data of Jocher et al.[25] who showed that in contrast to topical steroids pimecrolimus did not influence UV erythema.
There are conflicting data on an increased risk for non NMSC in patients with AD,[26] which need further confirmation. Nevertheless the anti-inflammatory effect of topical corticosteroids may simulate increased UV tolerance. This may expose AD patients to higher UV dosages thereby increasing the risk for NMSC.

Calcineurin has a role in the DNA repair pathway.[8, 27] In mononuclear cells inhibition of DNA repair by TACR has been linked to inhibition of the DNA repair enzyme DNA polymerase-β. This enzyme is primarily implicated in base excision repair, while in UV damage induced repair, nucleotide excision repair (NER) is primarily involved. So far it is unknown whether CI directly interact with this DNA repair mechanism. Apart from DNA-damage *per se*, the dysregulated repair of cell-cycle and apoptosis may also be involved in photocarcinogenesis. A recent in vitro study demonstrated that genetic and pharmacological suppression of calcineurin (by CI treatment) promotes tumour formation. This was demonstrated in mouse skin, in xenografts, in immune compromised mice, in primary human keratinocytes (HRas1) and keratinocyte-derived squamous carcinoma cells. This was associated with ATF3 expression (an activating transcription factor) and P53 (oncogene) down modulation.[28] Another in vitro model for skin cancer in organ transplant recipients showed that cyclosporin A induced inhibition of the mitochondrial permeability transition pore resulting in a suppression of keratinocyte cell death after UV irradiation, thus suggesting skin cancer promotion.[29] This interaction was not shown in tacrolimus treated cells, emphasizing differences in carcinogenetic potential between CI.

T cells are the main therapeutic target cells of CI, whereas calcineurin inhibition finally results in inhibition of T cell activation. T-cell mediated activation of DNA repair implicates primarily base-excision repair. The effects of tacrolimus on the nucleotide excision repair, which is the predominant mechanism in the repair of DNA damage, is however, still unclear.[12]

In conclusion, our data do not show an immediate effect of the topical CI tacrolimus on UVB induced DNA damage repair in patients with AD. However the mechanisms involved in photocarcinogenesis are complex and it may take decades before NMSC develop. Therefore long-term cohort studies in AD patients are necessary before it can be stated that maintenance therapy with topical CI on UV exposed skin sites is safe concerning the risk of NMSC.

Reference List

1 Allen BR. Tacrolimus ointment: its place in the therapy of atopic dermatitis. J.Allergy Clin.Immunol. 2002 Mar.

2 Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. N Engl J Med 2003 Apr 24;348(17):1681-91.

3 Douki T. Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. 2001 Feb 27.

4 de Gruijl FR. Skin cancer and solar UV radiation. Eur.J.Cancer 1999 Dec.

5 Matsumura Y. Molecular mechanisms of photocarcinogenesis. Front Biosci. 2002 Apr 1.

6 Yarosh D. Pyrimidine dimer removal enhanced by DNA repair liposomes reduces the incidence of UV skin cancer in mice. Cancer Res. 1992 Aug 1.

7 Canning MT, Nay SL, Pena AV, Yarosh DB. Calcineurin inhibitors reduce nuclear localization of transcription factor NFAT in UV-irradiated keratinocytes and reduce DNA repair. J Mol Histol 2006 Sep;37(5-7):285-91.

8 Herman M, Weinstein T, Korzets A, Chagnac A, Ori Y, Zevin D, et al. Effect of cyclosporin A on DNA repair and cancer incidence in kidney transplant recipients. J Lab Clin Med 2001 Jan;137(1):14-20.

9 Yarosh DB. Calcineurin inhibitors decrease DNA repair and apoptosis in human keratinocytes following ultraviolet B irradiation. J.Invest Dermatol 2005 Nov.

10 Lan CC, Yu HS, Huang SM, Wu CS, Kuo HY, Lee CH, et al. Topical tacrolimus has a limited direct effect on ultraviolet B-irradiated keratinocytes: implications for its photocarcinogenic potential. Clin Exp Dermatol 2010 Mar;35(2):173-9.

11 Lerche CM, Philipsen PA, Poulsen T, Wulf HC. Topical pimecrolimus and tacrolimus do not accelerate photocarcinogenesis in hairless mice after UVA or simulated solar radiation. Exp Dermatol 2009 Mar;18(3):246-51.

12 Tran C, Lubbe J, Sorg O, Doelker L, Carraux P, Antille C, et al. Topical calcineurin inhibitors decrease the production of UVB-induced thymine dimers from hairless mouse epidermis. Dermatology 2005;211(4):341-7.

13 Doelker L. Production and clearance of cyclobutane dipyrimidine dimers in UV-irradiated skin pretreated with 1% pimecrolimus or 0.1% triamcinolone acetonide creams in normal and atopic patients. 2006 May.

14 Gambichler T, Schlaffke A, Tomi NS, Othlinghaus N, Altmeyer P, Kreuter A. Tacrolimus ointment neither blocks ultraviolet B nor affects expression of thymine dimers and p53 in human skin. J Dermatol Sci 2008 May;50(2):115-22.

15 Yan AC, Honig PJ, Ming ME, Weber J, Shah KN. The safety and efficacy of pimecrolimus, 1%, cream for the treatment of Netherton syndrome: results from an exploratory study. Arch Dermatol 2010 Jan;146(1):57-62.

16 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch.Dermatol. 1988 Jun.

17 Hanifin JM. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Suppl.) (Stockholm) 1980.

18 Caproni M. The comparative effects of tacrolimus and hydrocortisone in adult atopic dermatitis: an immunohistochemical study. 2007 Feb.

19 Hoetzenecker W, Meindl S, Stuetz A, Stingl G, Elbe-Burger A. Both pimecrolimus and corticosteroids deplete plasmacytoid dendritic cells in patients with atopic dermatitis. J Invest Dermatol 2006 Sep;126(9):2141-4.

20 Simon D, Vassina E, Yousefi S, Kozlowski E, Braathen LR, Simon HU. Reduced dermal infiltration of cytokine-expressing inflammatory cells in atopic dermatitis after short-term topical tacrolimus treatment. J Allergy Clin Immunol 2004 Oct;114(4):887-95.

21 Meads DM. The quality of life of parents of children with atopic dermatitis: interpretation of PIQoL-AD scores. Qual.Life Res. 2005 Dec.

22 Boonstra HE. Polymorphous light eruption: A clinical, photobiologic, and follow-up study of 110 patients. J.Am.Acad.Dermatol.2000 Feb.

23 http://www.nih.gov/. 2010.

24 ten Berge O, van Weelden H, Bruijnzeel-Koomen CAFM, de Bruin-Weller MS, Sigurdsson V. Throwing a light on photosensitivity in atopic dermatitis: a retrospective study. Am J Clin Dermatol 2009;10(2):119-23.

25 Jocher A, Kessler S, Hornstein S, Schulte MJ, Schempp CM. The UV erythema test as a model to investigate the anti-inflammatory potency of topical preparations--reevaluation and optimization of the method. Skin Pharmacol Physiol 2005 Sep;18(5):234-40.

26 Arana A, Wentworth CE, Fernandez-Vidaurre C, Schlienger RG, Conde E, Arellano FM. Incidence of cancer in the general population and in patients with or without atopic dermatitis in the U.K. Br J Dermatol 2010 Jun 9.

27 Fruman DA, Klee CB, Bierer BE, Burakoff SJ. Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. Proc Natl Acad Sci U S A 1992 May 1;89(9):3686-90.

28 Wu X, Nguyen BC, Dziunycz P, Chang S, Brooks Y, Lefort K, et al. Opposing roles for calcineurin and ATF3 in squamous skin cancer. Nature 2010 May 20;465(7296):368-72.

29 Norman KG, Canter JA, Shi M, Milne GL, Morrow JD, Sligh JE. Cyclosporine A suppresses keratinocyte cell death through MPTP inhibition in a model for skin cancer in organ transplant recipients. Mitochondrion 2010 Mar;10(2):94-101.

Summary and general discussion
(Met Nederlandse samenvatting)

Acknowledgements

Curriculum vitae

List of abbreviations

List of publications

Summary and general discussion

The overall aim of this thesis was to evaluate adverse effects of ultraviolet irradiation (UV) in atopic dermatitis (AD). We focused on the two important adverse effects of UV: photosensitivity and skin cancer risk associated with calcineurin inhibitor treatment.

In **chapter 2** and **3** we found that photosensitivity affects approximately 3% of our AD population, being mainly females. Phototesting distinguishes two photosensitivity reaction patterns. Based hereupon, patients were diagnosed with photosensitive AD (PhAD) or AD with co-existing polymorphic light eruption (AD + PLE). Children with AD and photosensitivity showed the same two clinical reaction patterns as demonstrated in adults in the same 50-50% ratio.
In **chapter 4**, we analyzed the inflammatory cell infiltrate after a single UVB irradiation in these two patient groups compared to non-photosensitive AD patients. There was no difference between the 3 groups concerning the influx of neutrophils, eosinophils and T-cells, the degranulation of mast cells or the quantity of epidermal dendritic cells.

In **chapter 5** we described a digital quantification method for immunohistochemical assessment of UV induced thymine cyclobutane pyrimidine dimers (TT-CPDs) in human skin. This method increased the linear measurement range compared to visual assessment and hence improved immunohistochemical quantification of DNA photo damage; it is more sensitive and efficient in measuring the extent of DNA-damage per nucleus. This method was eventually used to assess the difference in UV induced DNA damage repair in AD patients treated with either topical triamcinolone acetonide 0,1 % ointment (corticosteroid) or tacrolimus 0,1 % ointment (calcineurin inhibitor) described in **chapter 6**. Our study did not show differences in DNA damage repair rate of topical tacrolimus treated skin after a single UVB irradiation in human AD skin compared to non-treated or triamcinolone treated skin.

Is photosensitivity in atopic dermatitis a major issue?

Atopic dermatitis (AD) has a considerable influence on the quality of life of the patient and, when concerning a child, the quality of life of its family.[1, 2] The understanding of the disease epidemiology and pathophysiology will aid in the rational approach to its treatment. Control of potential environmental triggers and pharmacotherapy are first line treatments. Patient education is essential for successful disease management.[3] The chronic and intermittent course of AD requires professional and long term aimed treatment and counselling.[2] It affects large numbers of patients, considering that the worldwide estimate of AD in children is around 3-10% and 1-3 % in adults.[4-7]

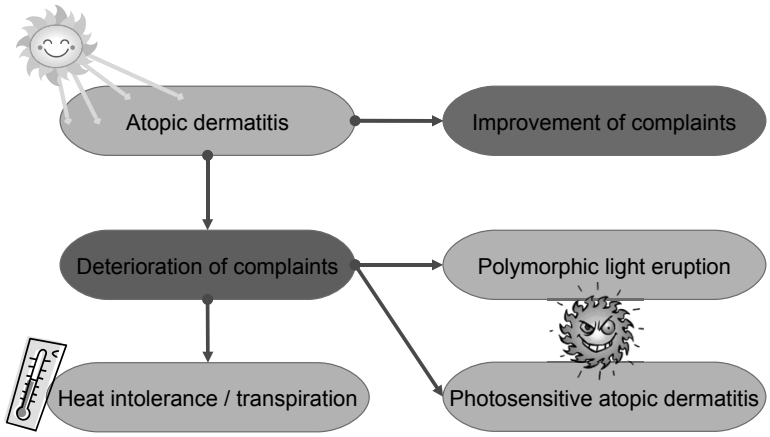
The majority of AD patients benefit from sunlight or ultraviolet radiation (UVR) therapy (UVB/UVA1/PUVA).[8] Unfortunately, a small group of patients experience an adverse reaction to sunlight or artificial UVR. Photosensitivity in AD is a known phenomenon, but frequently troubled by discordant nomenclature. In the literature photosensitivity in AD is reported as photoaggravation of AD,[9-21] photosensitive eczema,[22-24] light exacerbated eczema,[25, 26] photoexacerbated AD,[27] or sometimes even chronic actinic dermatitis (CAD).[28-31] There are also reports of the co-existence of polymorphic light eruption (PLE) with AD.[32-35] Even though photosensitivity in AD has often been referred to in literature, a thorough description of this phenomenon is lacking. Furthermore it is important to discriminate photosensitivity in AD from CAD, since these are different entities both clinically and photobiologically. Particularly the UK nomenclature tends to lump photosensitivity problems in AD together with CAD.[26] We believe this is indiscriminate and incorrect since patients with CAD (synonymous with persistent light eruption and actinic reticuloid) do usually not have a history of AD. Furthermore they suffer from a chronic photosensitive dermatitis, arising in the context of multiple (photo)contact allergies or (photo)allergy for drugs with diminished MEDs for UVB, UVA and often visible light.[36, 37]

It is evident that the skin of AD patients can be deteriorated by hot and sunny weather. This is probably due to heat intolerance and transpiration and not photosensitivity, whereas these patients have negative phototest results.[33] This phenomenon should be referred to as heat intolerance.

Then there are AD patients who show photo activation of non-lesional AD skin. Phototests distinguishes two clinically different reaction patterns to UV. Based on the outcome of these tests, patients are diagnosed either as having AD with co-existing polymorphic light eruption (AD + PLE) or photosensitive atopic dermatitis (PhAD). Both patients with PhAD and AD + PLE show normal minimal erythemal doses (MED) for UVB, UVA and Visible light. Half of the AD patients with photosensitivity has PLE, the other half slowly develops eczema after sun exposure without preceding PLE. The same ratio is present in children with AD and photosensitivity.
Repeated exposures to UVR in patients with AD + PLE induce a pruritic, erythematous and papulous reaction resembling the reaction seen after UVR provocation in patients with PLE.
Repeated exposures to UVR in patients with PhAD induce a pruritic reaction with erythema, papules, scales, vesiculation, and excoriations. This reaction is eczematous and takes longer (days) to develop. In addition, it has a more persistent character compared to patients with AD + PLE.

The UV wavelengths to which the patients react is the same in AD + PLE and PhAD. The majority react to UVA and UVB, about 17% only to UVA and another 17% to UVB. Figure 1 depicts a classification of the influence of sunlight on the skin of patients with AD, as described above.

Figure 1. Sunlight related effects on atopic dermatitis.



In this thesis and in earlier literature it is shown that in approximately 3-10% of patients with AD the disease is deteriorated or triggered by sunlight.[33] Other chronic dermatoses can also be exacerbated by sunlight exposure. From 2000 psoriasis patients in Sweden 4.4% stated that their psoriasis always worsened upon solar exposure and 7.3% stated that this sometimes occurred.[38] After phototesting an estimated prevalence of 5.5% photosensitivity was obtained. Half of the psoriasis patients were diagnosed with PLE and the other half slowly developed psoriasis after UV exposure without preceding PLE.
The prevalence of PLE in Europeans was recently estimated to be around 18%.[39, 40] The majority however being only mild cases and the prevalence was based on questionnaires and not on actual phototest outcome, which may have caused overestimation. Comparing the prevalence data for PLE in the general population with those in AD and psoriasis suggests a lower prevalence in the latter patients groups. However these prevalence data may be confounded since mild cases of photosensitivity may be underreported in patients with chronic disorders such as AD and psoriasis. Furthermore, photosensitivity may be difficult to diagnose in a chronic skin disease like AD, with an intermittent, unpredictable and often seasonally influenced course.

In conclusion, AD patients with photosensitivity show two different reaction patterns after phototesting, 50% shows a pattern similar to that observed in PLE and the other 50% develops eczema. This pattern is observed in adults and children. The terminology of light exacerbated or photosensitive eczema may give confusion since they may be used in the context of CAD. Therefore we propose to diagnose patients with AD and photosensitivity as patients with AD and coexistent PLE and patients with photosensitive AD. CAD rarely occurs in AD patients and has different clinical and photobiological characteristics.[28-31]

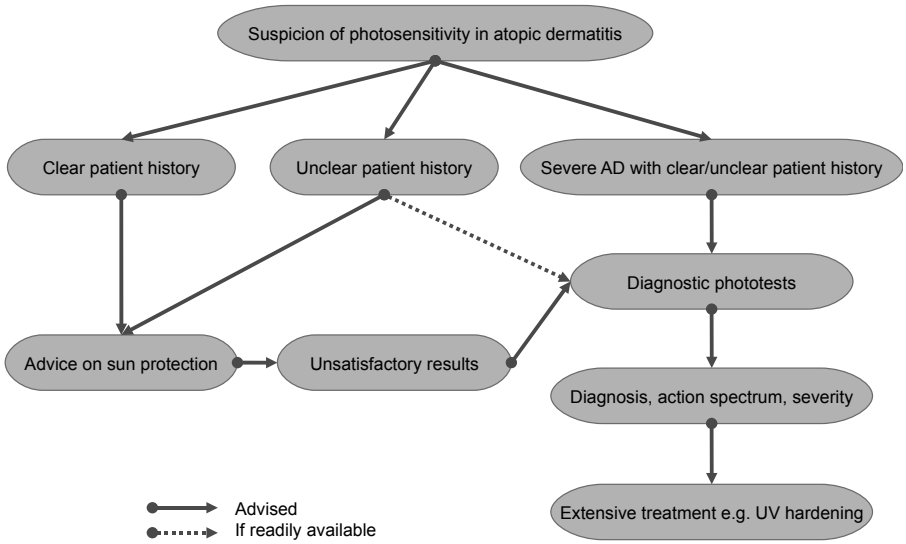
Should we phototest atopic dermatitis patients?

The diagnosis of photosensitivity can be easily missed in AD patients since a clear correlation between complaints and exposure to sun light is frequently absent. Firstly, for the majority of patients AD is a chronic disorder with remissions and

exacerbations, which may be season dependent and in many patients photo-exposed skin regions are also the predilection sites for AD. For that reason, a clear seasonal limitation of sunlight induced complaints may be absent. Nevertheless AD+PLE patients frequently recognize photosensitivity and clearly differentiate sun related skin symptoms from lesional AD skin. Their patient history is often identical to a patient with PLE: symptoms starting in spring or summer with itching papules and plaques after exposure, which disappear without scarring within days. The symptoms diminish during summer due to natural hardening. However, photosensitivity may be missed in UVA sensitive patients, in whom lesions can be induced on cloudy days. Secondly, a change in temperature is one of the many environmental factors which may influence the course of AD. For that reason, patients may find it difficult to differentiate between a deterioration of their eczema due to heat and transpiration or to UV exposure. Unfortunately, whereas most of the PhAD patients have more severe eczema it is difficult to differentiate from patient's history whether deterioration is induced by heat or by sun exposure. Especially in children the diagnosis may be delayed for several years or even missed, because of the absence of a clear relation between UV exposure and objective cutaneous manifestations.

In cases where patient history and/or clinical presentation are clear enough to diagnose photosensitivity phototesting is not necessary. Photoprotection can directly be advised for these patients. However, in cases without a clear-cut relation between cutaneous symptoms and sun exposure, but with exacerbations during spring and summer months for which no other factors may be responsible, the clinician should consider photosensitivity and consequently, advice on sun exposure and sun protection. If phototests are available it may be considered.
If there are persistent complaints despite adequate sun protection and topical treatment or if the patient requires specialized care as a consequence of the severity of their disease, phototesting is necessary.
Furthermore, phototesting may be helpful to prevent unnecessary sun avoidance. Especially parents of children with AD are on crusade to find an avoidable cause for the chronic skin disease of their child. This was shown in *chapter 3*, in which the diagnostic potential of phototesting is demonstrated by the number of children in whom photosensitivity could not be confirmed with phototesting (39%) despite sun related complaints. A decision protocol for testing photosensitivity in AD is shown in figure 2.

Figure 2. Clinical decision protocol for photosensitivity diagnosis in atopic dermatitis



The majority of AD patients with photosensitivity may benefit from advice concerning sun exposure, the use of sunscreen(s) and/or protective clothing. The success of UV-hardening therapy[41] in photodermatoses is mainly due to thickening of the stratum corneum, resulting in a decrease of UV penetration. In our clinical experience the use of UVB hardening therapy in AD patients is often complicated by aggravation of existing lesions and is more successful if the eczema is adequately treated with anti-inflammatory drugs before the start of UVB therapy Therefore, this therapy should only be tried in severely photosensitive AD patients who do insufficiently respond to UV avoidance advices and sunscreens

In conclusion, diagnosing photosensitivity based on patient's history only may be difficult, especially in patients with severe AD and in children with AD. In these patients phototesting is an important diagnostic tool. Differentiating between the clinical reaction patterns occurring in PhAD and AD+PLE may demand for experience in phototesting. However, since the majority of AD + PLE and PhAD will clinically benefit from avoidance and protection advices differentiation between these phototest patterns is not necessary. This is only demanded in patients who do not have a favourable response to these prevention measures.

What did we learn from a single dose UVB irradiation in atopic dermatitis patients with photosensitivity?

The presence of two clinically different UV reaction patterns in AD may be due to different pathological mechanisms. Therefore we analysed the inflammatory cell composition in the skin after a single exposure to UV.

UV-exposure of the skin of healthy controls induces damage and apoptosis of keratinocytes, immigration of neutrophils and monocytes, mast cell degranulation and emigration of dendritic cells from the epidermis into the dermis and the draining lymph nodes.[42-47]After a single radiation with 4MED UVB neutrophils may express the cytokines IL-4 and IL-10.[48, 49] It was hypothesized that the expression of these cytokines might induce immune tolerance to UV light.
Compared to healthy controls, patients with polymorphic light eruption showed a decreased number of skin infiltrating neutrophils after a single UVB irradiation (Philips TL12, 3x MED).[50] Since neutrophils express immunoregulatory cytokines TNF-alpha, IL-4, and IL-10,[48, 49, 51] it was hypothesized that a decrease in the number of infiltrating neutrophils upon UVB would mean a decrease in immunosuppression and would lead to an inflammatory skin response in PLE patients.
We hypothesized that aberrances in neutrophil infiltration upon UV irradiation might also be responsible for photosensitivity in patients with AD.
In contrast to the data of Schornagel et al.[50], we found no negative correlation between photosensitivity and neutrophil numbers in the AD patients with photosensitivity in our study with the same 3x MED UVB irradiation (Philips TL12) and similar time points. All AD patients (with and without photosensitivity) showed an infiltration of neutrophils, which was in the same range as the numbers observed in healthy controls.[18, 52] Moreover, AD + PLE patients even showed a tendency towards even higher number of neutrophils 24 hours after irradiation compared to patients with either AD or PhAD.

Our data suggest that photosensitivity in AD is not related to the number of neutrophils infiltrating the skin. However, we could not quantify the production of IL-4 and IL-10 by skin infiltrating neutrophils. Therefore we can not exclude

that decreased immunoregulatory cytokines, such as IL-4 and IL-10, might be responsible for breaking immune tolerance to UVB.

Neutrophil infiltration into inflammatory tissue is critically influenced by mast cells.[53] There were no aberrances in mast cell degranulation in the photosensitive population, nor in the subsequent inflammatory cellular infiltrate denying a pathomechanistic role for the mast cell in photosensitivity associated with AD. Chronic inflammation in AD skin is dominated by CLA-CCR4 positive T cells, producing mainly IL-4 in acute and IL-13 and IFN-γ in chronic eczematous skin lesions.[54-56] UV irradiation of the skin induces immunosuppression. For that reason UVB and UVA are used in the treatment AD. The immunosuppressive effect of phototherapy in AD is thought to be via the changes in the morphology and a decrease in the number of T cells, due to apoptosis.[8] Recently it was found *in vitro* that UV irradiation can induce regulatory T-cells which can alter antigen presenting cells from stimulatory into regulatory phenotypes, hence down regulating immune responses via dendritical cell types.[57] This suggest that immunomodulation of T cells via the upregulation of the number of T regulatory cells might be the mechanism by which repeated irradiation with increasing doses of UVB (starting with 75% of 1 MED as occurs in phototherapy) induces clinical improvement of lesional AD skin. A single 3 MED exposure shows an increase in the number of T-cells in both photosensitive and non-photosensitive AD skin.
It can be hypothesized that there is recruitment of pro-inflammatory T-cells in AD patients with photosensitivity after repeated exposures caused by a failure in regulatory T-cell activation, which down regulates the UVR induced immune response in non-photosensitive patients. It would therefore be interesting to unravel if differences in the induction of skin infiltration by regulatory T cells would explain differences in skin responses to UV in patients with AD.

In AD an impaired barrier function of the skin is suggested to contribute to its pathogenesis. About 20 % of all AD patients and about 50% of the patients with severe AD have a loss of function mutation of the filaggrin (FLG) gene.[58-65]
FLG is a key protein in the formation of the cornified cell envelope and is critical for an effective skin barrier. Common loss-of-function variants of FLG is a predisposing factor for AD.[60] Trans-urocanic acid (trans-UCA) is formed from FLG protein breakdown products in the corneal layer. Urocanic acid is a major UV absorbing component of the skin.[66] Upon UV irradiation photo-isomerization from trans- to

cis-UCA is induced. Importantly, cis-UCA is associated with an immunosuppressive effect in the skin.[67] We hypothesized that a decrease in the production of UCA, and consequently a different photostationary state of photo-isomerization due to FLG loss of function mutations, was responsible for a change in UV induced skin dynamics. However there was no difference in FLG protein expression between photosensitive and non-photosensitive AD patients in non-lesional skin. Kezic et al.[68] did not find differences in concentrations of UCA in the stratum corneum between 11 patients with a FLG mutation (the most common FLG mutations: R501x and R2447x) and 10 wild type controls. Recently the knockdown of FLG in a human skin model did however demonstrate an increase of UV sensitivity.[69] They showed a decrease of UCA concentration and an increase of cyclobutane pyrimidine dimers and caspase-3 activation. Furthermore Barresi et al. found an increased sensitivity for UVB in histidinemic mice, suggesting a crucial role of endogenous UCA in photoprotection.[66] It would therefore be interesting to assess UCA values in the skin upon UV irradiation in correlation to photosensitivity.

As mentioned before UV hypersensitivity has also been observed in other chronic dermatoses, such as psoriasis. Analysis of skin infiltrating cells after a high dose UV did not reveal differences between patients with psoriasis and PLE and patients with UV induced psoriasis.[38] The fact that UV can induce PLE in both psoriasis and AD patients suggests that the mechanism behind a PLE reaction is independent from the immunological mechanism at the level of T-cell cytokine production, since psoriasis is a Th1/Th17 dominated and AD a Th2/Th1 dominated skin disease. Furthermore, it shows that UV induces lesional skin in clinically and immunologically distinct skin diseases. This suggests that the induction of lesional skin by UV in both psoriasis and AD is comparable to a Koebner phenomenon for which a rather indirect than a direct immune mechanism may be responsible.
Recently, data are generated on the innate immune system of the upper part of the epidermal skin.[70] For that reason it would be interesting to use UV as a model to compare the physiochemical activation of the innate immune response of the epidermis and the subsequent activation of the adaptive between psoriasis and AD.
In conclusion, we studied a single exposure to UVB, which did not unravel differences in cellular inflammatory reaction patterns in our patient groups.
Ideally, future studies regarding photosensitivity (in AD) should be focussed on the inflammatory cell phenotype after UV exposure over a longer period of time and also include cellular interactions. An in vivo UV-summation model with more time

point analyses is however hard to standardize, since the number of irradiations needed for each individual patient widely varies, making it difficult to compare individual patients.

Are topical calcineurin inhibitors (CI) safe to use for inflammatory skin diseases such as atopic dermatitis?

Non melanotic skin cancer is a major complication of treatment with systemic CIs in organ recipients and raised questions about the topical use of these agents.[71] However, the assessment of a putative photo-carcinogenesis risk associated with the (topical) use of CIs is hampered by a principal obstacle: cutaneous photocarcinogenesis in humans is a stepwise process that progresses over decades before eventually resulting in clinically noticeable skin cancer.[72-74]

Hence, the most important factor for accumulation of carcinogenic events is time.[75] During a human life, damage accumulates from free radical attacks, viruses,[76] and direct carcinogens, such as UV irradiation, that cause point mutations favouring the development of cancer.[77, 78] Oncogenetic gatekeepers like p53 can be damaged and become less efficient at eliminating damaged cells.[79] Furthermore, a decrease in immunosurveillance favours carcinogenesis.[78, 80, 98]
One way to examine the very early effects of topically applied CI, is to study local molecular events involved in skin photo-carcinogenesis.[72] Quantitative assessment of photoproducts in irradiated epidermis at different points in time reflects both the amount of absorbed radiations and the rate of dimer removal as a result of DNA repair, apoptosis, cell cycle delay or other biological mechanisms.[81] Deficiencies in, or ineffectiveness of the DNA repair mechanisms can lead to the formation of mutations over time and increase the risk of developing skin cancer.[82-84]
In vitro studies have shown a decrease in DNA repair rate or apoptosis in CI treated keratinocytes and peripheral blood mononuclear cells after UV irradiation.[85, 86, 95] There is also data disaffirming these results; denying a significant effect of treatment with CIs on the induction and repair rate of UV induced DNA damage.[87] Since topical treatment has practically no systemic absorption,[88-90] it is conceivable that the tentative potential carcinogenesis of topical treatment might be effective either through local immunosuppression or by affecting DNA damage repair.

UV irradiation of topically CI treated mice showed a decrease in induction of DNA damage but failed to show a decrease in DNA damage repair rate.[91, 92] Enhanced photocarcinogenesis was demonstrated by Niwa et al. in mice treated with topical CI in combination with UV irradiation, but this could not be confirmed by Lerche et al.[91, 93] Two in vivo studies in healthy volunteers treated with CIs showed no effect on induction or repair of UV induced DNA damage or on P53 expression within the first 24 hours after irradiation.[94] However, differences in radiation sources, CI compounds and study protocols make it difficult to compare all these results. Furthermore, the topical CI are applied on diseased skin, which has different absorption kinetics compared to healthy skin,[95] it is not known how this affects carcinogenetic potential.

We used the technique described in chapter 5 to evaluate the DNA damage repair rate after UVB irradiation in the in vivo skin of AD patients; non-treated compared to tacrolimus (TACR) treated. We paralleled it with triamcinolone acetonide 0.1% ointment (TCA) treatment to evaluate the local immunosuppressive effect on DNA damage repair (chapter 6).
In this study we could not reveal a significant difference in UVB induced TT-CPDs repair rate within 24 hours after irradiation between non-treated skin and treated skin sites with TCA or TACR. This suggests no, or at most a minor discriminative effect of topical tacrolimus on thymine dimer removal 24 hours after UVB irradiation.

T cells are the main therapeutic target cells of CI, whereas calcineurin inhibition finally results in inhibition of T cell activation. T-cell originated activation of DNA repair enzymes implicates primarily base-excision repair. The effects of CI on the nucleotide excision repair, the predominant mechanism in DNA damage repair is however still unclear.[92]

A recent in vitro study demonstrated that genetic and pharmacological suppression of calcineurin (by CI treatment) promotes tumour formation. This was furthermore associated with cancer cell senescence and oncogene down modulation in tacrolimus promoted tumour formation in vitro and in mice.[96] Another in vitro model for skin cancer in organ transplant recipients showed a cyclosporin-A induced inhibition of the mitochondrial permeability transition pore resulting in a suppression of keratinocyte cell death after UV irradiation, suggesting skin

cancer promotion.[97] This interaction was not found in tacrolimus treated cells,[97] emphasizing different carcinogenetic properties between CI.

Assuming that, as shown in our study, the direct effect of topical TACR or its indirect immunosuppressive effect on the DNA repair (after UV induction) is negligible, we can speculate that changes in oncogene-induced senescence in CI treated patients is of far greater importance in the (UV) carcinogenesis than solitary changes in DNA damage repair mechanisms within the epidermal keratinocytes.
The extent of immunosuppression and effect on cancer cell senescence or oncogene down modulation of topical use of CIs in vivo is unknown at present time and therefore remains a concern. Consequently, there is a great need for additional research and long-term registry data of the population observed in controlled studies are required to define maintenance treatment safety in the long run of topical treatment with CIs. Until then short-term or intermittent long-term topical use of these compounds is advised. When used as maintenance, UV protection is favourable.

Concluding remarks

- Photosensitivity in patients with AD consists of two, by phototest distinguishable, clinical reaction patterns. Patients should be diagnosed with either photosensitive AD or with AD and co-existing polymorphic light eruption. This pattern is observed in adults and children.

- A single 3 MED UVB irradiation did not show differences in cell kinetics between AD patients with and without photosensitivity.

- Our *in vivo* data did not show an effect of topical tacrolimus treatment on UVB induced DNA damage repair in the skin of patients with AD within 24 hours.

- Patients with photosensitivity avoid solar irradiation and frequently use solar protection; they represent a population in which it should be regarded safe to use topical CIs on short or long term and intermittent or continuous therapy.

- Long-term registry data in controlled studies are required to define maintenance treatment safety in the long run in general.

Reference List

1 Alvarenga TM, Caldeira AP. Quality of life in pediatric patients with atopic dermatitis. J Pediatr (Rio J) 2009 Sep;85(5):415-20.

2 Bruijnzeel-Koomen CA, Sillevis Smitt JH, Boukes FS, van Everdingen JJ. [The practice guideline 'atopic dermatitis']. Ned Tijdschr Geneeskd 2007 Jun 23;151(25):1399-402.

3 Wasserbauer N, Ballow M. Atopic dermatitis. Am J Med 2009 Feb;122(2):121-5.

4 Hanifin JM. Epidemiology of atopic dermatitis. Acta Derm Venereol (Suppl.) (Stockholm): 21 ;116-131 1987.

5 Leung DY. Atopic dermatitis. Lancet 2003 Jan (361) 9352; 151-160.

6 Russell SC. The photosensitivity dermatitis and actinic reticuloid syndrome (chronic actinic dermatitis) occurring in seven young atopic dermatitis patients. Br.J.Dermatol. 138(3); 496-501 1998 Mar.

7 Schultz LF. The epidemiology of atopic dermatitis. Monogr Allergy 31; 9-28 1993.

8 Berneburg M, Rocken M, Benedix F. Phototherapy with narrowband vs broadband UVB. Acta Derm Venereol 2005;85(2):98-108.

9 Harber, Bickers. Photoaggravated dermatoses, including lupus erythematosus; atopic dermatitis. Photosensitivity diseases: principles and treatment Dermatol.Clin. 1989 4(2); 167-169.

10 photomedicine; abnormal responses to UV radiation: idiopathic and photoexacerbated. Fitz-Patrick's Dermatology in General Medicine section eighteen: chapter 135; 1294. 2004 .

11 Amblard P. [Skin diseases with photosensitivity]. Rev.Prat. 1992; 42(11); 1365-1368.

12 Crouch RB. Analysis of patients with suspected photosensitivity referred for investigation to an Australian photodermatology clinic. J.Am.Acad.Dermatol. 2003; 48(5); 714-720.

13 Deguchi H. Sun exposure is an aggravating factor responsible for the recalcitrant facial erythema in adult patients with atopic dermatitis. Dermatology 2002; 204; 23-38.

14 Frain-Bell W. Photoaggravated dermatoses; Atopic dermatitis. Cutaneous photobiology 1985; chapter 3; 60-61.

15 Keong CH. Photosensitivity in atopic dermatitis: demonstration of abnormal response to UVB. J.Dermatol. 1992; 19(6); 342-7.

16 Kerker BJ. The photoaggravated dermatoses. Semin.Dermatol. 1990; 9(1); 70-7.

17 Murphy GM. Diseases associated with photosensitivity. J Photochem.Photobiol.B 2001 64(2-3) 93-8.

18 Murphy GM. Investigation of photosensitive disorders. Photodermatol.Photoimmunol.Photomed.2004; 20(6); 305-11.

19 Pigatto PD. Atopy and photosensitivity in children. Photodermatol. 1986; 3(5); 303.

20 Tajima T. A variety of skin responses to ultraviolet irradiation in patients with atopic dermatitis. J.Dermatol. Sci. 1998 17(2); 101-7.

21 V.A.deLeo. other photoaggravated diseases. photosensitivity: topics in clinical dermatology 1992 chapter 11; 155-7.

22 Magnus. Other photodermatoses; Photosensitive eczema. Dermatological photobiology: clinical and experimental aspects 1976; 14; 194-5.

23 Ramsay CA. Photosensitive eczema. Trans.St.Johns.Hosp.Dermatol.Soc. 1973; 59(2) 152-8.

24 Yokel BK. Management of chronic photosensitive eczema. Arch.Dermatol. 1990; 126(10); 1283-5.

25 Harber LC. Photosensitivity. Classification. Dermatol.Clin. 1986; 4(2); 167-9.

26 Hawk. Diseases cause by environmental exposure or trauma; Chronic actinic dermatitis; Photosensitive eczema. Dermatology 1996; 2(13); 559.

27 Morison WL. Clinical practice. Photosensitivity. N.Engl.J.Med. 2004; 350(11); 1111-7.

28 Creamer D. Chronic actinic dermatitis occurring in young patients with atopic dermatitis. Br.J.Dermatol. 1998; 139(6); 1112-3.

29 Ogboli MI. Chronic actinic dermatitis in young atopic dermatitis sufferers. Br.J.Dermatol. 2000; 142(4); 845.

30 Russell SC. The photosensitivity dermatitis and actinic reticuloid syndrome (chronic actinic dermatitis) occurring in seven young atopic dermatitis patients. Br.J.Dermatol. 1998; 138(3); 496-501.

31 Yones SS. Exacerbation of presumed chronic actinic dermatitis by cockpit visible light in an airline pilot with atopic eczema. Photodermatol.Photoimmunol.Photomed. 2005; 21(3); 152-153.

32 Pearson IC. Atopic dermatitis - a simple entity? Clin.Exp.Dermatol. 2002; 27(1); 88-9.

33 Rajka G. clinical aspects; other essential features; photosensitvity. Essential Aspects of Atopic Dermatitis 2006; chapter 2.6.2; 27.

34 Tajima T. A variety of skin responses to ultraviolet irradiation in patients with atopic dermatitis. J.Dermatol. Sci. 1998; 17(2); 101-7.

35 Vieluf D. Complications and Diseases associated with Atopic Eczema. Handbook of Atopic Eczema 1991; 54-79.

36 Lim HW. Chronic actinic dermatitis. An analysis of 51 patients evaluated in the United States and Japan. Archives of Dermatology 1994; 36(1); 84-90.

37 Roelandts R. Chronic actinic dermatitis. J.Am.Acad.Dermatol 1993; 28(1); 240-9.

38 Ros AM, Eklund G. Photosensitive psoriasis. An epidemiologic study. J Am Acad Dermatol 1987 Nov;17(5 Pt 1):752-8.

39 Pao C, Norris PG, Corbett M, Hawk JL. Polymorphic light eruption: prevalence in Australia and England. Br J Dermatol 1994 Jan;130(1):62-4.

40 Rhodes LE. Polymorphic light eruption occurs in 18% of europeans and does not show higher prevalence with increasing latitude: multicenter survey of 6,895 individuals residing from the Mediterranean to Scandinavia. J.Invest Dermatol. 2010; 130(2); 626-8.

41 Boonstra HE. Polymorphous light eruption: A clinical, photobiologic, and follow-up study of 110 patients. J.Am.Acad.Dermatol. 2000; 42(2); 199-207.

42 Hawk JL. The presence of neutrophils in human cutaneous ultraviolet-B inflammation. Br.J.Dermatol. 1988; 118(!); 27-30.

43 Kim MS. Acute exposure of human skin to ultraviolet or infrared radiation or heat stimuli increases mast cell numbers and tryptase expression in human skin in vivo. Br.J.Dermatol. 2009; 160(2); 292-402.

44 Kolgen W. Epidermal langerhans cell depletion after artificial ultraviolet B irradiation of human skin in vivo: apoptosis versus migration. J.Invest Dermatol. 2002; 118(5); 812-7.

45 Lee PL. Neutrophil infiltration in normal human skin after exposure to different ultraviolet radiation sources. Photochem.Photobiol. 2008; 84(6); 1528-34.

46 Soter NA. Acute effects of ultraviolet radiation on the skin. Semin.Dermatol. 1990; 9(1); 11-5.

47 Walsh LJ. Ultraviolet B irradiation of skin induces mast cell degranulation and release of tumour necrosis factor-alpha. Immunol.Cell Biol. 1995; 73(3); 226-33.

48 Piskin G, Bos JD, Teunissen MB. Neutrophils infiltrating ultraviolet B-irradiated normal human skin display high IL-10 expression. Arch Dermatol Res 2005 Jan;296(7):339-42.

49 Teunissen MB, Piskin G, di NS, Sylva-Steenland RM, de Rie MA, Bos JD. Ultraviolet B radiation induces a transient appearance of IL-4+ neutrophils, which support the development of Th2 responses. J Immunol 2002 Apr 15;168(8):3732-9.

50 Schornagel IJ. Decreased neutrophil skin infiltration after UVB exposure in patients with polymorphous light eruption. J.Invest Dermatol. 2004; 123(1); 202-206.

51 Kolgen W. Differential expression of cytokines in UV-B-exposed skin of patients with polymorphous light eruption: correlation with Langerhans cell migration and immunosuppression. Arch.Dermatol. 2004; 140(3); 295-302.

52 Rijken F, Kiekens RC, van den WE, Lee PL, van WH, Bruijnzeel PL. Pathophysiology of photoaging of human skin: focus on neutrophils. Photochem Photobiol Sci 2006 Feb;5(2):184-9.

53 Kneilling M, Rocken M. Mast cells: novel clinical perspectives from recent insights. Exp Dermatol 2009 May;18(5):488-96.

54 Hijnen D, Haeck I, van Kraats AA, Nijhuis E, de Bruin-Weller MS, Bruijnzeel-Koomen CA, et al. Cyclosporin A reduces CD4(+)CD25(+) regulatory T-cell numbers in patients with atopic dermatitis. J Allergy Clin Immunol 2009 Oct;124(4):856-8.

55 Hijnen D, Nijhuis E, Bruin-Weller M, Holstege F, Koerkamp MG, Kok I, et al. Differential expression of genes involved in skin homing, proliferation, and apoptosis in CD4+ T cells of patients with atopic dermatitis. J Invest Dermatol 2005 Dec;125(6):1149-55.

56 Hijnen D, de Bruin-Weller M, Oosting B, Lebre C, De JE, Bruijnzeel-Koomen C, et al. Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell- attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. J Allergy Clin Immunol 2004 Feb;113(2):334-40.

57 Schwarz A, Noordegraaf M, Maeda A, Torii K, Clausen BE, Schwarz T. Langerhans cells are required for UVR-induced immunosuppression. J Invest Dermatol 2010 May;130(5):1419-27.

58 Howell MD. Cytokine modulation of atopic dermatitis filaggrin skin expression. J.Allergy Clin.Immunol. 2007; 120(1); 150-155.

59 Irvine AD, McLean WH. Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. J Invest Dermatol 2006 Jun;126(6):1200-2.

60 Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006 Oct;118(4):866-71.

61 Palmer CN. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat.Genet. 2006; 38(4); 441-6.

62 Weidinger S. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J.Allergy Clin.Immunol. 2006; 118(1); 214-9.

63 Weidinger S. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J.Invest Dermatol. 2007; 127(3); 724-6.

64 Weidinger S. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J.Allergy Clin.Immunol. 2008; 121(5); 1203-9.

65 Jungersted JM, Scheer H, Mempel M, Baurecht H, Cifuentes L, Hogh JK, et al. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. Allergy 2010 Jul;65(7):911-8.

66 Barresi C, Stremnitzer C, Mlitz V, Kezic S, Kammeyer A, Ghannadan M, et al. Increased Sensitivity of Histidinemic Mice to UVB Radiation Suggests a Crucial Role of Endogenous Urocanic Acid in Photoprotection. J Invest Dermatol 2010.

67 Noonan FP, De Fabo EC. Immunosuppression by ultraviolet B radiation: initiation by urocanic acid. Immunol Today 1992 Jul;13(7):250-4.

68 Kezic S, Kammeyer A, Calkoen F, Fluhr JW, Bos JD. Natural moisturizing factor components in the stratum corneum as biomarkers of filaggrin genotype: evaluation of minimally invasive methods. Br J Dermatol 2009 Nov;161(5):1098-104.

69 Mildner M, Jin J, Eckhart L, Kezic S, Gruber F, Barresi C, et al. Knockdown of Filaggrin Impairs Diffusion Barrier Function and Increases UV Sensitivity in a Human Skin Model. J Invest Dermatol 2010 May.

70 De Benedetto A, Agnihothri R, McGirt LY, Bankova LG, Beck LA. Atopic dermatitis: a disease caused by innate immune defects? J Invest Dermatol 2009 Jan;129(1):14-30.

71 Euvrard S, Kanitakis J, Claudy A. Skin cancers after organ transplantation. N Engl J Med 2003 Apr 24;348(17):1681-91.

72 Doelker L. Production and clearance of cyclobutane dipyrimidine dimers in UV-irradiated skin pretreated with 1% pimecrolimus or 0.1% triamcinolone acetonide creams in normal and atopic patients. Exp.Dermatol 2006; 15(5); 342-6.

73 Brash DE, Ziegler A, Jonason AS, Simon JA, Kunala S, Leffell DJ. Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion. J Investig Dermatol Symp Proc 1996 Apr;1(2):136-42.

74 Brash DE. Sunlight and the onset of skin cancer. Trends Genet 1997 Oct;13(10):410-4.

75 Anisimov VN. Carcinogenesis and aging 20 years after: escaping horizon. Mech Ageing Dev 2009 Jan;130(1-2):105-21.

76 Struijk L, van der ME, Kazem S, ter SJ, de Gruijl FR, Steenbergen RD, et al. Specific betapapillomaviruses associated with squamous cell carcinoma of the skin inhibit UVB-induced apoptosis of primary human keratinocytes. J Gen Virol 2008 Sep;89(Pt 9):2303-14.

77 Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer 2007 Dec 1;121(11):2381-6.

78 Fulop T, Kotb R, Fortin CF, Pawelec G, de AF, Larbi A. Potential role of immunosenescence in cancer development. Ann N Y Acad Sci 2010 Jun;1197:158-65.

79 Salvioli S, Capri M, Bucci L, Lanni C, Racchi M, Uberti D, et al. Why do centenarians escape or postpone cancer? The role of IGF-1, inflammation and p53. Cancer Immunol Immunother 2009 Dec;58(12):1909-17.

80 Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol 2006 Oct;6(10):715-27.

81 Bykov VJ. In situ repair of cyclobutane pyrimidine dimers and 6-4 photoproducts in human skin exposed to solar simulating radiation. 1999; 112(3); 326-31.

82 de Gruijl FR. Skin cancer and solar UV radiation. Eur.J.Cancer 1999; 35(14); 2003-9.

83 Matsumura Y. Molecular mechanisms of photocarcinogenesis. Front Biosci. 2002; 7; 765-83.

84 Yarosh D. Pyrimidine dimer removal enhanced by DNA repair liposomes reduces the incidence of UV skin cancer in mice. Cancer Res. 1992; 52(15); 4227-31.

85 Canning MT, Nay SL, Pena AV, Yarosh DB. Calcineurin inhibitors reduce nuclear localization of transcription factor NFAT in UV-irradiated keratinocytes and reduce DNA repair. J Mol Histol 2006 Sep;37(5-7):285-91.

86 Herman M, Weinstein T, Korzets A, Chagnac A, Ori Y, Zevin D, et al. Effect of cyclosporin A on DNA repair and cancer incidence in kidney transplant recipients. J Lab Clin Med 2001 Jan;137(1):14-20.

87 Lan CC, Yu HS, Huang SM, Wu CS, Kuo HY, Lee CH, et al. Topical tacrolimus has a limited direct effect on ultraviolet B-irradiated keratinocytes: implications for its photocarcinogenic potential. Clin Exp Dermatol 2010 Mar;35(2):173-9.

88 Bos JD. Non-steroidal topical immunomodulators provide skin-selective, self-limiting treatment in atopic dermatitis. Eur J Dermatol 2003 Sep;13(5):455-61.

89 van Leent EJ, Ebelin ME, Burtin P, Dorobek B, Spuls PI, Bos JD. Low systemic exposure after repeated topical application of Pimecrolimus (Elidel), SD Z ASM 981) in patients with atopic dermatitis. Dermatology 2002;204(1):63-8.

90 van Leent EJ, De Vries HJ, Ebelin ME, Burtin P, Scott G, Bos JD. Blood concentrations of pimecrolimus in adult patients with atopic dermatitis following intermittent administration of pimecrolimus cream 1% (Elidel) for up to 1 year. J Dermatolog Treat 2007;18(1):19-22.

91 Niwa Y, Terashima T, Sumi H. Topical application of the immunosuppressant tacrolimus accelerates carcinogenesis in mouse skin. Br J Dermatol 2003 Nov;149(5):960-7.

92 Tran C, Lubbe J, Sorg O, Doelker L, Carraux P, Antille C, et al. Topical calcineurin inhibitors decrease the production of UVB-induced thymine dimers from hairless mouse epidermis. Dermatology 2005;211(4):341-7.

93 Lerche CM, Philipsen PA, Poulsen T, Wulf HC. Topical pimecrolimus and tacrolimus do not accelerate photocarcinogenesis in hairless mice after UVA or simulated solar radiation. Exp Dermatol 2009 Mar;18(3):246-51.

94 Gambichler T, Schlaffke A, Tomi NS, Othlinghaus N, Altmeyer P, Kreuter A. Tacrolimus ointment neither blocks ultraviolet B nor affects expression of thymine dimers and p53 in human skin. J Dermatol Sci 2008 May;50(2):115-22.

95 Yan AC, Honig PJ, Ming ME, Weber J, Shah KN. The safety and efficacy of pimecrolimus, 1%, cream for the treatment of Netherton syndrome: results from an exploratory study. Arch Dermatol 2010 Jan;146(1):57-62.

96 Wu X, Nguyen BC, Dziunycz P, Chang S, Brooks Y, Lefort K, et al. Opposing roles for calcineurin and ATF3 in squamous skin cancer. Nature 2010 May 20;465(7296):368-72.

97 Norman KG, Canter JA, Shi M, Milne GL, Morrow JD, Sligh JE. Cyclosporine A suppresses keratinocyte cell death through MPTP inhibition in a model for skin cancer in organ transplant recipients. Mitochondrion 2010 Mar;10(2):94-101.

98 Buell et al. Malignancy after transplantation. Transplantation, 2005; 80; 254-64.

Samenvatting

Constitutioneel eczeem (CE) of atopische dermatitis is een chronische ontstekingsziekte van de huid, welke verloopt met exacerbaties en remissies. Ultraviolette straling (UV) speelt een belangrijke ambivalente rol bij deze ziekte. Door haar immunosuppressieve effect kan UV eczeem verbeteren. Maar ook kan UV het ziektebeeld verslechteren, door irritatie door zweten en of warmte, of door fotosensitiviteit, waarbij de huid geactiveerd wordt door zonlicht. Daarnaast kan UV de behandeling van het eczeem compliceren door interactie met geneesmiddelen. Dit proefschrift focust zich op deze ongunstige effecten van UV bij CE.

In hoofdstuk 2 en 3 wordt ingegaan op de klinische definiëring van fotosensitiviteit bij CE, bij volwassenen en kinderen. Er wordt beschreven welke reacties er optreden na provocaties met verschillende soorten UV, te weten UVB en UVA. Fotosensitiviteit bij CE patiënten bestaat uit twee verschillende door middel van fotoprovocatie te induceren klinische reactie patronen, die op basis van hun fenotype gediagnosticeerd werden als fotosensitief CE of CE met bijkomstige polymorphic light eruption ((PLE) de meest voorkomende vorm van zonlichtovergevoeligheid). Het komt in 3% voor van onze CE populatie en het betreft voornamelijk vrouwen.

Hierna wordt er in hoofdstuk 4 ingegaan op de ontstekingsreacties in de huid van deze 2 typen lichtovergevoelige CE patiënten. Deze patiënten werden belicht met een enkele dosis UVB op niet lesionale eczeemhuid (3x de minimale erythema dosis (MED) TL-12 Philips). Hierop volgend werden er in biopten op chronologische tijdstippen van deze belichtte huid gekeken naar cellulaire ontstekingsinfiltraten (onbelichte controle, direct na belichting, 6 en 24 uur na belichting). Patiënten werden vergeleken op basis van mest cel degranulatie, neutrofiele en eosinophiele granulocyten influx, T- en dendritische cel aantallen en filaggrine eiwit expressie. Er konden met deze uitleesparameters geen verschillen gevonden worden tussen deze typen patiënten of in vergelijking met CE patiënten zonder fotosensitiviteit.
Locale corticosteroïden vormen al vele jaren de hoeksteen van de behandeling van CE. In de afgelopen jaren zijn nieuwe topicale imuunosuppressieve middelen (TIMs) op de markt gekomen, zoals tacrolimus 0.1% zalf. De werking van deze middelen is gebaseerd op de remming van calcineurin en is in systemische vorm geassocieerd

met het verhoogde voorkomen van niet- melanocytaire huidkankers op chronisch zonlicht blootgestelde huid. Deze incidentie bij systemische behandeling riep vragen op over de mogelijke toegenomen carcinogenesis van de huid bij topicaal gebruik. Hoewel tot op heden geen causaal verband tussen het gebruik van TIM's en het optreden van maligniteiten is gevonden, is er wel *in vitro* evidence dat het herstel van UV-geïnduceerde DNA schade in keratinocyten geremd kan worden.

Het effect van topicaal gebruik van tacrolimus op de UVB geïnduceerde DNA schade en het herstel werd geïnventariseerd middels een hernieuwde digitale quantificatie methode. Deze methode, beschreven in hoofdstuk 5, is gebaseerd op een antilichaam dat de door UVB geïnduceerde thymine dimeren (de meest voorkomende vorm van UV geinduceerde DNA schade) in blootgestelde celkernen van keratinocyten in de huid immunohistochemisch kan aankleuren. Deze aankleuring wordt vervolgens digitaal gefotografeerd en daarna met een speciaal geschreven software programma geanalyseerd op intensiteit van het aangekleurde antilichaam.

Hoofdstuk 6 beschrijft een studie waarin het effect op UVB geïnduceerde DNA schade en het herstel daarvan na 2 weken topicaal gebruik van tacrolimus wordt geëvalueerd (met de in hoofdstuk 5 beschreven methode). Daarnaast werd het effect van deze behandeling vergeleken met huid die op eenzelfde wijze behandeld is met triamcinolon 0,1% zalf. De behandelde huid werd blootgesteld aan 2x de MED UVB en hierop volgend werd direct volgend een huidbiopt afgenomen en na 24 uur. Deze procedure werd bij dezelfde patiënt op niet behandelde huid herhaald, ter vergelijking. Deze kleinschalige gerandomiseerde en gecontroleerde observer geblindeerde *in vivo* pilot-studie liet geen effect zien van 2 weken topicaal gebruik van tacrolimus op de inductie en herstel van DNA schade in de huid na UVB blootstelling in CE patiënten in de eerste 24 uur.

De belangrijkste inzichten die dit proefschrift geeft zijn (hoofdstuk 7):

- Fotosensitiviteit bij patiënten met CE bestaat uit twee, met lichttesten te differentiëren, klinische reactie patronen. Patiënten werden gediagnosticeerd als fotosensitief CE of CE met bijkomstig PLE. Dit patroon is gelijk in volwassenen en kinderen.

- Een enkele dosis 3 MED UVB toonde geen verschillen aan in celdynamiek van de huid tussen CE patiënten met of zonder fotosensitiviteit.

- Onze *in vivo* data toonde geen effect van topicaal gebruik van tacrolimus op de UVB geïnduceerde DNA schade en het herstel daarvan in de huid van CE patiënten in de eerste 24 uur.

- Patiënten met CE en fotosensitiviteit komen weinig in de zon en gebruiken frequent zonlicht (UV) protectie; het kan verondersteld worden dat het gebruik van TIMs in deze groep zonder verdere consequenties gebruikt zou kunnen worden.

- Lange termijn gecontroleerde studies zijn nodig om de veiligheid van onderhoudsbehandeling met TIMs te definiëren.

Acknowledgements

I would like to acknowledge everyone who contributed, professionally or personally, to the realizations of this thesis.

Prof. dr. C.A.F.M. Bruijnzeel-Koomen, Beste Carla,
Nadat ik dagenlang bij jou op de stafgang heb gebivakkeerd, omdat ik zeker wist dat ik dermatoloog wilde worden, heb je mij de kans gegeven in mijn studententijd al met onderzoek in deze richting te starten. Jouw volhardende geloof dat in een ieder een onderzoeker schuilt, ondanks mijn aanvankelijke ontkenning, heeft uiteindelijk geresulteerd in dit proefschrift. Ik bewonder jouw grenzeloos enthousiasme voor de wetenschap en altijd kritische benadering. Het "L'exactitude est la politesse des rois" zal nog lang door mijn brein galmen. Ik wil je bedanken voor de kans die je me hebt gegeven en voor alle tijd en energie die je in mij hebt geinvesteerd.

Dr. E.F. Knol, Beste Edward,
In de eindsprint van dit proefschrift heb je getoond hoe grenzeloos ook jouw enthousiasme voor de wetenschap is. Ondanks dat ik altijd met meer ideeën en oude proefschriften je kamer uitkwam dan me soms lief was, heb je bij mij een blijvende interesse in het research getriggerd. Dank voor je prettige begeleiding en de translationele inzichten.

Dr. V. Sigurdsson, Beste Vigfus
De door jouw opgelegde KISS-methode (keep it simple, stupid) heeft van mijn warrige langdradigheid vaak leesbare stukken tekst gemaakt. Ik hoop dat we in de toekomst nog vaak over de semantiek van nomenclatuur mogen schermen. Ik vind het een eer om onder jouw opleiderschap mijn opleiding af te mogen ronden.

Drs. H. van Weelden, Beste Huib,
Zonder jou was dit proefschrift niet tot stand gekomen. Jouw kennis en ervaring betreffende het licht zijn van onschatbare waarde. Op jouw terrein ben je een alles(door)ziend oog, bij voorkeur op het laatste moment. Ik hoop nog vaak je kamer binnen te struinen veel licht-nuances van je te leren.

Dr. S.G.M.A. Pasmans, Beste Suzanne,
Tegen de verwachting in schreven wij een stuk de JAAD in. Je gaf mij groot inzicht ten aanzien van het promoveren. Ik ben je daarvoor zeer dankbaar. Ik hoop in de toekomst met je te blijven samenwerken, misschien zelfs wel in de kinderdermatologie.

Dr. M.S. de Bruin-Weller, Beste Marjolein,
Onder jouw begeleiding, "de moeder van de eczeem kinderen", zette ik de eerste stapjes als klinisch onderzoeker. Dat was heel prettig en ik heb veel van je geleerd, vooral ook het "meeveren". Je hebt nu een goede balans gevonden tussen werk en vrije tijd, dat werkt aanstekelijk en scherpt mijn ambities.

C. Guikers, Beste Kees,
Zonder jou geen goed gefundeerde statistiek en geen controle van de controle. Jouw ervaring in het lab en statistiek zijn onmisbaar geweest voor de analyse van onze hypothesen. Daarvoor wil ik je danken.

I. Bihari, Beste Ilse,
Altijd als ik zonder energie zat toverde jij iets eetbaars tevoorschijn, meestal zelfgemaakt. Daarnaast heb je bergen immunohistochemisch werk verzet.
Je verschijning tovert altijd een glimlach op mijn gezicht, dank voor al je inspanningen.

Dr. B. Giovannone, Dear Barbara,
The amount of work you did on the DNA damage repair rates is incredible!! The way you finally handled our staining troubles earns great respect. I greatly enjoy working with you and I hope to continue our collaboration in the future.

I. Sybesma, Beste Ina,
Al mijn patiënten zijn een keer door jouw handen gegaan, dat is een ongelooflijke prestatie. Je draagt de patiënten een warm hart toe; ik jou ook.

Drs. I. Haeck, Beste Inge,
Bijna synchroon gaan wij door ons geneeskundig traject. Samen nieuw curriculum doorlopen, samen onderzoek bij de dermatologie, samen de opleiding in, in dezelfde maand onze promoties afronden. Daarnaast deelden wij wetenschappelijk

pieken en dalen. Je bent een bijzondere vriendin. Ik hoop nog heel lang samen het geneeskundig pad te blijven bewandelen.

Drs. S.G.A. van Velsen, Beste Sara,
Dank voor je inspanningen omtrent de DNA schade analyse. Succes met het afronden van je eigen proefschrift.

Bete Maarten, Peter en Jonas,
"Popular hypothesis testing" zou een goede term zijn voor onze korte brain(be) stormende koffiepauzes. Onze wetenschappelijke vriendschap is mij zeer dierbaar.

Beste Jantine, Miranda en Sawita,
Door alle jaren heen weten jullie heel goed wat het betekent om te promoveren parallel aan de opleiding. Jullie ruim(d)en mijn logistieke imperfecties geruisloos op. Waarvoor grote dank en respect!

Beste collega AIOS, dermatologische verpleegkundigen, polisecretaresses en doktersassistentes. Een promotie traject naast de opleiding kent altijd zijn weerslag op de klinisch beschikbare tijd. Vanaf nu ben ik er weer 120% bij! Dank voor jullie tijd en begrip.

Beste onderzoekers van de dermatologie,
Dank voor alle uitleg en gezelligheid die jullie gaven, ik heb met veel plezier met jullie gewerkt.

Mijn Paranimfen, drs. Hoogwater en drs. Lee,
Hetzelfde lot zullen jullie in de toekomst ook ondergaan. Dat is een goede reden om paranimf te worden.
Beste Frederik je bent mijn beste vriendje, daar hoeft verder niets aan toegevoegd te worden: Dank.
Beste Peter, je bent mijn research buddy, neutroman van het eerste uur en vaste bewoner van labland: Dank.

Lieve Carla en Pieter, dank voor de zorgzame opvang van uw schoonzoon te allen tijde.

Lieve pappa en mamma, dank voor alle liefde die heeft geleid tot deze prestatie! Mijn respect voor jullie is groot en groeit nog steeds. 2010 is een sleuteljaar! Ik houd van jullie.

Lieve Josine,
Je bent helaas niet een van mijn paranimfen geworden, ondanks *alle* inspanning achter de schermen om dat voor elkaar te krijgen. Je bent wel mijn lieve vrouw en moeder van onze prachtige kinderen. Met jou wil ik lachen om de streken van onze stoute jongetjes, heel oud worden en dan samen achter de geraniums klagen over de jongere generaties en doen alsof we onze kinderen niet meer herkennen als ze eindelijk weer eens langskomen. Je bent alles voor me.

Curriculum Vitae

Onno ten Berge was born 26 March 1978 in Utrecht. After finishing grammar school at the Stedelijk Gymnasium Utrecht, he started studying Pharmacy at Utrecht University. After one year he changed to studying medicine at the Utrecht University. During a scientific elective period at the dermatology department of the University Medical Centre Utrecht in 2005 he started the research on UV related problems in atopic dermatitis. This work finally resulted in this thesis. In 2008 he started his dermatological residency at the department of dermatology of the UMC Utrecht.

List of abbreviations

6-4 PP	6-4 photoproduct
AD	Atopic Dermatitis
AD + PLE	Atopic dermatitis with co-existing PLE
AP	Actinic prurigo
$CD4^+$	Cluster of differentiation type 4 glycoprotein
CAD	Chronic Actinic Dermatitis
CI	Calcineurin inhibitor
CPD	Cyclobutane pyrimidine dimer
DNA	Deoxyribonucleic acid
EPP	Erythropoietic protoporphyria
HC/HV	Healthy control / volunteer
IHC	Immunohistochemical
IL	Interleukin (cytokine)
LE	Lupus erythematosus
MED	Minimal erythemal dose
NER	Nucleotide excision repair
NFAT	Nuclear factor of activated T-cells
PBS	phosphate-buffered saline
PhAD	Photosensitive Atopic Dermatitis
PLE	Polymorphic Light Eruption
PUVA	Psoralen + UVA
RT	Rothmund-Thomson syndrome
SU	Solar urticaria
T cell	Thymus cell, lymphocyte
Th2	Thymus helper cell type 2
TIM	Topical immunomodulator
TL	Tube luminescent
TNF	Tumor necrosis factor
TT-CPD	Thymine dimer
UV (A/B/R)	Ultra Violet (A/B/irRadiation)
XP	Xeroderma Pigmentosum

List of Publications

Efficacy and safety of long-term treatment with cyclosporin A for atopic dermatitis. Hijnen DJ, **ten Berge** O, Timmer-de Mik L, Bruijnzeel-Koomen CA, de Bruin-Weller MS. J Eur Acad Dermatol Venereol. 2007 Jan;21(1):85-9.

Throwing a light on photosensitivity in atopic dermatitis: a retrospective study. **ten Berge** O, van Weelden H, Bruijnzeel-Koomen CA, de Bruin-Weller MS, Sigurdsson V. Am J Clin Dermatol. 2009;10(2):119-23.

Photosensitivity testing in children. Onno **ten Berge**, MD a, Vigfus Sigurdsson, MD, PhD a, Carla A. F. M. Bruijnzeel-Koomen, MD, PhD a, Huib van Weelden, MSc a, and Suzanne G. M. A. Pasmans, MD, PhD. Journal of the American Academy of Dermatology 2010; in press and poster ESPD Lausanne 2010.

Enteric-Coated Mycophenolate Sodium versus Cyclosporine A as long-term treatment in adult patients with severe atopic dermatitis: a randomized controlled trial. I.M. Haeck, MD, M.J. Knol, PhD, O. **ten Berge**, MD, S.G.A. van Velsen, MD, M.S. de Bruin-Weller, MD, PhD, C.A.F.M. Bruijnzeel-Koomen, MD, PhD. Journal of the American Academy of Dermatology 2010, in press.

Topicale immunosuppressieve middelen bij de behandeling van constitutioneel eczeem. M.S. de Bruin-Weller, O. **ten Berge**, C.A.F.M. Bruijnzeel-Koomen. Nederlands tijdschrift voor allergie 2007;7:43-8 **&** Huisartsen editie 2007; 2:38-43.

Gram-negatieve bacteriële interdigitale infectie van de voet. O. **ten Berge**, C.J. Sanders. Nederlands tijdschrift voor dermatology en venereologie 2009.

UVB responses in atopic dermatitis patients with photosensitivity. Onno **ten Berge**, Edward F. Knol, Huib van Weelden, Marjolein S. de Bruin-Weller, Carla A. F. M. Bruijnzeel-Koomen. Submitted.

Assessment of cyclobutane pyrimidine dimers by digital photography in human skin. Onno **ten Berge**, Sara G.A. van Velsen, Barbara Giovannone, Carla A. F.

M. Bruijnzeel-Koomen, Edward F. Knol, Kees Guikers, Huib van Weelden. Poster Nederlandse vereniging voor experimentele dermatologie 2009 and Submitted.

No effect on DNA damage repair after UVB irradiation in patients with atopic dermatitis treated with topical tacrolimus. Onno **ten Berge**, Barbara Giovannone, Marjolein de Bruin-Weller, Kees Guikers, Huib van Weelden, Carla Bruijnzeel-Koomen, Edward Knol. Submitted.

Moderate correlation between quality of life and disease activity in adult patients with atopic dermatitis. I.M. Haeck, O. **ten Berge**, S.G.A. van Velsen, M.S. de Bruin-Weller, C.A.F.M. Bruijnzeel-Koomen and M.J. Knol. Submitted.

Erythropoietic Protoporphyria: Free Erythrocyte Protoporphyrin, phototesting, DNA-mutation analysis and genotype-phenotype correlation. Renske J. Schimmel MD; Jorge Frank MD, PhD Onno **ten Berge** MD; Anne-Moon van Tuyll van Serooskerken MD; Carla A.F.M. Bruijnzeel-Koomen MD, PhD; Peter M. Steijlen MD, PhD; Huib van Weelden MD, PhD; Vigfus Sigurdsson MD, PhD; Suzanne G.M.A. Pasmans MD, PhD. Submitted 2010.