Actas Dermosifiliogr. 2009;100:Supl. 2:55-8

Field Treatment of Actinic Keratoses – Focus on COX-2-Inhibitors

M. Ulrich and E. Stockfleth
Department of Dermatology, Venerology and Allergy. Charité Universitätsmedizin. Berlin

Abstract. Actinic keratoses (AK) represent the most common carcinoma *in situ* of the skin and show continuously increasing incidences worldwide. Clinically, AK occur as multiple lesions in sun-exposed areas, which has been referred to as field cancerization. Novel treatment modalities for actinic field cancerization include 3% diclofenac in 2.5% hyaluronic acid (HA). Recent investigations have gained insights in the mode of action of diclofenac in HA, showing that the induction of apoptosis is the major mode of action of this treatment.
Herein, we give an overview about actinic keratosis focusing on treatment with the COX-2 inhibitor diclofenac 3% gel and summarize current concepts of its antineoplastic mode of action.

Key words: actinic keratoses, carcinoma *in situ*, diclofenac, hyaluronic acid, apoptosis, COX-2.

TRATAMIENTO DEL CAMPO DE QUERATOSIS ACTÍNICAS: ESPECIAL INTERÉS EN LOS INHIBIDORES DE COX-2

Resumen. Las queratosis actínicas representan el carcinoma intraepidérmico más frecuente de la piel y muestran un incremento continuo de la incidencia mundialmente. Clínicamente, las queratosis actínicas aparecen de forma múltiple en áreas fotoexpuestas, lo que se conoce como campo de cancerización. Las nuevas opciones para el tratamiento del campo de cancerización actínico incluyen diclofenaco al 3% en ácido hialurónico al 2,5%. Investigaciones recientes han revelado el principal mecanismo de acción de diclofenaco, que consiste en la inducción de la apoptosis.
En este artículo aportaremos una visión de conjunto sobre las queratosis actínicas, centrándonos en el tratamiento con un inhibidor de la enzima COX-2, diclofenaco al 3%, y resumiremos los conceptos actuales sobre su mecanismo de acción antineoplásico.

Palabras clave: queratosis actínicas, carcinoma intraepidérmico, diclofenaco, ácido hialurónico, apoptosis, COX-2.

Introduction

Actinic keratoses (AK) represent the most common carcinoma *in situ* of the skin showing continuously increasing incidences worldwide[1-3]. However, as AK are not recorded in tumor registries, the real incidence remains unknown and seems to be underestimated[4]. Risk factors driving this rise in AK numbers include UV exposure and increasing numbers of older people in the population. The importance of UV radiation in skin carcinogenesis is reflected in the geographic distribution that shows a correlation of increased prevalence of AK in areas of high UV exposure[3]. Furthermore, the increase of vacational and recreational sun exposure during the past decades has contributed to the current epidemiologic developments. Other risk factors for AK include fair skin type, immunodeficiency, e.g. after organ transplantation, arsenic exposure and hereditary disorders (xeroderma pigmentosum). Immunocompromised individuals show a significant increase of AK with a 250-fold higher risk for AK and 100-fold increase for invasive squamous cell carcinoma (SCC) when compared to the normal population. Moreover, progression of AK to invasive SCC is more common in organ transplant recipients, with 40% of AKs developing into invasive SCC[5,6].

Clinically, AK appear as hyperkeratotic, rough lesions that occur on areas of chronically sun damaged skin including the scalp, face, ears, forearms and dorsum of the hands. AK have been previously referred as preneoplastic lesions, but are nowadays defined as carcinoma *in situ* of the skin[7,8].

Correspondence:
Eggert Stockfleth.
Department of Dermatology, Venerology and Allergy.
Skin Cancer Centre Charité.
Charitéplatz 1.
10117 Berlin, Germany.
eggert.stockfleth@charite.de

Histopathologically, AKs are characterized by an epidermal proliferation of atypical keratinocytes starting from the basal cell layer. Morphologically, the cells of AK and SCC may be indistinguishable. According to the epidermal involvement of dysplasia, three subtypes may be differentiated. A recent classification proposed by Roewert-Huber et al classifies AK into early *in situ* SCC type AK I (mild), early *in situ* SCC type AK II (moderate) and *in situ* SCC type AK III (severe). This classification describes the disease continuum from AK to SCC in the context of field cancerization[9].

Skin Carcinogenesis

The most important factor in the pathogenesis of AK is chronic UV exposure. UV-B radiation (290-320 nm) has been shown to directly induce DNA and RNA mutations via thymidine dimer formation. In this regard, the mutation of p53 is of special importance. This tumor suppressor gene is located on chromosome 17p132 and leads to cell cycle arrest, allowing repair of DNA damage. Dysregulation of p53 results in uncontrolled growth and proliferation of damaged keratinocytes and potentially neoplastic cells, representing an early step in the carcinogenesis of AK and SCC[10]. These mutations are detected in both AK and SCC as they occur in the early phase in the continuum from early keratinocyte dysplasia to AK and SCC[11]. Other molecular markers that may indicate an increased likelihood of malignancy include the expression of $p16^{ink4}$, CD95 ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and loss of herterozygosity[12]. These findings indicate that AK are not precancerous lesions, but represent early SCC *in situ*[9]. UV-A radiation (320-400 nm) contributes to the development of AK by inducing oxidative stress that leads to tissue damage. Furthermore, UV is known to have an immunosuppressant effect by depleting Langerhans cells in the epidermis, an effect that has also been used in the light treatment of psoriasis with PUVA therapy. Patients who have received high-dose PUVA therapy have been shown to have an increased incidence of AK and SCC[13]. Human papilloma viruses (HPV) have been proposed as a co-carcinogen in the etiopathogenesis of AK. In patients with the hereditary disorder epidermodysplasia verruciformis, the association between special cutaneous HPV types and the development of nonmelanoma skin cancer is well established. In the past decade, cutaneous HPV have also been detected in AKs, especially in organ transplant recipients. The anti-apoptotic effect of the E6 protein in HPV has been proposed as a tumor inducing factor[14,15]. However, HPV can also be detected in normal sun-exposed skin of healthy individuals[16] and the correlation between HPV and skin cancer is less strong than in cervical cancer. Therefore, it has been proposed that HPV rather acts as a co-carcinogen in skin carcinogenesis via anti-apoptotic pathways and delay of DNA repair mechanisms in response to UV radiation[17].

AK usually present as multiple lesions in areas of chronically sun-exposed skin reflecting the actinic damage of the field, an observation that has been described as field cancerization[18].

The Role of Apoptosis in Carcinogenesis

Apoptosis is defined as programmed cell death and represents a critical point in tumor cell formation. In normal cells, apoptosis leads to the ordered destruction of damaged cells via two different pathways. The extrinsic pathway is initiated by binding of cell death ligands (TNF α, TRAIL, CD 95/FasL) on cellular receptors and activation of the caspase pathway. The intrinsic pathway involves p53 and mitochondria and leads to the release of pro-apoptotic factors including cytochrome c. The mitochondrial pathway of apoptosis is controlled by pro- and anti-apoptotic Bcl-2 proteins[19]. Inactivation of apoptotic pathways leads to uncontrolled proliferation of cells and, therefore, represents a critical step in cancerogenesis. Furthermore they may be responsible for treatment resistance. Apoptosis-inducing therapeutic strategies represent an important approach in the development of effective cancer therapy. These treatment approaches aim to restore p53 activity, downregulate anti-apoptotic Bcl-2 or NF-κB and upregulate extrinsic, death receptor-mediated pathways as the main course for tumourigenesis are defects in pro-apoptotic signal pathways[20].

The Role of COX-2 in the Pathogenesis of Skin Cancer

Cyclooxygenases (COX) are enzymes which activate the release of several prostaglandins with different biological properties. Of these, prostaglandin E2 (PGE_2) represents the main mediator of inflammation and tumor growth[21,22]. Prostaglandins belong to the class of eicosanoids and are ubiquitously present in most cell types. The initial step of prostaglandin synthesis is the COX mediated synthesis of prostaglandins PGH_2, PGI_2, PGD_2, $PGH_2\alpha$ and thromboxane A_2 (TXA_2). Two different classes of COX can be distinguished, COX-1 and COX-2. Whereas COX-1 is constitutively expressed and plays a role in physiological effects such as cytoprotection of gastric mucosa or renal circulation, COX-2 is induced by pro-inflammatory cytokines, growth factors and tumour promoters including protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and NFκB[23-25]. The upregulation of COX-2 is strongly related to non physiological conditions like inflammation and cancer and high expression of COX-2 has

been demonstrated in several solid tumours including colon, lung, breast and epithelial skin cancer. In this regard, COX-2 upregulation was shown in AK, SCC and BCC[26,27].

Mode of Action of COX-2 Inhibitors in the Treatment of Actinic Keratosis

Recently, the COX-2 inhibitor diclofenac has been introduced in a formulation with hyaluronic acid (3% diclofenac in 2,5% hyaluronic acid) for the treatment of actinic keratoses. The main mode of action of this COX-2 inhibitor seems to be the induction of apoptosis, which has been demonstrated in several cancer models[19]. Furthermore, a strong correlation between COX-2 activity and expression of the anti-apoptotic protein of the Bcl-2 family has been demonstrated. Apoptosis induced by diclofenac and other non steroidal anti-inflammatory drugs (NSAIDs) involves the extrinsic pathway and activation of caspases, mainly caspase 9. Xenograft models have shown the pro-apoptotic effects of NSAIDs *in vivo*[28,29] and topical application of diclofenac 3% gel in colon adenocarcinoma xenografts in nude mice inhibited prostaglandin synthesis and increased the apoptotic index[30]. A recent study by Eberle et al[20] investigated the effects of 3% diclofenac in 2.5% hyaluronic acid (diclofenac /HA) on induction of apoptosis in four cutaneous SCC cell lines (SCL-1, SCL-II, SCC-12 and SCC-13) derived from SCC lesions of the face, and three cultures of normal human keratinocytes (NHK). Apoptosis was significantly induced by diclofenac /HA in three of four SCC cell lines (SCL-II, SCC-12 and SCC-13) and activation of the caspase cascade was shown. Furthermore, the study showed that diclofenac was responsible for the induction of apoptosis, whereas hyaluronic acid did not show any anti-apoptotic effects. Hyaluronic acid is a high molecular polysaccharide chain, which is part of the normal extracellular matrix. In diclofenac 3% gel, it may provide a more sustained delivery of diclofenac to the skin cells. Furthermore, it has been shown that HA binds the CD44 receptor of keratinocytes which may lead to an increased bioavailability of diclofenac within the epidermis, resulting in prolongation of its half-life[31].

Besides the induction of apoptosis as the main mode of action, other mechanisms seem to be involved in the antitumoral effectivity of NSAIDs such as antiangiogenesis and direct inhibition of tumor cell proliferation[32,33].

Treatment of Actinic Keratosis with 3% Diclofenac in Hyaluronic Acid

Several randomized, double-blind, HA gel vehicle-controlled clinical studies have evaluated the efficacy of topical diclofenac HA gel in patients with AK. The 30-day interval between the end of treatment and the evaluation of efficacy was due to earlier findings stating a significant advantage for diclofenac HA gel over placebo, when efficacy was evaluated 4 weeks after the end of treatment. The product significantly reduced lesions when applied for 60 or 90 days, however, the efficacy was significantly increased after 90 days of application. A double-blind, randomized, placebo-controlled multicenter study showed response rates of 79% (treatment group) versus 45% in the placebo group; a complete healing was seen in 50% (treatment group) versus 20% in the control group ($p < 0{,}001\%$)[34]. Other controlled studies showed similar effects[35,36].

Adverse effects were skin related and mild to moderate in severity (pruritus, erythema, dry skin, hypo and paresthesia). Systemic bioavailability of diclofenac was demonstrated to be considerably lower after topical application than after systemic administration and the drug demonstrated a good safety profile.

Recent investigations have shown the efficacy of 3% diclofenac in HA for the treatment of actinic keratosis in organ transplant recipients with response rates comparable to those seen in the immunocompetent population, and an excellent safety profile[37]. Furthermore, diclofenac 3% gel has also shown efficacy in the treatment of actinic cheilitis. A recently published case series showed histological clearance in 4/6 patients[38].

Conclusion

Actinic keratoses are currently defined as carcinoma *in situ* of the skin and represent a continuum from keratinocyte dysplasia to invasive squamous cell carcinoma. Diclofenac 3% gel is a novel treatment approach for field cancerization which has shown efficacy for AK in several studies. Recent insight in the mode of action of diclofenac 3% gel indicates that apoptosis represents the major mode of action of this treatment. The efficacy of diclofenac 3% gel has also been shown for AK in organ transplant recipients and for the treatment of actinic cheilitis.

Conflict of interest
Authors have no conflict of interest to declare.

References

1. Memon AA, Tomenson JA, Bothwell J, Friedmann PS. Prevalence of solar damage and actinic keratosis in a Merseyside population. Br J Dermatol. 2000;142:1154-9.
2. Salasche SJ. Epidemiology of actinic keratoses and squamous cell carcinoma. J Am Acad Dermatol. 2000;42:4-7.
3. Frost C, Williams G, Green A. High incidence and regression rates of solar keratoses in a queensland community. J Invest Dermatol. 2000;115:273-7.

4. Trakatelli M, Ulrich C, del Marmol V, Euvrard S, Stockfleth E, Abeni D. Epidemiology of nonmelanoma skin cancer (NMSC) in Europe: accurate and comparable data are needed for effective public health monitoring and interventions. Br J Dermatol. 2007;156 Suppl 3:1-7.
5. Stockfleth E, Ulrich C, Meyer T, Christophers E. Epithelial malignancies in organ transplant patients: clinical presentation and new methods of treatment. Recent Results Cancer Res. 2002;160:251-8.
6. Ulrich C, Christophers E, Sterry W, Meyer T, Stockfleth E. [Skin diseases in organ transplant patients]. Hautarzt. 2002; 53:524-33.
7. Ackerman AB. Solar keratosis is squamous cell carcinoma. Arch Dermatol. 2003;139:1216-7.
8. Heaphy MR Jr, Ackerman AB. The nature of solar keratosis: a critical review in historical perspective. J Am Acad Dermatol. 2000;43:138-50.
9. Röwert-Huber J, Patel MJ, Forschner T, Ulrich C, Eberle J, Kerl H, et al. Actinic keratosis is an early in situ squamous cell carcinoma: a proposal for reclassification. Br J Dermatol. 2007;156 Suppl 3:8-12.
10. Park WS, Lee HK, Lee JY, Yoo NJ, Kim CS, Kim SH. P53 mutations in solar keratosis. Hum Pathol. 1996;27: 1180-4.
11. Kushida Y, Miki H, Ohmori M. Loss of herterozygosity in actinic keratosis, squamous cell carcinoma and sun-exposed normal appearing skin: difference between Japanese and Caucasians. Cancer. 1999;140:169-75.
12. Nelson MA, Einspahr JG, Alberts DS, Balfour CA, Wymer JA, Welch KL, et al. Analysis of the p53 gene in human precancerous actinic keratosis lesions and squamous cell cancers. Cancer Lett. 1994;85:23-9.
13. Chuang TY, Heinrich LA, Schultz MD, Reizner GT, Kumm RC, Cripps DJ. PUVA and skin cancer. A historical cohort study on 492 patients. J Am Acad Dermatol. 1992;26 2 Pt 1: 173-7.
14. Jackson S, Storey A. E6 proteins from diverse cutaneous HPV types inhibit apoptosis in response to UV damage. Oncogene. 2000;19:592-8.
15. Jackson S, Harwood C, Thomas M, Banks L, Storey A. Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. Genes Dev. 2000;14: 3065-73.
16. Hazard K, Karlsson A, Andersson K, Ekberg H, Dillner J, Forslund O. Cutaneous human papillomaviruses persist on healthy skin. J Invest Dermatol. 2007;127:116-9.
17. Nindl I, Gottschling M, Stockfleth E. Human papillomaviruses and non-melanoma skin cancer: basic virology and clinical manifestations. Dis Markers. 2007;23:247-59.
18. Braakhuis BJM, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. Cancer Res. 2003;63:1727-30.
19. Fecker LF, Stockfleth E, Nindl I, Ulrich C, Forschner T, Eberle J. The role of apoptosis in therapy and prophylaxis of epithelial tumours by nonsteroidal anti-inflammatory drugs (NSAIDs). Br J Dermatol. 2007;156 Suppl 3:25-33.
20. Eberle J, Fecker LF, Forschner T, Ulrich C, Röwert-Huber J, Stockfleth E. Apoptosis pathways as promising targets for skin cancer therapy. Br J Dermatol. 2007;156 Suppl 3:18-24.
21. Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. Inflamm Res. 1995;44:1-10.
22. Wang D, DuBois RN. Prostaglandins and cancer. Gut. 2006; 55:115-22.
23. DuBois RN, Abrahamson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, et al. Cyclooxygenase in biology and disease. FASEB J. 1998;12:1063-73.
24. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular and molecular biology. Ann Rev Biochem. 2000;69:145-82.
25. Yu W, Murray NR, Weems C, Chen L, Guo H, Ethridge R, et al. Role of Cyclooxygenase 2 in protein kinase C beta II-mediated colon carcinogenesis. J Biol Chem. 2003;278: 11167-74.
26. Muller-Decker K. [Cyclooxegenases in the skin]. J Dtsch Dermatol Ges. 2004;2:668-75.
27. Nijsten T, Colpaert CG, Vermeulen PB, Harris AL, Van Marck E, Lambert J. Cyclooxegenase-2 expression and angiogenesis in squamous cell carcinoma of the skin and its precursors: a paired immunohistochemical of 35 cases. Br J Dermatol. 2004;151:837-45.
28. Johnsen JI, Lindskog M, Ponthan F, Pettersen I, Elfman L, Orrego A, et al. Cyclooxygenase-2 is expressed in neuroblastoma, and nonsteroidal anti-inflammatory drugs induce apoptosis and inhibit tumor growth *in-vivo*. Cancer Res. 2004;64:7210-15.
29. Johnsen JI, Lindskog M, Ponthan F, Pettersen I, Elfman L, Orrego A, et al. NSAIDs in neuroblastoma therapy. Cancer Lett. 2005;228:195-2001.
30. Seed MP, Brown JR, Freemantle CN, Papworth JL, Colville-Nash PR, Willis D, et al. The inhibition of colon-26 adenocarcinoma development and angiogenesis by topical diclofenac in 2.5 % hyaluran. Cancer Res. 1997;57: 1625-9.
31. Wang C, Tammi M, Tammi R. Distribution of hyaluran and its CD 44 receptor in the epithelia of human skin appendages. Histochemistry. 1992;98:105-12.
32. Jain RK. Tumor angiogenesis and accessibility: role of vascular endothelial growth factor. Semin Oncol. 2002;29: 3-9.
33. Gallo O, Franchi A, Magnelli L, Sardi I, Vannacci A, Boddi V, et al. Cyclooxygenase pathway correlates with VEGF expression in head and neck cancer. Implications for tumour angiogenesis and metastasis. Neoplasia. 2001; 3:53-61.
34. Rivers JK, Arlette J, Shear N, Guenther L, Carey W, Poulin Y. Topical treatment of actinic keratoses with 3.0% diclofenac in 2,5 % hyaluronan gel. Br J Dermatol. 2002;146: 94-100.
35. Gebauer K, Brown P, Varigos G. Topical diclofenac in hyaluronan gel for the treatment of solar keratoses. Austr J Dermatol. 2003;44:40-5.
36. Wolf JE Jr, Taylor JR, Tschen E, Kang S. Topical 3,0 % diclofenac in 2,5 % hyaluronan gel in the treatment of actinic keratoses. Int J Dermatol. 2001;40:709-13.
37. Ulrich C, Hackethal M, Ulrich M, Howorka A, Forschner T, Sterry W, et al. Treatment of multiple actinic keratosis with topical diclofenac 3 % gel (Solaraze™) in organ transplant recipients: a series of 6 cases. Br J Dermatol. 2007;156 Suppl 3:40-2.
38. Ulrich C, Forschner T, Ulrich M, Stockfleth E, Sterry W, Termeer C. Management of actinic cheilitis using diclofenac 3 % gel: a report of six cases. Br J Dermatol. 2007;156 Suppl 3:43-6.