LETTER TO THE EDITOR

Actinic keratoses treated with cold atmospheric plasma

Editor

Plasma is a partially or fully ionized gaseous state of matter containing chemically active species, such as ions, electrons, photons, reactive oxygen and nitrogen species, and UV light. Cold atmospheric plasma (CAP) can be applied onto vital, heat-sensitive surfaces and is currently used in surgery, endoscopic procedures, and wound care with proven antimicrobial efficacy and the ability to deactivate pathogens, stop bleeding and stimulate cell proliferation.[1,2] Recent preclinical observations suggested an antitumoral potential, for example in melanoma, glioma and colorectal carcinoma cells.[3,4] Hitherto, harmful clinical effects have not been reported, and *in vitro* studies did not see increased genotoxicity in cultured cells after repetitive argon plasma treatment.[5,6]

AK typically appears as singular lesions or field cancerization in chronically sun-exposed fair-skinned individuals with a reported prevalence of 11–26%.[7] This intraepidermal keratinocytic dysplasia may transform into invasive squamous cell carcinoma (SCC) in up to 20% within 10–25 years.[8] Although various therapy options exist, they often bear undesirable side-effects.[7] To see whether CAP could be an effective therapy option, we treated seven patients (two females, five males) with Fitzpatrick skin types II or III and clinically diagnosed AK in this proof of concept study. Written informed consent was obtained after detailed explanation according to institutional standards. In each patient, a circular area of 19.64 cm^2 containing multiple AK was defined, photo-documented, and the lesions were classified according to Olsen grading: (I) slightly palpable, better felt than seen; (II) moderately thick, easily felt and seen; (III) very thick and hyperkeratotic. In patient number 6, two areas were treated. Seven CAP treatments per patient were performed with a CE-certified microwave-driven argon plasma jet (Adtec Steri-Plas™). It produces non-thermal plasma below 40°C, which was applied twice a week, 120 s per treatment. No concomitant AK-therapy was used. Photo-documentation in steady conditions was performed before, during and after treatments (Fig. 1). All patients showed promising responses after seven applications. Clinical downgrading according to Olsen, including a decline of AK characteristics such as erythema, scaling, crusts and thickness, was found in all treated areas. In six areas, the total lesion number decreased (Table 1). No adverse events, commonly observed for other AK treatments, such as pain or inflammation, were seen.[8] Thus, unlike other common AK therapies, CAP might not be limited to a maximum treatment area (e.g. 5% imiquimod is limited to 25 cm^2) and could be an attractive option for patients with extensively sun-damaged skin or even immune-compromised patients. Additionally, rejuvenating effects similar to that reported in dermal remodelling procedures like photodynamic therapy might be ascribed to CAP. A refined complexion, discrete reduction of hyper or mottled pigmentation and palpatory firmer skin were observed in patients 3, 4, 6 (site no. 1) and 7.

The biological mode of action regarding the antitumorigenic potential of CAP remains unclear. Effects on the cell cycle and induction of senescence, apoptosis and necrosis by reactive nitrogen and oxygen species have been discussed.[3,4] Interestingly, the tumour-selectivity and apoptotic effect of CAP are especially high in p53-mutated cancer cells.[9] Dysregulation of the p53 pathway plays an important role in inducing clonal keratinocytic expansion in AK and SCC.[7] As human papilloma viruses are considered cocarcinogenic in AK and SCC development,[7] and infected keratinocytes frequently express HPV

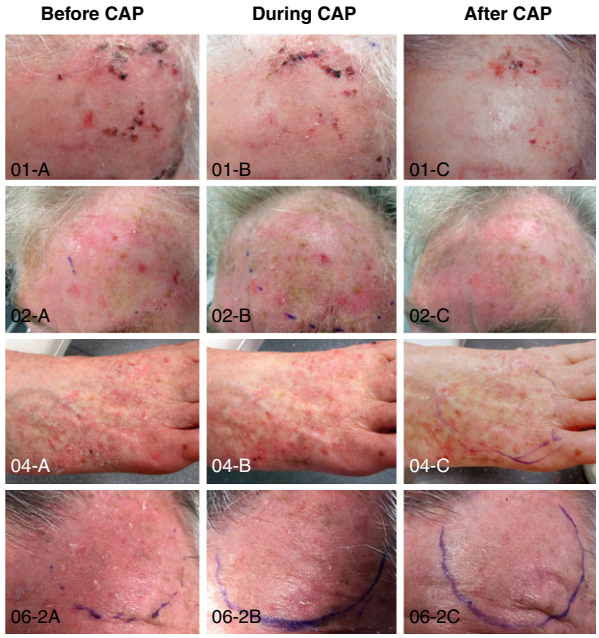

Figure 1 Cold atmospheric argon plasma treatment of actinic keratoses. Clinical images of four patients (01, 02, 04 and 06-2) with actinic keratoses prior to (1st column), during (after three CAP treatments, 2nd column) and after seven CAP treatments (3rd column).

Table 1 Lesion count and clinical observations

Patient	Olsen Grade	No. at baseline	No. after treatment	Total change from baseline No. (%)	Clinical observations
01	I	4	14	+10 (+250.00)	• Massive haemorrhagic crusts at baseline, decrease during treatments (cf. Fig. 1/01)
	II	5	5	0 (0.00)	
	III	19	1	−18 (−94.74)	
	Total	28	20	−8 (−28.57)	
02	I	1	4	+3 (+300.00)	• Decrease of erythematous maculae and plaques (cf. Fig. 1/02)
	II	5	2	−3 (−60.00)	
	III	0	0	0 (0.00)	
	Total	6	6	0 (0.00)	
03	I	3	6	+3 (+100.00)	• Refined complexion • Reduction of hyperpigmentation • Reduction of hyperkeratosis
	II	8	7	−1 (−12.50)	
	III	2	0	−2 (−100.00)	
	Total	13	13	0 (0.00)	
04	I	8	22	+14 (+175.00)	• Reduction of hyperkeratosis • Firmer complexion (cf. Fig. 1/04)
	II	21	6	−15 (−71.43)	
	III	3	0	−3 (−100.00)	
	Total	32	28	−4 (−12.5)	
05	I	2	7	+5 (+250.00)	• Reduction of hyperpigmentation
	II	7	1	−6 (−85.71)	
	III	0	0	0 (0.00)	
	Total	9	8	−1 (−11.11)	
06-1	I	2	2	0 (0.00)	• Firmer skin • Reduction of scaling
	II	4	2	−2 (−50.00)	
	III	1	0	−1 (−100.00)	
	Total	7	4	−3 (−42.86)	
06-2	I	2	4	+2 (+100.00)	• Reduction of scaling (cf. Fig. 1/06-2)
	II	13	4	−9 (−69.23)	
	III	0	0	0 (0.00)	
	Total	15	8	−7 (−46.67)	
07	I	2	1	−1 (−50.00)	• Refined complexion • Reduction of hyperpigmentation • Reduction of scaling
	II	3	0	−3 (−100.00)	
	III	0	0	0 (0.00)	
	Total	5	1	−4 (−80.00)	

oncoproteins, virocide properties of CAP might work synergistically.[10]

In conclusion, even though there exists a variety of AK therapies, CAP might represent a novel and safe treatment because of its lack of side-effects. Certainly, the small case number reported here requires additional research in a standardized, prospective clinical trial, especially concerning application schemes, the biological mode of action and long-term follow-up.

Adtec provided the CAP instrument without further financial compensation.

M. Wirtz,[1,2] I. Stoffels,[1,2] J. Dissemond,[1,2] D. Schadendorf,[1,2] A. Roesch[1,2,*]

[1]Department of Dermatology, University Hospital Essen, Hufelandstr. 55, 45122, Essen, Germany, [2]German Cancer Consortium (DKTK), Essen, Germany

*Correspondence: A. Roesch. E-mail: alexander.roesch@uk-essen.de

References

1 Fridman G, Friedman G, Gutsol A, *et al.* Applied plasma medicine. *Plasma Processes Polym* 2008; **5**: 503–533.
2 Isbary G, Shimizu T, Li YF, *et al.* Cold atmospheric plasma devices for medical issues. *Expert Rev Med Devices* 2013; **10**: 367–377.
3 Vandamme M, Robert E, Lerondel S, *et al.* ROS implication in a new antitumor strategy based on non-thermal plasma. *Int J Cancer* 2012; **130**: 2185–2194.
4 Keidar M, Walk R, Shashurin A, *et al.* Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. *Br J Cancer* 2011; **105**: 1295–1301.
5 Wende K, Bekeschus S, Schmidt A, *et al.* Risk assessment of a cold argon plasma jet in respect to its mutagenicity. *Mutat Res, Genet Toxicol Environ Mutagen* 2016; **798–799**: 48–54.
6 Maisch T, Bosserhoff AK, Unger P, *et al.* Investigation of toxicity and mutagenicity of cold atmospheric argon plasma. *Environ Mol Mutagen* 2017; **58**: 172–177.
7 Werner RN, Stockfleth E, Connolly SM, *et al.* Evidence- and consensus-based (S3) Guidelines for the Treatment of Actinic Keratosis - International League of Dermatological Societies in cooperation with the

European Dermatology Forum – Short version. *J Eur Acad Dermatol Venereol* 2015; **29**: 2069–2079.
8 Vegter S, Tolley K. A network meta-analysis of the relative efficacy of treatments for actinic keratosis of the face or scalp in Europe. *PLoS ONE* 2014; **9**: e96829.
9 Ma Y, Ha CS, Hwang SW, *et al.* Non-thermal atmospheric pressure plasma preferentially induces apoptosis in p53-mutated cancer cells by activating ROS stress-response pathways. *PLoS ONE* 2014; **9**: e91947.
10 Weiss M, Daeschlein G, Kramer A, *et al.* Virucide properties of cold atmospheric plasma for future clinical applications. *J Med Virol* 2017; **89**: 952–959.

DOI: 10.1111/jdv.14465