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Clinical and Experimental Dermatology

Assessment of the efficacy of a new formulation for plantar wart mummification: new experimental design and human papillomavirus identification

C. Viennet,[1] T. Gheit,[2] P. Muret,[1,3] F. Aubin,[4] J. Cabou,[5] A. Marchal,[5] M. Tommasino[2] and P. Humbert[1,4]

[1]*Engineering and Cutaneous Biology Laboratory, INSERM UMR1098, SFR FED 4234, University of Franche-Comte, Besançon, France;* [2]*International Agency for Research on Cancer, Lyon, France; Departments of* [3]*Clinical Pharmacology,* [4]*Dermatology, University Hospital, Besançon, France; and* [5]*Auriga International, Braine-L'Alleud, Belgium*

doi:10.1111/ced.12025

Summary

Cutaneous warts are caused by infection of the epidermis with human papillomavirus (HPV). Cryotherapy using liquid nitrogen is one of the most common local treatments. In this study, we used a novel *ex vivo* approach to compare the efficacy of a new product with conventional liquid-nitrogen cryotherapy by studying epidermal histology and assessing the presence of HPV types 1 and 2 DNA in plantar warts. The studied formulation, which acts by tissues mummification, is a combination of nitric acid, organic acids and metallic salts. We found that, similar to liquid nitrogen, the studied product induced alterations in the wart structure. In addition, unlike liquid nitrogen, this product also reduced the amount of HPV DNA. The results suggest that there is a poor correlation between the histological response and the antiviral efficacy of standard wart treatment.

Warts are the cutaneous presentation of human papillomavirus (HPV). They may exist in different forms, depending on the epithelial surface involved and the HPV type responsible for the infection.[1] Currently, the most frequently detected HPV types in cutaneous warts are types 1, 2, 3, 4, 10, 27 and 57.[2,3] In benign warts, the virus infects the basal layer of the epidermis, and, as keratinocytes differentiate and migrate to the suprabasal layer, they are triggered to undergo replication and maturation.[4] Active HPV replication stimulates keratinocyte proliferation and contributes to the development of cutaneous excrescences. The structure of warts (i.e. whether they show hyperkeratosis, parakeratosis and papillomatosis), depends upon the clinical morphology and location. In benign warts, koilocytes with pyknotic small nuclei surrounded by a halo and clear cytoplasm are present, characteristic of HPV infection. This abnormal epithelial histology is associated with hyperproliferation and changes in keratin expression.[5]

Correspondence: Dr Céline Viennet, Engineering and Cutaneous Biology Team, University of Franche-Comte, 19 Rue Ambroise Paré, Les Hauts de Chazal, Besançon 25000, France
E-mail: celine.viennet@univ-fcomte.fr

Conflict of interest: This work was supported by Auriga International.

Accepted for publication 7 June 2012

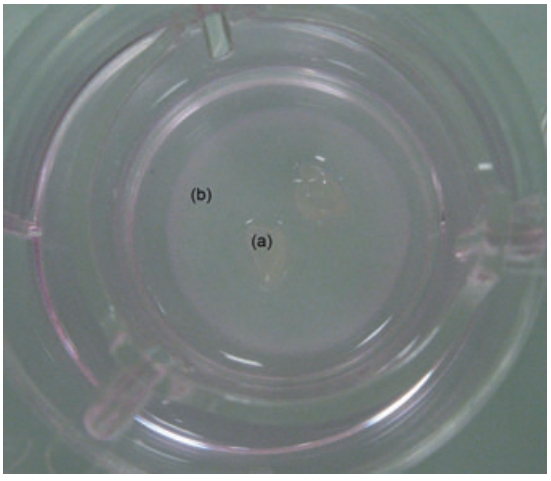

Figure 1 Illustration of wart fragments (a) placed at the air-liquid interface on three-dimensional fibroblast collagen matrix, and (b) cultured in an insert for six-well plates.

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Table 1 Primers used for PCR assays.

Primer	Direction	Sequence 5′ 3′	Size, bp
HPV_1 L1	Forward	GTCTGGTTACCAGCGCAG	314
	Reverse	CTACCTATCTCTATCCCTCTT	
HPV_2 E1	Forward	CCCTAATGATAACAACCAACAC	371
	Reverse	GACTCGTGTCATTCCACATT	

HPV, human papillomavirus.

A wide range of local treatments is available for treating warts, but no single therapy has proven to be effective in achieving complete remission in all patients.[6,7] Most of the trials on local treatments of cutaneous warts were strongly dependent on the methods used. However, the two most common treatments showing a beneficial effect are cryotherapy with liquid nitrogen and topical formulations containing salicylic acid.[8]

The aim of this study was to evaluate *ex vivo* the efficacy of a new product (a combination of nitric acid, organic acids and metallic salts) by studying the epidermal histology and the presence of HPV types 1 and 2, and to compare the efficacy of this treatment with that of liquid nitrogen.

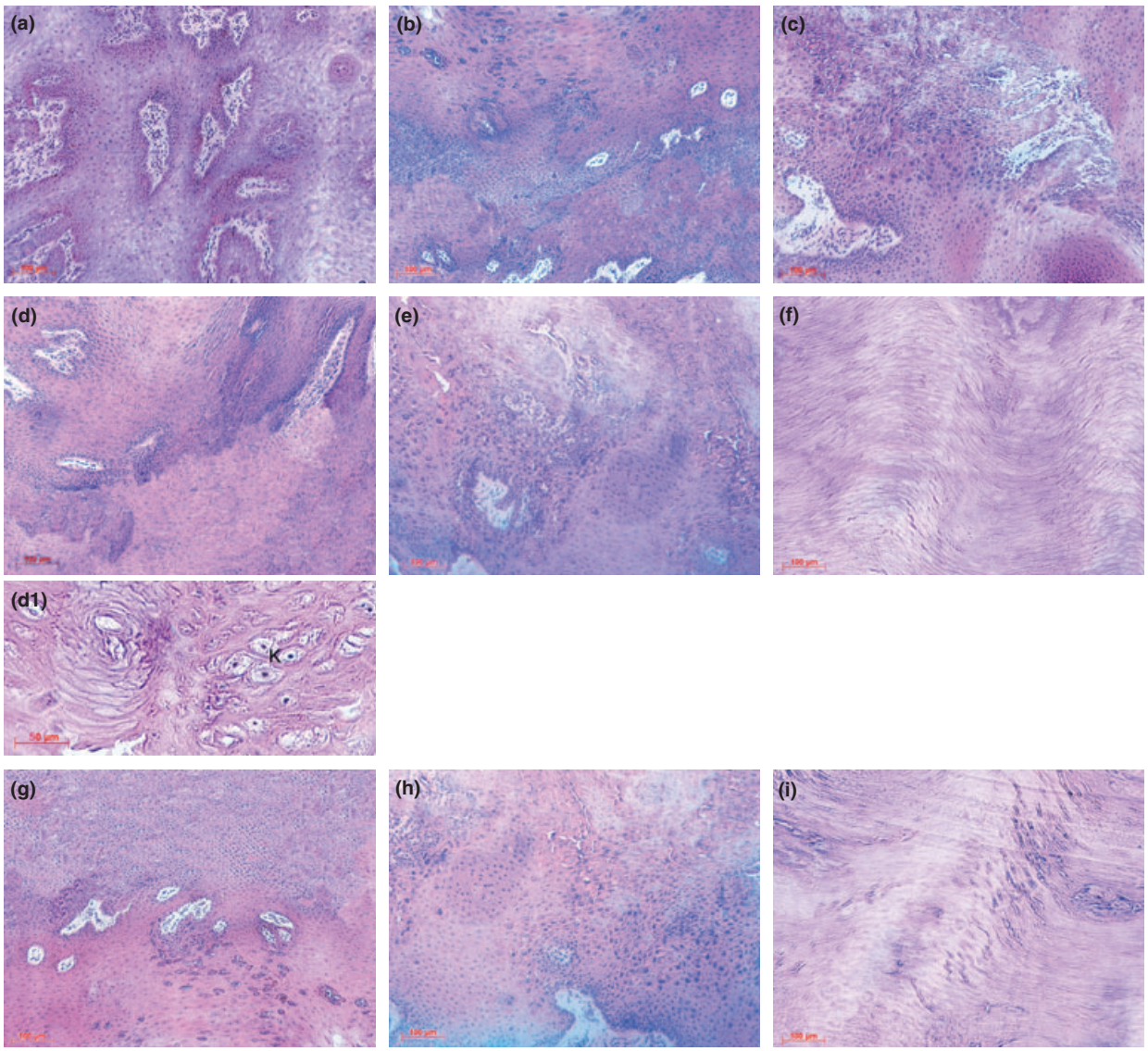

Figure 2 Wart number 9. Wart fragment: (a–c) untreated at (a) baseline, (b) week 1 and (c) week 2; (d–f) treated with the formulation at (d) baseline, (e) week 1 and (f) week 2; and (g–i) treated with liquid nitrogen at (g) baseline, (h) week 1 and (i) week 2. Haematoxylin and eosin, original magnification × 10. K, koilocytes.

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Table 2 HPV 1, HPV2 and β-globin DNA detection in plantar warts from 10 patients.

Wart no.	Time		HPV1	HPV2	ββ-globin
1	Baseline	Untreated	+	–	+
		LN	+	–	+
		Formulation	+	–	+
	Week 1	Untreated	+	–	+
		LN	+	–	+
		Formulation	+	–	+
	Week 2	Untreated	+	–	+
		LN	+	–	+
		Formulation	+	–	+
2	Baseline	Untreated	+	+	+
		LN	+	+	+
		Formulation	+	+	+
	Week 1	Untreated	+	+	+
		LN	+	+	+
		Formulation	+	+	+
	Week 2	Untreated	+	+	+
		LN	+	+	+
		Formulation	+	+	+
3	Baseline	Untreated	+	+	+
		LN	+	+	+
		Formulation	–*	–*	+
	Week 1	Untreated	+	+	+
		LN	+	+	+
		Formulation	–*	–*	+
	Week 2	Untreated	+	+	+
		LN	+	+	+
		Formulation	–*	–*	+
4	Baseline	Untreated	+	+	+
		LN	+	+	+
		Formulation	+	+	+
	Week 1	Untreated	+	+	+
		LN	+	+	+
		Formulation	+	+	+
	Week 2	Untreated	+	+	+
		LN	+	+	+
		Formulation	+	–*	+
5	Baseline	Untreated	+	+	+
		LN	+	+	+
		Formulation	+	+	+
	Week 1	Untreated	+	+	+
		LN	+	+	+
		Formulation	–*	–*	+
	Week 2	Untreated	+	+	+
		LN	+	+	+
		Formulation	–	–	+
6	Baseline	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 1	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 2	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+

Table 2. (continued).

Wart no.	Time		HPV1	HPV2	ββ-globin
7	Baseline	Untreated	+	+	+
		LN	+	+	+
		Formulation	–*	+	+
	Week 1	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 2	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
8	Baseline	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 1	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 2	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
9	Baseline	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 1	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 2	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	–*	+
10	Baseline	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	+	+
	Week 1	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	–*	+
	Week 2	Untreated	–	+	+
		LN	–	+	+
		Formulation	–	–*	+

HPV, human papillomavirus; LN, liquid nitrogen. β-globin was used as the reference gene. Antiviral effect.*

Report

In total, 10 plantar warts were excised from 10 different patients, and were placed on a sterile compress soaked with physiological saline. Each of the 10 warts was cut into 9 fragments: 3 fragments were treated with the experimental product (Verrutop®; Auriga International, Braine-l'Alleud, Belgium), 3 fragments were treated with liquid nitrogen, and the remaining 3 fragments were not treated. The formulation and liquid nitrogen were applied for 30 s with a cotton bud at different times [time 0 (baseline), 1 week and 2 weeks]. Two hours after each application, one fragment was kept for histological analysis and DNA extraction, while the others were placed at the air–liquid interface on three-dimensional fibroblast colla-

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gen matrices cultured in cell inserts with Dulbecco modified Eagle medium at 37 °C in 5% CO_2 (Fig. 1).

For histological examination, the wart-tissue samples were fixed and stained with haematoxylin and eosin.

For detection of HPV, DNA samples were sent to the International Agency for Research on Cancer (Lyon, France). DNA extraction was performed (BioRobot EZ1 with the EZ1 DNA tissue kit and EZ1 DNA tissue card; Qiagen, Hilden, Germany), and the DNA quality of samples was checked by amplifying a fragment of the housekeeping β-globin gene. PCR was performed with a commercial kit (HotstarTaq; Qiagen), in accordance with the manufacturer's instructions. The presence of HPV1 and 2 DNA was determined by PCR using HPV type-specific primers (Table 1).[9] The PCR amplification products were separated by electrophoresis in 2% agarose gels.

In our study, the collagen-feeder cell matrix maintained the typical plantar wart histology for 14 days; beyond this time, signs of damaged wart structure appeared. On histological examination of untreated wart fragments under light microscopy, histological features characteristic of HPV-associated lesions were seen. The structure was completely disorganized with strongly staining areas of hyperkeratosis, and a granular layer with large koilocytes. This histology did not change over time (Fig. 2a–c). After one and two applications of the studied product, hyperkeratosis was still visible, with large numbers of stained nuclei (Fig. 2d,e). After the third application of the formulation, the staining decreased, and there were no visible keratinocyte nuclei present (Fig. 2f). There was also a decrease in the thickness of the wart fragment. A similar pattern was seen in the wart fragments treated with liquid nitrogen (Fig. 2g–i).

The DNA typing results showed that all samples were positive for β-globin, indicating successful PCR amplification and good DNA quality (Table 2). The PCR products also indicated an effect of the studied formulation in 60% of cases, which was independent of the application number. HPV DNA was not detectable in warts 3, 5, 9 and 10, while in warts 4 and 7, the studied product had a partial antiviral effect in that one of the HPV DNA types disappeared. The PCR products showed no effect of liquid nitrogen, as there was no HPV DNA regression in any patient.

Liquid nitrogen is known to have an effect on wart clearance through necrotic destruction of HPV-infected keratinocytes.[10] With the new formulation, the specific combination of ingredients uses an oxidoreduction mechanism, resulting in the preservation of an active concentration of nitrites in the formulation, and this produces a chemical fixation with protein denaturation by nitric acid and maintenance of the tissue structure, but also produces discoloration and mummification of the treated area.

In conclusion, our study confirms that application of both this new formulation and of liquid nitrogen induces damage to the plantar wart structure. However, application of the studied product reduced the amount of HPV DNA, whereas liquid nitrogen did not induce HPV DNA regression. The persistence of HPV DNA after liquid nitrogen might be due to insufficient keratinocyte necrosis and modulation of cytokine expression in skin.[11] Our study suggestes that there is a poor correlation between the histological response and the antiviral efficacy of standard wart treatments. The new formulation seems to be an effective treatment for plantar warts, resulting in thinning of the tissue and histological and DNA changes.

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