No effect of topical digoxin and furosemide gel for patients with external anogenital warts

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Editor

Anogenital warts (AGW) are caused by low-risk HPV types. Current therapies for AGW have notable side-effects and high recurrence rates.[1] As DNA viruses such as HPV rely on cellular K+ influx and ionic contra-viral therapy (ICVT) comprised of digoxin and furosemide inhibits the K+ influx, ICVT might be a potential new treatment for AGW. Recently, a phase 2 randomized controlled trial showed a reduction in size and viral load of HPV-induced cutaneous warts after 6 weeks of treatment with topical ICVT.[2] A randomized, controlled trial was performed from October 2017 until July 2018 to assess safety and tolerability and explore pharmacodynamics and clinical efficacy of ICVT in patients with AGW. Twenty-four patients with at least three external AGW were randomized to either ICVT or placebo (ratio 3 : 1) and administered the gel once daily for 42 consecutive days. To assess safety and tolerability, laboratory safety testing was performed and adverse events, vital signs and ECGs were monitored. Clinical efficacy was assessed by lesion count and dimensions, measurement of viral load, HPV expression and histology. ICVT was well tolerated as there were no clinically relevant safety findings and no serious adverse events. All adverse events ($N = 17$) were of mild severity and self-limiting. No between-group differences in lesion count, dimensions, viral load and HPV6 expression were observed after treatment (Table 1). This demonstrates that ICVT has a favourable safety profile in patients with AGW, but that no pharmacological nor clinical activity occurred upon the once daily administration of ICVT for 42 consecutive days.

Our findings are inconsistent with the proven 6-week reductions in wart size and viral load in cutaneous warts.[2] As cutaneous warts are hyperkeratotic lesions often associated with callus growth, while AGW are commonly more smooth lesions, it is reasonable to think that the uptake and delivery of the drug in AGW are not responsible for the lack of efficacy. Two explanations can now be given for the lack of pharmacological and clinical activity of ICVT in our study. One explanation might be the difference in biological properties of the HPV type that causes AGW (HPV6) and cutaneous warts (HPV2, HPV27 and HPV57). Both are members of a different species within the alpha-papillomavirus group.[3] In plantar warts, the HPV genotype has been found to influence natural history and treatment response.[4] Clinical practice shows that not all treatments effective in cutaneous warts are also effective in AGW and vice versa.[5–7] On the other hand, ICVT showed to inhibit other viruses such as herpes simplex and varicella zoster which makes

Table 1 Clinical efficacy of ICVT compared to placebo

Assessment		AGW			
		Predose	EOT†	EOS‡	*P*-value
Lesion count – mean (SD)	ICVT	15 (12.7)	15 (12.2)	9.9 (8.1)	0.89
	Placebo	12.8 (5.9)	13.3 (7.4)	6.6 (4.5)	
Long diameter in mm – mean (SD)	ICVT	5.1 (2.7)	5.5 (3.0)	4.1 (3.5)	0.49
	Placebo	6.4 (1.8)	6.5 (1.7)	5.9 (3.8)	
Short diameter in mm – mean (SD)	ICVT	3.1 (1.8)	3.5 (1.9)	2.6 (2.1)	0.84
	Placebo	3.9 (1.4)	3.7 (1.4)	3.4 (2.3)	
Height in mm – mean (SD)	ICVT	1.8 (2.2)	1.7 (2.6)	1.6 (2.9)	0.43
	Placebo	1.6 (0.6)	1.8 (1.3)	1.1 (0.7)	
Viral load swab in LN copies/μL – mean (SD)	ICVT	2.1 (5.1)	2.4 (4.8)	1.8 (4.4)	0.68
	Placebo	2.7 (4.9)	3.3 (2.1)	−0.3 (5.0)	
Relative HPV6 E6 expression biopsy – mean (SD)	ICVT	0.41 (0.05)	0.34 (0.12)	0.68 (0.08)	0.27
	Placebo	0.90 (0.06)	0.74 (0.06)	0.08 (0.001)	
Histology	ICVT	AGW 11/18 Other 7/18§ Normal 0/18	AGW 8/18 Other 10/18§ Normal 0/18	AGW 13/18 Other 2/18§ Normal 0/18 No biopsy 3/18¶	–
	Placebo	AGW 6/6 Other 0/6 Normal 0/6	AGW 3/6 Other 3/6§ Normal 0/6	AGW 5/6 Other 1/6§ Normal 0/6	

†After 6 weeks of treatment. ‡After 12 weeks of follow-up. §Other = seborrhoeic verruca, fibro-epithelial polyp, hyperkeratotic papilloma or reactive changes. However, all biopsies taken predose were HPV-positive. ¶Three patients refused the biopsy at the end of study.
EOS, end of study; EOT, end of treatment; ICVT, ionic contra-viral therapy.

it less plausible that the type of HPV influences this process.[8] A second explanation might be related to treatment resistance. In the current study, 79% of patients had undergone a minimum of one previous treatment for AGW and 50% had undergone 2–6 different previous treatments indicating treatment resistance. Knowing that warts were generally present for a long time (median 3.8 years), it can therefore be anticipated that ICVT could have shown slight efficacy in subjects with recently developed, treatment-naive AGW. Dose or treatment duration could also be responsible for the negative results of this trial; however, these were similar to those in the previous cutaneous warts trial.

In conclusion, ICVT demonstrates to be safe to administer in patients with AGW but shows no pharmacodynamic activity or clinical efficacy after 6 weeks of treatment. The observed lack of pharmacodynamic activity of ICVT in this early-phase clinical trial, involving viral load as a relevant biomarker, facilitates further rational drug selection for AGW and might therefore compress timelines for future drug development.

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Capsule-like naevus: digital 3D reconstruction of its steric structure

Editor

Observation of histopathology is usually based on vertical two-dimensional (2D) images. While this approach is sufficient for lesions with a relatively simple structure, a steric analysis could be useful for the one with a more complex structure. In this report, we present an unusual 'capsule-like naevus' and implemented three-dimensional (3D) analysis using a digital scanner and a 3D reconstruction software.

A 28-year-old woman without a remarkable past medical history visited our department because of black spots on her buttock. Dermoscopy revealed several evenly black-coloured spots with a diameter of 0.7–1.0 mm (Fig. 1a). Some of them were aggregated together to form a large cluster. The dermoscopic features of melanocytic naevi, including pigmented network, dots, globules and streak, were not found. The whole lesion was totally resected. The histopathology revealed naevus cells closely aggregated around hair follicles. Rete ridges were elongated around a hair follicle, forming a capsule-like peculiar structure (Fig. 1b). The inside of the capsule was filled with pigmented naevus cells and vessels, suggesting that the inside contents are connected to the dermis. The outside of capsules was also enveloped with naevus cells. Therefore, there were layered structures, consisting of hair follicle, naevus cells, capsule-like rete ridges and naevus cells, from the inside to the outside. In some area, prominent epidermal projections were observed (Fig. 1c).

To clarify the formation of this capsule-like structure, we reconstructed a digital 3D image volume from serial sections of the sample. All procedures were approved by the ethical committee of Hamamatsu University School of Medicine (IRB 18-082) and were in accordance with the Helsinki declaration guidelines. After the specimen was fixed, sections with a thickness of 5 μm were stained with Fontana–Masson (FM, Melanin Stain). One hundred sixty serial sections for the lesion were scanned by a digital scanner. The scanned 2D images were used to reconstruct 3D image by a 3D visualization software, Voloom (Micro Dimensions, Munich, Germany).[1–3] Voloom is an automated software for 3D alignment enabling examination of tissue slices as a 3D volume.

Digitally reconstructed 3D images showed that epidermal projection progressed deep into the dermis and wrapped around the hair follicles (Fig. 1d). This structure was not closed but left open at the dermis (Fig. 1d, black square, white arrow). Therefore, we found that this structure is shaped like a cylinder provided by the rete ridges as shown in Fig. 1e.

The distribution of naevus cells around hair follicle is frequently seen in congenital naevus.[4] To our knowledge, however,