Early phase clinical drug development for HPV-induced disorders: novel tools and treatments

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Chapter 6

TOPICAL DIGOXIN AND FUROSEMIDE GEL FOR PATIENTS WITH EXTERNAL ANOGENITAL WARTS, RESULTS OF A PHASE 2 STUDY

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Abstract

BACKGROUND Anogenital warts (AGW) are caused by low-risk HPV types and represent the most common sexually transmitted viral disease. Current therapies for AGW have notable side effects and high recurrence rates. DNA viruses such as HPV rely on cellular K^+ influx. Ionic contra-viral therapy (ICVT) comprised of digoxin and furosemide inhibits the K^+ influx and is therefore a potential new treatment for AGW.

OBJECTIVES A randomized, controlled trial was performed to assess safety and tolerability and explore pharmacodynamics and clinical efficacy of ICVT in patients with AGW.

METHODS Twenty-four patients with at least 3 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. Patient-reported outcomes and quality of life (QOL) were assessed with use of an e-diary and paper questionnaires.

RESULTS 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, patient-reported outcomes and QOL were observed after treatment.

CONCLUSION ICVT is safe to be administered in patients with AGW but shows no pharmacodynamic activity or clinical efficacy after 6 weeks of treatment.

Introduction

Anogenital warts (AGW) are caused by the human papilloma virus (HPV), mostly type 6 and represent the most common sexually transmitted viral disorder.[1] AGW cause pruritus, irritation or pain and most patients experience substantial psychological burden.[2] Current treatment options are associated with low efficacy rates, serious side effects and high recurrence rates.[3,4] Therefore the development of novel effective treatments with acceptable side effects is crucial for patients with AGW. Ionic contra-viral therapy (ICVT), comprised of digoxin and furosemide, inhibits the cellular K^+ influx.[5] 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.[6,7] Based on these findings we hypothesized that ICVT could show clinical activity in another HPV-induced disease, i.e. AGW. The objectives of this study were to evaluate safety and tolerability and to explore pharmacodynamics and clinical efficacy of ICVT in patients with AGW.

Materials and Methods

STUDY DESIGN

A randomized, double-blind, placebo-controlled, phase 2 trial was conducted from October 2017 until July 2018 at the Centre for Human Drug Research (CHDR), Leiden, The Netherlands. The study was approved by the Dutch independent Medical Ethics Committee of the Foundation BEBO (Assen, the Netherlands) prior to any procedure. Patients ≥18 years were considered eligible for the study if they had a minimum of 3 external AGW and were otherwise healthy. Patients were prohibited to use active treatment for AGW within 28 days prior to enrolment until the end of study. The patients were randomly assigned by a computer-generated list prepared by an independent statistician to either a fixed dose of a topical gel of ICVT, containing digoxin and furosemide (0.125%, w/w), or placebo in a 3:1 ratio. The study drug was applied once daily for 42 consecutive days with a follow-up period of 12 weeks. Patients, study personnel and investigators were blinded for allocated treatment throughout the study. Patients could be enrolled in an open label extension study, with 8 weeks treatment and 12 weeks follow-up, if no safety or tolerability issues occurred in the double-blind study part.

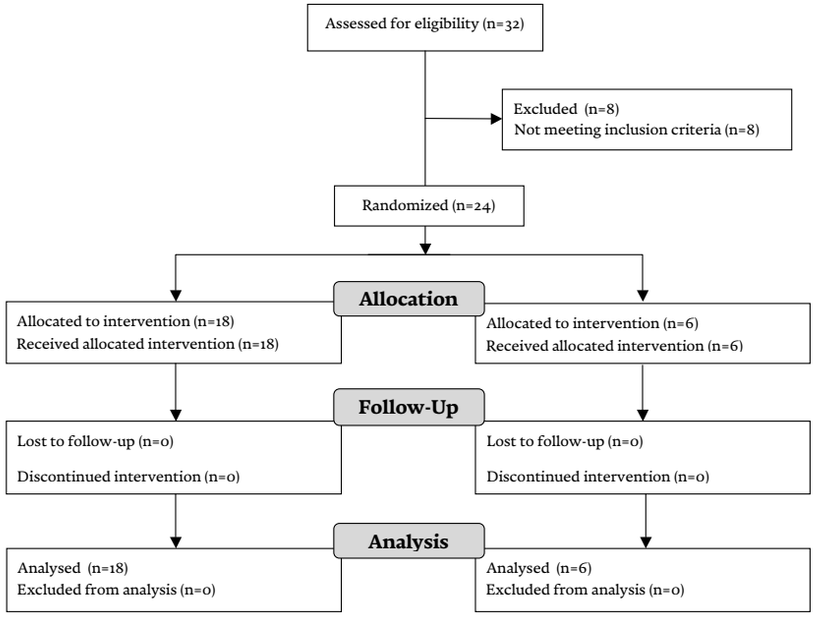
STUDY PROCEDURES

Safety and tolerability were monitored by tracking of adverse events (AEs), physical examination, vital signs, ECG and laboratory blood and urine tests. Systemic exposure of digoxin was measured during the treatment period at week 3 and 6. All warts were counted and the diameter and height (mm) of three selected warts, i.e. target wart (TW), biopsy wart 1 (BW1) and biopsy wart 2 (BW2), were measured using a digital calliper (HBM Machines B.V., Moordrecht, the Netherlands). Swabs were taken at each study visit and were analyzed in a single batch at the end of study. The HPV genotype was identified in baseline swabs using SPF10-LIPA25 version 1 (Labo Bio-medical Products B.V., Rijswijk, The Netherlands).[6–8] Viral load of HPV6 and HPV11 was determined by qPCR in all swabs. Biopsies were taken at baseline (BW1), end of treatment (BW2) and end of study (TW) and were cut in two equal pieces. One piece of the biopsy was assessed according to histopathological analyses by the LUMC department of pathology.[9,10] HPV genotyping was performed of BW1 using INNO-LIPA HPV genotyping Extra II (INNO-LIPA; Fujirebio Europe, Ghent, Belgium). From the other piece, the expression of the HPV6 E6 gene was determined using real-time quantitative reverse transcriptase PCR. Patient-reported outcomes were determined with use of an e-diary during the treatment period and quality of life (QOL) by paper questionnaires at each study visit.[11] The questionnaire was based on adapted questions from a vulvar HSIL questionnaire.[12] Treatment adherence, the actual administrations divided by the expected administrations, was monitored by the e-diary to register daily dose administration and to remind patients; in case patients did not fill in the e-diary, they were contacted and asked whether they applied the drug.

STATISTICS

Due to the exploratory nature of the trial the sample size was determined empirically. Safety analyses were conducted in the pre-defined intention-to-treat (ITT) population, comprising all enrolled patients who received at least one dose of study treatment. Pharmacodynamic and clinical efficacy analyses were conducted in the per protocol population, which consisted of the ITT population with at least one post-baseline assessment and no major protocol deviation. All efficacy and pharmacodynamic endpoints were analyzed with a mixed model analysis of covariance (ANCOVA) using treatment, time and treatment by time as fixed factors, patient as random factor and the baseline value as covariate with SAS 9.4 for Windows (SAS institute Inc., Cary, NC, USA). A two-sided Fisher's exact and a two-sided Wilcoxon exact rank test were used to analyze wart clearance. Graphs were made using GraphPad Prism (version 6.05 for Windows, GraphPad Software, La Jolla, California, USA). All statistical tests were two-tailed with α-level of 0.05.

Figure 1. Flow diagram of the study. Thirty-two subjects were screened of whom 24 (75%) were enrolled. Of the included subjects, 18 were randomly assigned to treatment with ICVT and 6 to placebo. All subjects completed the study.



Results

Twenty-four patients were enrolled and all subjects completed the trial (Fig. 1). Patient characteristics are shown in Table 1. The most frequently present HPV-type in baseline biopsy specimens was HPV6 (92%) and most patients (79%) had undergone one or more previous treatments. No serious AEs occurred and there were no study discontinuations. All AEs (N=17) were of mild severity and self-limiting. The most frequently reported AE was a burning

sensation at the application site directly after application of the gel, which was reported by 6 (33%) patients in the ICVT group and 3 (50%) patients in the placebo group. All other AEs were considered as unrelated to treatment. Safety laboratory testing, vital signs and electrocardiograms showed no differences between the treatment groups. There was no difference upon treatment in number, dimensions, viral load, HPV6 expression and histology between the ICVT and placebo group (Table 2). There was no statistical significant difference in pain scores between both treatment groups (+1.3; 95% CI -1.3 to 4.0; p=0.30). When comparing the itch scores, there was a statistically significant difference between the ICVT and placebo group (+5.9; 95% CI 0.7 to 11.2; p=0.03), because of a decrease in the mean itch score in the placebo group. There was no difference in total score of the QOL between the treatment groups (+22.4; 95% CI -70.1 to 114.9; p=0.62). Treatment adherence was 99%. Twelve subjects were enrolled in the open label extension study, which showed no differences upon treatment.

Table 1. Patient characteristics at baseline.

Characteristics	ICVT (N=18)	Placebo (N=6)	Total (N=24)
GENDER			
Female	4 (22%)	1 (17%)	5 (21%)
Male	14 (78%)	5 (83%)	19 (79%)
Age in years - median (range)	27.5 (21-44)	33 (22-67)	28 (21-67)
Number of lesions – median (range)	10 (4-51)	14 (6-19)	10 (4-51)
TARGET WART SIZE IN MM – MEDIAN (RANGE)			
Long diameter	4.6 (2.7-14.2)	6.1 (3.8-8.5)	4.8 (2.7-14.2)
Short diameter	2.8 (1.3-9.3)	3.9 (2.1-5.5)	2.8 (1.3-9.3)
Height	1.3 (0.4-9.7)	1.6 (0.8-2.3)	1.4 (0.4-9.7)
Disease duration in years – median (range)	3 (0.1-11.8)	5.4 (0.4-7.7)	3.8 (0.1-11.8)
HPV GENOTYPE BIOPSY – N (%)			
HPV6	17 (94)	5 (83)	22 (92)
HPV44	1 (6)	1 (17)	2 (4)
HPV73	0	1[1] (17)	1[1] (8)
PREVIOUS TREATMENT			
No – N (%)	3 (17)	2 (33)	5 (21)
Yes – N (%)	15 (83)	4 (67)	19 (79)
Cryotherapy	8	2	10
Surgical[2]	7	2	9
Medical[3]	13	4	17
SMOKING			
No	10	3	13
1-15/day	5	3	8
15+/ day	3	0	3
SYMPTOMS[4]			
no	10	4	14
yes	8	2	10

1: One subject had a co-infection of HPV6 and HPV73 / 2: Surgical excision and laser / 3: Podofyllotoxine, imiquimod, sinetachins, 5-FU / 4: Pain and/or pruritus / ICVT: ionic contra-viral therapy

Discussion

This study was performed to evaluate safety and tolerability and to explore pharmacodynamics and clinical efficacy of ICVT in patients with AGW. ICVT has demonstrated a favorable safety profile in patients with AGW, and that no pharmacological nor clinical activity occurred upon the once daily

Table 2. Clinical efficacy of ICVT compared to placebo.

Assessment		AGW			
		Pre-dose	EOT[1]	EOS[2]	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[3] Normal 0/18	AGW 8/18 Other 10/18[3] Normal 0/18	AGW 13/18 Other 2/18[3] Normal 0/18 No biopsy 3/18[4]	-
	Placebo	AGW 6/6 Other 0/6 Normal 0/6	AGW 3/6 Other 3/6[3] Normal 0/6	AGW 5/6 Other 1/6[3] Normal 0/6	

1: After 6 weeks of treatment / 2: After 12 weeks of follow-up / 3: Other= seborrheic verruca, fibro epithelial polyp, hyperkeratotic papilloma or reactive changes. Although, all biopsies taken pre-dose were HPV positive. / 4: Three patients refused the biopsy at the end of study / ICVT: ionic contra-viral therapy, EOT: end of treatment, EOS: end of study

administration of ICVT for 42 consecutive days. As expected, HPV6 was the most frequently present (92%) HPV-type.[13,14] In our previous trial with cutaneous wart patients ICVT showed clear reduction in wart size and viral load after 6 weeks of treatment.[15] 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 is 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 the Alpha-papillomavirus group.[16] In plantar warts, the HPV genotype has been found to influence natural history and treatment response.[17] Clinical practice shows that not all treatments effective in cutaneous warts are also effective in AGW, and vice versa. For example, imiquimod is registered for the use in AGW and shows an efficacy of 27-54%.[18] Several small and non-controlled trials performed to investigate the efficacy of imiquimod in cutaneous warts showed limited evidence for its efficacy.[19] A Cochrane review reported no difference in treatment with imiquimod compared to placebo, based on data from two unpublished RCTs in 391 patients with cutaneous warts.[20] On the other hand ICVT showed to inhibit other viruses such as herpes simplex and varicella zoster which makes it less plausible that the type of HPV influences this process.[5] 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 of 3.8 years), we can therefore anticipate 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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