Topical ionic contra-viral therapy comprised of digoxin and furosemide as a potential novel treatment approach for common warts

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SHORT REPORT

Topical ionic contra-viral therapy comprised of digoxin and furosemide as a potential novel treatment approach for common warts

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Abstract

Background DNA viruses such as HPV rely on K^+ influx for replication. Both digoxin and furosemide inhibit the K^+ influx by interacting with cell membrane ion co-transporters (Na^+/K^+-ATPase and Na^+-K^+-$2Cl^-$ co-transporter-1, respectively). We therefore hypothesized that these two compounds in a topical formulation may be valuable in the treatment of HPV-induced warts. This new approach is called Ionic Contra-Viral Therapy (ICVT).

Objective To evaluate systemic exposure, safety and tolerability of ICVT with a combination of furosemide and digoxin after repeated topical application in subjects with common warts. Furthermore, we aimed to evaluate pharmacodynamics effects of ICVT.

Methods Twelve healthy subjects with at least four common warts on their hands were included in the study and treated with a fixed dose of 980 mg topical gel containing 0.125% (w/w) digoxin and 0.125% (w/w) furosemide for 7 consecutive days on their lower back to assess safety and systemic exposure. Two warts were treated with 10 mg each and two served as negative controls to obtain preliminary evidence of treatment effect.

Results ICVT was well tolerated topically, and there was no evidence of systemic exposure of digoxin or furosemide. There were no clinical relevant safety findings and no serious adverse events (SAEs). A rapid and statistically significant reduction in diameter, height and volume of the warts was already observed at day 14.

Conclusion ICVT was found to be safe for administration to humans and 7 days of active treatment showed a statistical significant wart reduction compared to untreated control lesions, clearly indicating pharmacological activity.
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Short report

Verrucae vulgaris, commonly referred to as common warts, have a high prevalence of 3%–13% in the general population and up to 33% in primary school children.[1–5]

These warts are caused by the human papillomavirus (HPV), with the most prevalent HPV genotypes being HPV 1, HPV 2, HPV 27 and HPV 57.[6,7] Efficacy rates of current treatment options, for example cryotherapy, salicylic acid and monochloroacetic acid, are low (cryotherapy 39%; salicylic acid 24%;

monochloroacetic acid 46%), and cure rates are dependent on HPV type.[8–11] As efficacy rates of current treatment options are not optimal, side-effects are common, and recurrences often occur, there is an unmet need to develop new therapeutics for common warts.

As it has been shown that DNA viruses such as HPV rely on K^+ influx for replication (Hartley *et al.*, 1993), this could provide a therapeutic option. Both digoxin and furosemide inhibit the K^+ influx by interacting with cell membrane ion co-transporters (Na^+/K^+-ATPase and Na^+-K^+-$2Cl^-$ co-transporter-1, respectively). It can therefore be hypothesized that these two

compounds in a topical formulation may be valuable in the treatment of HPV-induced warts. This new approach, called Ionic Contra-Viral Therapy (ICVT), has already shown inhibitory effects on DNA replication *in vitro*, with the strongest effect when digoxin and furosemide were combined.[12]

We conducted an open-label, first-in-human (FIH), monocentre study to evaluate systemic exposure, safety and tolerability of ICVT with a combination of furosemide and digoxin after repeated topical application in subjects with common warts that were otherwise healthy. In addition, exploratory pharmacodynamic effects on wart morphology and HPV load were included. The protocol of this study was approved by the Medical Ethics Committee of the Foundation BEBO (Assen, the Netherlands). The study was conducted according to the Dutch Act on Medical Research involving Human Subjects (WMO). Written informed consent was obtained from all participants prior to participation. Twelve (12) healthy subjects with at least four (4) common warts on their hands were included in the study. Subjects with a clinical significant disease as judged by the investigator and pregnant or breastfeeding women could not participate. Included subjects were treated with a fixed dose of 980 mg topical gel containing 0.125% (w/w) digoxin and 0.125% (w/w) furosemide for 7 consecutive days on their lower back to assess systemic exposure. Furthermore, two warts were selected based on wart location, to enable pharmacodynamics measurements and biopsy, and treated with 10 mg each (target wart 1 and 2). Two volume-matched warts served as negative controls (target wart 3 and 4) to obtain preliminary evidence of treatment effect.

Safety assessment was performed throughout the study by monitoring the occurrence of adverse events, physical examination, recording of ECGs, vital signs, urinalysis and standard laboratory tests. Blood samples were also taken to perform therapeutic drug monitoring (TDM) of digoxin.

Swab and biopsy specimens were tested for the presence of 23 cutaneous wart-associated HPV types by HSL-PCR/MPG assay, as previously described.[13] Swab samples positive for HPV2, 27 or 57 were further tested with a new exploratory quantitative biomarker namely the HPV load. This was used as preliminary efficacy read-out for all target warts and assessed predose, on days 4, 7 and 14 (end-of-study). As comparison, biopsies of one treated and one untreated wart were taken for HPV genotyping and load assessment at end-of-study. Clinical assessment consisted of change in wart volume (mm³) as calculated out of diameter and height as measured by a clinical ruler. Results are presented as mean with standard deviation (±SD) and differences are given as point estimate with 95% confidence interval (95%CI), unless otherwise specified.

All enrolled subjects completed the study. The study group consisted of nine male and three female subjects with a mean age of 24.3 (±5.3) years (Table 1). The warts were most often described as sharp bounded solitary papules of lenticular size, white-to-normal skin colour. Baseline characteristics of the target

warts ranged from 4.1 mm to 6.4 mm in diameter, 1.0 mm to 2.0 mm in height and 125.4 mm³ to 286.9 mm³ in volume (Table 1). ICVT was well tolerated topically, and there was no evidence of systemic exposure of digoxin or furosemide. There were no clinical relevant safety findings and no serious adverse events (SAEs). Most frequently occurring mild adverse events (AEs) were headache (3/12, 25.0%), application site erythema and pruritus (both 2/12, 16.7%). Two AEs of moderate severity (dysmenorrhoea and dizziness) occurred. These resolved without intervention and were considered unrelated to treatment.

Surprisingly, a rapid and statistically significant reduction in diameter, height and volume of the warts was already observed at day 14, (diameter end-of-study, -0.29 mm, 95%CI −0.43/

Table 1 Baseline characteristics of the study group

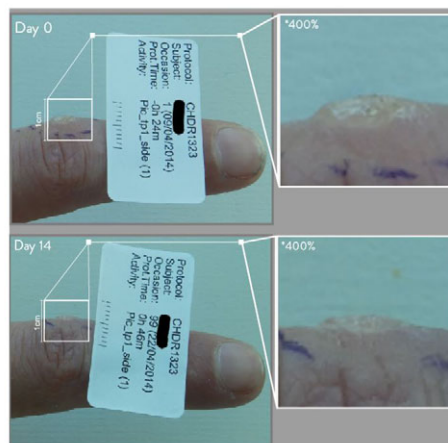| Characteristics	N = 12
Age – mean (y) (SD)	24.3 (5.3)
Sex – no. (%)	
Men	9 (75%)
Woman	3 (25%)
ICVT (target wart 1&2)	
Wart diameter – mean (mm) (SD)	5.31 (2.037)
Wart height – mean (mm) (SD)	1.75 (0.794)
HPV by Swab	
HPV 2 – no. (%)	4 (17)
HPV 57 – no. (%)	8 (33)
Untreated (target wart 3&4)	
Wart diameter – mean (mm) (SD)	4.56 (2.902)
Wart height – mean (mm) (SD)	1.33 (0.868)
HPV by Swab	
HPV 2 – no. (%)	4 (17)
HPV 57 – no. (%)	7 (29)

Figure 1 Clinical photography of a common wart treated with ICVT, day 0 (predose) vs day 14 (after treatment).
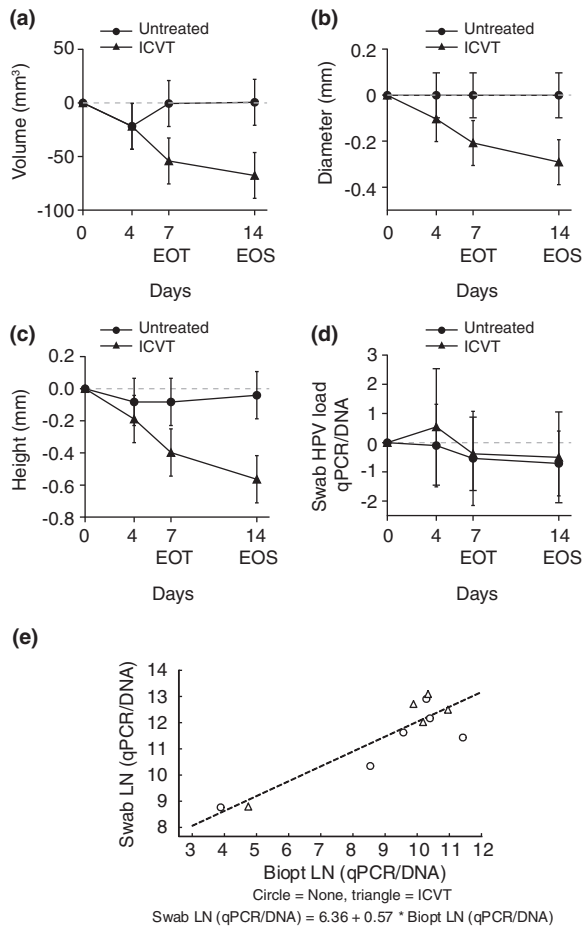
Figure 2 Effect of ICVT on wart volume and HPV load. a) Change from baseline in wart volume (mm³), mean ± SD. b) Change from baseline in wart diameter (mm), mean ± SD. c) Change from baseline in wart height (mm), mean ± SD. d) HPV load by swab from baseline to end-of-study, mean ± SD. e) Correlation in viral load between swab and biopsy.

observed during this 7-day treatment period and follow-up (Fig. 2d).

The correlation between qPCR in swab samples and biopsy was investigated using a mixed model analysis. The linear regression model of log-transformed swab (qPCR/DNA concentration) versus biopsy (qPCR/DNA concentration) showed a significant correlation between the two assessments (slope = 0.5675, *P*-value = 0.0033; Fig. 1e).

In conclusion, ICVT was found to be safe for administration to humans and 7 days of active treatment showed a statistical significant wart volume reduction compared to untreated control lesions, clearly indicating pharmacological activity. HPV load by swab correlated well with biopsy measurements and is therefore a potential new biomarker to monitor treatment effects on common warts. Currently, a phase II, randomized, placebo-controlled, double-blind study in subjects with common and/or plantar warts with a longer treatment and follow-up period is ongoing which will shed more light on the potential efficacy of ICVT.

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−0.15, *P*-value = 0.0003; height end-of-study, −0.52 mm, 95% CI −0.75/−0.30, *P*-value = <0.0001; volume end-of-study, −68.3 mm³, 95%CI −105.4/−31.2, *P*-value = 0.0007) when comparing treated warts with untreated warts (Fig. 1, Fig. 2a,b, c). However, an observer bias cannot be excluded in this open-label study.

Human papillomavirus was detected in all wart swab samples at baseline. Evaluation restricted to genotypes with viral load data showed the presence of HPV 2 (8/48, 17%) and HPV 57 (15/48, 31%) and this did not change in the subsequent swab samples. Analysis of biopsies at day 14 (*n* = 24) also showed 100% positivity for any HPV (Table 1). This profile is in accordance with previous findings.[7,13] Viral load was higher in the biopsy samples when compared to the swab samples. No clear trend towards viral load reduction in the treated warts was