Evaluation of the Efficacy, Safety, and Tolerability of 3 Dose Regimens of Topical Sodium Nitrite With Citric Acid in Patients With Anogenital Warts
A Randomized Clinical Trial

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IMPOR TANCE  Anogenital warts are a common disorder associated with significant physical and mental distress and a substantial cause of health care costs.

OBJECTIVE To assess the efficacy of the topical application of nitric oxide delivered using acidified nitrite.

DESIGN, SETTING, AND PARTICIPANTS A multicenter, randomized, controlled, dose-ranging clinical trial was conducted in European genitourinary medicine clinics between December 20, 2001, and January 14, 2003. Analysis was by intent to treat for all individuals initiating therapy. Participants included male and female volunteers older than 18 years with between 2 and 50 external anogenital warts. A total of 299 individuals from 40 centers were randomized to a control arm and a treatment arm that received 3 doses of acidified nitrite applied topically for 12 weeks with an additional 12 weeks of follow-up, with the final follow-up visit on January 14, 2003.

INTERVENTIONS Placebo nitrite cream and placebo citric acid cream were applied twice daily. Active treatment was divided as low dose (sodium nitrite, 3%, with citric acid, 4.5%, creams applied twice daily), middle dose (sodium nitrite, 6%, with citric acid, 9%, creams applied once daily at night, with placebo applied in the morning), and high dose (sodium nitrite, 6%, with citric acid, 9%, creams applied twice daily).

MAIN OUTCOMES AND MEASURES The primary outcome was proportion of patients with complete clinical clearance of target warts; secondary outcomes were reduction in target wart area and safety.

RESULTS Complete clinical clearance at 12 weeks occurred in 10 of 74 patients (14%; 95% CI, 6%-21%) with placebo; 11 of 72 (15%; 95% CI, 7%-24%) with low-dose treatment; 17 of 74 (23%; 95% CI, 13%-33%) with middle-dose treatment; and 22 of 70 (31%; 95% CI, 21%-42%) with high-dose treatment (P = .01). Reduction in target wart area, time to clearance, and patient and investigator assessments supported the superiority of the high-dose therapy vs placebo. There were no systemic or serious adverse events associated with treatment. However, there was a dose-related increase in itching, pain, edema, and staining of the anogenital skin associated with the active treatment. Overall, 21 patients withdrew from active treatment because of adverse events compared with none using placebo.

CONCLUSIONS AND RELEVANCE Use of sodium nitrite, 6%, with citric acid, 9%, twice daily is more effective than placebo in the treatment of anogenital warts. Treatment was associated with local irritant adverse effects.

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Anogenital warts are a common sexually transmitted disease (STD). The prevalence is reported as ranging from 160 to 289 per 100,000 individuals, with a median of 194.5 per 100,000.¹ The warts are caused by infection with human papillomavirus (HPV) types 6 and 11 in more than 90% of the cases.²,³ Although not directly associated with cancer, lesions often are coinfectied with multiple HPVs, including carcinogenic HPV types, such as HPV16.⁴ Anogenital warts are sexually transmitted at rates of approximately 60% in sexual partners² and impose a significant burden on resources⁵,⁶ and in the quality of life of patients;⁶ they also pose a psychological burden.⁷

Topical therapies and surgical removal of anogenital warts are associated with local adverse reactions, including itching, burning, pain, and erosions; recurrence rates with existing therapies are approximately 30%.⁵,⁸ Several treatments, including cryotherapy, trichloracetic acid, and surgical removal, require frequent visits to physicians.

Mixing inorganic nitrite with an organic acid (1) forms nitrous acid, which is (2) converted to dinitrogen trioxide, which then (3) dissociates into nitrous oxide and nitric oxide (NO) as follows:

(1) \[ \text{NO}_2^- + \text{H}^+ \rightarrow \text{HNO}_2 \]
(2) \[ 2\text{HNO}_2 \rightarrow \text{H}_2\text{O} + \text{N}_2\text{O}_3 \]
(3) \[ \text{N}_2\text{O}_3 \rightarrow \text{NO} + \text{NO}_2 \]

Nitric oxide can freely diffuse in the skin to act against a wide range of pathogens.⁹ Nitric oxide is an important component of the innate immune defense against infection, with broad antimicrobial effects against bacterial,⁹-¹¹ fungal,¹² and parasitic organisms.¹² Nitric oxide also has antiviral effects against several viral pathogens.¹³-²² Furthermore, depending on the context, NO can modulate host responses²¹,²² or, in skin, activate immune responses.²³-²⁵

Many platforms have been designed to release NO for antimicrobial effects including nanoparticles,²⁶ gaseous delivery,²⁷-²⁸ NO donors,²⁹-³³ and probiotic film devices.³⁴,³⁵ We have developed a simple NO delivery system comprising separately prepared creams containing an acid and an inorganic nitrite that are coapplied to the site of infection. With the hypothesis that NO has activity against HPV infections, the objectives of this study were to compare the efficacy, safety, and tolerability of 3 dose regimens of topical acidified nitrite with placebo in patients with anogenital warts and establish the optimal dose of citric acid and sodium nitrite in this population.

Methods

Trial Design
This was a double-blind, placebo-controlled, randomized, dose-ranging, parallel-group multicenter study. The study comprised a 12-week treatment phase followed by 12 weeks of follow-up. The study was conducted from December 20, 2001, to the final follow-up on January 14, 2003. The trial was reviewed and approved by the ethics committee at the State Medical Chamber of Baden-Württemberg and appropriate reciprocal ethics committees covering 40 centers. The full study protocol is found in the trial protocol in Supplement 1. Written informed consent was obtained from all participants. Participants did not receive financial compensation.

Participants
Individuals 18 years or older with 2 to 50 external warts in the anogenital region were recruited. Women with child-bearing potential were required to use a nonbarrier method of contraception at study entry and for the duration of the study; all patients had to use barrier contraception throughout the study period. Patients meeting the following criteria were excluded from the study: clinically relevant abnormal hematology or biochemistry laboratory test results, active use of local therapy for anogenital warts within 2 weeks of randomization, other abnormality of anogenital skin or skin that had not healed following surgery, concomitant sexually transmitted disease (STD), internal warts, types 1 and 2 diabetes mellitus, human immunodeficiency virus, immunosuppressed condition, and alcohol and/or drug abuse.

Populations Analyzed
The safety population consisted of all patients screened and randomized except documented as not receiving any study treatment. The intent-to-treat population for efficacy determination consisted of all patients randomized who had received at least 1 postbaseline assessment. The follow-up population was monitored for an additional 12 weeks, excluding patients who used alternative treatments during this time.

Interventions
There were 4 arms to the study: 1 placebo arm and 3 dose-ranging acidified nitrite intervention arms. Participants in the placebo arm applied sodium nitrite placebo with citric acid placebo twice daily. The acidified nitrite doses in the other 3 arms were as follows: low dose: sodium nitrite, 3%, with citric acid, 4.5%; creams applied twice daily; middle dose: sodium nitrite, 6%, with citric acid, 9%, creams applied once daily at night (with placebo applied in the morning); and high dose: sodium nitrite, 6%, with citric acid, 9%, creams applied twice daily. The 2 creams were applied topically; sodium nitrite was applied first, then citric acid, and the 2 creams were mixed. When mixed, citric acid reacts with nitrite to form the active molecule (NO). The patients returned 1, 2, 4, 6, 8, 10, and 12 weeks later for assessment of outcome measures, and medication was dispensed at each visit.

Outcomes
The primary efficacy variable was the proportion of patients with complete clinical clearance of target warts in the intent-to-treat population. Secondary outcomes were time to clearance, area of target warts, patient assessment of overall efficacy at week 12 or withdrawal if before week 12, and a similar investigator assessment of efficacy.
Safety and tolerability measures included (1) patient assessment of itching, pain, and burning (categorized as none, mild, moderate, or severe) at the treatment site; (2) investigator assessment of erythema or eschar and of edema (using modified Draize scales\(^16\) from 0 (nonirritating) to 4 (very irritating)); and (3) an investigator assessment of the presence of staining. Adverse events were recorded throughout the treatment period; heart rate and blood pressure were taken and laboratory tests were performed at screening and week 12 or at withdrawal if before week 12.

Adherence to therapy was recorded in a patient diary and returned medication was weighed at the start and finish of the study. To assess improvement after stopping therapy and recurrence of cleared warts, patients were evaluated monthly for a 12-week period after stopping treatment.

**Sample Size**

Previous controlled studies\(^{37}\) with imiquimod in the treatment for anogenital warts had shown a clearance rate ranging from 37% to 52% and from 0% to 11% in patients who received the active formulation and the vehicle, respectively. To determine the sample size for the present study, we assumed that the clinically significant clearance rates would be 40% with the active formulation and 15% with the placebo treatment. A sample size of 62 patients per treatment group had 90% power to demonstrate this difference with \(\alpha = .05\).

**Randomization and Blinding**

Randomization to treatment was stratified by sex and evenly distributed 1:1:1:1 in the 4 arms. Randomization was centrally generated by the contract research organization for the study. Blinded, numbered medication supplies were distributed to participating centers in block sizes of 4 for each sex. Each of the 4 treatments appeared once per block of 4 in a random order. Patients were randomized to 1 of the 4 treatment groups by receiving the next numbered medication supply as appropriate from the randomization list for men or women. Allocation was concealed in sealed envelopes provided to the investigators.

The active and placebo citric acid creams were identical in packaging and labeling and had the same appearance and odor. Investigators and participants were blinded to treatment allocation.

**Statistical Analysis**

The primary efficacy analysis was a comparison of the proportion of patients with complete clinical clearance of their target warts in the intent-to-treat population and was analyzed using logistic regression, fitting a model consisting of treatment, total number of warts at baseline, and the target wart area recorded at baseline, center, and sex.

Pairwise comparisons were performed for each high dose compared with placebo and presented as the odds ratio (OR) for each of the treatment comparisons. There were 3 comparisons of interest (each active vs placebo) and so the 5% significance level was adjusted to allow for this: an adjusted significance level of 1.67% was used.

Time to healing was analyzed by a Cox proportional hazards regression model, and patient and physician assessments of efficacy were analyzed by logistic regression, fitting a model consisting of treatment, center, and sex. The fit of all models was evaluated using the Hosmer-Lemeshow test. The Wilcoxon rank sum test was used to determine the percentage of reduction in the wart area. Sex, the total number of warts at baseline, and the target wart area were recorded at baseline and included as covariates in the analyses.

**Results**

Patients were screened by 40 centers in the Netherlands, Germany, Poland, United Kingdom, and Sweden. Recruitment took place December 20, 2001, to October 1, 2002, and was stopped when recruitment was completed on October 1, 2002. A total of 299 individuals were randomized to a control arm and a treatment arm.

Participant flow is presented in the Figure; eTable 1 in Supplement 2 summarizes data on participants who withdrew. Patient demographics, as well as the previous history of anogenital wart infection, baseline numbers, and areas of warts, are summarized in Table 1.

**Numbers Analyzed**

Placebo cream was used by 75 patients, low-dose treatment by 74 patients, middle-dose treatment by 77 patients, and high-dose treatment by 73 patients. Nine patients were excluded from analysis because they failed to return for any follow-up visit. The treatment phase of the study was completed according to protocol by 48 patients (64%), 40 patients (54%), 43 patients (56%), and 46 patients (63%), respectively. The reasons for withdrawal are listed in eTable 1 in Supplement 2.

**Outcomes and Estimation**

The primary outcome, complete clinical clearance of target warts, was achieved in 10 patients (14%) in the placebo group 11 patients (15%) in the low-dose group, 17 patients (23%) in the middle-dose group, and 22 patients (31%) in the high-dose group (Table 2). The comparison was significant for the high-dose vs placebo group \(P = .01\).

For the secondary outcome measures, high-dose treatment had a shorter time to clearance compared with placebo \(P = .03\). Reduction in wart area decreased during the study in the high-dose treatment group \(P = .02\). Similarly, the time to clearance was significantly reduced in the high-dose group \(P = .03\).

At the final visit, the proportion of patients self-assessed as having complete clinical clearance or significant improvement was greatest for the high-dose group (OR, 3.007; 95% CI, 1.249-7.242; \(P = .01\)) (Table 2 in Supplement 2). Similarly, the investigators’ assessment of efficacy at the final visit (OR, 2.805; 95% CI, 1.158-6.791; \(P = .02\)) (Table 2 in Supplement 2) showed greater efficacy for the high-dose group. There were no recurrences in the 63 patients assessed after 12 weeks of follow-up.
Harms
Before treatment began, itching was common, being recorded for 27% to 30% of all patients in each treatment group. At the final visit, the proportions of participants with itching were 14%, 35%, 35%, and 38% for patients in the placebo, low-dose, middle-dose, and high-dose groups, respectively. Pain was reported by 4% to 11% of each treatment group at screening, and it increased more with active treatment than with placebo. At the final follow-up visit, the proportions of patients with pain were 4%, 11%, 20%, and 21% for patients in the placebo, low-dose, middle-dose, and high-dose groups, respectively. The symptom of burning at baseline was variably reported in 6% to 18% of the patients. At the final visit, the proportions of participants with burning were 10%, 26%, 30%, and 28% for patients in the placebo, low-dose, middle-dose, and high-dose groups, respectively.

Ancillary Analyses
Drug use derived from days of treatment recorded in patient diaries is summarized in eTable 4 in Supplement 2. Data on weighing of the cream tubes are reported in eTable 5 in Supplement 2.

Reasons for discontinuing the intervention are presented in eTable 1 in Supplement 2. Participants who failed to return for at least 1 visit after baseline were excluded from the intent-to-treat analysis.

Table 1. Participant Demographics by Treatment Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo</th>
<th>Sodium Nitrite With Citric Acid Dosea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>No. of patients</td>
<td>74</td>
<td>72</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>35.2 (13.1)</td>
<td>31.9 (10.7)</td>
</tr>
<tr>
<td>Men No. (%)</td>
<td>48 (65)</td>
<td>46 (64)</td>
</tr>
<tr>
<td>Duration, mean (SD), mo</td>
<td>7.3 (13.5)</td>
<td>9.2 (15.1)</td>
</tr>
<tr>
<td>First episode, No. (%)</td>
<td>34 (71)</td>
<td>28 (61)</td>
</tr>
<tr>
<td>No. of warts, mean (SD)</td>
<td>14.1 (13.6)</td>
<td>14.0 (13.5)</td>
</tr>
<tr>
<td>Area, mean (SD), mm²</td>
<td>117.31 (133.61)</td>
<td>90.04 (97.96)</td>
</tr>
<tr>
<td>Women No. (%)</td>
<td>26 (35)</td>
<td>26 (36)</td>
</tr>
<tr>
<td>Duration, mean (SD), mo</td>
<td>16.1 (28.3)</td>
<td>19.2 (43.5)</td>
</tr>
<tr>
<td>First episode, No. (%)</td>
<td>18 (69)</td>
<td>14 (53)</td>
</tr>
<tr>
<td>No. of warts, mean (SD)</td>
<td>7.2 (7.7)</td>
<td>10.0 (11.2)</td>
</tr>
<tr>
<td>Area, mean (SD), mm²</td>
<td>68.17 (69.36)</td>
<td>75.90 (118.69)</td>
</tr>
</tbody>
</table>

a Low dose: sodium nitrite, 3%, with citric acid, 4.5%, creams applied twice daily; middle dose: sodium nitrite, 6%, with citric acid, 9%, creams applied once daily at night (with placebo applied in the morning); and high dose: sodium nitrite, 6%, with citric acid, 9%, creams applied twice daily.

Figure. CONSORT Diagram of Participant Flow Through Each Stage of the Randomized Clinical Trial

328 Assessed for eligibility
29 Excluded
3 Did not meet inclusion criteria
6 Refused to participate
20 Other reasons
Table 2. Primary End Point: Clearance of Anogenital Warts

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo</th>
<th>Sodium Nitrite With Citric Acid Dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall clearance of anogenital warts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete clearance at 12 wk, % (95% CI)</td>
<td>14 (6 to 21)</td>
<td>15 (7 to 24)</td>
<td>23 (13 to 33)</td>
<td>31 (21 to 42)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.11 (0.43 to 2.86)</td>
<td>1.65 (0.67 to 4.01)</td>
<td>2.97 (1.25 to 7.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.83</td>
<td>.27</td>
<td>.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to clearance, hazard ratio (95% CI)</td>
<td>1.05 (0.42 to 2.59)</td>
<td>2.04 (0.88 to 4.74)</td>
<td>2.46 (1.11 to 5.44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.91</td>
<td>.10</td>
<td>.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction in area of target warts at final visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (median)</td>
<td>22.5 (15.7)</td>
<td>23.8 (34.4)</td>
<td>22.8 (34.4)</td>
<td>41.4 (72.5)</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>72</td>
<td>70</td>
<td>73</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Median difference (95% CI)</td>
<td>4.3 (~9.2 to 23.4)</td>
<td>3.3 (~9.3 to 23.3)</td>
<td>16.8 (0.0 to 40.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.46</td>
<td>.46</td>
<td>.02</td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviation: OR, odds ratio.

* Overall clearance intent-to-treat (ITT) population: linear regression, active vs placebo; time to complete clearance ITT population: Cox proportional hazards regression active treatment vs placebo; and reduction in area of target warts at final visit ITT population (Wilcoxon rank sum test, active treatment vs placebo). The Hosmer-Lemeshow test showed that the model consisting of treatment, total number of warts at baseline, target wart area at baseline, and sex was a good fit to the data.

<sup>a</sup> Low dose: sodium nitrite, 3%, with citric acid, 4.5%, creams applied twice daily; middle dose: sodium nitrite, 6%, with citric acid, 9%, creams applied once daily at night (with placebo applied in the morning); and high dose: sodium nitrite, 6%, with citric acid, 9%, creams applied twice daily.

Table 3. Overall Incidence of AEs From Baseline to Week 12

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
<th>Placebo</th>
<th>Sodium Nitrite With Citric Acid Dose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>75</td>
<td>74</td>
<td>77</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with AEs</td>
<td>41 (55)</td>
<td>58 (78)</td>
<td>54 (70)</td>
<td>67 (92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe AEs</td>
<td>3 (4)</td>
<td>19 (26)</td>
<td>13 (17)</td>
<td>29 (40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment-related AEs</td>
<td>36 (48)</td>
<td>55 (74)</td>
<td>50 (65)</td>
<td>66 (90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment-site AEs</td>
<td>36 (48)</td>
<td>57 (77)</td>
<td>52 (68)</td>
<td>67 (92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEs leading to withdrawal</td>
<td>0</td>
<td>9 (12)</td>
<td>5 (6)</td>
<td>7 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related AEs leading to withdrawal</td>
<td>0</td>
<td>7 (9)</td>
<td>5 (6)</td>
<td>7 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serious AEs</td>
<td>1 (1)</td>
<td>2 (3)</td>
<td>1 (1)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Related serious AEs</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total No. of AE reports</td>
<td>90</td>
<td>217</td>
<td>238</td>
<td>316</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: AE, adverse event.

* Low dose: sodium nitrite, 3%, with citric acid, 4.5%, creams applied twice daily; middle dose: sodium nitrite, 6%, with citric acid, 9%, creams applied once daily at night (with placebo applied in the morning); and high dose: sodium nitrite, 6%, with citric acid, 9%, creams applied twice daily.

Overall, 3% to 12% of each group had erythema or eschar recorded at baseline. At the final visit, the proportions of individuals with erythema or eschar were 10%, 16%, 25%, and 40% for patients in the placebo, low-dose, middle-dose, and high-dose groups, respectively. Edema was observed in 0% to 4% of each group at baseline. At the final follow-up visit, the proportions of patients with edema were 3%, 8%, 13%, and 16% for patients in the placebo, low-dose, middle-dose, and high-dose groups, respectively.

Staining, manifesting as yellow to brown superficial skin discoloration, was more common with active treatment than with placebo and has been documented in previous studies.38,39 At the final follow-up visit, the proportions of participants with staining were 0%, 13%, 11%, and 25% for patients in the placebo, low-dose, middle-dose, and high-dose groups, respectively.

Adverse events are summarized in Table 3. There were no treatment-related serious adverse events. Overall, 21 patients withdrew from active treatment because of adverse events compared with none using placebo. The percentage of patients with at least 1 adverse event was higher for active treatment (70%-92% for each group) than for placebo (55%). Local application site adverse events were common in all 3 active treatment groups (68%-92%). Adverse events were more common in the active treatment groups, with the highest incidence in the high-dose group vs placebo (OR, 9.275; 95% CI, 3.583-24.008). The most common adverse events were treatment-site reactions reported in 49% of the placebo group and 66% to 92% with active treatment, with itching being the most common.

Results of biochemistry, hematology, and urinalysis tests were similar across the treatment groups, with minimal change in mean values over time. No patient became pregnant. No abnormal physical examination characteristics were noted during the study for any of the treatment groups. The types of adverse events seen in this study were those commonly associated with current treatments.5 At the end of the 12-week follow-up, most of the adverse events had resolved.

Discussion

Acidified nitrite leading to NO release has been an effective treatment for tinea pedis39 and tinea versicolor40 and was benefi-
Efficacy, although less so, in cutaneous leishmaniasis. Salicylic acid, 5%, with sodium nitrite, 5%, was effective in treating molluscum contagiosum. Although ascorbic acid, 2%, with sodium nitrite, 5%, caused significant irritation of the skin, citric acid, 9%, with sodium nitrite, 6%, were well tolerated in treating ulcerated skin lesions with *Mycobacterium ulcerans* infection (Buruli ulcer). Furthermore, citric acid, 4.5%, with sodium nitrite, 3%, was tolerated well in treating wounds infected with *methicillin-resistant Staphylococcus aureus*. This dose-ranging study demonstrated that the highest dose (sodium nitrite, 6%, with citric acid, 9%) twice daily was effective. This study proves the concept that acidified nitrite is effective in treating anogenital warts and has identified the dose required for efficacy. Trial design and power sufficiently robust to demonstrate the superiority of topical NO and the multicenter setting (across 40 European centers) predict good generalizability of the results. Even allowing for a correction for 3 comparator groups to a significant value of $P < .02$, the primary outcome remains statistically significant for the high-dose treatment, area-reduction patient-rated benefit and is consistently supported by all other secondary outcomes. Because the treatment effect did not plateau, a longer duration of treatment may be indicated in future studies. In addition, a higher concentration or more sustained-release application of NO, such as nanoparticles, might be more effective, with the caveat that irritation might be greater. Similar to other therapies for anogenital warts, there is a high incidence of local adverse effects in the sensitive skin of the genital area, but recurrence rates were not detectable in patients who achieved clearance, suggesting the efficacy to be curative in responders.

A quantitative systematic review of randomized trials of imiquimod concluded that imiquimod was more effective in women, with a 72% clearance rate, than in men, with a 37% clearance rate, although the overall proportion of warts cured and not recurring was 37% (31%-43%) [27], which compares with the high-dose treatment in this study.

Although the response rate observed in this study was lower than expected, the placebo rate was in line with published placebo rates and was similar to that assumed in the sample size calculation. Recurrence of the warts is a problem, whichever form of therapy is used. Recurrence rates of 6% to 26% have been reported with imiquimod, 13% to 100% with podophyllotoxin, 7% to 11% with sinecatechins, and 21% to 42% with cryotherapy. Considering the high recurrence rates with podophyllotoxin and cryotherapy, the results with the highest dose of acidified nitrite are comparable to those of current therapies.

There was a high incidence of application site adverse events in this study. Overall, the severity of these, including pruritus, burning, erythema, staining, pain, and edema, was dose related. However, similar effects are observed with other self-applied and office-based treatments and are common at the effective dose with this delivery system. A total of 8% of patients withdrew from the study owing to treatment-associated adverse events. There was, however, no evidence of systemic effects of treatment, as demonstrated by the absence of systemic adverse events and the lack of statistically significant changes in vital signs or laboratory test results.

Because anogenital warts occur at a site that has more sensitive skin, including mucosal surfaces, skin irritation was a limitation to therapy. However, adherence to therapy and retention of participants were good for studies addressing this indication. Self-directed topical therapy can be difficult in areas hard to visualize and could impair accurate application. Staining of the skin potentially could unblind the patient and attending physician, but was reported in only 25% of patients receiving the highest dose and would be unlikely to confound the primary outcome of clearance of the warts.

**Conclusions**

Sodium nitrite, 6%, with citric acid, 9%, twice daily is more effective than placebo in the treatment of anogenital warts. Treatment in the present study was associated with local irritant adverse effects. Lower doses were not more efficacious than placebo. For the sensitive anogenital application site, this dose probably represents the optimal one for further evaluation. For future research, extending the duration of treatment might improve the efficacy. This treatment could be tested on more resilient application sites, such as palmar, plantar warts, where a stronger formulation may be better tolerated and more effective.

**ARTICLE INFORMATION**

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**Conflict of Interest Disclosures:** Dr Ormerod has received past honoraria and royalty payments from ProStrakan and has patents filed on acidified nitrite for treatment of skin infections. Dr Benjamin has received royalty payments from ProStrakan and has patents filed on acidified nitrite. Drs Majewski and Vanscheidt received honoraria from ProStrakan for performing this study, and Dr van der Meijden received nonpersonal fees from ProStrakan for participation in the study. No other disclosures were reported.

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Research Original Investigation

Evaluation of Topical Nitric Oxide Treatment for Anogenital Warts

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NOTABLE NOTES

José Gay Prieto
Teacher of Dermatologists

Leyre A. Falto-Aizpurua, MD; Brian J. Simmons, BS; Robert D. Griffith, MD; Fleta N. Bray, BS; Keyvan Nouri, MD

José Gay Prieto, born on January 17, 1905, was one of the most renowned Spanish dermatologists. He greatly contributed to the development of dermatology in Spain and around the world. He was educated in Madrid, where he excelled academically and stood out for his great communicative skills. He then pursued a dermatology residency under the mentorship of Dr José Sánchez Covisa and Dr Julio Bejarano at San Juan de Dios Hospital, which was dedicated to the treatment of leprosy, dermatologic and venereal diseases. Throughout his academic career he worked under the mentorship of Civatte, Pautrier, Jadasshon, and Bloch. In 1933, at the age of 28, he was appointed professor and chairman of dermatology at Granada University, and in 1940 he was subsequently named chairman at University of Madrid.

Gay Prieto was interested in leprosy, and, along with Felix Contreras, he would go on to organize the government leprosy campaign around the country. In 1957, he was asked by the World Health Organization to organize and conduct the World Leprosy Campaign and was chief of the leprosy unit from 1959 to 1961. He was one of the first clinicians to observe the histological lesions of rectal lymphogranulomatosis, and he described 2 new variants of the disease. He was also 1 of the first to prove the efficacy of the intravenous administration of the Frei antigen. With his colleagues, he also studied in depth urticarial reactions, syphils treatments, pyoderma gangrenosum, and proposed the viral etiology of leroaocاصت. Along with López Acona, Prieto discovered the wavelengths of radiation that were pathogenic to skin and that copper chloride selectively absorbed this wavelength, protecting skin from its damage. Gay Prieto also organized a cutaneous anticancer center and adapted the radiotherapeutic technique of Chaul using Roentgen tubes.

Gay Prieto was president of the Spanish Academy of Dermatology from 1948 to 1951 and was also an honorary member of numerous dermatological societies. He founded dermatology and serology schools in Spain, which rapidly gained an international recognition. He was fluent in Spanish, French, and German, writing over 400 publications, with the 2 most important ones being the books Dermatología and Tropenmiasis y Enfermedades Venéreas.

Gay Prieto retired in 1975. In 1978 he experienced an acute myocardial infarction with secondary decreased brain blood flow and left hemiparesis; he died in 1979. Gay Prieto is remembered as the teacher of dermatologists given his constant, devoted work in dermatology as well as his role in improving and expanding medical education.

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