Genital human papillomavirus infection is one of the most common sexually transmitted diseases. Polyhexamethylene biguanide is a new agent, that has been demonstrated to have potent in vivo antiviral effects in animal and in human models. The present prospective, double-blind, randomized, placebo (vehicle-controlled) trial evaluated the efficacy and safety of daily patient-applied polyhexamethylene biguanide for up to 16-weeks for the treatment of external genital warts. Wart recurrence was investigated during a 12-week treatment-free follow-up period. In the intent-to-treat analysis, baseline warts cleared from 49 of 94 (52%) patients treated with polyhexamethylene biguanide cream versus 3 of 95 (4%) placebo patients; the differences between the groups treated with placebo and polyhexamethylene biguanide were significant (P < 0.0001). For subjects who completed the follow-up period, recurrence rates after a complete response were 19% (9 of 48 patients) in the polyhexamethylene biguanide cream group, 17% cream group, and 0% (0 of 3) in the placebo group. There were no systemic reactions, although local skin reactions (generally of mild or moderate severity) were common in the polyhexamethylene biguanide cream group. Local reactions caused two patients to discontinue treatment. The most frequently reported local skin reactions were erythema, excoriation or flaking, and erosion. Patient-applied polyhexamethylene biguanide cream is effective for the treatment of external genital warts and has a favorable safety profile.

**Key Words:**
Polyhexamethylene biguanide, Papillomavirus infection, Genital warts.

**Introduction**

Human papillomaviruses (HPVs) belong to a family of small (8-kb pairs) double-stranded circular DNA viruses that infect squamous epithelia of the genital tract, anal, and perianal areas, and mucosal epithelium of the larynx. The most frequent clinical manifestation of infection is genital warts. Low-risk HPVs, such as HPV-6 and HPV-11, cause benign genital warts, whereas high-risk types, such as HPV-16 and HPV-18, are associated with the development of high-grade squamous intraepithelial lesions and cervical cancer. It is estimated that HPV-16 accounts for approximately 60% of cervical cancers, with HPV-18 adding another 10%-20%. Other high-risk types include types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 73. An estimated 30 to 50% of sexually active adults in the United States are infected with HPV, although only 1% may have visible genital warts. The natural history of genital warts in humans appears to be that they may persist, progress, or regress. Regression of genital warts is thought to be due to an immune response. Most treatments currently available for genital warts (e.g., cryotherapy, excision, electro-surgery, coagulation, laser surgery, trichloroacetic acid treatment, and podophyllin resin treatment) are directed at the lesions and not at the etiologic agent, HPV. Interferons (IFNs) and patient-applied podofilox are currently available therapies that have been evaluated in well-controlled trials. Polybiguanide-based compounds (PBGs) are polycationic comprised of biguanide repeat units separated by hydrocarbon chain spacers of variable length. PBGs are normally present as chloride or other salts at physiologic pH. PBGs represent a unique class of compounds with a convergence of safety, broad-spectrum activity, and structural diversity. Chlorhexidine diglu-
conate (CHG), a bis-biguanide (two biguanide groups), has been used as a safe, general vaginal disinfectant for over thirty years\textsuperscript{14,15} and has more recently been evaluated as a microbicidal agent effective against chlamydial infection\textsuperscript{16}. Biguanide-based drugs, which have also been used safely and successfully for decades, include Porguanil, which is used as an anti-malarial agent\textsuperscript{17}, and Metformin, which is used to treat type 2 diabetes. The PBG compound polyhexamethylene biguanide (PHMB) is used as the broad-spectrum active ingredient in anti-bacterial contact lens solutions\textsuperscript{18}, as a treatment for Acanthamoeba keratitis\textsuperscript{12}, as a topical antiseptic mouthrinse\textsuperscript{8}, and as a potential anti-septic agent\textsuperscript{19}. PBGs such as PHMB have many characteristics that are consistent with the attributes of an ideal microbicide\textsuperscript{19}, including (1) ease of synthesis and preparation, (2) the absence of odor, and (3) chemical stability. The antibacterial activity of PHMB is attributed to interactions with cellular membrane components, specifically anionic phospholipids\textsuperscript{20,21} and perhaps proteins and lipopolysaccharides. To ability to interact with membranes suggests that PHMB may also be effective against HIV-1, since infection of cells susceptible to HIV-1 is mediated by components in both the cellular membrane and viral envelope. Indeed, PHMB was demonstrated to be effective \textit{in vitro} against herpes simplex virus type 1 (HSV-1) at concentrations as 0.01\%\textsuperscript{5}. The current study was designed to evaluate the efficacy and safety of polyhexamethylene biguanide for the treatment of external genital warts.

**Materials and Methods**

This double-blind, randomized, placebo study assessed the efficacy and safety of daily applications of polyhexamethylene biguanide cream placebo in patients with external genital warts. Otherwise healthy males and females, aged 18-years and older, were enrolled if they had at least 2 but not more than 50 external genital warts (defined as warts in the genital, anal, perineal, or perianal area) with a biopsy diagnostic or suggestive of condyloma acuminatum and a bidimensional wart area of at least 10 mm\textsuperscript{2}. All patients were seronegative for the human immunodeficiency virus. Female patients could not be pregnant or lactating and had to agree to use effective birth-control measures. Exclusion criteria included genital wart therapy in the 4-weeks prior to treatment initiation and, for female patients, a pre-study Pap smear showing a high-grade squamous intraepithelial lesion. All patients gave written informed consent before enrollment. The protocol and consent forms were reviewed and approved by appropriate institutional review boards. Patients were randomized to receive daily applications of either polyhexamethylen biguanide cream, or placebo (vehicle cream) for a maximum of 16-weeks. Before bedtime patients rubbed the study cream into clean, dry, wart-area skin until it disappeared and washed the area with soap and water 8 ± 2 h after application. To investigate wart recurrence, patients who had complete clearance of their baseline warts at any time during the treatment period stopped treatment and entered a 12-week treatment-free follow-up period. Patients were evaluated weekly for the first 4-weeks and every 2-weeks thereafter for the remainder of the 16-week treatment period as well as during the 12-week follow-up period. A detailed wart assessment, including photographs, measurements, counts, and location, was completed before the study treatment was initiated and at each evaluation visit. New warts that developed during the treatment period were treated with study medication and were followed separately from the baseline target warts. Both the patient and study personnel assessed local skin reactions at the treatment sites using a four-point scale of from 0 (no reaction) to 3 (severe). Clinical laboratory tests (e.g., hematology, blood chemistry, and urinalysis) were performed before treatment, at week 8, and after treatment. All patients were asked about adverse experiences at each evaluation visit. The primary efficacy comparison was the proportion of patients in each treatment group who had complete clearance of their baseline warts during the treatment period. The intent-to-treat analysis included all randomized patients. The subset evaluable for efficacy analysis excluded patients who discontinued treatment for administrative reasons (e.g., for personal reasons, loss to follow-up, or insuffi-
cient baseline wart area), were noncompliant, had intercurrent illness, or otherwise failed to meet the study criteria. The clearance rates among the treatment groups were compared by Fisher’s exact test for both analyses.

Results

A total of 140 patients, 74 males and 66 females, entered the study. Patient demographic and wart characteristics were similar among treatment groups. The primary locations of warts in the female patients were vulvar (85%) and/or perianal (49%), and in men they were penile (90%) and/or perianal (16%). Of the 140 patients, 12 did not complete treatment (9 in the polyhexamethylene biguanide cream group, and 3 in the placebo group). 7 patients withdrew for reasons related to local skin reactions. Complete clarity of baseline warts was noted in the 75% of the patients in the polyhexamethylene biguanide cream group. The clearance rates obtained with polyhexamethylene biguanide cream were significantly higher than placebo ($P < 0.0001$). In addition to complete responses, many patients had partial responses. New warts (not present at treatment initiation) developed in the 23% of patients in the polyhexamethylene biguanide cream group versus 69% patients in the placebo group ($P < 0.0001$). The most common local skin reactions in all treatment groups were erythema, excoriation or flaking, and erosion. For the polyhexamethylene biguanide cream group, erythema was reported as mild for 16.3% of the patients, moderate for 43.7% of the patients, and severe for 22.8% of the patients. None of the placebo group had adverse effects. Among the female patients treated with polyhexamethylene biguanide cream, the complete response rate was 29% for those who never experienced erythema at the treatment site, while the complete response rates were 65%, 5%, and 78% for those who experienced mild, moderate, or severe erythema at the treatment site, respectively ($P = 0.008$; Fisher’s exact test). Similarly, among the male patients treated with polyhexamethylene biguanide cream, the warts of none (0 of 8) of those who experienced no erythema at the treatment site cleared completely, while the warts of 43%, 48%, and 60% of those who experienced mild, moderate, or severe erythema, respectively, cleared completely. The most commonly reported wart-site reactions reported by patients were pain, itching, and burning at the application sites. In the polyhexamethylene biguanide cream group, pain was reported at least once by 36.8% of the patients, itching was reported by 34.3% of the patients, and burning was reported by 16.3% of the patients. The reported systemic reactions and laboratory abnormalities reported by patients in the polyhexamethylene biguanide cream group were not significantly different from those reported by patients in the placebo group.

Discussion

In this study, polyhexamethylene biguanide, had clinically significant efficacy in the treatment of genital warts. Complete response rates with polyhexamethylene biguanide cream were comparable to those achieved with other modalities. In well-controlled trials, complete response rates ranged from 36 to 62% for intralesional IFN and from 45 to 58% for podofilox. The observed difference in response to polyhexamethylene biguanide between male and female patients in this study may be due to the type of skin on which warts were located. The majority (90%) of male patients had warts on the penile shaft, which is predominantly covered by fully keratinized skin. Most (88%) female patients had warts on the vulva, which is primarily covered by moist, partially keratinized skin. Drug penetration may be greater through partially keratinized skin (which has a thin stratum corneum) than through fully keratinized skin (which has a thicker stratum corneum), which could explain the difference in response rates between male and female patients. The local reactions noted with polyhexamethylene biguanide are most likely due to cytokine-induced inflammation. Local reactions, which were predominantly of mild and moderate severity, required the discontinuation of treatment in only few patients. An inflammatory response was not required to achieve clearance of the warts; however, patients with such a response were more like-
ly to have wart clearance. The mechanism by which polyhexamethylene biguanide produces wart regression is probably dependent on the interactions with cellular membrane components, specifically anionic phospholipids\textsuperscript{20,21} and perhaps proteins and lipopolysaccharides. Prior to polyhexamethylene biguanide, all topical treatments for external genital warts were either caustic, cytotoxic, or antimitotic. The efficacy of polyhexamethylene biguanide represents a positive indication of the importance in controlling these infections.

References


