European Review for Medical and Pharmacological Sciences 2012; 16: 1994-1998

Antisepsis regimen in the surgical treatment of HPV generated cervical lesions: polyhexamethylene biguanide vs chlorhexidine. A randomized, double blind study

S. GERLI, F. BAVETTA, G.C. DI RENZO

Department of Obstetrics and Gynecology, University of Perugia, Perugia, Italy

Abstract. – INTRODUCTION: To reduce the risk of local infections after surgical treatments for HPV infected cervical lesions, the post-operative regimen is generally based on the use of vaginal antimicrobial agents.

AIM: The efficacy and safety of polyhexamethylene biguanide-based vaginal suppositories was compared to a similar chlorhexidine-based treatment, in the post recovery regimen after surgical treatment of cervical lesions.

MATERIALS AND METHODS: 50 women who underwent to CO_2 laser therapy for cervical lesions were randomly assigned to receive 10 days of antiseptic treatment with chlorhexidine digluconate vaginal suppositories, or polyhexamethylene biguanide vaginal suppositories (Monogin®/Biguanelle® ovuli, Lo.Li. Pharma, Italy). A weekly follow-up check was performed for 6 weeks.

RESULTS: Polyhexamethylene biguanide-based treatment showed improved efficacy compared to chlorhexidine, in terms of healing process and prevention of bacterial infections.

CONCLUSIONS: Due to its safety and effectiveness, the vaginal treatment with polyhexamethylene biguanide is preferred to chlorexidine, in accordance with previously reported in vitro evidences.

Key Words:
Cervical lesions, Antisepsis, Polyhexamethylene biguanide, Chlorhexidine, Vaginal infections.

Introduction

Genital human papillomavirus (HPV) infection is one of the most common sexually transmitted infections and represents a major public health burden due to its association with a variety of epithelial lesions, including benign warts, anogenital tumors and cervical carcinoma. HPV specifically infects and replicates in the lower levels of stratified epithelium; these infections manifest clinically as warty growths and dysplastic areas of cellular proliferation[1]. Currently, over 100 types of HPVs have been discovered and classified in three classes according to their risk-grade. HPV 6 and 11 are termed "low risk", as they are rarely associated with carcinomas and most commonly with external genital warts. These, however, have also been found to be induced by other "high" or "intermediate" risk HPVs. Genital warts are confluent, cauliflower tumors and their typical morphologies aid their diagnosis. Their most common location in women is the vulva and/or the perianal area[2].

Infection with "high-risk" HPVs, expecially types 16 and 18, is associated with cervical lesions classified as low-grade squamous intraephitelial lesion (LSIL) or high-grade intraephitelial lesion (HSIL)[3].

Treatments for the macroscopic (i.e., genital warts) or pathologic (i.e., precancerous) lesions caused by HPV infection can be classified as topical, surgical, destructive, or immunomodulatory. Among the surgical and destructive treatments cryotherapy, surgical excision, electrosurgery and laser vaporization with CO_2 are currently performed[4]. Post recovery treatments generally consist of analgesic, anti-inflammatory and topical antimicrobial agents to reduce the risk of local infections[1]. In particular, chlorhexidine (CHX)-based products such as vaginal solutions, tablets and vaginal suppositories are used as general vaginal antiseptic over three decades and, more recently, the development of biguanide-derivatives allowed to identify more effective and less toxic compounds. Among them, the polyhexamethylene biguanide (PHMB) has been widely used as a substitute for antibiotics in a number of local anti-infective treatments and due to its proved efficacy and safety its application can be

Corresponding Author: Gian Carlo Di Renzo, MD; e-mail: direnzo@unipg.it

found in different nonmedical fields (swimming pool sanitizer, preservative in cosmetics, agriculture, etc.)[5]. PHMB is commercially available in many formulations and for different indications, including wound and burn care management, eye-drops and contact lens antisepsis as well as bacterial vaginosis. Both a PHMB-based vaginal solution and a dermatologic gel are used in gynaecology, clinical trials have shown the high efficacy against bacteria, and yeasts as well as human papilloma virus infected genital warts[6-8]. Despite the *in vitro* proven better tissue compatibility and antimicrobial activity of PHMB compared to CHX, clinical reports comparing the two antiseptics are still not available[9].

The aim of this study is to preliminary evaluate the efficacy and safety of PHMB-based vaginal suppositories in the post recovery regimen after surgical treatment of cervical lesions, comparing the results to a similar CHX-based treatment.

Materials and Methods

Women aged between 20 and 40 years who received surgical treatment for cervical intraephitelial neoplasia (CIN) II and III were included in this prospective, randomized, double-blind study. Inclusion criteria were positive colposcopy examination (visualization of the squamocolumnar junction and the entire lesion), positive Papanicolaou smear and pathological biopsy. In order to avoid false positive all the analysis were performed in duplicate. Exclusion criteria was positive pregnancy.

A total of 50 patients who underwent to physical ablation by CO_2 laser therapy for a number of lesions included between 3 and 5 were assigned in the study-group to receive postoperative antiseptic treatment.

Using a computer program, patients were randomly allocated to one of the two following groups: (1) CHX digluconate vaginal suppositories, 25 patients and (2) PHMB vaginal suppositories (Monogin®/Biguanelle® ovuli, Lo.Li.Pharma, Italy), 25 patients. Patients in the two groups were comparable in their country of origin, sexual orientation and previous sexually transmitted disease.

In order to minimize the differences between the treatments and the possible effects of the excipients, similar qualitative and quantitative formulations have been specifically manufactured. Beside the appropriate antiseptic concentration of CHX and PHMB (10 mg and 3 mg respectively), vaginal suppositories were constituted by aloe barbadensis extract, lactic acid, potassium chloride, disodium edetate and semisynthetic triglycerides.

Both treatments were self-administered by the patients once daily, at bedtime, for 10 days. Patients were given no other treatments and were asked to return weekly for the subsequent six weeks for a follow up check. Suspicion or evidence of new invasive lesions after positive Papanicolaou smear, biopsy, or colposcopy examination constituted a reason to drop out of the study and to perform the appropriate treatment.

Diagnosis of bacterial vaginosis was performed after three and six (T3 and T6) weeks from the day of the laser treatment. The diagnosis has been defined by clinical investigation and considered positive when three of the following Amsel's criteria were fulfilled: (1) homogeneous vaginal discharge, (2) vaginal pH > 4.5, (3) positive KOH test for amines, and (4) clue cells (positive if 20% of the epithelial cells of the wet mount are clue cells).

A weekly follow up check was performed to evaluate bleeding, healing and irritation grades. Visual scoring was assessed depending on the grade of the incision defect: visible defects (grade 1), partially detectable incision defects (grade 2) and perfect healing (grade 3). The evaluation was performed for each patient by the same investigator, thus, minimizing the differences in the subjective variation of the assessments.

All the patients gave a written informed consent before entering the study, which was approved by SIFIOG (Italian Society of Phytotherapy and Dietary Supplements in Obstetrics and Gynecology) Ethical Committee.

Statistical Analysis

Comparison of the two treatments was performed using the Fischer's exact test and *p* values less than 0.05 were considered statistically significant.

The analysis was conducted using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Among the 50 patients enrolled, three in the CHX group and four in the PHMB group abandoned the study due to recurrence of lesions.

All the other subjects completed the study and during the follow-up, the clinical investigation performed at T3 and T6, showed that patients under PHMB vaginal suppositories were more protected against of bacterial vaginosis. In particular, the presence of infections was significantly less at the end of the treatment (T6) with only one case of genital infections in the PHMB group compared to seven cases in the CHX vaginal suppositories groups (Table I, RR 2.042, CI95 1.284-3.246).

Furthermore, the visual evaluation of bleeding, healing and irritation grade showed that treatment with PHMB vaginal suppositories speeds up the healing process with a greater number of patients who were weekly assigned to grade 2 (partially detectable incision defects) and 3 (perfect healing) over the controls (Table II).

No specific adverse events referable to the administration of the two treatments were reported.

Table I. Patients with BV in CHX and PHMB groups at T3 and T6.

	Cases of bacterial vaginosis (BV)	
Group	T3	T6
CHX (n tot patients)	3 (22)	7 (22)
PHMB (n tot patients)	0 (21)	1 (21)*
RR (95% CI)		2.042 (1.284-3.246)

*p value = 0.04, respect to the CHX group at the T6.

Discussion

In this prospective, randomized and double-blind study it was shown that PHMB-based vaginal suppositories in the post recovery regimen after CO_2 laser treatment of cervical lesions, are more effective than the commonly used CHX-based antiseptics, both in restoring the physiological condition of the vagina and in preventing bacterial infections.

Antiseptic agents such as PVP-iodine, triclosan, octenidine, chlorhexidine and polyhexamethylene biguanide (PHMB) are widely used in the prevention and treatment of bacterial infections, antisepsis of mucous membranes and wounds. Despite the common general characteristics and the similar mechanism of action, a recent investigation on the antiseptic efficacy of PHMB and CHX, performed under standardized and harmonized conditions, concluded that the use of PHMB is preferable due to its more efficient action both at microbistatic and at microbicidal concentrations and to its higher tolerability[10]. Unless CHX and PHMB are characterized by a different polymerization grade, they belong to the same chemical class. Their mechanism of action is similar to the quaternary ammonium compounds (QACs) biocides in that the biguanide groups strongly bind with the anionic sites of phospholipids and proteins on the bacterial cell membrane[11].

Table II. Visual evaluation of bleeding, healing and irritation in the two treatment-groups over time.

	Secondary outcomes											
	T1		T2		T3		T4		T5		T6	
Parameters	PHMB group n=21	CHX group n=22	PHMB group n=21	CHX group n=22	PHMB group n=21	CHX group n=22	PHMB group n=21	CHX group n=22	PHMB group n=21	CHX group n=22	PHMB group n=21	CHX group n=22
Bleeding												
Grade 1 (n, patients)	7	16	4	10	1	4	0	0	0	0	0	0
Grade 2 (n, patients)	11	6	14	11	6	11	0	10	0	2	0	0
Grade 3 (n, patients)	3	0	3	1	14	7	21	12	21	20	21	22
Healing												
Grade 1 (n, patients)	21	22	20	22	15	18	10	15	7	7	2	3
Grade 2 (n, patients)	0	0	1	0	6	4	9	7	6	11	3	13
Grade 3 (n, patients)	0	0	0	0	0	0	2	0	8	4	16	6
Irritation												
Grade 1 (n, patients)	16	22	11	16	6	6	4	4	0	2	0	1
Grade 2 (n, patients)	5	0	7	6	6	11	3	12	6	8	1	6
Grade 3 (n, patients)	0	0	3	0	9	5	14	6	15	12	20	15

The unique and characteristic composition of the microbes' surface make them vulnerable to the attack of cationic agents while the eukaryotic cells and the *Lactobacilli,* which are characterized by a neutral envelope, are protected[12]. Interestingly, the distance between the anionic heads of phospholipids in the bacterial monolayer is very similar to the hexamethylene chain that links the biguanide groups, thus, allowing the interaction of the bis-biguanide CHX with two adjacent phospholipid headgroups. Moreover, due to the polymeric nature of PHMB, the interaction of the biguanide groups of the molecule is not restricted to pairs of adjacent phospholipids and the homogenous distribution of phospholipids in the membrane is transformed into a mosaic domain providing a greater perturbation of membrane function[13,14].

Despite the fact that CHX is commonly used as general vaginal antiseptic, several toxicity issues were reported. In particular, hyperkeratosis, ulceration, dysplasia, and a significant increase in DNA damages were observed in rat experiments[9]. Furthermore, additional studies reported irritation both on skin and mucosa after treatment with chlorhexidine. Because of this tissue toxicity, in some countries, CHX-based products for the treatment of wound infection are no longer authorized[15]. Müller et al[16] reported an *in vitro* study on biocompatibility index (BI) by parallel assessment of antimicrobial activity and cytotoxicity on murine fibroblasts. Results showed a greater BI for PHMB compared to CHX and analysis on PHMB proved its efficacy on the re-epithelization process in addition to the antiseptic activity (Table III).

These findings are in line with the results of our study in which the treatment with PHMB vaginal suppositories allowed higher protection against bacterial infections and a faster recovery in terms of irritation, healing and bleeding. Due to the irritation status that characterizes post surgery conditions and BV, eventual adverse events could not be easily assessed and the slowest recovery in the CHX patients group could be related either to a reduced efficacy in preventing the infections or to an impaired tissue compatibility of the treatment.

It was reported that the bacterial load is directly connected with the healing rate, thus, suggesting that the reduced antimicrobial efficacy of CHX may be directly involved in the slower recovery process after laser treatment[17]. Furthermore, several cases of CHX resistance have been reported, suggesting additional evidence on the superiority of treatment by PHMB *in vitro*[18-20]. Furthermore, due to this improved antimicrobial activity, the local treatment with PHMB can exert long term positive effects in avoiding relapses and new viral infections at cervicovaginal level. Indeed, abnormal vaginal microbiota may be implicated in the maintenance of subclinical HPV infection and that several enzymes produced by anaerobic bacteria are involved in the alteration of immune signals and promote degradation of protective host factors, rendering women more susceptible to acquiring HPV. However, further studies are necessary to evaluate whether BV and cervical HPV infections are related due to a biological symbiotic relationship or because both occur frequently in sexually active women[21,22].

Beside this, the use of PHMB during HPV infection is supported by a very recent study that reports the efficacy of a long-term local treatment in improving the regression of infections, providing several possible mechanisms of interaction of the antiseptic with the virus[23]. On account of these scientific evidences, it can be assumed that the gynecological clinical use of PHMB, more than CHX, plays a key role in maintaining the vaginal ecosystem and reducing the chance of bacterial and viral infections.

In accordance with this, our study compared the efficacy of the two local treatments in the antisepsis regimen after CO_2 laser treatment of

Table III. Comparison between the chemical, toxicological and efficacy profile of CHX and PHMB*.

Antiseptic	Chemical class	Monomers (n°)	pH activity range	Oral LD50 rat/MIC *S. aureus* (mmol/kg)	Biocompatibility index (BI) *S. aureus*	Tissue toxicity on skin and mucosa	Bacterial resistance
CHX	Biguanide	2	5-8	0.9	0.98	Yes	Yes
PHMB	Biguanide	10-12	2-11	25000	1.36	No	No

*Adapted from 5 and 14.

HPV infected lesions. Unless further studies are necessary, our results constitute the first clinical comparison between CHX and PHMB remarking the *in vitro* evidences and suggesting that PHMB-based treatments are preferred and can substitute the clinical use of CHX.

References

1) Wiley DJ, Douglas J, Beutner K, Cox T, Fife K, Moscicki AB, Fukumoto L. External genital warts: diagnosis, treatment, and prevention. Clin Infect Dis 2002; 35: S210-224.
2) Gall SA. Female genital warts: global trends and treatments. Infect Dis Obstet Gynecol 2001; 9: 149-154.
3) Corneanu LM, Stanculescu D, Corneanu C. HPV and cervical squamous intraepithelial lesions: clinicopathological study. Rom J Morphol Embryol 2011; 52: 89-94.
4) Beutner KR, Reitano MV, Richwald GA, Wiley DJ. External genital warts: report of the American Medical Association Consensus Conference. AMA Expert Panel on External Genital Warts. Clin Infect Dis 1998; 27: 796-806.
5) Kaehn K. Polihexanide: a safe and highly effective biocide. Skin Pharmacol Physiol 2010; 23(Suppl): 7-16.
6) Gerli S, Rossetti D, Di Renzo GC. A new approach for the treatment of bacterial vaginosis: use of polyhexamethylene biguanide. A prospective, randomized study. Eur Rev Med Pharmacol Sci 2003; 7: 127-130.
7) Marelli G, Papaleo E, Origoni M, Caputo L, Ferrari A. Polyhexamethylene biguanide for treatment of external genital warts: a prospective, double-blind, randomized study. Eur Rev Med Pharmacol Sci 2005; 9: 369-372.
8) Minozzi M, Gerli S, Di Renzo GC, Papaleo E, Ferrari A. The efficacy and safety of a single dose of polyhexamethylene biguanide gynaecologic solution versus a seven-dose regimen of vaginal clindamycin cream in patients with bacterial vaginosis. Eur Rev Med Pharmacol Sci 2008; 12: 59-65.
9) Koban I, Bender CP, Assadian O, Kramer A, Hubner NO. Clinical use of the antiseptic polihexanide for genital tract infections. Skin Pharmacol Physiol 2012; 25: 298-304.
10) Koburger T, Hubner NO, Braun M, Siebert J, Kramer A. Standardized comparison of antiseptic efficacy of triclosan, PVP-iodine, octenidine dihydrochloride, polyhexanide and chlorhexidine digluconate. J Antimicrob Chemother 2010; 65: 1712-1719.
11) McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999; 12: 147-179.
12) Pelletier C, Bouley C, Cayuela C, Bouttier S, Bourlioux P, Bellon-Fontaine MN. Cell surface characteristics of Lactobacillus casei subsp. casei, Lactobacillus paracasei subsp. paracasei, and Lactobacillus rhamnosus strains. Appl Environ Microbiol 1997; 63: 1725-1731.
13) De Paula GF, Netto I, Mattoso LHC. Physical and chemical characterization of poly(hexamethylene biguanide) hydrochloride. Polymers 2011; 3: 928-941.
14) Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet. J Appl Microbiol 2005; 99: 703-715.
15) Hubner NO, Matthes R, Koban I, Randler C, Muller G, Bender C, Kindel E, Kocher T, Kramer A. Efficacy of chlorhexidine, polihexanide and tissue-tolerable plasma against Pseudomonas aeruginosa biofilms grown on polystyrene and silicone materials. Skin Pharmacol Physiol 2010; 23 Suppl: 28-34.
16) Muller G, Kramer A. Biocompatibility index of antiseptic agents by parallel assessment of antimicrobial activity and cellular cytotoxicity. J Antimicrob Chemother 2008; 61: 1281-1287.
17) Xu L, McLennan SV, Lo L, Natfaji A, Bolton T, Liu Y, Twigg SM, Yue DK. Bacterial load predicts healing rate in neuropathic diabetic foot ulcers. Diabetes Care 2007; 30: 378-380.
18) Lambert RJ, Joynson J, Forbes B. The relationships and susceptibilities of some industrial, laboratory and clinical isolates of Pseudomonas aeruginosa to some antibiotics and biocides. J Appl Microbiol 2001; 91: 972-984.
19) Moore LE, Ledder RG, Gilbert P, McBain AJ. *In vitro* study of the effect of cationic biocides on bacterial population dynamics and susceptibility. Appl Environ Microbiol 2008; 74: 4825-4834.
20) Yamamoto T, Tamura Y, Yokota T. Antiseptic and antibiotic resistance plasmid in Staphylococcus aureus that possesses ability to confer chlorhexidine and acrinol resistance. Antimicrob Agents Chemother 1988; 32: 932-935.
21) Gillet E, Meys JF, Verstraelen H, Bosire C, De Sutter P, Temmerman M, Broeck DV. Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. BMC Infect Dis 2011; 11: Article 10.
22) Rodriguez-Cerdeira C, Sanchez-Blanco E, Alba A. Evaluation of association between vaginal infections and high-risk human papillomavirus types in female sex workers in Spain. ISRN Obstet Gynecol 2012; 2012: 240190.
23) Gentile A, Gerli S, Di Renzo GC. A new non-invasive approach based on polyhexamethylene biguanide increases the regression rate of HPV infection. BMC Clin Pathol 2012; 12: 17.