Taylor & Francis
Taylor & Francis Group

Journal of Dermatological Treatment

ISSN: 0954-6634 (Print) 1471-1753 (Online) Journal homepage: http://www.tandfonline.com/loi/ijdt20

A randomized trial of efficacy of beta-sitosterol and its glucoside as adjuvant to cryotherapy in the treatment of anogenital warts

Christina Stefanaki, Xara Fasoulaki, Anargyros Kouris, Chrysseis Caroni, Konstantina Papagianaki, Panagiota Mavrogianni, Electra Nicolaidou, Stamatis Gregoriou & Christina Antoniou

To cite this article: Christina Stefanaki, Xara Fasoulaki, Anargyros Kouris, Chrysseis Caroni, Konstantina Papagianaki, Panagiota Mavrogianni, Electra Nicolaidou, Stamatis Gregoriou & Christina Antoniou (2015) A randomized trial of efficacy of beta-sitosterol and its glucoside as adjuvant to cryotherapy in the treatment of anogenital warts, Journal of Dermatological Treatment, 26:2, 139-142, DOI: 10.3109/09546634.2014.925535

To link to this article: http://dx.doi.org/10.3109/09546634.2014.925535

Journal of Dermatological Treatment

http://informahealthcare.com/jdt
ISSN: 0954-6634 (print), 1471-1753 (electronic)

J Dermatolog Treat, 2015; 26(2): 139–142
 DOI: 10.3109/09546634.2014.925535

informa healthcare

ORIGINAL ARTICLE

A randomized trial of efficacy of beta-sitosterol and its glucoside as adjuvant to cryotherapy in the treatment of anogenital warts

Christina Stefanaki[1], Xara Fasoulaki[1], Anargyros Kouris[1], Chrysseis Caroni[2], Konstantina Papagianaki[1], Panagiota Mavrogianni[3], Electra Nicolaidou[1], Stamatis Gregoriou[1], and Christina Antoniou[1]

[1]*Department of Dermatology, Andreas Sygros Hospital, University of Athens, Athens, Greece,* [2]*Department of Mathematics, National Technical University of Athens, Athens, Greece, and* [3]*Gastrenterology Unit, Army Mutual Fund Hospital of Athens, NIMTS, Athens, Greece*

Abstract

Introduction: All available treatments for anogenital warts have substantial failure rates. An immunomodulating treatment that enhances the patient's own immunity could be valuable as an adjuvant to conventional methods.
Methods: About 123 patients were enrolled in this study and were randomized either to undergo only cryotherapy every 3 weeks ($n = 60$ patients) or to undergo cryotherapy and to receive a mixture containing 20 mg sterol and 0.2 mg sterolin (BSS–BSSG mixture), known for its immunomodulating properties ($n = 63$).
Results: A complete response after 3 months was demonstrated by 18.3% of the patients in the first group and 30.2% of the second group, while 61.7% of the first group and 79.4% of the second group were lesion free at the end of the 6-month follow-up period. Cox regression analysis of the time until response showed a significant advantage to the mixture treatment group (hazard ratio 2.76, 95% confidence interval 1.61–5.67).
Limitations: The study was not placebo controlled.
Conclusion: The BSS–BSSG mixture gave promising results as an adjuvant to cryotherapy and may be used in patients with refractory warts.

Keywords

Anogenital warts, cryotherapy, beta-sitosterol, HPV, treatment, sterolins

History

Received 19 March 2014
Accepted 3 May 2014
Published online 9 June 2014

Introduction

Anogenital HPV infection is the most common sexually transmitted disease (1). No effective treatment for it exists to date, despite the various therapeutic modalities available for anogenital warts. Most of the available treatments for genital warts rely on tissue destruction by heat (electrosurgery), cold (cryotherapy), acids (bi/trichloroacetic acid), or light absorbed by water (laser), or by arresting cell division (podophyllotoxin). All available treatments have substantial failure rates and it has been estimated that 20–30% of patients develop new lesions at the same or new sites (2). Recurrence rates as high as 50% have been reported in patients treated with cryotherapy, a widely used method for the destruction of genital warts (3). Among all the available treatments, only imiquimod cream 5% has been found to have a low recurrence rate (9–19%) (4,5) and for that reason it has been used as an adjuvant after surgical ablation (6). Imiquimod, an immune response modifier, is the only treatment to date that induces the patient's own immunity to fight against HPV virus and to control the infection (7,8).

It is obvious that there is a need for new immunostimulating treatments that might reduce the recurrence rate after ablative methods. Prompted by the promising immunostimulating properties of sterols and sterolins that have been reported in the literature (9–12), we conducted the present study to access their efficacy in combination with cryotherapy, as an adjuvant treatment for genital warts.

Materials and methods

This open label, non-placebo-controlled, randomized study was conducted at the Sexually Transmitted Diseases Unit of the Andreas Sygros University Hospital for Skin and Sexually Transmitted Diseases in Athens, Greece. The study protocol was approved by the Ethical Committee of the Institutional Review Board of the Hospital and an informed consent was obtained from all patients prior to enrollment. Only immunocompetent patients diagnosed with anogenital warts were included in the study. Pregnant patients or patients with Bowenoid papulosis or a severe medical condition (hematological, hepatic- Hepatitis B or C-, neurological, renal, endocrine, collagen, and gastrointestinal) or with drug or alcohol dependency were excluded from the study. Patients were routinely screened for syphilis and HIV and only negative individuals were enrolled. Furthermore, patients who had been treated recently with steroids, cytotoxic drugs, and interferons (INFs) were also excluded.

Patients were randomized either to undergo only cryotherapy every 3 weeks or to undergo cryotherapy and additionally to receive modullon, a mixture containing 20 mg sterol and 0.2 mg sterolin (BSS–BSSG mixture) available as a medicinal product in Greece, one tablet three times daily. Cryotherapy was employed by the same dermatologist with a spray gun and freezing was applied to each wart for 10–20 s or until the wart and a small

Correspondence: Anargyros Kouris, Department of Dermatology, Andreas Sygros Hospital, University of Athens, I. Dragoumi 5 Street, Athens 14969, Greece. Tel: +30 210 7265100. E-mail: kouris2007@yahoo.com

margin of surrounding normal skin was frozen. For large lesions, two freeze cycles were performed. At the baseline visit prior to treatment, the warts were measured in cm^2 and the exact number of warts, their location on the genitalia and the duration of the disease were recorded.

Follow-up appointments for both groups, to inspect the treatment outcome, were arranged at 3 and 6 months. At each visit, any remaining warts were counted and measured, and adverse events were recorded. Response was defined as 100% resolution of the lesions for 1 month.

Statistical analysis

The time until response to treatment was analyzed by Cox's semi-parametric proportional hazards model, examining treatment group, gender, age, history of disease, previous treatment, baseline number of warts, and baseline duration of disease as potential predictors of time to response (13). Because the patients are not under continual observation, the time to response is not known exactly; it is known only to fall within the time interval from the last examination to the present one. Data of this form are known as interval censored (REF) and special software is required in order to fit Cox's model to them. We used the program *intcox* written in the R language by V. Henschel, C. Heiss and U. Mansmann (2009) intcox: Compendium to apply the iterative convex minorant algorithm to interval censored event data (available from http://cran.r-project.org/web/packages/intcox/vignettes/intcox.pdf, accessed 12 June 2010). The estimated survivor function – that is, the probability that the patient has not responded, as a function of time – is estimated and plotted by another R routine written by R. Gentleman and A. Vandal (2010) Package ''Icens''. Version 1.20.0 (available from http://cran.r-project.org/web/packages/Icens/index.html, accessed 14 June 2010).

Results

Of the 123 patients enrolled in this study, 111 were males and 12 were females. After randomization 60 patients were included in the cryotherapy-only treated group and 63 in the group receiving cryotherapy and beta-sitosterol. Patients in the first group were aged from 18 to 75 years (mean 30.8 ± 9.5) in the first group and from 19 to 76 years (mean 30.0 ± 9.0) in the second. The duration of disease before enrollment ranged from < 1 month to 60 months in each group, with mean duration σ of 7.5 months in the first group and 12 months in the second group. Patients had up to nine warts on their genitalia; most (65.1%) had at least three. Almost a third (32.8%) had newly diagnosed genital warts, while the rest reported at least one recurrence before attending our department. Gender, age, disease duration, number of lesions, and recurrences of the two groups of patients are presented in Table 1. The only notable difference in the distribution of characteristics between the two groups as a result of the randomization was that patients in the cryotherapy-only group tended to have fewer lesions than patients in the cryotherapy and b-sitosterol group (Table 1). Irrespective of group, for males, the majority of the lesions were located on the shaft of the penis (77.5%), whereas for females on the mons pubis (75%). Wart distribution on the genitalia for the two groups is presented in Table 2. Thirty percent of patients in the first group and 20.6% of the second group had never received any other form of treatment prior to enrollment (Table 3), whereas all the other patients had tried various therapeutic modalities including podophyllotoxin cream or solution, cryotherapy, laser, electrosurgery, and imiquimod.

All patients were invited to return for review 3 months after enrollment; however, six patients in the cryotherapy group and

Table 1. Gender, age, disease duration, number of lesions, and recurrences in the two groups of patients.

	Treatment group			
	Cryotherapy only ($n = 60$)		Cryotherapy + BSS–BSSG mixture ($n = 63$)	
	n	%	*n*	%
Gender				
Male	55	91.7	56	88.9
Female	5	8.3	7	11.1
Age				
<25	9	15.5	11	17.5
25–29	23	39.7	30	47.6
30–34	15	25.9	9	14.3
35–44	7	12.1	10	15.9
45+	4	6.9	3	4.8
Disease duration before enrollment (months)				
<6	21	35.0	17	27.0
6–11	11	18.3	10	15.9
12–23	19	31.6	19	30.2
24+	9	15.0	17	27.0
Recurrences before enrollment				
0	22	36.7	18	29.0
1	9	15.0	11	17.7
2	14	23.3	9	14.5
3+	15	25.0	24	38.1
Number of lesions at baseline				
1–2	11	18.3	3	4.8
3–4	20	33.3	15	23.8
5–9	19	31.7	26	41.3
10–14	7	11.7	7	11.1
15+	3	5.0	12	19.0

Table 2. Location of warts on the genitalia in the two groups.

	Treatment group			
	Cryotherapy only ($n = 55$)		Cryotherapy + BSS–BSSG mixture ($n = 56$)	
	n	%	*n*	%
Male				
Shaft of penis	41	74.5	45	80.4
Mons pubis	12	21.8	17	30.4
Glans penis	12	21.8	17	30.4
Base of penis	8	14.5	7	12.5
Anus	6	10.9	8	14.3
Scrotum	5	9.1	7	12.5
Genital crurae	2	3.6	4	7.1
Colonal sulcus	2	3.6	1	1.8
Bridle	1	1.8	1	1.8
Prepuce	0	0.0	1	1.8
Foreskin	1	1.8	0	0.0
Perineum	1	1.8	0	0.0
Bridle	1	1.8	1	1.8
	($n = 5$)		($n = 7$)	
	n	%	*n*	%
Females				
Mons pubis	3	60.0	6	85.7
Vulva	3	60.0	4	57.1
Anus	2	40.0	2	28.6

Percentages total more than 100 because multiple locations in the same patient are possible.

one patient in the cryotherapy and b-sitosterol group did not attend. Eighteen percent of the patients (18.3%) in the first group and 30.2% of the second group demonstrated a complete response after 3 months. At the end of the 6-month follow-up period, 61.7% of the first group and 79.4% of the second group were lesion free. The association of time until response with age (25, 26–30, >30 years), gender (male, female), disease duration (≤3 months, 4–12, >12), previous treatments (yes–no), number of lesions (1–4, 5–9, 10+), and type of treatment (only cryotherapy versus cryotherapy and b-sitosterol) was examined in Cox's semi-parametric proportional hazards regression analysis adapted to interval-censored data. Only the type of treatment (hazard ratio 2.76, 95% confidence interval 1.61–5.67) and the initial number of lesions (hazard ratio 1.05, 95% confidence interval 0.57–1.78 for 5–9 lesions, and hazard ratio 0.31, 95% confidence interval 0.10–0.62 for 10 + lesions) were found to influence the complete response rate. Thus the time to complete response was significantly shortened if the patient was taking b-sitosterol but was longer if the patient presented initially with a large number of lesions. Figure 1 presents the estimated probability of complete response in relation to time in both groups of patients, with respect to their number of lesions.

No patient in the combination group reported any gastrointestinal or any other adverse events. Blood was not routinely taken to assess any changes in blood count, urea, and electrolytes or liver function tests in the combination group.

Table 3. Treatments received by the patients before enrollment in the study.

	Treatment group			
	Cryotherapy only ($n = 60$)		Cryotherapy + BSS–BSSG mixture ($n = 63$)	
	n	%	*n*	%
Cryotherapy	35	58.3	32	50.8
Imiquimod	17	28.3	25	39.7
Podophyllotoxin	4	6.7	10	15.9
Laser	2	3.3	5	7.9
Electrosurgery	1	1.7	2	3.2
None	18	30.0	13	20.6

Discussion

The major plant sterol b-sitosterol (BSS), first described in 1922, has enjoyed biological importance only as a natural approach to control cholesterol plasma levels (14). The glycoside of sterols, particularly b-sitosterol glycoside (BSSG), has not received biological attention because of its presence in low quantities in the plasma of humans (15). However, these underestimated molecules have demonstrated immunomodulating properties (9,11). Bouic et al. (11) showed that a proprietary mixture of sterols and sterolins (BSS–BSSG mixture) has the ability to enhance the cellular response of T lymphocytes both *in vitro* and *in vivo* and to enhance the cytotoxic ability of natural killer cells (NK) against the target cell line NK562. They also demonstrated that the mixture preferentially enhanced the activity of CD4 + cells belonging to the TH1 phenotype. These results implied the potential ability of the mixture to restore the TH1 and TH2 balance in conditions where it is disrupted and the immune response is insufficient. A bias towards the TH2 phenotype results in chronic viral infections including HIV and HPV infection. The immune system plays a central role in regression of genital HPV disease via migration of effector cells (16). Spontaneously regressing genital warts show a significant epidermal and dermal influx of CD4 + activated memory lymphocytes compared to non-regressing lesions (17). Moreover, it has been found that HPV is capable of inducing a local immune deficiency, manifested by depletion of intraepithelial lymphocytes, Langerhans cells, and CD4 + cells with down-regulation of cytokine production (18–21). The importance of an intact immune system for the regression of genital warts is best demonstrated in immunocompromised patients, particularly HIV positive patients, who are known to have a higher incidence of HPV disease and larger and multifocal lesions (18). It is obvious that, from a theoretical point of view, the BSS–BSSG mixture might be effective in helping the patient's own immunity to fight against HPV infection. A previous open-labeled study in HIV positive patients with no access to antiretroviral drugs because of their high cost showed that patients receiving a BSS–BSSG mixture demonstrated a significant decrease in their plasma viral loads and stable CD4 cell counts over a period of 40 months, by maintaining a favorable TH1 response (22).

Our results are similarly promising in the treatment of genital warts as we have demonstrated a more favorable response in patients treated with cryotherapy and simultaneously taking the

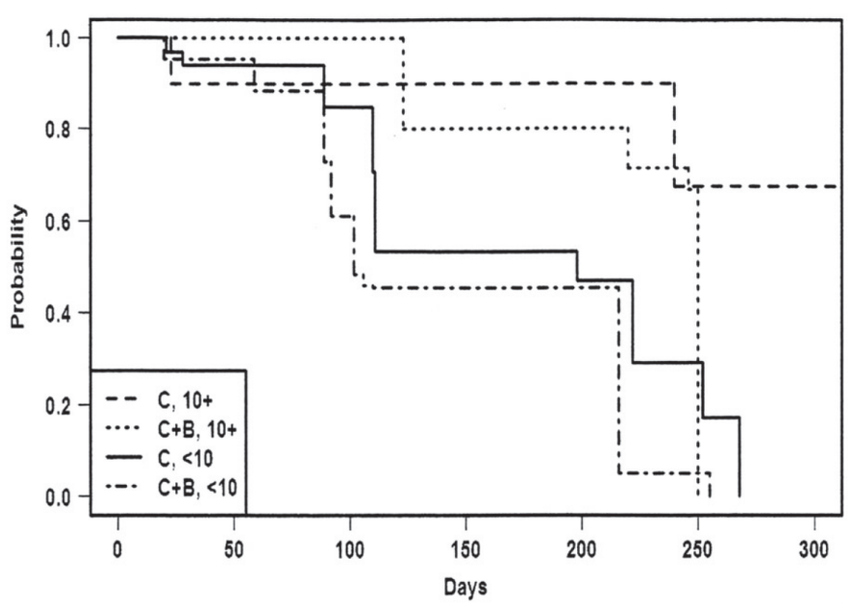

Figure 1. Estimated probability that the patient has not responded to treatment, as a function of time in days since enrollment, by treatment group, and number of lesions at enrollment.

BSS–BSSG mixture in comparison with those treated only with cryotherapy. Only 18% of the cryotherapy group demonstrated a sustained response by the end of 3 months, whereas 30% of the patients in the cryotherapy and BSS–BSSG mixture group responded. The difference in the therapeutic outcome was still evident at the end of the 6-month follow-up period, when 62% of the first group but 80% of the second group were found lesion free. An important issue in our study is that patients treated with the mixture started from a worse position than the others, as they suffered from a greater number of lesions on average.

Cryotherapy uses liquid nitrogen to freeze the affected tissues, which eventually become inflamed and slough before they heal. Three treatment sessions are needed on an average before warts are cleared but occasionally multiple sessions may be required. The reported clearance rates range between 27 and 88% after multiple treatments (2,3,16,23–26). The low clearance rate that we observed in our cryotherapy group, in contrast to our previous observation (3), is striking but could be explained by our different study methodology as we defined a complete response as not only the resolution of the individual lesions but also a wart-free interval of at least 1 month.

The optimal dose of the mixture of sterols and sterolins has been addressed before by Bouic et al. (10,11), and the standardized mixture of 20 mg of sterol and 0.2 mg of sterolin in a specific ratio of 100:1, given three times daily, has shown immunomodulating activity *in vitro* and *in vivo*. The safety of the BSS-BSSG mixture has not been addressed in our study as the safe and non-toxic profile of the plant molecules, which has enabled previous clinical trials, is known (9,10,27,28).

The main limitation of our study is that it is not placebo controlled and the placebo effect of BSS–BSSG mixture cannot be excluded. However, spontaneous regression has rarely been noted in placebo-controlled trials and only a few studies have recorded spontaneous clearance, particularly among women (5,16,29). In conclusion, in our clinical study using a sterol and sterolin mixture, we have shown promising results for the treatment of patients with anogenital warts. This natural plant derived immune modulator may offer a cheap therapeutic adjuvant, particularly in patients with refractory anogenital warts. Certainly, more studies are needed before any definite conclusions can be drawn but natural remedies cannot be overlooked and might be revisited as important immunomodulating molecules.

Declaration of interest

The authors report no conflicts of interest.

References

1. Cates W. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. Sex Transm Dis. 1999;26:S2–7.
2. Wiley DJ, Douglas J, Beutner K, et al. External genital warts: diagnosis, treatment, and prevention. Clin Infect Dis. 2002;35:S210–24.
3. Stefanaki C, Katzouranis I, Lagogianni E, et al. Comparison of cryotherapy to imiquimod 5% in the treatment of anogenital warts. Int J STD AIDS. 2008;19:441–4.
4. Beutner KR, Spruance SL, Hougham AJ, et al. Treatment of genital warts with an immune response modifier (imiquimod). J Am Acad Dermatol. 1998;38:230–9.
5. Edwards L, Ferenczy A, Eron L, et al. Self-administered topical 5% imiquimod cream for external anogenital warts. Arch Dermatol. 1998;134:25–30.
6. Kaspari M, Gutzmer R, Kaspari T, et al. Application of imiquimod by suppositories (anal tampons) efficiently prevents recurrences after ablation of anal canal condyloma. Br J Dermatol. 2002;147:757–9.
7. Hemmi H, Kaisho T, Takekuchi O, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 2002;3:196–200.
8. Tyring SK, Arany I, Stanley MA, et al. A randomized, controlled, molecular study of condylomata acuminata clearance during treatment with imiquimod. J Infect Dis. 1998;78:551–5.
9. Breytenbach U, Clark A, Lambrecht J, Bouic P. Flow cytometric analysis of the TH1-TH2 balance in healthy individuals and patients infected with the human immunodeficiency virus (HIV) receiving a plant sterol/sterolin mixture. Cell Biol Int. 2001;25:43–9.
10. Bouic P. Sterols and sterolins: new drugs for the immune system? Drug Discov Today. 2002;7:775–8.
11. Bouic PJD, Etsebeth S, Liebenberg RW, et al. Beta-sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. Int J Immunopharmacol. 1996;18:693–700.
12. Bouic PJD, Lamprecht J. Plant sterols and sterolins: a review of their immune-modulating properties. Alt Med Rev. 1999;4:170–7.
13. Collett D. Modelling Survival Data in Medical Research, 2nd ed. Boca Raton, FA: Chapman and Hall/CRC.
14. Pollak OJ. Effect of plant sterols on serum lipids and atherosclerosis. Pharmacol Ther. 1985;31:177–208.
15. Pegel RH, The importance of sitosterol and sitosterolins in human and animal nutrition. S Afr J Sci. 1997;93:263–8.
16. Lacey CJN. Therapy for genital human papillomavirus-related disease. J Clin Virol. 2005;328:S82–90.
17. Coleman N, Birley HDL, Renton AM, et al. Immunological events in regressing genital warts. Am J Clin Pathol. 1994;102:768–74.
18. Gunter J. Genital and perianal warts: new treatment opportunities for human papillomavirus infection. Am J Obstet Gynecol. 2003;189:S3–11.
19. Tyring SR. Human papillomavirus infections: epidemiology, pathogenesis and host immune response. J Am Acad Dermatol. 2000;43:S18–26.
20. Altmann A, Jochmus I, Rosl F. Intra- and extracellular control mechanisms of human papillomavirus infections. Intervirology. 1994;37:180–8.
21. Arany I, Rady P, Tyring SR. Alterations in cytokine/antioncogene expression in the skin lesions caused by low-risk types of human papillomavirus. Viral Immunol. 1993;6:255–65.
22. Bouic P, Lambrecht JH, Freestone M, et al. Plant sterol and sterolin supplement use in a cohort of SA HIV-infected patients: effects on immunological and virological surrogate markers. S Afr Med J. 2001;91:848–50.
23. Abdullah AN, Walzman M, Wade A. Treatment of external genital warts comparing cryotherapy and trichloacetic acid. Sex Transm Dis. 1993;20:344–5.
24. Godley MJ, Bradbeer CS, Gellan M, Thin RNT. Cryotherapy compared with trichloroacetic acid in treating genital warts. Genitourin Med. 1987;63:390–2.
25. Stone K, Becker T, Hagdu A, Kraus S. Treatment of external genital warts: a randomized clinical trial comparing podophyllin, cryotherapy, and electrodessication. Genitourin Med. 1990;66:16–19.
26. Maw R. Critical appraisal of commonly used treatment for genital warts. Int J STD & AIDS. 2004;15:357–64.
27. Donald PR, Lamprecht JH, Freestone M, et al. A randomized placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis. Int J Tuberc Lung Dis. 1997;1:518–22.
28. Bouic PJD, Clark A, Lamprecht JH, et al. The effects of b-sitosterol (BSS) and b-sitisterol glucoside (BSSG) mixture on selected immune parameters of marathon runners: inhibition of post marathon immune suppression and inflammation. Int J Sports Med. 1999;20:258–62.
29. Tying S, Edwards L, Cherry K, et al. Safety and efficacy of 0.5% podophyllox gel in the treatment of anogenital warts. Arch Dermatol. 1998;134:33–8.


Modücare®