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ORIGINAL ARTICLE

A double-blind placebo-controlled study of 5-fluorouracil:cyclodextrin complex loaded thermosensitive gel for the treatment of HPV induced condyloma

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Abstract Objective of this double-blind placebo-controlled study was to determine the efficacy of thermosensitive mucoadhesive gel loaded with 5-fluorouracil (5-FU): hydroxypropyl-ß-cyclodextrin (HP-ß-CD) complex via topical administration or intralesional injection for the treatment of human papilloma virus induced condyloma in 44 women. The diagnosis of human papilloma virus was established with clinical, histopathological and polymerase chain reaction techniques. Subjects were randomized into four parallel groups to evaluate topical or intralesional administration of drug-loaded or blank gel. The formulation used in the study consisted of 20% Pluronic PF 127 and 0.2% hydroxypropylmethylcellulose (HPMC) to render thermosensitive and mucoadhesive properties to the blank and drug-loaded gels. 5-FU was complexed to hydroxypropyl-ß-cyclodextrin to improve its solubility and this complex was loaded into thermosensitive gel to obtain controlled release of the cytotoxic drug in administration site over a two-week period cure regimen aiming therapeutic efficacy with lower 5-FU doses. Complete response was achieved in 61% of patients through intralesional administration while topical administration resulted in only 29% complete cure. Relapse rates of all therapy groups were significantly low in the 6-month follow-up time.

Keywords HPV · 5-Fluorouracil · Cyclodextrin · Thermosensitive gel · Condyloma

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Introduction

Anogenital warts are benign epithelial tumors mostly found on cutaneous surfaces and are caused by one of the many human papilloma virus (HPV) types. HPV types 16 and 18 are most often associated with intraepithelial neoplasia (cervical, vulvar, vaginal) whereas types 6 and 11 are recovered from benign anogenital warts [1]. Cervical cancer induced by HPV remains as the most common cause of death among women under 50 years of age [2].

There is currently no virus specific drug therapy available for HPV infections. Present practice of therapy of HPV infections relies on nonspecific destruction or removal of infected tissue by often ablative procedures. The surgical procedures for HPV induced diseases include cryotherapy with dry ice or liquid nitrogen, carbon dioxide laser therapy, electrocauterisation and local excisions. Various localized topical or intralesional treatments have been used including acids (salicylic acid, bi- and trifluoroacetic acid) and chemotherapeutic agents such as podophyllin, colchicine, bleomycin, cantharidin and 5-fluorouracil as well as antivirals such as cidofovir and immunomodulators such as imiquimod have been studied in the literature [3–5]. The treatment choice should be guided by the preference of the patient, the available sources and the experience of the health care provider. However an ideal treatment is still not reached and recurrence of warts are still frequently observed.

5-Fluorouracil (5-FU) is a powerful cytotoxic drug that acts as an antimetabolite and has been shown to be successful in treating genital, urethral and intravaginal condylomata. However experience has shown that when 5-FU is applied topically to treat vaginal lesions, a patient may experience local discomfort due to chemoinflammation or epithelial erosion probably due to concentration (5%) of 5-FU as well as inadequate vehicle formulation causing irritation [6]. Researchers proposed lower dose for 5-FU to reduce the side effects associated with 5-FU topical therapy.

Thermosensitive drug delivery systems possess characteristic properties of existing in liquid state at room temperature and gelling at biological temperature 37 °C and allow the incorporated drug a controlled release profile by slowing down the diffusion of the drug from the gel. We proved that 5-FU:HP-ß-CD complex loaded thermosensitive gel was demonstrated to be equally effective as thermosensitive gel containing 1% free 5-FU against HeLa human cervical epithelial carcinoma cell line in anticancer efficacy cell culture studies indicating that even with a 10-fold lower 5-FU dose (0.1% when administered as CD complex), the effectiveness of this drug remains the same upon formation of cyclodextrin complexes which increases the water solubility of the drug and improves its stability as well as a 5-day release profile of 5-FU from the inclusion complex loaded into the gel system [7].

The objective of this double-blind placebo-controlled study was to evaluate the effectiveness and safety of thermosensitive gel prepared from PF127 loaded with 5-FU:HP-ß-CD inclusion complex. The scientific approach was to lower the drug dose and provide a controlled release system for the treatment of condyloma acuminata through two different administration types; topical and intralesional. Thus, our goal was to compare the efficacy in terms of treatment response, relapse rate and safety for side-effects associated with the therapy. Low dose 5-FU complexed to cyclodextrin applied in a thermosensitive gel via different administration routes that are practiced in clinics for this purpose were evaluated in a clinical trial on volunteer patients.

Methods

Prior to the study, Local Ethical Committee Approval (31032008-003) was obtained. Women with external biopsy proven genital warts were included in the study. Presence of HPV infection was confirmed by polymerase chain reaction (PCR). Patients enrolled in the study were patients consulting at the STD Clinic in Zekai Tahir Burak Women's Hospital, Ankara. Use of an adequate means of birth control during the study was required and partners were asked to use barrier contraception. Patients were excluded from the study if they had any wart exceeding 10 mm in height or more than 20 warts in total; if they had any dermatological condition in the anogenital area; if they had a history within the last 12 months of significant renal, hepatic or hematological abnormalities or of substance abuse; if they had serum creatinine level >2 mg/dl; if they were women with current evidence of vulvar or cervical intraepithelial neoplasia (CIN) grade II or III; if they had internal warts requiring immediate treatment; if they were known to be seropositive with HIV or had a history of underlying immunodeficiency; if they had been treated within the 4 previous weeks with any drug with known or potential anti-HIV activity; if they had been treated within the 8 previous weeks with interferon; and if they were women who were pregnant, lactating or planning to become pregnant.

This was a double-blind placebo-controlled study of the efficacy and safety of 5-FU:HP-ß-CD complex loaded gel for the treatment of patients with HPV induced condyloma acuminata. Only randomized patients who received at least 1 cycle or treatment and observation were assigned for the efficacy analysis. All patients who received at least 1 dose of the study formulation were included in the safety analysis.

5-FU:HP-ß-CD gel or blank (placebo) gel was applied on days 1, 3 and 5 every other week for a maximum of 6 cycles. A cycle was defined as 1 week of gel application followed by 1 week of observation. The composition of the gel was as follows:

Pluronic F127 20%, HPMC 0.5%, methyl paraben 0.02%, propyl paraben 0.02%, disodium edetate 0.02% for the placebo and with the addition of 1% 5-FU:HP-ß-CD (1:1 M ratio) complex for the active formulation. Topical gel was applied at bedtime on days 1, 3 and 5. On day 7, patient reported to the clinic to receive next weekly dosage for the therapy. The first administration of the drug was made under the supervision of a doctor. Later applications were self-administered on an outpatient basis. The gel was applied with a cotton-tipped swab or a rubber glove in a thin layer sufficient to cover the wart area and to extend beyond the edge of each wart by a margin of 5 mm. The patients were advised to keep the gel on the tested area for at least 4 h. Occlusive bandages or dressings were not used. The treated areas, particularly those occluded by skin folds were washed the next morning to remove the residual gel minimizing the potential for local skin reactions.

Intralesional gel application was performed in the clinic by health care professionals on day 1, 3 and 5 of every other week. Dosage cycles were similar to the topical administration groups and consisted of 1 week of treatment and 1 week of observation. The treatment and observation period extended for a planned duration of <12 weeks of active treatment depending on lesion response.

Table 1 Baseline characteristics of patients enrolled in the clinical study of 1% 5-FU:HP-ß-CD complex in thermosensitive gel for HPV induced condyloma

Variable	Blank topical ($n = 6$)	Drug-loaded topical ($n = 14$)	Blank intralesional ($n = 6$)	Drug-loaded intralesional ($n = 18$)	Total ($n = 44$)
Age in years (median, range)	28 (20–48)	27 (21–50)	27 (21–50)	27 (20–50)	27.2
Previous wart therapy, %	33	27	33	37	32.5
Baseline wart area in mm^2 (median)	56	54	58	58	56.5
Number of warts (median, range)	10 (1–20)	11 (2–20)	11 (1–18)	10 (1–20)	

"Complete response" was defined as total healing; if a complete response was achieved any time during the 12 weeks of active treatment, the patient continued treatment for an additional 1 cycle and then proceeded to the follow-up group.

"Partial response" was defined as <50% decrease or >25% increase in total surface area; the patient continued the 12 cycles before being removed from the study.

*"Progression" was defined as ≥25% increase in total surface area; the patient completed 3 cycles of therapy and removed from the study for follow-up.

Study endpoints are as follows; tolerance to 5-FU topical or intralesional gel was the primary safety endpoint and was assessed throughout the treatment period by clinical examination. Laboratory tests were performed on a regular basis throughout the treatment and observation periods. Primary efficacy endpoint was the proportion of patients with lesion regression which was determined on the basis of change in overall surface area of treated lesions compared with baseline for the final evaluation after the 12 cycles. New lesions appearing after the baseline assessment were treated and quantified but were not included as part of the primary efficacy endpoint.

Secondary endpoints included time to best response, duration of response for those achieving a complete regression, and recurrence rate in those who had achieved a complete response. One month after completion of the treatment period or after removal from study, patients underwent a complete follow-up evaluation including a physical examination and blood and urine analyses.

For patients who responded completely to treatment, a 6-month follow-up was performed to assess the duration of response. The proportion of patients achieving the primary efficacy endpoint was compared by Wilcoxon rank sum test for statistical analysis of the treatment groups.

Results

A total of 44 patients were enrolled in this study randomized into 4 groups:

(a) Topical 5-FU:HP-ß-CD gel,
(b) Topical placebo gel;
(c) Intralesional 5-FU:HP-ß-CD gel;
(d) Intralesional placebo gel.

The baseline characteristics of the patients are given in Table 1 regarding age, previous wart therapy, baseline wart area in mm^2 and number of warts. The median age in each group were similar. The percentage of patients who had undergone previous wart therapy, the median baseline wart area and the median number of warts in each group were statistically not different (Table 1).

In terms of safety, none of the patients encountered adverse effects that were graded as severe. After intralesional injection, a temporary oedema was observed in the injection area which deteriorated rapidly. Patients also reported a sensation of mild burning and pressure during the injection procedure. No significant hemorrhage was observed since the injections were performed with small PPD syringes. During the treatment, observation and follow-up periods, no ulcerative, corrosive and infective lesions were observed for both topical and intralesional gel groups with or without active drug. On the contrary, at the end of the study, patients who did not show complete or total response were treated with chemical cauterisation and experienced ulcerative and hemorrhaging lesions. Figure 1

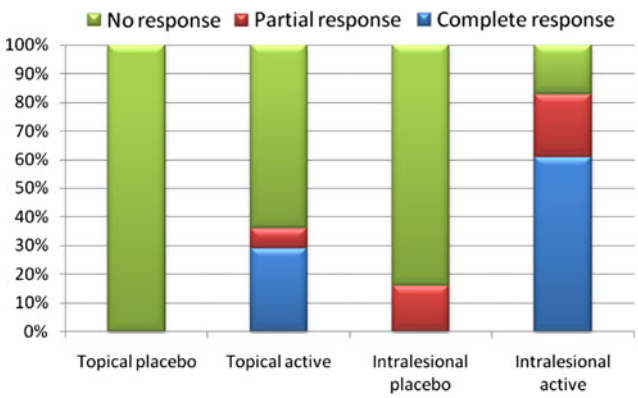

Fig. 1 Therapeutic outcome of the double blind placebo controlled study for the efficacy of thermosensitive gel in condyloma treatment

Table 2 Therapeutic efficacy of topically applied placebo and drug-loaded gel in patients

Response	Patient number per therapy group (%)		
	5-FU:ß-CD loaded thermosensitive gel ($n = 14$)	Placebo (blank) thermosensitive gel ($n = 6$)	P
Complete response	4 (29%)	0	$P < 0.005$*
Partial response	1 (7%)	0	$P > 0.05$
No response	9 (64%)	6 (100%)	$P < 0.05$*
Progression	0	0	$P > 0.05$

* Represents statistically significant difference between treatment groups

summarizes the response rates obtained during the clinical study.

Intralesional 5-FU:HP-ß-CD gel yielded a 61% complete response to the therapy as seen in Table 2 and a significant difference existed between the complete response obtained with the intralesional placebo group and the intralesional active group. No progression was found in both groups and a total of 24 patients were treated with intralesional active or placebo groups.

On the other hand, topical 5-FU:HP-ß-CD gel group resulted in a significantly lower complete response of 29% as seen in Table 3. Difference between the number of patients with no response to therapy were statistically not significant ($P > 0.05$). A total of 15 patients who did not respond to the topical gel therapy were treated with chemical cauterisation and these patients were characterized with rapid relapse of warts and formation of new lesions. However, 4 patients who completely responded to the topical 5-FU:HP-ß-CD gel did not show any relapse during the 12 cycle treatment period and the 6 month follow up period.

Table 3 Therapeutic efficacy of intralesionally injected placebo and drug-loaded gel in patients

Response	Patient number per therapy group (%)		
	5-FU:ß-CD loaded thermosensitive gel ($n = 18$)	Blank (placebo) thermosensitive gel ($n = 6$)	P
Complete response	11 (61%)	0	$P < 0.05$*
Partial response	4 (22%)	1 (16%)	$P < 0.05$*
No response	3 (17%)	5 (84%)	$P < 0.05$*
Progression	0	0	$P > 0.05$

* Represents statistically significant difference between treatment groups

Side effects associated with therapy and the relapse rates in treatment groups are given in Table 4. Relapse rates are significantly higher in groups with no response to therapy that received chemical cauterisation after the treatment period was terminated. On the other hand, relapse rates in thermosensitive gel formulation treated groups are markedly lower. No relapse has been observed for intralesional injection group.

Discussion

HPV-related genital lesions have been traditionally treated with superficial procedures, chemical agents or administration of antineoplastic drugs such as 5-FU, podophyllin, antivirals like cidofovir or immunomodulators such as imiquimod. Main challenges associated with vaginal drug delivery are attributed to the drug delivery system, in other words, the vehicle which forms the platform for the drug to be present at the site of action in sufficient concentrations for a prolonged period of time.

Vaginal delivery systems comprise of creams, gels, foams, tablets, pessaries and irrigations which are believed to exert their effectiveness depending on the residence time in the genital tract. Therapeutic efficacy of conventional systems for the treatment of HPV induced lesions are also highly dependent on the residence time, occlusivity and drug release behavior of such systems including solutions, gels or creams which are in clinical practice currently [8].

Another important challenge in vaginal drug delivery is patient compliance during drug administration and throughout the dosage regimen which lasts for several weeks. Patients are reported to tolerate gels as optimum vaginal delivery systems [9]. Overall, a patient-compliant delivery system capable of retaining considerable drug concentration

Table 4 Recurrence rate of condyloma in patients randomized into different treatment groups after 6-month follow-up period

Response	Intralesional active	Topical active	Intralesional placebo	Topical placebo
Complete response	0	0	0	0
Partial response/cauterisation	3	0	1	1
No response/cauterisation	3	8	5	5
Total	6/18 (33%)	8/14 (57%)	6/6 (100%)	6/6 (100%)

at administration site can be beneficial for the effective therapy of genital lesions and reduce recurrence rate.

Drug: cyclodextrin complexes are reported to establish significant changes in the physicochemical characteristics of a poorly-soluble drug and/or drug with stability problems. Cyclodextrin derivatives were also reported to act as mucosal penetration enhancers [10]. Inclusion of cytotoxic drug in a water-soluble cyclodextrin derivative was believed to improve the water solubility of the drug while the presence of cyclodextrin provided a controlled release platform for the drug from first the complex and then the gel to the surrounding biological medium.

In this study, administration of 5-FU complexed to HP-ß-CD led to 10-fold reduction in administered drug dose, provided a controlled drug release and resulted in equivalent cytotoxic effect to free 5-FU in 10-fold higher dose against HeLa human cerval epithelial carcinoma cells as previously reported by our group [7]. Tables 2 and 3 represent therapeutic efficacy of blank and 5-FU:HP- ß-CD complex loaded thermosensitive gel for intralesional and topical administration. Clearly, intralesional administration results in significantly higher rate of complete response and lower recurrence rate. With a more invasive route of administration such as intralesional injection, the response to therapy is markedly higher as expected. It is believed that thermosensitive gel system which is in liquid form in room temperature and inside the syringe but eventually gels once it is injected into the lesion or applied topically forms a drug reservoir which liberates the drug in a controlled and prolonged release profile [11, 12]. The effectiveness of the therapy is also characterized by the significantly low recurrence rates. No recurrence was observed for patients who showed complete response to topical or intralesional gel therapy and lower recurrences were observed for patients with partial response.

Topical administration was found to be associated with a lower complete response which can be attributed to the impaired residence time of the drug at administration site and limited cutaneous absorption of 5-FU after its diffusion from the gel. 5-FU is known to penetrate better into mucous membranes and damaged tissue than normal skin [6, 13].

Conclusion

In the light of data obtained with a model drug delivery system for 5-FU, it can be concluded that a thermosensitive gel can be a clinically effective delivery system upon intralesional injection of the drug loaded gel. This approach can be applied to poorly soluble antiviral drugs like cidofovir or labile molecules such as imiquimod that are in clinical practice for the therapy of genital warts associated with HPV infection. Thermosensitive gel is capable of controlling the release profile of the drug as well as cyclodextrin complex which acts as a second rate limiting parameter for drug release affecting the therapeutic effectiveness of the formulation.

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