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Short communication

A randomized open-label clinical trial of an anti-HPV biological dressing (JB01-BD) administered intravaginally to treat high-risk HPV infection

Xuetao Guo [a,1], Lixia Qiu [c,1], Yue Wang [d,1], Yonghong Wang [d,1], Qian Wang [b], Lei Song [e], Yali Li [e], Ke Huang [e], Xinxin Du [e], Wensheng Fan [e], Shufang Jiang [e], Qianqing Wang [f], Haoyang Li [b], Yi Yang [g], Yuanguang Meng [e,2], Yun Zhu [h,2], Lu Lu [b,2], Shibo Jiang [b,i,*,2]

[a] *First Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001, China*
[b] *Key Laboratory of Medical Molecular Virology of Ministries of Education and Health, Shanghai Public Health Clinical Center and Shanghai Medical College, Fudan University, Shanghai 200032, China*
[c] *Shanxi Medical University, Shanxi 030001, China*
[d] *Second Hospital of Shanxi Medical University, Taiyuan, Shanxi 030001, China*
[e] *Chinese PLA General Hospital, Beijing 100036, China*
[f] *Xinxiang Central Hospital Affiliated with Medical School, Henan 453000, China*
[g] *Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Beijing 100730, China*
[h] *National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*
[i] *Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY 10065, USA*

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Abstract

Currently, there is no specific antiviral therapy for HPV infection. We conducted a randomized open-label clinical trial of JB01-BD, an anti-HPV biological dressing from Shanxi Jinbo Pharmaceutical Co., Ltd., China, for treatment of HPV infection. Seventy-seven women with cervical infection by high-risk HPV were randomly divided into a treatment group and a non-treatment group. After treatment, about 60.5% (23/38) of HPV-positive women in the treatment group became HPV-negative compared with 13.5% (5/37) of women in the non-treatment group becoming HPV-negative ($P < 0.001$). These data suggest that JB01-BD is an effective topical biological agent for the treatment of cervical HPV infection.

Keywords: Cervical cancer; Human papilloma virus (HPV); Antiviral therapy; Clinical trial

1. Introduction

As the fourth most common cancer in women worldwide, cervical cancer has caused more than 266,000 deaths each year [1]. About 80% of cervical cancer occurs in developing countries, where it has become the second leading cause of cancer mortality in women between 15 and 44 years of age [2]. Each year in China, about 135,000 women develop cervical cancer, and 50,000 die from it [3]. This statistic indicates a rapid increase in the incidence of cervical cancer with a correspondingly increased threat to women's health. This calls for the development of effective and safe biological agents to prevent cervical cancer.

The induction of cervical cancer by infection with high-risk human papillomavirus (HPV) is well established [4]. As a

* Corresponding author. Key Laboratory of Medical Molecular Virology of Ministries of Education and Health, Shanghai Public Health Clinical Center and Shanghai Medical College, Fudan University, Shanghai 200032, China. Tel.: +86 21 54237673; fax: +86 21 54237465.
E-mail address: shibojiang@fudan.edu.cn (S. Jiang).
[1] These authors contributed equally to the study.
[2] These authors contributed equally to the study.

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double-stranded DNA, non-enveloped virus, HPV has a genome consisting of three regions: E (early genes E1-E7), L (late genes L1 and L2) and LCR (long control region). HPV have been divided into high-risk and low-risk subsets [5]. HPV always infects epithelial cells or mucosal tissues, mainly around the mouth, hands, feet and genitals. When the skin or mucosa is injured, HPV particles can penetrate the human barrier, exposing the target tissue cells to direct contact. At the same time, however, this process also affords the best opportunity for blocking HPV infection and preventing cervical cancer. So far, there has been no specific antiviral therapy for treatment of HPV infection. Although HPV vaccines have been licensed in many countries, they are not available in some developing countries, such as China, and their application is still limited by high cost and the large number of HPV subtypes [6,7], making it necessary to find a specific and effective agent to treat and prevent HPV infection.

It has been reported that anhydride-modified proteins have broad-spectrum inhibitory activities against several viruses [8–12]. In particular, the 3-hydroxyphthalic anhydride-modified bovine beta-lactoglobulin has exhibited potent antiviral activity against infection by high-risk and low-risk HPV subtypes [11]. It is inexpensive and highly stable in aqueous solution, thus being able to be easily formulated into a topical gel [8,11,12].

Recently, this chemically modified protein, desingated JB01, was formulated into biological dressing (JB01-BD) by the technologists at the Shanxi Jinbo Pharmaceutical Co., Ltd., Taiyuan, China. Here, we conducted a randomized open-label clinical trial of JB01-BD in women infected with HPV of high-risk types to evaluate the efficacy of JB01-BD in treatment of HPV infection.

2. Materials and methods

2.1. Materials

JB01-BD, which contains 0.01% (w/w) JB01, the chemically modified bovine beta-lactoglobulin [11], in a biological dressing, was obtained from Shanxi Jinbo Pharmaceutical Co., Ltd., JB01-BD was produced under GMP conditions. The main ingredients of the biological dressing are 2% (w/w) carbomer and 2.5% (w/w) glycerol. Both are generally recognized as safe (GRAS) excipients under U.S. FDA guidelines, and both have been used as inactive pharmaceutical additives. According to the treatment guidelines of HPV infection in China, there is no corresponding drug for control group. Moreover, producing a placebo biological dressing without JB01, but with the same look and feel as JB01-BD, proved impractical; therefore, the subjects in the control group received no treatment in this trial.

2.2. Participants

The study was approved by the Ethics Committees of the participating hospitals. All enrolled subjects signed a written informed consent. The inclusion criteria are as follows: women aged 25–65 years old infected by high-risk HPV, such as HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 [13], but without a high level of cervical lesions (Thinprep cytologic test). The exclusion criteria included the following: allergic reaction to product components, liver or kidney dysfunction, tumor malignancy, immunodeficiency, active vaginal inflammation (trichomoniasis, mold, bacteria), as well as subjects presenting with an STD or other disease which, in the clinical experience of the doctor, would disallow participation in this study. Clinical trials were scheduled to avoid the menstrual period, pregnancy and lactation. Subjects volunteered to participate in this clinical trial and signed an informed consent form before testing.

2.3. Study design

A randomized open-label phase I/IIa clinical trial was conducted at two hospitals in China to evaluate the efficacy of JB01-BD as a topical treatment of cervical infection specifically derived from high-risk HPV types. The trial was registered with the Clinical Trial Registry (ChiCTR-TRC-12002016). The study participants were randomly divided into a treatment group and a control group by using a computer-generated randomized numbering system to achieve equal sample size in both groups. Before treatment, each participant was tested for HPV-DNA loads in accordance with the manufacturer's instructions (Digene Hybrid Capture 2 High-Risk HPV DNA Test, Digene Corporation, USA). The signal from liquid-based cervicovaginal specimens was measured in relative light units (RLU) and compared with the average signal of positive controls provided by the manufacturer. The RLU/cutoff (RLU/CO) ratio value is considered positive when greater than, or equal to, 1, corresponding to a concentration of 1 pg/mL [14]. For participants in the treatment group, JB01-BD (3 g per dose) was administered intravaginally every other day for 3 months, except during the menstrual period. Those in the non-treatment group received no treatment in this trial. All participants were followed up monthly. After 3 months, HPV-DNA loads were tested again.

2.4. Data analysis

All subjects enrolled and randomized were described using medians and frequency counts. All statistical analyses were performed using SPSS version 13.0. The between-group differences were compared using Rank Sum test or Chi-square test. A p-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Enrollment

The total enrollment was 77 participants, including 38 women receiving JB01-BD and 39 women without treatment (Table 1). Thirty-eight participants completed the trial in the treatment group, while 37 participants completed the trial in

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Table 1
The HPV-positive rate in HPV-infected women before and after treatment with JB01-BD.

	Treatment group	Non-treatment group	χ^2	*P*
HPV-positive subjects enrolled in the study	38	39		
HPV-positive subjects completed the study	38	37		
Mean age (range)	43.6 (30–60)	45.0 (30–59)		0.479
HPV-positive subjects before treatment (%)	38 (100)	37 (100)	17.71	< 0.001
HPV-positive subjects after treatment (%)	15 (39.5)	32 (85.5)		

the non-treatment group with 2 dropouts. No significant difference in the age of subjects between the JB01-BD and the non-treatment groups was noted ($P = 0.479$).

3.2. Significant reduction of HPV-positive rate in HPV-infected women treated with JB01-BD

As shown in Table 1, for 38 HPV-positive participants in the treatment group receiving JB01-BD, 15 (39.5%) were still positive for HPV (HPV-DNA load-positive), suggesting that 60.5% of the HPV-positive participants became negative for HPV after the treatment with JB01-BD. In the non-treatment group, 32 out of 37 (85.5%) HPV-infected participants were still positive for HPV, indicating that only 13.5% of the HPV positive women without treatment became negative. The Chi-square test showed that $\chi^2 = 39.62$, and the difference between the two groups was statistically significant ($P < 0.001$). Thus, JB01-BD is effective in inhibiting HPV infection.

3.3. Significant reduction of HPV-DNA load in HPV-infected women treated with JB01-BD

HPV-DNA loads for each participant were detected with normal HC-2 methods [14]. As shown in Table 2, before the trial, the mean HPV-DNA load of participants in the JB01-BD-treatment group was 19.80 RLU/CO, compared with 13.16 RLU/CO in the non-treatment group. According to the rank-sum test, the difference between the two groups was comparable with no statistical difference (Z score = 0.618; $P = 0.537$). However, after the trial, all listed parameters between the two groups had a statistical significance of $P < 0.001$. For the 38 participants receiving JB01-BD, the mean HPV-DNA load was reduced to 0.76RLU/CO (negative). In contrast, the mean HPV-DNA load in the non-treatment group was still as high as 22.25 RLU/CO (positive). In the treatment group, the HPV-DNA load value had been reduced by an average of 88.42%, while in the non-treatment group, the HPV-DNA load value had even increased by an average of 5.55%.

Table 3
Change of HPV-DNA load in cervical scrapes of HPV-infected women before and after treatment with JB01-BD.

HPV-DNA load (RLU/CO)	Before the trial (%)		After the trial (%)	
	Treatment group	Non-treatment group	Treatment group	Non-treatment group
<1	0	0	60.5	13.5
1 ~ 9.9	36.8	35.1	18.4	24.3
10 ~ 99.9	31.6	37.8	13.2	32.4
100 ~ 999.9	26.3	21.6	5.3	18.9
≥1000	5.4	5.3	2.6	10.8
Total	100	100	100	100
χ^2	1.227		42.326	
P	0.746		< 0.001	

As shown in Table 3, HPV-DNA loads could be divided into five categories according to magnitude: <1 (negative); 1 ~ 9.9; 10 ~ 99.9; 100 ~ 999.9, and equal to, or greater than, 1000 RLU/CO. Before the trial, no HPV-DNA load-negative participant was found in either group. Moreover, the distribution of participants in the other four categories showed no statistical difference in either group ($\chi^2 = 1.227$, $P = 0.746$). In contrast, after the trial, the distribution of participants in all five categories showed significant difference between the treatment and the non-treatment groups ($\chi^2 = 42.326$, $P < 0.001$). In the group receiving JB01-BD, 38 participants were mainly distributed in the low viral load categories, and 60.5% had HPV-DNA loads of less than 1 RLU/CO (negative). In the non-treatment group, 37 participants were mainly distributed in the high viral load categories (32.4% in 10 ~ 99.9 RLU/CO and 24.3% in 1 ~ 9.9 RLU/CO), thus proving that JB01-BD treatment resulted in both negative RLU/CO and HPV-DNA load reduction, as reflected in the lower categories.

Table 2
The effect of JB01-BD on reducing HPV-DNA loads of women with HPV infection.

	Groups	Cases	Mean HPV-DNA load (RLU/CO)	Z	*P*
Before the trial	Treatment group	38	19.80	0.618	0.537
	Non-treatment group	37	13.16		
After the trial	Treatment group	38	0.76	5.837	<0.001
	Non-treatment group	37	22.25		
Reduction of viral load	Treatment group	38	88.42%	22.54	<0.001
	Non-treatment group	37	−5.55%		

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4. Discussion

So far, no specific antiviral drug has been developed to treat HPV infection [15]. For diseases caused by HPV infection, such as genital warts and cervical cancer, treatments are available, including surgical excision, chemical ablation, and cryotherapy [15]. However, these diseases may recur if HPV infection is not controlled. Three U.S. FDA-approved HPV vaccines (Cervarix, Gardasil, and Gardasil 9) have been recommended for all females aged 9–26 years [16], but they cannot be used to prevent HPV infection in women older than 26 years, nor can they be used for the treatment of women who are already infected by HPV. Furthermore, these vaccines are not available in many developing countries, including China. Therefore, it is essential to develop effective antiviral agents for the treatment and/or prevention of HPV infection.

Our previous studies have shown that JB01 blocks HPV entry into the target cells *in vitro,* possibly through interaction between the negatively charged residues on JB01 and the positively charged residues on the L1 and/or L2 proteins [11]. However, it was unknown whether it is effective and safe for JB01 administered intravaginally to treat high-risk HPV infection. Here we conducted a randomized open-label clinical trial of JB01-BD, an anti-HPV biological dressing provided by Shanxi Jinbo Pharmaceutical Co., Ltd., to evaluate its efficacy and safety in high-risk HPV-infected women aged 25–60 years. Unlike orally or intravenously administered antiviral drugs, JB01-BD, which is administered intravaginally, is not expected to cause systemic toxicity because JB01 protein cannot enter into the blood circulation. Indeed, no obvious side effect was observed in this randomized clinical trial when JB01-BD (3 g per dose) was administered intravaginally to 38 people every other day for 3 months.

JB01-BD was shown to be effective in controlling HPV infection because about 60.5% (23/38) of HPV-positive women in the treatment group became HPV-negative compared with 13.5% (5/37) in the non-treatment group ($P < 0.001$) (Table 1). JB01-BD was also found to be very effective in reducing HPV-DNA viral load. HPV-positive (or HPV infection) was defined as detection of HPV-DNA loads $\geq$1.00 RLU/CO, while HPV-negative was defined as detection of HPV-DNA loads <1.00 RLU/CO. The mean HPV-DNA load in women receiving JB01-BD decreased from 19.8 RLU/CO to 0.76 RLU/CO, compared with those in the non-treatment group whose values increased from 13.16 RLU/CO to 22.25 RLU/CO ($P < 0.001$) (Table 2). Although about 39.5% of the women remained positive after the treatment with JB01-BD, their viral loads were still significantly lower than those in the non-treatment group ($\chi^2 = 42.326$, $P < 0.001$) (Table 3), proving that treatment of HPV-infected patients with JB01-BD results in significant reduction of HPV positive rate and/or HPV-DNA viral loads. Therefore, these results suggest that JB01-BD is an effective and safe microbicide for treatment, and also possible for prevention, of HPV infection.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.micinf.2015.10.004.

Conflict of interest

Lu Lu and Shibo Jiang are co-inventors of the related patent (Chinese patent number: ZL 2012 1 0066696.9), which has been assigned to Fudan University and Shanxi Jinbo Bio-pharmaceutical Co., Ltd. Other authors: No potential conflicts of interest.

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