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

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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
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, designated 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., Jinbo. JB01-BD was produced under GMP conditions. 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 the control group.

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:

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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, 37 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 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%.

As shown in Table 3, HPV-DNA loads could be divided into five categories according to magnitude: $<1$ (negative); $1 \sim 9.9$; $10 \sim 99.9$; $100 \sim 999.9$, 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 $10 \sim 99.9$ RLU/CO and 24.3% in $1 \sim 9.9$ 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.

### 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 in the four categories had a statistical significance of $P < 0.001$. For the 38 participants receiving JB01-BD, the mean HPV-DNA load was reduced to 0.76 RLU/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%.

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

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Non-treatment group</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-positive subjects enrolled in the study</td>
<td>38</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>HPV-positive subjects completed the study</td>
<td>38</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>43.6 (30–60)</td>
<td>45.0 (30–59)</td>
<td>0.479</td>
</tr>
<tr>
<td>HPV-positive subjects before treatment (%)</td>
<td>38 (100)</td>
<td>37 (100)</td>
<td>17.71</td>
</tr>
<tr>
<td>HPV-positive subjects after treatment (%)</td>
<td>15 (39.5)</td>
<td>32 (86.5)</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Mean HPV-DNA load (RLU/CO)</th>
<th>$Z$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before the trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>38</td>
<td>19.80</td>
<td>0.618</td>
<td>0.537</td>
</tr>
<tr>
<td>Non-treatment group</td>
<td>37</td>
<td>13.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After the trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>38</td>
<td>0.76</td>
<td>5.837</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Non-treatment group</td>
<td>37</td>
<td>22.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction of viral load</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment group</td>
<td>38</td>
<td>88.42%</td>
<td>22.54</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Non-treatment group</td>
<td>37</td>
<td>−5.55%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.

Acknowledgments

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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.

References


