Safety Evaluation of Chemically Modified Beta-Lactoglobulin Administered Intravaginally

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Currently, there is no specific antiviral therapy for treatment of HPV infection. Jiang and colleagues previously reported that anhydride-modified proteins have inhibitory activities against multiple viruses including HPV. Here, we evaluated the safety of 3-hydroxyphthalic anhydride-modified bovine beta-lactoglobulin, designated JB01, vaginally applied in women infected by high-risk HPV. After the vaginal application of JB01 in 38 women for 3 months, no serious adverse events were reported, and normalization of the vaginal microenvironment has been observed. It can be concluded that JB01-BD is safe for vaginal use in HPV-infected women, suggesting its potential application for the treatment of HPV infection. J. Med. Virol. © 2015 Wiley Periodicals, Inc.

KEY WORDS: human papilloma virus (HPV); antiviral therapy; clinical trial; safety evaluation

INTRODUCTION

Human papillomavirus (HPV) is a DNA virus belonging to the papillomavirus family. HPV could establish persistent infections through human skin or mucous membranes. There are about 200 different subtypes of HPV, and more than 40 of them could transmit through sexual contact and infect the anogenital region like anus and genitals [Alani and Munger, 1998]. As we known, persistent infections with high-risk HPV could induce cervical cancer-[Stanley et al., 2012], which has become the second leading cause of cancer mortality in women between 15 and 44 years of age [Leeson et al., 2014]. About 80% of cervical cancer occurs in developing countries, and about 135,000 women develop cervical cancer each year in China [Chen et al., 2012]. The rapid increase in the incidence of cervical cancer calls for the development of effective and safe biological agents to prevent it. 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 [Tran et al., 2014]. It is quite necessary to find a specific agent to treat and prevent HPV infection.

Jiang and colleagues previously reported that anhydride-modified proteins have broad-spectrum inhibitory activities against multiple viruses that can be transmitted sexually, such as human immunodeficiency virus

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(HIV), herpes simplex virus (HSV), and human papillomavirus (HPV) [Neurath et al., 1996; Lu et al., 2013; Sun et al., 2015]. Therefore, we believed that these modified proteins could be developed as microbicides for vaginal or anal application to prevent infection by sexually transmitted viruses [Li et al., 2010; Li et al., 2013]. Due to the broad antiviral activity against HIV, HPV, etc., this kind of microbicide has significant advantage for multiple usages. However, to our knowledge, there are no studies to evaluate the safety of anhydride-modified proteins in human body up to now, which largely limits the development of such microbicide. Here, we evaluated the safety of 3-hydroxyphthalic anhydride-modified bovine beta-lactoglobulin, designated JB01, vaginally applied in women infected by high-risk HPV. JB01 was formulated into biological dressing (JB01-BD) by the technologists at the Shanxi Jinbo Pharmaceutical Co., Ltd., Taiyuan, China.

MATERIALS AND METHODS

Materials

JB01-BD, which contains 0.01% (w/w) JB01, the chemically modified bovine beta-lactoglobulin, 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.

Ethics Statement

This study was approved by Ethics Committees of participating hospitals. All enrolled subjects signed a written informed consent. The study was scheduled to avoid the menstrual period, pregnancy, and lactation. The trial was registered with the Clinical Trial Registry (ChiCTR-TRC-12002016).

Participants

The inclusion criteria are as follows: women aged 25–65 years old infected by high-risk HPV, 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.

Study Design

In the study, 77 participants were randomly divided into a JB01-BD group (n = 38) and a blank control group (n = 39) by using a computer-generated randomized numbering system to achieve equal sample size in both groups. For participants in the JB01-BD group, JB01-BD (3 grams per dose) was administered intravaginally every other day for 3 months, except during the menstrual period. Those in the control group received no treatment. All participants were followed up monthly. Thirty eight and 37 participants in the JB01-BD and control groups, respectively, completed the trial, while two participants in the control group dropped out.

Safety Evaluation

Blood samples were collected from each participant at the first visit (before the trial) and at the last visit 3 months later (after the trial) for hemoglobin B (Hb) testing, white blood cell (WBC) count, and platelet (PLT) count, as well as tests to evaluate liver and kidney functions, including alanine transaminase (ALT), aspartate transaminase (AST), and creatinine (Cr). Urine samples were collected for urine protein (PRO) testing, white blood cell (WBC) count, and red blood cell (RBC) count. Moreover, the vaginal microenvironment was examined at the first visit (before the trial) and at the last visit (after the trial) for vaginal pH, vaginal cleanliness, signs of bacterial vaginosis, in addition to leukocyte esterase (LE) activity in vaginal lavage fluid and WBC in vaginal discharge. Vaginal pH greater than, or equal to, 4.5 was considered abnormal. Any patient complaints, such as burning, tingling, or severe adverse events during the treatment period, were recorded immediately after occurrence.

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 \( \chi^2 \) test. A \( P \)-value of less than 0.05 was considered to be statistically significant.

RESULTS

No serious adverse events, including burning, tingling, or other serious symptoms, were reported by participants in this study. JB01-BD, as applied, was reported as soft, moist, and relatively comfortable inside the body. In the blood and urine test (Table I), no significant difference was observed in the percent of participants with abnormal blood and urine parameters between the two groups (\( P > 0.05 \)), except for the urine red blood cell (RBC) count (\( P = 0.019 \)). Interestingly, some initial abnormal parameters, such as blood hemoglobin B (HB), returned to normal in the JB01-BD group after treatment. The percent of abnormal white blood cells (WBC), blood platelets (PLT), blood creatinine (Cr) and urine red blood cells (RBC) in the JB01-BD group significantly decreased, when compared with the control group. These data
suggest that the vaginal application of JB01-BD does not cause any adverse effects; instead, some abnormal parameters tended to return to normal values in the JB01-BD group.

Next, we investigated the effect of JB01-BD on the vaginal microenvironment. As shown in Table II, before treatment, participants in both treatment and control groups had an abnormal vaginal microenvironment, possibly caused by HPV infection, including bacterial vaginosis, elevated vaginal pH, increased abnormal rate of vaginal cleanliness, WBCs in vaginal discharge, and leucocyte esterase activity. After 3 months of JB01-BD treatment, the percent of abnormal vaginal pH largely decreased from 63.2% to 7.9%, compared with 54.1–35.1% in the control group. Similarly, the percent of abnormal values in other tests of JB01-BD vaginal samples was significantly lower than that of control (\(P < 0.05\)). These results suggest that JB01-BD could normalize the vaginal microenvironment.

**DISCUSSION**

So far, no antiviral drug has been developed to treat HPV infection. For diseases caused by HPV infection, such as genital warts and cervical cancer, treatments are available, including surgical excision, chemical ablation, and cryotherapy. However, these diseases may recur if HPV infection is not controlled. To develop safe antiviral agents for the treatment and/or prevention of HPV infection, for the first time, we conducted a randomized open-label clinical trial of JB01-BD to evaluate its safety in high-risk HPV-infected women. The results showed that, intravaginal administration of JB01-BD is safe by the absence of serious adverse events reported by participants. There were no significant differences in the percent of participants with abnormal blood and urine parameters between the treatment and the non-treatment groups before and after the treatments (\(P > 0.05\)). 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.

In general, intravaginal application of a biological gel product may cause itching and odor. However, in this clinical trial, no serious adverse events, including itching, odor, burning, tingling, or other serious symptoms, were reported by participants in this study. Besides, none of the participants reported to be allergic to the chemically modified JB01 protein; therefore, no one was excluded from the trial because of allergy to JB01-BD. More interestingly, the rate of abnormal vaginal micro-environment, such as elevated vaginal pH, possibly caused by HPV infection, was significantly decreased after the

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**TABLE I. Blood and Urine Parameters of Participants before and after Treatment With JB01-BD**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Before</th>
<th>After</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood hemoglobin (Hb)</td>
<td>JB01-BD group 5 (13.2%)</td>
<td>Control group 3 (8.1%)</td>
<td>0.479</td>
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<tr>
<td>Blood white blood cells (WBC)</td>
<td>JB01-BD group 6 (15.8%)</td>
<td>Control group 3 (8.1%)</td>
<td>0.306</td>
</tr>
<tr>
<td>Blood platelets (PLT)</td>
<td>JB01-BD group 5 (13.2%)</td>
<td>Control group 6 (16.2%)</td>
<td>0.708</td>
</tr>
<tr>
<td>Blood alanine transaminase (ALT)</td>
<td>JB01-BD group 3 (7.9%)</td>
<td>Control group 1 (2.7%)</td>
<td>0.317</td>
</tr>
<tr>
<td>Blood aspartate transaminase (AST)</td>
<td>JB01-BD group 2 (5.3%)</td>
<td>Control group 0 (0.0%)</td>
<td>0.157</td>
</tr>
<tr>
<td>Blood creatinine (Cr)</td>
<td>JB01-BD group 9 (23.6%)</td>
<td>Control group 6 (16.2%)</td>
<td>0.933</td>
</tr>
<tr>
<td>Urine protein (PRO)</td>
<td>JB01-BD group 1 (2.6%)</td>
<td>Control group 0 (0.0%)</td>
<td>0.321</td>
</tr>
<tr>
<td>Urine white blood cells (WBC)</td>
<td>JB01-BD group 0 (0.0%)</td>
<td>Control group 0 (0.0%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Urine red blood cells (RBC)</td>
<td>JB01-BD group 5 (13.2%)</td>
<td>Control group 6 (16.2%)</td>
<td>0.708</td>
</tr>
</tbody>
</table>

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**TABLE II. Effect of JB01-BD Treatment on Vaginal Microenvironment**

<table>
<thead>
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<th>After</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal pH</td>
<td>JB01-BD group 24 (63.2%)</td>
<td>Control group 20 (54.1%)</td>
<td>0.423</td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>JB01-BD group 8 (21.1%)</td>
<td>Control group 9 (24.3%)</td>
<td>0.679</td>
</tr>
<tr>
<td>Vaginal cleanliness</td>
<td>JB01-BD group 31 (81.6%)</td>
<td>Control group 30 (81.1%)</td>
<td>0.992</td>
</tr>
<tr>
<td>WBCs in vaginal discharge</td>
<td>JB01-BD group 32 (84.2%)</td>
<td>Control group 33 (89.2%)</td>
<td>0.814</td>
</tr>
<tr>
<td>Leucocyte esterase</td>
<td>JB01-BD group 29 (76.3%)</td>
<td>Control group 34 (91.9%)</td>
<td>0.112</td>
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treatment with JB01-BD, suggesting that inhibition of HPV infection by JB01-BD lead to the normalization of the vaginal micro-environment, including the recovery of vaginal pH. Furthermore, the ratio of some abnormal blood tests (such as HB and WBC in blood) and urine test (urine RBC) in the patients caused by HPV infection was also decreased, possibly because the HPV infection had been controlled after the treatment with JB01-BD. Our recent report has shown that after the treatment of JB01-BD, 60.5% of HPV-positive women in the treatment group became HPV-negative, while in the control group, only 13.5% women becoming HPV-negative [Guo et al., 2015].

In conclusion, after vaginal application of 3-hydroxyphthalic anhydride-modified bovine beta-lactoglobulin based JB01-BD in 38 women for three month, no serious adverse events were reported. Comparing with control group, many detected parameters had trend to return to normality after the treatment with JB01-BD. Based on study results, it can be concluded that JB01-BD is safe for vaginal use in HPV-infected women, suggesting its potential application for the treatment of HPV infection and prevention of sexually transmitted viruses, such as HPV, HIV, and HSV. Further data are needed to confirm its efficiency and safety in larger populations.

ACKNOWLEDGMENTS

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REFERENCES