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A randomized, double-blind, controlled clinical trial to evaluate the efficacy and safety of CJ-50300, a newly developed cell culture-derived smallpox vaccine, in healthy volunteers☆

Hee-Chang Jang[a], Choong Jong Kim[a], Kye Hyoung Kim[a], Kwang-Hee Lee[b], Young-Ho Byun[b], Baik-Lin Seong[b], Giulietta Saletti[c], Cecil Czerkinsky[c], Wan Beom Park[a], Sang-Won Park[a], Hong-Bin Kim[a], Nam Joong Kim[a], Myoung-don Oh[a,*]

[a] Department of Internal Medicine, Seoul National University College of Medicine, 101 Daehangno, Jongno-gu, Seoul 110-744, Republic of Korea
[b] Department of Biotechnology, College of Engineering, Yonsei University, Seoul, Republic of Korea
[c] International Vaccine Institute, Seoul, Republic of Korea

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ABSTRACT

A randomized, double-blind, controlled clinical trial was conducted to evaluate the efficacy and safety of CJ-50300, a newly developed cell culture-derived smallpox vaccine, and to determine its minimum effective dose. The overall rates of cutaneous "take" reaction and humoral and cellular immunogenicity in CJ-50300 vaccinees were 100% (123/123), 99.2% (122/123), and 90.8% (109/120), respectively, and these rates did not differ significantly between the conventional-dose and the low-dose CJ-50300 (1.0×10^8 and 1.0×10^7 plaque-forming units/mL, respectively) ($P > 0.05$ for each). No serious adverse reaction was observed. However, one case of possible generalized vaccinia occurred in the conventionally dosed group [ClinicalTrials.gov Identifier: NCT00607243].

1. Introduction

Smallpox vaccination has been reinitiated during the past decade, because of the increased possibility of bioterrorism using this organism [1]. Until 1981, smallpox vaccines were composed of live attenuated vaccinia viruses, isolated from calf lymph [2]. However, the lack of available stocks, the need to prevent bovine prion transmission, and the desire to avoid unwanted immune responses to bovine material have limited the use of these traditional first-generation smallpox vaccines.

A few smallpox vaccine products derived from cell culture have been developed and evaluated for efficacy and safety [3–6]. However, of these second-generation products, only one, ACAM2000, has been approved by the United States (US) Food and Drug Administration [7]; it has been produced on a large scale, but only in the US. Most countries do not have smallpox vaccine stocks sufficient to respond to the urgent need that would arise from a bioterrorist attack. Thus, stocks of efficacious and safe alternative vaccine products are needed for better preparation against smallpox bioterrorism.

Here, we performed a randomized trial to evaluate the efficacy and safety of CJ-50300, a newly developed cell culture-derived smallpox vaccine (CJ CheilJedang Corporation, Seoul, Republic of Korea) in healthy vaccinia-naïve adults and to estimate its minimum effective dose.

2. Methods

2.1.1. Vaccine preparation

CJ-50300 was derived from vaccinia virus strain ATCC VR-118 (originating from the New York City Board of Health vaccinia strain), adapted to replicate in MRC-5 cells under serum-free conditions without plaque purification. Preclinical studies in mice and cynomolgus monkeys have shown that CJ-50300 is similar in cutaneous reactogenicity, immunogenicity, protection, and

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* Corresponding author. Tel.: +82 2 2072 2945; fax: +82 2 762 9662.
E-mail address: mdohmd@snu.ac.kr (M.-d. Oh).

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neurovirulence to the first-generation vaccine Lancy-Vaxina (Berna Biotech, Bern, Switzerland), and tests of MRC-5 cells and the final vaccine products have revealed no evidence of adventitious agents [8]. In a phase I clinical trial, CJ-50300 vaccinees exhibited cutaneous take reactions, acquired adequate humoral and cellular immunity, and experienced no serious adverse reactions [6].

Conventional-dose and low-dose CJ-50300 vaccines containing 1.0×10^8 and 1.0×10^7 plaque-forming units (pfu)/mL, respectively, were prepared. The two preparations appeared to be visually identical. The vaccine preparations for each vaccinee were prepared in duplicate to allow for possible loss or breakage or the need for revaccination.

2.2. Study design and participants

A randomized, double-blind, controlled clinical trial was conducted at the Clinical Research Institute of Seoul National University Hospital from January through April 2008. The study was approved by the institutional review board of Seoul National University Hospital and the Korea Food and Drug Administration (KFDA). All volunteers attended a briefing, received an informational brochure, and provided written informed consent before screening.

Healthy volunteers between 20 and 60 years of age with no history of previous smallpox vaccination were enrolled. Exclusion criteria for this trial included the contraindications for smallpox vaccination noted in the guidelines published by the US Centers for Disease Control and Prevention (CDC) [9]. Medical histories, physical examinations, and laboratory tests were used to confirm that the participants were healthy and satisfied all inclusion and exclusion criteria. Screening laboratory tests included measurements of glucose in serum, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, phosphorus, uric acid, total bilirubin, total protein, albumin, aspartate transaminase, alanine transaminase, alkaline phosphatase, γ-glutamyl transferase, total cholesterol, lactate dehydrogenase, creatine kinase, creatine kinase-MB, surface antigen of the hepatitis B virus, antibody to the hepatitis C virus, antibody to the human immunodeficiency virus, urinalysis, urine human chorionic gonadotrophin for woman, prothrombin time, activated partial thromboplastin time, complete blood cell count with differential count, chest radiograph and electrocardiogram (ECG).

All volunteers were assigned to experimental groups to receive CJ-50300. Ethical considerations of the possibilities of bovine prion contamination and transmission or unwanted immune responses to bovine materials prohibited the administration of traditional first-generation vaccines to any volunteer. To estimate the minimum effective dose of CJ-50300, volunteers were randomly assigned to the conventional-dose (CD) and low-dose (LD) groups in a 2:1 ratio. The randomization list was generated by SAS (version 9.1; SAS Institute Inc., Cary, NC). The vaccinator and the data-collecting study nurse, all of the study volunteers, clinical and laboratory investigators, outcome analyzers, and pharmacists, were all blinded to dosage until data acquisition was complete.

2.3. Vaccine administration, follow-up procedures, and safety evaluation

Freeze-dried CJ-50300 was reconstituted with 0.3 mL glycerin–phenol diluent and used within 1 h. One vaccinator administered the reconstituted vaccine to all subjects, according to the recommended method [9], using a bifurcated needle (BD, Franklin Lakes, NJ) to create 15 percutaneous punctures over the deltoid muscle. To appropriately evaluate the efficacy of the vaccine, technical errors occurring during the process of inoculation were excluded by revaccination of subjects who exhibited no evidence of vesicle formation by post-vaccination days (PVDs) 7–9; these subjects were revaccinated between PVDs 7 and 17 [10,11]. All of the vaccinees were examined on PVDs 3, 7, 14, 21, and 28. The vaccination site was photographed, and a semi-permeable dressing of Tegaderm (3M Corporate, St. Paul, MN) and gauze were applied to the site until a scab formed.

At each visit, the vaccinees returned a completed daily diary containing a questionnaire about adverse reactions and received a new diary. Symptoms were assessed as mild (present, but not bothersome), moderate (bothersome, but not precluding the performance of routine activities), or severe (precluding the performance of normal activity). At each visit, a new medical history was taken, and a physical examination and laboratory tests, including creatine kinase, creatine kinase-MB, and lactate dehydrogenase levels, were performed. ECGs and blood samples were obtained before and 28 days after vaccination. Additional ECGs were obtained for vaccinees reporting chest pain or dyspnea. Serious adverse reactions were defined as encephalitis, acute myopericarditis, eczema vaccinatum, progressive vaccinia, and death [1].

2.4. Outcome measures

The primary endpoints of the study were the cutaneous take reaction rate and humoral immunogenicity. The “take reaction” was defined as a vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion (a crust or ulcer) occurring at the vaccination site at any of PVDs 6–8 [9]. Humoral immunogenicity was evaluated by plaque-reduction neutralization (PRN) testing, as described previously [6]. The 50% PRN titer ($PRNT_{50}$) was defined as the serum dilution yielding a 50% reduction in vaccinia virus concentration (pfu/mL). Humoral immunogenicity was defined as successfully induced if the $PRNT_{50}$ increased by a factor of 4 or more.

Cellular immunogenicity was the secondary endpoint of the study. The cell-mediated immune (CMI) response was evaluated as the immediate vaccinia-specific interferon-γ-producing T-cell response using an enzyme-linked immunosorbent spot (ELISPOT) assay, as described previously [6]. The number of spot-forming cells (SFC) per 1 million peripheral blood mononuclear cells (10^6 PBMC) was determined. Cellular immunogenicity was defined as successfully induced if the SFC/10^6 PBMC ratio at least doubled or increased by at least 10. Each endpoint (take reaction, humoral immunogenicity, and cellular immunogenicity) was measured independently, blinded to the findings of the other two endpoints.

2.5. Statistical analyses

We calculated a sample size sufficient for a lower 95% confidence limit (CI) of the take reaction of at least 96% (the take reaction rate for ACAM2000 in a Phase III trial [7]), assuming a true rate of 99.15%, derived from the results of previous studies [3–7]. Allowing for a 10% loss to follow-up, a sample size of at least 82 was required for a statistical power of 80%. The sample size of 82 was also sufficient for the limit of the PRN response to be greater than 90%, assuming a true rate of 96.8% derived from the results of previous studies [3–7]. The sample size of the LD group, 41, was selected as sufficient to provide preliminary data for estimating the minimum effective dose of CJ-50300.

Categorical variables were compared using Fisher’s exact test or Pearson χ^2 test, as appropriate. Continuous variables were compared using Student’s t test or analysis of variance (ANOVA), as appropriate. All tests of significance were two-tailed, and differences were deemed to statistically significant at $P \leq 0.05$. Statistical analyses of data were performed using SAS (version 9.1).