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Smallpox vaccine, ACAM2000: Sites and duration of viral shedding and effect of povidone iodine on scarification site shedding and immune response

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ABSTRACT

The U.S. Department of Defense vaccinates personnel deployed to high-risk areas with the vaccinia virus (VACV)-based smallpox vaccine. Autoinoculations and secondary and tertiary transmissions due to VACV shedding from the vaccination site continue to occur despite education of vaccinees on the risks of such infections. The objectives of this study were to investigate, in naïve smallpox vaccinees, (a) whether the vaccination site can remain contagious after the scab separates and (b) whether the application of povidone iodine ointment (PIO) to the vaccination site inactivates VACV without affecting the immune response. These objectives were tested in 60 individuals scheduled to receive smallpox vaccine. Thirty individuals (control) did not receive PIO; 30 subjects (treatment) received PIO starting on post-vaccination day 7. Counter to current dogma, this study showed that VACV continues to shed from the vaccination site after the scab separates. Overall viral shedding levels in the PIO group were significantly lower than those in the control group ($p = 0.0045$), and PIO significantly reduced the duration of viral shedding (median duration 14.5 days and 21 days in the PIO and control groups, respectively; $p = 0.0444$). At least 10% of control subjects continued to shed VACV at day 28, and 3.4% continued to shed the virus at day 42. PIO reduced the proportion of subjects shedding virus from the vaccination site from day 8 until days 21–23 compared with control subjects. Groups did not differ significantly in the proportion of subjects mounting an immune response, as measured by neutralizing antibodies, IgM, IgG, and interferon-gamma enzyme-linked immunospot assay. When applied to the vaccination site starting on day 7, PIO reduced viral shedding without altering the immune response. The use of PIO in addition to a semipermeable dressing may reduce the rates of autoinoculation and contact transmission originating from the vaccination site in smallpox-vaccinated individuals.

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1. Introduction

ACAM2000, a cell culture–based live vaccinia virus (VACV) vaccine [1], is the only vaccine licensed by the U.S. Food and Drug Administration for prophylaxis against variola virus, the causative agent of smallpox [2,3]. The renewed threat of bioterrorism following September 11, 2001, led the U.S. Department of Defense (DoD) in 2002 to reinstate vaccinations against smallpox for troops deploying to high-risk areas [4].

The multiple adverse events associated with live VACV vaccines, including autoinoculation and secondary and tertiary (contact) transmissions, were well documented during the worldwide Smallpox Eradication Program (1966–1980) [5–7], which used first-generation VACV vaccines like Dryvax (Wyeth Laboratories, Inc., Marietta, PA) [1,8]. The rate of such adverse events during the DoD smallpox vaccination program, which has used the second-generation vaccine ACAM2000 since March 2008 [9,10], has been lower than that experienced during the eradication effort [11–17]. This reduced rate is due, in part, to the pre-vaccination screening and education program implemented by the medical departments of the U.S. Armed Services. Nevertheless, unintended transmission continues to occur, with approximately 5.4 cases of contact transmission and 10.7–20.6 cases of autoinoculation reported per 100,000 vaccinees [15,17,18]. Though most complications are minor, each case of inadvertent transmission has the potential to result in serious adverse events.

Medical professionals are traditionally taught that the smallpox vaccination site is infectious only until the scab separates at approximately post-vaccination days 14–21 [3]. However, empirical evidence supporting this assertion has been lacking. A medical solution for such infections should sterilize and/or inactivate VACV at the vaccination site without affecting the immune response to the vaccine. To be feasible for use in the DoD vaccination program, treatment of the site would have to occur after documentation of the "take" reaction (a vesiculo-papular response) on post-vaccination days 6–8.

Hammarlund and colleagues found that the application of povidone iodine ointment (PIO) to the vaccination site, starting 7 days after Dryvax administration, effectively inactivated VACV without significantly altering the immune response [19]. However, these investigators conducted the virus shedding analysis with 35 re-vaccinees and only 11 primary vaccinees; the immunological analysis evaluated only the re-vaccinees. Although the Hammarlund et al. study presents a strong proof of concept, the small number of primary vaccinees limits its relevance in the context of pre-deployment vaccination of military personnel, the majority of whom are primary vaccinees.

In the present study, we investigated the duration of VACV shedding – and, in particular, whether detectable VACV shedding occurs after scab separation – in primary vaccinees. Additionally, we determined whether PIO, starting at day 7 in a vaccinia-naïve population, can reduce VACV shedding from the vaccination site without affecting the antibody or cellular immune response to vaccination.

2. Methods

2.1. Vaccine

ACAM2000 (Sanofi Pasteur Biologics, Cambridge, MA) is a live (unadjuvanted) VACV smallpox vaccine [1,3]. The vaccine used in this study (lot no. VV04-003A) was reconstituted according to the product package insert using the diluent provided [3]. ACAM2000 was administered percutaneously to the upper arm over the deltoid muscle area with 15 jabs using a bifurcated needle.

2.2. Design

This study was approved by the U.S. Army Medical Research Institute of Infectious Diseases Human Use Committee. Per DoD policy, all military personnel deploying to the Republic of Korea for more than 15 days must be vaccinated against smallpox. A synopsis of the study was presented to soldiers being in-processed into South Korea at Yongsan Garrison. Each interested soldier participated in a one-on-one discussion of the study. After all questions were answered, each soldier still interested and eligible (e.g., with no allergy to iodine), signed the informed consent document.

Enrolled subjects were assigned to either the PIO group or the control group. All vaccinations were administered at the 1st Replacement Company (1RC), Brian Allgood Army Community Hospital (BAACH), Yongsan Garrison. Subjects in the PIO group were provided with PIO, disposable gloves, bandages, and a plastic zip closure bag and were instructed to apply PIO at every dressing change (approximately every 1–3 days), beginning on post-vaccination day 7. Specifically, they were told to apply PIO to the bandage, then cover the scarification site with the bandage, ensuring that the ointment covered the "take" area. They were asked to place waste, such as used dressings, in the plastic bag and return the bag to the 1RC. Subjects in the control group were not given or told to apply PIO. To ensure that control subjects would not purchase and apply PIO to their vaccination sites, we used different informed consent documents for subjects in the control and PIO groups. We completed the control group first, both to further minimize the risk of PIO application by control group subjects and to establish the standard characteristics of vaccination in this population. Volunteers were required to wait at least 28 days after all other in-processing vaccines were administered before receiving the smallpox vaccine. After ACAM2000 administration, volunteers returned to BAACH for clinical examinations, dressing changes, vaccination site swabs, and blood draws.

2.3. Preparation of tissues

To assess VACV shedding, samples were obtained from the vaccination site, scabs, and blood.

On post-vaccination days 7, 8, 10, 14–16, 21–23, 28 (±2 days), and 42 (±7 days), the vaccination site was swabbed. Each vaccination site swab was placed in a labeled culture tube containing 1.0 mL sterile phosphate-buffered saline (PBS), swirled six to eight times, then pressed against the side of the tube to remove the fluid. The tube was recapped and stored at −70 °C.

Scabs were obtained within 72 h after desquamation, placed in a labeled cryotube (Sarstedt, Newton, NC), and stored at −70 °C.

Blood samples were collected from volunteers pre-vaccination and on post-vaccination days 7, 10, 14–16, 21–23, 28 (±2 days), and 42 (±7 days). Serum for a 50% plaque reduction neutralization titer assay ($PRNT_{50}$) and an enzyme-linked immunosorbent assay (ELISA) was separated and frozen at–70 °C using standard clinical laboratory procedures. Whole blood for an enzyme-linked immunospot (ELISPOT) assay was drawn in five 10-mL sodium heparin tubes pre-vaccination and on post-vaccination day 42 (±7 days).

2.4. Immunogenicity assessment

2.4.1. $PRNT_{50}$

Serum vaccinia-neutralizing ability was measured by $PRNT_{50}$. Ten-fold diluted sera in Eagle's minimum essential medium (MEM) with Earle's salts and L-glutamine (Cellgro-Corning, Manassas, VA) supplemented with 2.5% v/v heat-inactivated FBS (Hyclone, Thermo Fisher Scientific, Waltham, MA), 100 IU penicillin, 100 μg/mL streptomycin (Cellgro-Corning), and 2 mM L-glutamine