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Safety and immunogenicity of ricin vaccine, RV*Ec*^{TM}, in a Phase 1 clinical trial

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ABSTRACT

Ricin is a potent toxin and potential bioterrorism weapon for which no specific licensed countermeasures are available. We report the safety and immunogenicity of the ricin vaccine RVEc^{TM} in a Phase 1 ($N = 30$) multiple-dose, open-label, non-placebo-controlled, dose-escalating (20, 50, and 100 μg), single-center study. Each subject in the 20- and 50-μg dose groups ($n = 10$ for each group) received three injections at 4-week intervals and was observed carefully for untoward effects of the vaccine; blood was drawn at predetermined intervals after each dose for up to 1 year. RVEc^{TM} was safe and well tolerated at the 20- and 50-μg doses. The most common adverse events were pain at the injection site and headache. Of the 10 subjects who received a single 100-μg dose, two developed elevated creatine phosphokinase levels, which resolved without sequelae. No additional doses were administered to subjects in the 100-μg group. Immunogenicity of the vaccine was evaluated by measuring antibody response using the well standardized enzyme-linked immunosorbent assay (ELISA) and toxin neutralization assay (TNA). Of the subjects in the 20- and 50-μg dose groups, 100% achieved ELISA anti-ricin IgG titers of 1:500 to 1:121,500 and 50% produced neutralizing anti-ricin antibodies measurable by TNA. Four subjects in the 50-μg group received a single booster dose of RVEc^{TM} 20–21 months after the initial dose. The single booster was safe and well tolerated, resulting in no serious adverse events, and significantly enhanced immunogenicity of the vaccine in human subjects. Each booster recipient developed a robust anamnestic response with ELISA anti-ricin IgG titers of 1:13,500 to 1:121,500 and neutralizing antibody titers of 1:400 to 1:3200. Future studies will attempt to optimize dose, scheduling, and route of administration. This study is registered at clinicaltrials.gov (NCT01317667 and NCT01846104).

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1. Introduction

Ricin is a potent protein synthesis inhibitor and a potential biological weapon [1–4]. Currently, no medical countermeasures for ricin intoxication are licensed by the U.S. Food and Drug Administration (FDA).

The toxic consequences of ricin are attributed to the ricin toxin A-chain (RTA), a 32-kDa *N*-glycosidase. The B-chain (RTB), a 34-kDa galactose- or *N*-acetylgalactosamine-binding lectin, binds the toxin to host cell surface receptors. Following endocytosis, the disulfide bond linking the RTA and RTB is reduced in the endoplasmic reticulum, leaving free RTA and RTB. In the cytosol, the RTA attacks ribosomes, inhibiting protein synthesis [5–7]. The rapid rate at which ricin is taken up into host cells [1], coupled with the toxin's enzymatic efficiency [8], leaves a short window for treatment of ricin intoxication. Vaccination is therefore the preferred approach to biological defense preparedness with respect to ricin.

Ricin vaccine candidates, such as a ricin toxoid [9,10] and a native subunit deglycosylated RTA vaccine [11,12], showed promise in animal models but had a number of shortcomings, such as potential reversion to a toxic form, a potential to cause vascular leak syndrome (VLS), protein instability, and the lack of a reproducible and well-characterized manufacturing process [13–15]. Recombinant DNA technology has been employed to address the shortcomings noted above. For example, researchers at the University of Texas produced a recombinant RTA (residues 1–267) vaccine candidate, RiVax, with two amino acid substitutions: a Y80A mutation to reduce ribotoxicity and a V76M mutation to

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mitigate potential VLS [16]. A dose-escalating Phase 1 clinical study demonstrated the safety and immunogenicity of RiVax [17,18].

Investigators at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) employed computational and structure-guided methods and protein engineering technologies to redesign the RTA into a protein structure that is more stable and soluble than its parent RTA and unable to inhibit protein synthesis. Amino acid C-terminal residues 199–267 in the RTA, which constitute the hydrophobic interfacial surface interacting with the RTB and contributing to the ribosomal inactivating activity, were genetically removed, generating RTA1–198. A protein loop composed of residues 34–43 in the RTA1–198, which was predicted to unfavorably increase the overall solvent accessibility of the protein, was also removed to create the recombinant rRTA1–33/44–198 (RV*Ec*^{TM}) as a lead vaccine candidate [13]. These structural changes to the RTA did not alter a neutralizing epitope previously identified in residues 95–110 [19,20]. In fact, Mantis and colleagues reported that the regions deleted in RV*Ec*^{TM} (residues 199–267 and 34–43) corresponded to RTA regions that they identified as targets for non-neutralizing epitopes and did not contribute to protective immunity [21].

RV*Ec*^{TM} elicited protective immunity in rodents [13,22], New Zealand white rabbits [23], and African green monkeys (L. Smith, unpublished results). In cell binding studies and in an *in vitro* vascular leak assay, the vaccine did not bind to human umbilical vein endothelial cells (HUVECs) or disrupt monolayer integrity of cultured HUVECs at concentrations up to 9 μM, demonstrating that the vaccine lacked cytotoxicity toward primary human endothelial cells [24]. The ricin vaccine was further evaluated in a rabbit reticulocyte cell-free translation assay system [25]; at concentrations of up to 4 μM, no protein inhibition was detected, whereas the half maximal inhibitory concentration of control RTA in the assay was 2.3×10^{-11} M.

A scaled-up manufacturing process for the ricin vaccine, expressed and purified from *Escherichia coli*, was developed, along with associated analytical and biological assays to characterize both the manufacturing process and the ricin vaccine bulk drug substance (BDS) [26]. The vaccine was manufactured in compliance with current Good Manufacturing Practices (cGMP). In a current Good Laboratory Practices preclinical toxicity study conducted in New Zealand white rabbits, no treatment-related or toxicologically significant effects were observed with a dose of up to 200 μg vaccine on study days 1, 29, 57, and 85 [27].

Here, we describe a Phase 1 escalating, multiple-dose study in which we evaluated the safety and immunogenicity of RV*Ec*^{TM} in healthy adults [28]. We hypothesized that RV*Ec*^{TM} would (a) display an acceptable safety profile and (b) elicit enzyme-linked immunosorbent assay (ELISA) IgG titers ≥1:500 and anti-ricin toxin neutralizing antibody titers ≥1:50 in vaccinees.

2. Methods

2.1. RVEc^{TM} vaccine candidate

Recombinant RTA 1–33/44–198 vaccine BDS was manufactured under cGMP at the Biological Process Development Facility at the University of Nebraska (Lincoln, NE). The BDS was formulated into the final drug product (RV*Ec*^{TM}) and fill/finished at the Walter Reed Army Institute of Research Pilot Bioproduction Facility (Silver Spring, MD). Each 3-mL, single-use vial of RV*Ec*^{TM} (lot 1545) contained the following components (1 mL total volume): 0.2 mg rRTA 1–33/44–198 protein, 20 mM sodium succinate, 100 mM NaCl, 0.2% Alhydrogel, and 0.03% Tween-20. Each 5-mL, single-use vial of diluent (lot 1544) contained the following components (2 mL total volume): 20 mM sodium succinate, 100 mM NaCl, and 0.2% Alhydrogel. Both RV*Ec*^{TM} and diluent were stored at 5 °C (2–8 °C).

2.2. Study design

The protocol for this study (FY09-03) was approved by the USAMRIID Human Use Committee (HUC). Healthy, nonsmoker, ricin vaccine–naïve 18- to 50-year-old male and nonpregnant female volunteers were recruited from the military and civilian populations in and around Fort Detrick, Maryland. A synopsis of the study was presented to eligible volunteers; each individual still interested signed an informed consent document. Each enrolled subject was assigned to one of three groups; all subjects received RV*Ec*^{TM} as an intramuscular (IM) injection in the deltoid muscle. Group 1 received 20 μg IM × 3 doses, Group 2 received 50 μg IM × 3 doses, and Group 3 was scheduled to receive 100 μg IM × 3 doses but received only a single dose due to toxicity.

The primary endpoints for measuring safety were the frequency, nature, severity, and causality of adverse events (AEs). These endpoints were evaluated on a per-dose basis and overall on each vaccination day; days 1, 3, 7, 14, and 28 after each dose; and at 6 months after the first dose. Subjects in Groups 1 and 2 were additionally evaluated at 9 and 12 months after the first dose. The endpoints used to measure immunogenicity of the RV*Ec*^{TM} vaccine were total anti-ricin IgG antibody titers, as determined by ELISA, and anti-ricin toxin neutralizing antibody titers, as determined by colorimetric toxin neutralization assay (TNA), at all specified time points. Time points for subjects in Groups 1 and 2 included study days 7, 14, 28, 35, 42, 56, 63, 70, and 84 and months 6 and 9; subjects with detectable titers at month 9 were additionally evaluated at month 12. Time points for subjects in Group 3 included study days 7, 14, 28, 56, 84, and 112 and month 6.

To ensure the safety of subjects, doses were administered in a staggered fashion within and between groups. Specifically, three subjects in Group 1 (20 μg) received dose 1 and were followed for at least 14 days. When safety criteria (triggers for halting dose escalation or for halting the administration of consecutive doses within a dose level) were not observed during the 14-day interval, the remaining seven subjects in Group 1 received dose 1. Doses 2 and 3 were administered 4 weeks and 8 weeks, respectively, after administration of dose 1. When safety criteria were not observed for at least 14 days after 9 of 10 individuals in Group 1 received dose 2, the first three subjects in Group 2 (50 μg) received dose 1. Dose administration and escalation continued in this fashion through dose 3 for Groups 1 and 2 and through dose 1 for Group 3 (100 μg).

A separate protocol (FY12-16) for a single booster dose administered to a subset of subjects in this study was also approved by the HUC. Subjects in Group 2 (50 μg) were invited to participate in the booster study. Of the 10 eligible subjects, four (two males and two females) were available and consented to participate in the booster study. All booster recipients were white, non-Hispanic, and aged 28–49.

2.3. Ricin-specific IgG antibody by ELISA

We used a standardized ELISA to analyze serum specimens for total anti-ricin IgG endpoint titers. Briefly, polyvinyl chloride microtiter plates were coated with ricin toxin at 2.5 μg/mL in phosphate-buffered saline (100 μL per well) and allowed to adhere overnight at 4 °C. Plates were washed three times with ELISA wash buffer and blocked with assay buffer (phosphate-buffered saline with 5% skim milk and 0.5% Tween-20, pH 7.4) for 40–120 min at 37 °C. Unknown serum was diluted in assay buffer to produce seven 3-fold serial dilutions ranging from 1:500 to 1:364,500. A positive control (PC; pooled positive monkey serum, 1:4000 in assay buffer), two negative controls (NC; pooled non-vaccinated