Pilot Phase IB Clinical Trial of an Alhydrogel-Adsorbed Recombinant Ricin Vaccine

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There is no FDA-approved vaccine for the potent plant toxin ricin. We have developed a recombinant ricin vaccine, RiVax. Without adjuvant it is safe and immunogenic in mice, rabbits, and humans. Based on our studies in mice, we now report the results of a small clinical trial with Alhydrogel-adsorbed RiVax.

Ricin, an extremely lethal toxin found in castor beans (2), is comprised of a ribotoxic A chain (RTA) and a cell binding B chain (RTB) (2, 4, 11, 12, 14). It is widely available as a by-product of castor oil production, easy to purify, and highly stable as a liquid or powder (5). Ricin is classified by the CDC as a level B biothreat (17). There is no approved vaccine for ricin, and a lethal dose kills within a few days. We have previously described a recombinant RTA vaccine (15, 16) in which two amino acids in the protein have been genetically altered to inactivate both the ribotoxic (8, 12, 13) and vascular leak syndrome (VLS)-inducing sites (1). The mutant protein, Y80A/V76M or RiVax, retains all the immunodominant epitopes recognized by a panel of monoclonal antibodies (MAbs) (16). In addition, the crystal structure of RiVax revealed no significant perturbation in the molecule (9), and all known immunodominant linear B cell and HLA class II-restricted T cell epitopes were retained (3, 18). Without adjuvant, mice vaccinated intramuscularly (i.m.) or intradermally (i.d.) with three doses of as little as 1 μg each were uniformly protected from a subsequent ricin challenge (10 50% lethal doses [LD50s]) administered by injection, aerosol, or intragastric gavage (10, 15, 16).

After confirming the safety and immunogenicity of RiVax in a rabbit toxicology study (16), we conducted a pilot clinical trial to determine whether it was safe and immunogenic in humans (19). Volunteers received three monthly doses of 10, 33, or 100 μg per dose. Toxicities were mild and typical of i.m. injections of approved vaccines. Seroconversion rates were 1/5, 4/5, and 5/5 at the three dose levels. However, the duration of the antibody responses was short, lasting 14 to 127 days after the third vaccination.

Based upon mouse studies using RiVax/alum where responses were enhanced by approximately 10-fold and protective for at least a year (reference 10 and unpublished data), we have now conducted a second pilot phase I clinical trial using RiVax/alum. The vaccine was prepared in our GMP (good manufacturing practice) laboratory and tested as described previously (15, 16). We have modified the published formulation by adding Alhydrogel (Brenntag, Denmark) to a final concentration of 1.0 mg/ml in 10 mM histidine-HCl and 144 mM NaCl, pH 6.0. The manufacturing methods and data supporting activity and stability are similar to those reported previously (16). The final product was adsorbed to alum, vialed, stored at 4°C, and shipped to the clinical research organization (CRO), Arkios Biodevelopment International, Virginia Beach, VA.

This was an open label, intergroup dose escalation trial in

FIG 1 Anti-RiVax antibody serum titers of each volunteer throughout the trial. (A) Group 2 volunteers (10 μg RiVax/alum per dose). (B) Group 3 volunteers (100 μg RiVax/alum per dose). Arrows below the x axis indicate the days of vaccination (days 0, 42, and 180, respectively). Serum samples were unavailable for one individual on day 112, for two individuals on day 180, for one individual on day 210, and for one individual on day 364.
TABLE 1 Comparison of antibody titers after the third vaccination using RiVax without (19) or with (this trial) alum

<table>
<thead>
<tr>
<th>Dose level (μg)</th>
<th>Anti-RiVax titer (μg/ml)</th>
<th>Reciprocal of maximum neutralizing serum dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ Alum</td>
<td>− Alum</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>256</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
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<td>10</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>280</td>
<td>8.1</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>105</td>
<td>NA</td>
</tr>
</tbody>
</table>

- a Sera were collected 14 days after the third dose in the first trial without alum (19) and 28 days after the third dose in this trial with RiVax/alum.
- b This table compares the two dose levels common to the two trials: the 33-μg dose level from the first trial is not included here, and none of the volunteers receiving a 1-μg dose of RiVax/alum in the second trial seroconverted.
- c The reciprocal of the serum dilution that neutralized 50% of the toxicity of 5 × 10^{-10} M ricin.
- d NA, not applicable since the radioimmunoassay was 0.
- e NP, serum sample not provided on day 210; subsequent samples were negative, indicating that the subject did not seroconvert.
- f Geometric mean of seroconverters.

Healthy volunteers between 19 and 30 years of age (5 males and 13 females, including Caucasians and African Americans of each gender). Fourteen volunteers completed the study. No volunteers dropped out due to toxicity or other factors directly related to the study drug. The entry criteria were the same as described in the RiVax vaccine trial (19) and included physical examinations, complete blood counts (CBCs), routine blood chemistries, urinalysis, and tests for human immunodeficiency virus, hepatitis B virus, and hepatitis C virus. Physical examinations, CBCs, routine blood chemistries, and urinalyses were performed just prior to each injection (day 0) and on days 1, 3, and 7 following each injection. All volunteers signed consent forms. The vaccinations, safety monitoring and blood draws, decisions to move to the next dose level, and medical tests were carried out by Arkios. Serum samples were shipped to the University of Texas Southwestern for evaluation. There were three dose levels with four or five volunteers per group. The individual doses were 1, 10, or 100 μg (versus 10, 33, and 100 μg in the first trial [19]). Each volunteer received three identical i.m. doses of the vaccine, the first at entry and the second and third at 6 and 26 weeks after entry, respectively. Toxicities were graded according to the FDA’s Draft Guidelines for Toxicity Grading in Healthy Volunteers. Sera for the measurement of anti-RTA antibodies were obtained immediately prior to each i.m. injection and on days 70, 112, 182, 210, 252, and 364 following the first vaccination. Total and neutralizing antibodies against RTA were measured as described previously (19).

All volunteers experienced one or more toxicities associated with the i.m. injection of approved vaccines (6, 7). All but one of these were grade I. The exception was a volunteer in group 3 who experienced a grade II headache and grade III nausea after the second vaccination. There were no abnormal laboratory values following any vaccination. When serum samples were available, titers of anti-RiVax antibodies were measured immediately prior to entry, prior to each vaccination, on days 70 and 112 (which were between the 2nd and 3rd vaccination), on day 210, and on days 252 and 364. None of five volunteers in group 1 had positive titers of anti-RiVax antibodies (≥1 μg/ml as designated previously (19)) following the third vaccination (day 210) (data not shown). However, as shown in Fig. 1, 4 of 5 and 4 of 4 individuals in groups 2 and 3, respectively, had anti-RiVax titers. In group 2, titers were low or negative until after the third vaccination, and most returned to near baseline by day 364. In group 3, 2 of 4 volunteers had anti-RiVax titers after the second vaccination, but these decreased prior to the third vaccination. However, there was no statistically significant difference in total anti-RiVax titers in groups 2 and 3 (Student’s t test, P > 0.05) after the third vaccination. Eight of nine volunteers who seroconverted still had positive titers on day 252, and 5 of 8 were positive on day 364.

Table 1 compares the results of the volunteers who received identical doses of RiVax with (this report) and without (19) alum. Four of five volunteers injected with 10 μg RiVax/alum and one of five injected with 10 μg RiVax made anti-RiVax antibodies. In the volunteers vaccinated with 100 μg RiVax/alum, the geometric mean titer was 17-fold higher than that of the volunteers receiving 100 μg RiVax without alum. Table 1 also lists the reciprocal of the maximum in vitro neutralizing dilutions of the sera from volunteers injected with RiVax with or without alum. Compared to RiVax alone, the geometric mean was 4-fold higher when 100 μg RiVax/alum was used (1/24 compared to 1/6). Figure 2 shows that the concentrations of anti-RiVax antibody correlated with the maximum neutralizing antibody dilution with an R² of 0.81 following the exclusion of a single pair of outliers.

Taken together with the results of the first clinical trial using RiVax without alum (19), our studies suggest that RiVax/alum is also safe and that it induces higher titers of both total and neutralizing antibodies. In some volunteers, these titers are long lasting. At the highest dose level of RiVax/alum, seroconversion was induced after only two vaccinations in 2 of 4 individuals. Of interest, these two individuals seroconversion occurred earlier but higher titers of anti-RiVax antibodies were not made. From the
results of the first trial in which mice were passively protected with antibodies from the human sera, we postulated that we induced titers that should protect humans against 1 to 10 LD50s of ricin (19). Therefore, the results from this trial lead us to postulate that RiVax/alum should be protective against 4 to 40 LD50s of ricin. However, this must be proven in a non-human primate challenge model. In addition, larger clinical trials should be carried out to confirm and extend the conclusion of this pilot trial and to administer boosters or higher vaccine doses to induce a longer-lasting response.

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REFERENCES