Phase 1 Safety and Pharmacokinetic Study of Chimeric Murine-Human Monoclonal Antibody coStx2 Administered Intravenously to Healthy Adult Volunteers

Thomas C. Dowling,1* Pierre A. Chavaillaz,3 David G. Young,3 Angela Melton-Celsa,4 Alison O’Brien,4 Claire Thuning-Roberson,3 Robert Edelman,2 and Carol O. Tacket2

Pharmacy Practice and Science Department, University of Maryland School of Pharmacy, Allied Health Building, Room 540D, Baltimore, Maryland 212011; Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore Street, Baltimore, Maryland 212012; Sunol Molecular Corporation, 2810 North Commerce Parkway, Miramar, Florida 330253; and Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 208144

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Hemolytic-uremic syndrome (HUS) is a serious complication of infection by Shiga toxin-producing Escherichia coli. Shiga toxin type 2 (Stx2) is responsible for the renal toxicity that can follow intestinal infection and hemorrhagic colitis due to E. coli. A chimeric mouse-human antibody, designated coStx2, that has neutralizing activity in a mouse model was produced and tested in healthy adult volunteers. In this phase I dose escalation study, coStx2 was generally well tolerated. Pharmacokinetic studies indicated that clearance was stable over the dose range of 1.0 to 10 mg/kg of body weight (0.249 ± 0.023 ml/kg/h) but was higher for the 0.1-mg/kg dose (0.540 ± 0.078 ml/kg/h), suggesting saturable elimination. A similar nonlinear trend was observed for the volume of distribution, where average values ranged from 0.064 ± 0.015 liter/kg for the 1.0- to 10-mg/kg doses and 0.043 ± 0.005 for the 0.01-mg/kg dose. The relatively small volume of distribution suggests that the antibody is limited to the vascular (plasma) compartment. The mean half-life was 165 ± 66 h, with lowest values observed for the 0.1-mg/kg dose (56.2 ± 9.7 h) and the highest values reported for the 10.0-mg/kg dose (206.4 ± 12.4 h). Future studies are needed to confirm the safety of this coStx2, and innovative clinical trials will be required to measure its efficacy in preventing or treating HUS.

In 1982, Escherichia coli of a previously rare serotype (O157:H7) was recognized as the cause of hemorrhagic colitis, a food-borne disease characterized by severe abdominal cramps, grossly bloody stools, and colonic mucosal edema, erosion, or hemorrhage (10). Infection with this E. coli serotype sometimes results in the hemolytic-uremic syndrome (HUS), consisting of acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia (1), and which is a leading cause of acute renal failure in children. Widespread food-borne outbreaks of E. coli O157:H7 infections, such as have occurred in Japan and in the United States, are possible, and E. coli O157:H7 has been classified as a category B biological threat agent (2, 8). Strains of the O157:H7 serotype that cause illness produce Shiga toxin (Stx) type 1 and/or type 2. The Stxs produced by Stx-producing E. coli (STEC) play a central role in the pathogenesis of hemorrhagic colitis and HUS (1). The Stxs have a 1A:5B noncovalently associated subunit structure. The pentameric B polypeptide is responsible for binding to a eucaryotic glycolipid receptor, which is usually globotriaosylceramide (Gb3). The A subunit contains the N-glycosidase activity that causes depurination of a critical residue in the 28S rRNA of 60S ribosomes and, hence, inhibition of protein synthesis.

Few options are currently available for the prevention of HUS due to STEC infection. Antimicrobial agents do not have convincing efficacy in this disease and may even be contraindicated (1). Because of the overall low incidence of HUS, the syndrome may not be an appropriate target for prevention by vaccination. One approach for prevention of HUS is to use passive immunotherapy by administration of neutralizing anti-Stx monoclonal antibodies before the development of the syndrome. We developed a chimeric murine-human monoclonal antibody, designated coStx2, with neutralizing activity in mice against the Stx2 produced by strains of E. coli responsible for HUS (3, 9). The intended usage of this product is to protect children infected with E. coli from developing HUS; however, the pharmacokinetics and pharmacodynamics of this agent are yet to be evaluated in humans. The purpose of this phase I study was to determine the safety of escalating doses of intravenously administered coStx2 in healthy adults and to determine the pharmacokinetic characteristics of coStx2 after a single dose. As part of the safety evaluation, the frequency of development of human antichimeric antibodies (HACA) was evaluated in volunteers who received coStx2.

MATERIALS AND METHODS

Development of coStx2. The coStx2 antibody is a chimera in which the variable regions of the Stx2-neutralizing murine monoclonal antibody 11E10 (9) are genetically fused directly to the human kappa light chain constant domain sequence and to the human immunoglobulin G1 (IgG1) heavy chain constant domain sequence. Approximately 87% of the antibody sequences are human based. The coStx2 antibody recognizes the A subunit of Stx2 and neutralizes most of the Vero cell toxicity associated with the Stx2 variants Stx2c and Stx2d

* Corresponding author. Mailing address: Pharmacy Practice and Science Department, University of Maryland School of Pharmacy, Allied Health Building, Room 540D, Baltimore, MD 21201. Phone: (410) 706-0884. Fax: (410) 706-6580. E-mail: td bowling@rx.umaryland.edu.
The final chimeric antibody product is produced in Chinese hamster ovary cells.

In an in vitro cell cytotoxicity assay, 82.8 ng of cosStx2 neutralized 1 pg of pure Stx2 (1 pg of Stx2 is equivalent to a 50% cytotoxic dose (3)). In a mouse model, a dose of 0.1 mg/kg of body weight was sufficient to protect animals against a lethal dose of Stx2 or Stx2dact-producing E. coli administered orally (3). cosStx2 was manufactured by Sunol Molecular Corporation and Massachusetts Biological Laboratories under contract to the Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health. cosStx2 was supplied in a 10-ml vial containing 5 ml of a 10-mg/ml solution of cosStx2 in an acetate-buffered saline solution. The vials were maintained at 2 to 8°C.

**Clinical study.** A phase I, single-site, open-label, nonrandomized, dose escalation study of cosStx2 in 17 healthy adult volunteers was conducted at the Center for Vaccine Development and the General Clinical Research Center of the University of Maryland under U.S. FDA IND BB-10770. The research complied with all relevant federal guidelines and the policies of the University of Maryland, Baltimore. Healthy volunteers ages 18 to 50 years were hospitalized in the University of Maryland General Clinical Research Center during the infusion and for 12 hours afterwards. Four escalating-dose cohorts were evaluated: 0.1 mg/kg (n = 3), 1 mg/kg (n = 3), 3 mg/kg (n = 6), and 10 mg/kg (n = 3). The study medication was given as a single dose diluted in normal saline (total volume of 100 ml) by slow intravenous infusion for 1 h through an in-line filter and using a dedicated upper extremity peripheral intravenous line. An additional two volunteers received approximately half of a 3-mg/kg dose before the infusion was stopped due to asymptomatic hypotension. The study was designed to infuse three volunteers at each dose and then expand to include an additional three volunteers at the highest tolerated dose. One volunteer at a time was enrolled to assess safety before proceeding to the next volunteer.

Subjects were observed for adverse events, and vital signs were measured every 15 min during the infusion and at 60 and 120 min after the infusion ended. Volunteers completed a diary for the first 7 days following infusion with cosStx2. Complete blood counts with platelets, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, blood urea nitrogen, creatinine, and alkaline phosphatase and urinalysis were measured on days 1, 3, 7, 14, and 28 after the infusion.

**Pharmacokinetics.** Following a single dose (0.1, 1, 0.3, or 10.0 mg/kg) of cosStx2, blood samples for pharmacokinetic analysis were obtained at baseline and 15, 30, 60, 75, and 90 min and 2, 3, 4, 7, 9, 12, 24, 48, and 72 h after beginning the 1-hr infusion. Additional samples were drawn at 17, 24, 48, and 56 days after the infusion. Serum samples were stored at −20°C and tested for the level of IgG1 antibody to Stx2 by enzyme-linked immunosorbent assay (ELISA) at Sunol Molecular Corporation.

To measure serum cosStx2 levels, wells of a 96-well plate (MaxiSorp 8-well strip modules; Nunc, Roskilde, Denmark) were coated with 100 μl per well of 2 μg/ml Stx2 in phosphate-buffered saline (PBS; pH 7.2) and incubated overnight at 2 to 8°C. The wells were washed with 400 μl of Stx-ELISA wash buffer (0.5% Tween 20 in PBS; pH 7.2) four times and patted dry. The wells were then blocked with 300 μl of Stx-ELISA diluent (5% normal goat serum [Equitech Bio, Kerrville, TX], 1% gelatin [Norland Products, Cranbury, NJ], 0.5% Tween 20 in PBS [pH 7.2]) for 60 min at room temperature on a rotator table at 50 rpm. Standards, controls, and samples were diluted 1:300 in Stx-ELISA diluent using 96-well format cluster tubes (Bio-Rad, Hercules, CA). Samples whose anti-Stx2 concentrations exceeded the highest standard (30 ng/ml after dilution) were serially diluted to bring assayed concentrations within the standard curve range. The blocked plate was washed as described above, and 100-μl aliquots of diluted standards, controls, and samples were transferred to respective wells in triplicate. The plate was incubated for 60 min at room temperature, rotating at 50 rpm. Following the sample incubation period, the plate was washed as described above and 100 μl of 1:40,000 goat anti-human IgG (Fc) peroxidase conjugate (Sigma, St. Louis, MO) in Stx-ELISA diluent was added to each well. The plate was incubated for 30 min at room temperature, rotating at 50 rpm. At the end of conjugate incubation the plate was washed as described above and 100 μl of 3,3′,5,5′-tetramethylbenzidine substrate (BioFx, Owings Mills, MD) was added to each well. The plate was incubated for 15 min at room temperature, rotating at 50 rpm. The stop solution (100 μl of 0.2 N sulfuric acid; Mallinckrodt, Baker, Phillipsburg, NJ) was added at the completion of the color development step and the plate was read at 450 nm in a spectrophotometer. Results were calculated from a two-parameter (logit-log) curve-fit equation.

**Noncompartmental analysis of the cosStx2 plasma concentration data was accomplished using WinNonlin Professional (version 3.1; Pharsight Corp, Mountain View, CA).** Pharmacokinetic parameter estimates were obtained for maximum plasma concentration (Cmax), elimination rate constant (k), half-life (t1/2), and the area under the plasma concentration-time curve to the last observed data point (AUCt) and extrapolated to infinity (AUC∞). Where Cmax was calculated as AUCt + C0.t. The systemic clearance (CL) was calculated as follows: dose/AUCt.

**Assessment of HACA.** Development of HACA could have safety implications for volunteers, since such antibodies could be associated with an allergic reaction to cosStx2 or result in more rapid clearance of another chimeric antibody needed for therapy of another disease in the future. The HACA was measured before the infusion and 28 and 56 days after the infusion in a validated assay. Polystyrene beads, 6.4 mm (Precision Plastic Ball Co.), were coated with 2 μg/bead of anti-Stx2 recombinant antibody in PBS by gentle agitation at 80 rpm overnight. The beads were washed three times with phosphate-buffered bovine serum albumin-EdTA (PBE), blocked with PBE for 1 h at room temperature, and stored in PBE at 4°C. A sample of 100 μl of diluted normal human serum or goat anti-human IgG diluted in PBE, was added to a 12- by 75-mm borosilicate glass culture tube. After addition of a single anti-Stx2 antibody-coated bead to each tube, the tubes were gently agitated at 140 rpm for 1 hour at room temperature. Each bead was washed by adding and aspirating 4 ml of PBS. A 100-μl sample of 2 μg/ml 125I-labeled anti-Stx2 antibody (approximately 200,000 cpm) was added to all tubes and gently agitated at 140 rpm for 1 hour at room temperature. Each bead was washed again by adding and aspirating 4 ml of PBS. The beads were transferred to clean tubes and counted for 1 minute in order to determine the amount of 125I-labeled anti-Stx2 antibody bound to each bead. The assay result was calculated from the bound 125I-labeled anti-Stx2 antibody cpm and the known concentration of 125I-labeled anti-Stx2 antibody. The results were expressed as ng of anti-Stx2 antibody/mL. A positive sample was defined as a HACA concentration greater than the upper limit of normal (37 ng/ml) and twice as high as the predose sample (4).

**RESULTS**

**Safety in volunteers.** All volunteers kept symptom diaries for 7 days after the infusion. Six (35%) of 17 had no symptoms during the surveillance period. Headache was the most common symptom reported; 9 (52%) of 17 had a headache during the 7-day surveillance period (Table 1). Headache occurred between days 0 and 7 in all volunteers and was rated mild or moderate in severity. All three volunteers who received 10 mg/kg reported headache (all mild) on day 1 or 2. Two volunteers who received 10 mg/kg reported mild joint pain involving the knees on day 1 or days 1 and 3. This resolved spontaneously.

Clinical laboratory tests were performed on days 1, 3, 7, 14, and 28 after infusion. Mild elevations of AST and/or ALT occurred in 3 (17.6%). In one volunteer (who received 0.1 mg/kg), the AST rose to 42 and 46 U/liter on days 3 and 7, respectively, and was normal on day 28 (upper limit of normal
The pharmacokinetic results for each dose level are shown in Fig. 1. The maximum plasma concentrations obtained following single-dose administration at each dose level are shown in Table 2. The mean plasma concentration (C_{max}) values were 2.4 ± 0.2 mg/liter, 25.2 ± 6.1 mg/liter, 76.6 ± 20.2 mg/liter, and 215.3 ± 34.5 mg/liter at the 0.1-, 1.0-, 3.0-, and 10.0-mg/kg dose levels, respectively. The overall clearance and volume of distribution values determined by noncompartmental analysis were 0.311 ± 0.129 ml/kg/h and 0.064 ± 0.018 liter/kg, respectively. Clearance was stable over the dose range of 1.0 to 10 mg/kg and averaged 0.249 ± 0.023 ml/kg/h; however, clearance for the lowest dose (0.1 mg/kg) averaged 0.540 ± 0.078 ml/kg/h. A similar nonlinear trend was observed for the volume of distribution, with average values of 0.064 ± 0.015 liter/kg for the 1.0- to 10-mg/kg dose range and 0.043 ± 0.005 liter/kg for the 0.01-mg/kg dose. The mean half-life was 65 ± 66 h, with the lowest values observed for the 0.1-mg/kg dose (56.2 ± 9.7 h) and the highest values reported for the 10.0-mg/kg dose (206.4 ± 12.4 h).

**HACA.** Before dosing, all HACA levels were ≤20 ng/ml. Four (24%) of 17 volunteers developed HACA on day 56 only (range, 40 to 75 ng/ml). Two of the four volunteers who developed HACA received 3 mg/kg, one received 1 mg/kg, and one received about half of a 3-mg/kg infusion that had to be discontinued because of asymptomatic hypotension. None of those who received the highest dose of 10 mg/kg developed HACA. The presence of HACA did not appear to influence the clearance of coStx2 in this study.

**DISCUSSION**

In this phase I study, intravenous coStx2 was generally well tolerated at doses from 0.1 mg/kg to 10 mg/kg. At the highest dose, two of three volunteers developed mild joint pain on the day after the infusion that was associated with mild headache. These symptoms of arthralgia and headache in recipients of coStx2 were probably not related to the infusion of coStx2 but rather to expected decreases in blood pressure after bed rest in healthy adults. The significance of these clinical and laboratory findings and the safety of coStx2 will be further assessed in future controlled studies.

This is the first study reporting plasma concentrations and pharmacokinetics of coStx2 in humans. The small volume of
distribution reported in the present study (0.064 liter/kg) suggests that the distribution of the antibody is limited to the vascular (plasma) compartment, although a determination of tissue concentrations using biopsy or necropsy would be needed to confirm these findings. The clearance values for \( c_{/H9251} \) Stx2 are slightly higher than previous studies with human IgG antibodies, where values ranging from 0.042 to 0.125 ml/kg/day have been reported (5, 11). Clearance of antibodies typically requires receptor occupancy before internalization, and it is possible that this is the saturable mechanism of elimination for \( c_{/H9251} \) Stx2. The half-life values observed in the present study, although highly variable, are similar to values reported in \( E. coli \)-infected and healthy mice (109.1 to 145.7 h).

The serum concentration of \( c_{/H9251} \) Stx antibodies required to prevent or treat HUS is not known. However, after infection with STEC, 71% of patients develop an anti-Stx2 immune response (6). Furthermore, the lack of transmission of STEC to exposed household contacts who had preexisting anti-Stx and antilipopolysaccharide antibodies is indirect evidence that these antibody responses may have been protective (7). Finally, in our mouse model of STEC infection, \( c_{/H9251} \) Stx2 prevented death and illness in STEC-infected mice (3). Such protection was observed even if the antibody was administered as late as 72 h after infection in mice that would otherwise die in 5 days (data not shown). Since HUS generally begins 3 to 5 days after presentation with bloody diarrhea, we hypothesize that there may be a period after exposure to STEC or early in infection when an infusion of \( c_{/H9251} \) Stx might prevent HUS.

Trials of the efficacy of \( c_{/H9251} \) Stx as a prophylactic agent will be challenging to design and conduct because of the relatively low incidence of STEC infections and the need to provide the \( c_{/H9251} \) Stx infusion soon after exposure. Testing \( c_{/H9251} \) Stx as a prophylactic agent against HUS during an outbreak would be possible, since exposed individuals could be more readily identified, but such a study would require careful planning for feasibility and approval by regulatory agencies.

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