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Open-Label, Dose Escalation Phase I Study in Healthy Volunteers to Evaluate the Safety and Pharmacokinetics of a Human Monoclonal Antibody to *Clostridium difficile* Toxin A

Claribel P. Taylor[1], **Sanjeev Tummala**[1], **Deborah Molrine**[2], **Lisa Davidson**[3], **Richard J. Farrell**[1], **Anthony Lembo**[1], **Patricia L. Hibberd**[3], **Israel Lowy**[4], and **Ciaran P. Kelly**[1]

1 *Beth Israel Deaconess Medical Center, Boston, Massachusetts*

2 *Massachusetts Biologic Laboratories, University of Massachusetts Medical School, Jamaica Plain, Massachusetts*

3, *Tufts-New England Medical Center, Boston, Massachusetts*

4, *Medarex, Inc., Bloomsbury, New Jersey*

Abstract

Background—Recent data suggest that *Clostridium difficile*-associated diarrhea is becoming more severe and difficult to treat. Antibody responses to *C. difficile* toxin A are protective against symptomatic disease and recurrence. We examined the safety and pharmacokinetics (pk) of a novel neutralizing human monoclonal antibody against *C. difficile* toxin A (CDA1) in healthy adults.

Methods—Five cohorts with 6 subjects each received a single intravenous infusion of CDA1 at escalating doses of 0.3, 1, 5, 10, and 20 mg/kg. Safety evaluations took place on days 1, 2, 3, 7, 14, 28, and 56 post-infusion. Samples for pk analysis were obtained before and after infusion, and at each safety evaluation. Serum CDA1 antibody concentrations and human anti-human antibody (HAHA) titers were measured with enzyme-linked immunosorbent assays. A noncompartmental model was used for pk analysis.

Results—Thirty subjects were enrolled. The median age was 27.5 yrs. There were no serious adverse events related to CDA1. Twenty-one of the 48 reported non-serious adverse events were possibly related to CDA1, and included transient blood pressure changes requiring no treatment, nasal congestion, headache, abdominal cramps, nausea, and self-limited diarrhea. Serum CDA1 concentrations increased with escalating doses: mean C_{max} ranged from 6.82 mcg/ml for the 0.3 mg/kg cohort to 511 mcg/ml for the 20mg/kg cohort. The geometric mean values of the half-life of CDA1 ranged between 25.3 and 31.8 days, and the volume of distribution approximated serum. No subject formed detectable HAHA titers.

Conclusion—Administration of CDA1 as a single intravenous infusion was safe and well tolerated. C_{max} increased proportionally with increasing doses. A randomized study of CDA1 in patients with *C. difficile* associated diarrhea is underway.

Introduction

Clostridium difficile is an important cause of antibiotic-associated diarrhea, and results in a wide spectrum of disease, ranging from asymptomatic colonization to fulminant pseudomembranous colitis.[1] Pathogenic strains produce two protein exotoxins: toxin A and toxin B.[1] Experiments in rabbits, hamsters, mice, and rats have shown that toxin A causes a severe inflammatory response, epithelial damage, and hemorrhagic fluid accumulation in intestinal loops.[2–4] Toxin B induces cytotoxic effects on cultured intestinal epithelial cells, but lacks enterotoxicity in rodents in vivo.[5–7] Active immunization of hamsters with toxin A was sufficient to prevent fatal clindamycin-induced *C. difficile*-associated ileocecitis.[8] However, immunization with toxin B alone was not protective in this model.[8] Passive immunization with monoclonal antibodies against toxin A protected axenic mice from pseudomembranous cecitis.[9] Therefore, toxin A has been considered to be the principal mediator of *C. difficile*-associated disease in animals.

Conventional management of *C. difficile*-associated diarrhea consists of discontinuing the precipitating antimicrobial agent(s) if possible, and providing supportive care.[1] If improvement does not occur, therapy with oral metronidazole or vancomycin is recommended.[1, 10–13] Although the majority of cases respond to such therapy, 15–30% of patients experience recurrent diarrhea after treatment is discontinued.[12, 13] Treating recurrent disease is often problematic, and an optimal therapeutic approach remains to be elucidated.[12, 13]

Humoral immune responses to *C. difficile* toxins play an important role in determining the severity of disease induced by the organism.[14–17] Approximately 60% of healthy individuals produce serum immunoglobulin (Ig) G antitoxin A antibodies and intestinal secretory antitoxin A antibodies.[14] Patients with low antibody levels against toxin A are at risk of experiencing severe, prolonged, or recurrent *C. difficile*-associated disease.[15,–17] A prospective study of hospitalized patients who received antibiotics and were colonized by toxigenic *C. difficile* showed that those with low serum IgG antitoxin A antibody levels were 48 times more likely to develop *C. difficile*-associated diarrhea compared to individuals with high antibody levels.[15] Small case series have demonstrated that administering pooled human immunoglobulin to children and adults with severe or protracted *C. difficile*-associated disease can lead to clinical improvement.[18–21] Therefore, passive immunization may be a promising adjunct to standard therapies for severe or recurrent disease.

Given the above observations, a novel human monoclonal antibody against *C. difficile* toxin A designated CDA1 was developed by the Massachusetts Biologic Laboratories in partnership with Medarex, Inc. CDA1 is an IgG1κ molecule that was generated against *C. difficile* toxin A using genetically altered mice (Hco7 HuMAB mice) which produce human antibodies. CDA1 binds to the receptor binding domain of toxin A with high affinity and has demonstrated efficacy in reducing mortality in the established hamster model for *C. difficile* associated disease.[19] An open-label, dose escalation study was therefore undertaken to assess the safety and pharmacokinetics of single infusions of CDA1 in healthy adult volunteers.

Subjects and Methods

Subject Population

Thirty subjects were enrolled at 2 centers (Beth Israel Deaconess Medical Center, Boston, MA and Tufts-New England Medical Center, Boston, MA). Inclusion criteria were as follows: age between 18 and 55 years, inclusive; good general health without a history of any of the conditions listed in the exclusion criteria; and screening laboratory values that met the following criteria: 1) WBC >= 4500 and <=13,000 cells/μl;2)Platelet count >= 150,000 cells/μl; 3) hemoglobin level >= 12 gm/dl; 4)creatinine level and bilirubin level < 1.1 times the upper

limit of normal (ULN); 5) blood urea nitrogen, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase level < 1.25 times the ULN; and 6) nonfasting glucose level <= 115 mg/dl. Exclusion criteria included previous receipt of a humanized monoclonal antibody (licensed or investigational); receipt of a licensed vaccine or investigational agent within 30 days of enrollment; personal history of cancer, heart disease, diabetes mellitus, respiratory conditions (*e.g.*, asthma requiring daily medication), autoimmune disorders, or blood dyscrasias; any chronic condition requiring daily prescription or over-the-counter medications, except for birth control products; personal history of severe allergic reactions with generalized urticaria, angioedema, or anaphylaxis; clinically significant physical examination findings (*e.g.*, heart murmurs, hepatosplenomegaly, lymphadenopathy, or focal neurologic deficits); blood pressure > 160/100 or < 90/70 on two separate readings; urinalysis positive for protein, >5 red cells/hpf, or >5 white cells/hpf; positive serology for HIV-1 antibody or HCV antibody; positive hepatitis B surface antigen; positive urine pregnancy test during an initial screening visit or within 24 hours of the administration of CDA1; unwillingness to undergo pregnancy testing; breast-feeding; and any other condition that in the opinion of the investigators would jeopardize the safety or rights of the volunteer, or make study completion unlikely. Female subjects of childbearing potential and male subjects were required to use effective contraception during the study. Women were asked to avoid becoming pregnant during the study, and for at least three months after the infusion of CDA1.

This study followed human experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions. It was approved by the institutional review boards of both participating centers. Written informed consent was obtained from each subject prior to study participation.

Study Product Administration

Subjects were sequentially enrolled into 5 dosing cohorts of 6 subjects each. Subjects were admitted to the General Clinical Research Center at each study site for study drug administration and monitoring during the first 8 hours after infusion. CDA1, a humanized IgG1κ monoclonal antibody, was manufactured under GMP (Good Manufacturing Practice)-compliant conditions with appropriate chemistry, manufacturing and control information provided to the Food and Drug Administration at the time of filing and Investigational New Drug application. CDA1 was manufactured by Massachusetts Biologic Laboratories (MBL, 305 South Street, Jamaica Plain, MA 02130) and supplied at a concentration of 10 mg/ml as a sterile product in 10 mM PBS buffer, pH 6.0, containing 0.01% polysorbate 80 and 0.15 M NaCl. Each calculated dose of CDA1 was diluted to a total volume of 200 ml in 0.9% NaCl. For administration, CDA1 was injected through a sterile 0.22 micron filter with the infusion controlled by a volumetric pump. The first cohort received a single intravenous infusion of CDA1 at 0.3 mg/kg over 2 hours. Subsequent cohorts were administered increasing doses of CDA1 at 1 mg/kg, 5 mg/kg, 10 mg/kg, and 20 mg/kg. CDA1 was infused over 2 hours for the 1 mg/kg and 5 mg/kg cohorts. For the 10 and 20 mg/kg doses, a test dose was first given at 180 mg/hr for 30 minutes. If this rate was tolerated, the infusion rate was increased to a maximum rate of 550 mg/hr for a total infusion time of not less than 2 hours.

Safety Monitoring

Before escalation to the next dosing cohort was permitted, the day 7 safety data of all subjects in the prior cohort were reviewed by the principal investigator and sponsor. An independent safety monitoring committee reviewed the safety data collected through day 14 for the first 18 subjects, then after the day 14 evaluation of the next 12 subjects, and at study completion of all subjects. Adverse events (AE) were assessed and reported using the Division of Microbiology and Infectious Diseases (DMID) Adult Toxicity Table (May 2001, National Institute of Allergy and Infectious Diseases, National Institutes of Health)

Each study subject was monitored during the infusion of CDA1 and in the immediate 8 hour post-infusion periodfor vital signs and emergence of adverse events. Each subject was re-assessed at study visits on days 1, 2, 3, 7, 14(+/−1), 28(+/−3), and 56(+/−7). At each study visit, interim medical histories, concomitant medication use, and clinical adverse events were assessed, and physical examinations were performed. Safety labs (complete blood cell counts, serum electrolytes, blood urea nitrogen, creatinine, glucose, liver function tests, and urinalysis with microscopic examination) were measured on days 0 (baseline), 1, 3, 7, and 28+/−3. Day 56+/−7 safety labs were measured only if day 28 results were abnormal. All safety laboratory measurements were performed by the local clinical laboratories of the study centers.

Pharmacokinetics

The immunogenicity of CDA1 was determined by measuring serum concentrations of human anti-human antibody (HAHA) with a bridging enzyme-linked immunosorbent-assay (ELISA) on days 0 (prior to the infusion of CDA1), 14+/−1, and 56+/−7. Serum CDA1 antibody titers were measured using an ELISA for measurement of IgG antibody concentrations to *C. difficile* Toxin A. The assays for HAHA titers and CDA1 antibody concentrations were performed at the Massachusetts Biologic Laboratories.

Blood samples for pk analysis were obtained pre-infusion, and 0.5, 1, 2, 4, 6, and 8 hours post-infusion on day 0; and on days 1, 2, 3, 7, 14+/1, 28+/−3, and 56+/−7. It should be noted that the Tmax and time to Cmax were calculated with reference to the start, not the mid-point or end, of the infusions and that for all of the cohorts except the 20 mg/kg group, the infusion time was 2 hours.

Statistical Methods

Summary descriptive statistics were used to describe the study population and safety data. Pk parameters were calculated using noncompartmental methods with WinNonlin Professional software (version 4.1; Pharsight).

Results

Study Population Demographics

Thirty subjects were enrolled at 2 centers between September 2004 and April 2005. Overall, 33% of the subjects were men, and the median age was 27.5 years (range, 20–53 years). Seventy-three percent of subjects were white, 3% were African American, 13% were Asian, 3% were American Indian/Alaskan Native, and 7% were of other ethnicities. All subjects completed the study.

The majority of AE were mild to moderate in severity and assessed as unlikely related or unrelated to receipt of CDA1 (Table 1). There were no serious AE assessed as possibly, probably, or definitely related to the administration of CDA1. One serious AE occurred that was assessed to be unrelated to receipt of the study product: a subject who was enrolled in the 10 mg/kg dosing cohort was hospitalized on day 44 post-infusion following a prescription drug and alcohol overdose, and subsequently recovered. A total of 21 AE were assessed as possibly related to infusion of CDA1. Three of these events were of moderate severity, and included a transient drop in systolic blood pressure by more than 20 mmHg during the 8-hour post-infusion monitoring period that required no treatment (2 subjects), and diarrhea lasting 24 hours requiring treatment with loperamide (1 subject). The remaining 18 possibly related AE were mild in severity, and included headache (2 subjects), nasal congestion (1 subject), abdominal discomfort (1 subject), nausea and loose stools (1 subject), transient changes in blood pressure (13 subjects). Twenty-seven AE were deemed to be unlikely to be related or unrelated to the

study drug, and were mild to moderate in severity (Table 1) There were no laboratory abnormalities that were associated with clinical signs or symptoms or required therapy.

Pharmacokinetics

CDA1 exhibited dose-dependent pharmacokinetics at the doses administered (Table 2 and Figure). C_{max} (maximum serum concentration) increased proportionally with increasing doses as did the AUC (area under the serum concentration time curve to the last collection time). Mean C_{max} were 6.82 μg/ml, 26.3μg/m, 109μg/m, 223μg/m and 511μg/m for the 0.3, 1.0, 5.0, 10 and 20 mg/kg cohorts respectively. The volume of distribution approximated that of serum, suggesting that drug distribution was mostly confined to the intravascular space. The median T_{max} (time to C_{max}) was 3 hours. The mean residence time was long (28.5 days) consistent with a long terminal disposition phase of CDA1. Similarly, the median terminal elimination half-life ranged between 22.9 and 30.3 days. CDA1 clearance was low (median 0.0014 ml/min/kg) and was independent of the administered dose. In general, the variability of the pk parameters exhibited a coefficient of variance in the range of 10% to 30%, which is within the normal variability that is observed in human populations.

Discussion

C. difficile-associated diarrhea usually responds to conventional treatment with metronidazole or vancomycin.[1, 10–13] A significant proportion of patients, however, develop recurrent disease that is associated with significant morbidity.[12, 13] Humoral immune responses are thought to play an important role in determining the severity of *C. difficile*-associated disease,[14–17] which can range from asymptomatic carriage to severe colitis with life-threatening complications.[1] Inadequate antibody levels to *C. difficile* toxins predispose individuals to severe or protracted disease,[15–17] while passive immunotherapy with pooled human immunoglobulin can lead to resolution of diarrhea among patients who have not responded to conventional therapies.[18–21] However, IgG titers against *C. difficile* toxins in commercial immunoglobulin preparations have been shown to vary by as much as fourfold.[18] In addition, the pharmacokinetics of these preparations for treating *C. difficile*-associated infection has not been characterized.

CDA1 is a novel human monoclonal antibody against *C. difficile* toxin A that has been developed as a potential adjunctive therapy for *C. difficile*-associated disease. In this study we examined its safety and pharmacokinetics in healthy adults. We found that CDA1 was safe and well tolerated at single doses between 0.3 mg/kg and 20 mg/kg. The majority of observed AE were mild to moderate in severity and assessed as unrelated to receipt of CDA1. There were no reports of serious AE associated with study product.

A therapeutic protein such as a monoclonal antibody may be immunogenic and lead to the development of human anti-monoclonal antibodies (HAMA). Immunogencity and HAMA production is most frequent with murine monoclonals, there is less immuogenicity with chimeric monoclonals and least with humanized monoclonals such as CDA1. Higher monoclonal antibody doses and frequent administration are also important factors that contribute to immunogenicity. In this study where subjects received a single dose of a human monoclonal the development of HAHA was not detected in any subject as might be expected.

Passive antibody-based therapy dates back to the late 19th century, when Behring first discovered that a component of serum with anti-toxin activity could protect rabbits from lethal doses of tetanus toxin.[22] Subsequently, passive immunotherapy was developed to treat many infectious diseases, but was largely abandoned in the 1940s, when effective antimicrobial agents were discovered and became widely available.[23] Renewed interest in immunoglobulins to treat infections has occurred in recent years, however, due to the emergence of drug-resistant

microorganisms and new pathogenic strains with potentially increased virulence.[23] Although only one monoclonal antibody for an infectious agent has been approved in the United States (*i.e.*, palivizumab for respiratory syncytial virus),[24] a number of antibody molecules are currently being evaluated for various infections. For example, tefibazumab (a humanized monoclonal antibody against a surface protein of *Staphylococcus aureus*) has undergone preclinical and phase I testing.[25]

Recent data indicate that *C. difficile*-associated disease has become more prevalent, more aggressive and more difficult to treat. Geographically distributed medical centers and the National Nosocomial Infections Surveillance (NNIS) system in the United States have reported increases in the incidence and severity of *C. difficile*-associated disease.[27–29] NNIS data from 1987–2001 showed that intensive care units in large hospitals experienced significant increases in the frequency of *C. difficile*-associated disease.[29] Outbreaks of severe cases have also occurred in Canada, the United Kingdom, and the Netherlands.[30, 31] Typing techniques have revealed dominant epidemic genotypes in North America and the United Kingdom with strains that exhibit resistance to certain antibiotics (e.g. clindamycin and fluoroquinolones), and may produce greater amounts of toxin A and toxin B.[31–33] Collectively, these observations highlight the need for new treatment strategies, including those that target *C. difficile* toxins.

CDA1 was developed to neutralize *C. difficile* toxin A. Given its demonstrated safety in healthy adults in this study, a randomized double blind, placebo-controlled study has been undertaken to determine its safety and effectiveness as an adjunct to standard of care treatment in hospitalized patients with *C. difficile* associated diarrhea. We acknowledge, however, that neutralizing *C. difficile* toxin B may also be a necessary component of treating patients who do not respond to conventional therapies. Because of its lack of enterotoxic effects in animal intestine in vivo, toxin B was not thought to be an important mediator of *C. difficile*-associated disease in humans. However, recent evidence suggests otherwise.[34, 35] Toxin B is a potent cytotoxin on cultured mammalian cells.[5, 6] It induced cell rounding, and disrupted actin-containing myofilament bundles in human lung fibroblasts[6] and human intestinal epithelial cell monolayers that were derived from a colonic adenocarcinoma.[7] Toxin B was also found to be 10 times more potent than toxin A at causing electrophysiologic changes in human colonic strips in vitro.[34] It had potent inflammatory enterotoxic effects on human intestinal xenografts that were subcutaneously transplanted into immunodeficient mice.[35] In addition, an increasing number of reports have detected toxin-A negative, toxin-B positive strains of *C. difficile* among symptomatic individuals,[36–40] and outbreaks associated with such strains have occurred in Canada and the Netherlands.[41, 42] Patients have exhibited a wide spectrum of disease, including recurrent diarrhea and fatal pseudomembranous colitis.[36–42] Studies attempting to characterize such strains have detected deletions in the repetitive regions of the toxin A gene, and altered restriction sites in the toxin B gene.[39, 41] Certain strains have also been noted to produce functionally inactive forms of toxin A.[39, 41, 43] Therefore, future treatments for *C. difficile*-associated disease may require targeted therapies against both toxin A and toxin B. Our preclinical studies support this approach since the use of a monoclonal antibody against toxin B in addition to CDA1 resulted in substantially increased efficacy in a hamster CDAD model.[19]

The mechanisms by which systemic antibody responses help to minimize *C. difficile*-mediated enteric infections are not well understood. Toxin A is synthesized in the colonic lumen, where it elicits severe damage to the intestinal mucosa.[44] Investigators have proposed that IgG antitoxin may leak from the circulation into the colonic lamina propria or intestinal lumen.[44, 45] It may then bind to and neutralize *C. difficile* toxins directly, or prevent their binding to intestinal epithelial cell receptors.[44, 45] Data supporting the exudation of IgG across the colonic mucosa can be found in animal models[8] and in observational studies in which investigators measured fecal IgG levels of infected patients.[17] Hamsters which were

immunized against *C. difficile* toxin A or B and subsequently challenged with the toxins were found to have antibodies against the immunizing toxin in their sera and cecal homogenates.[8] ELISAs of the cecal homogenates detected the presence of only the toxin against which the animals were not immunized.[8] In a human study patients with *C. difficile*-associated diarrhea who had high serum IgG antitoxin A titers were also found to have high levels of fecal IgG to toxin A.[17] Accordingly, an examination of the colonic contents of treated subjects for the presence of CDA1 may be instructive in determining whether or not the monoclonal antibody is also secreted or exuded into the colonic lumen during CDAD.

The clinical indications for passive immune-based therapies for *C. difficile*-associated disease are not fully defined. Pooled human immunoglobulin preparations have been administered to patients with recurrent *C. difficile* associated diarrhea.[16,20–21] Patients with severe, refractory and fulminant CDAD are another patient population that may benefit substantially from passive immunotherapy.[18] The clinical outcomes in this situation are often grim including multi-organ failure, colectomy and death. There is no available treatment with proven efficacy beyond antibacterial therapy with vancomycin and metronidazole. Normal pooled immunoglobulin infusion has been used for this indication but its efficacy is unclear since no prospective, controlled clinical trials have been reported.[18] Aside from treating refractory and recurrent disease, passive immunotherapy could also be administered prophylactically to hospitalized patients at high risk of developing nosocomial *C. difficile* diarrhea. Future studies will also need to address how best to apply what may prove to be a useful new form of therapy for this increasingly difficult to treat nosocomial infectious disease.

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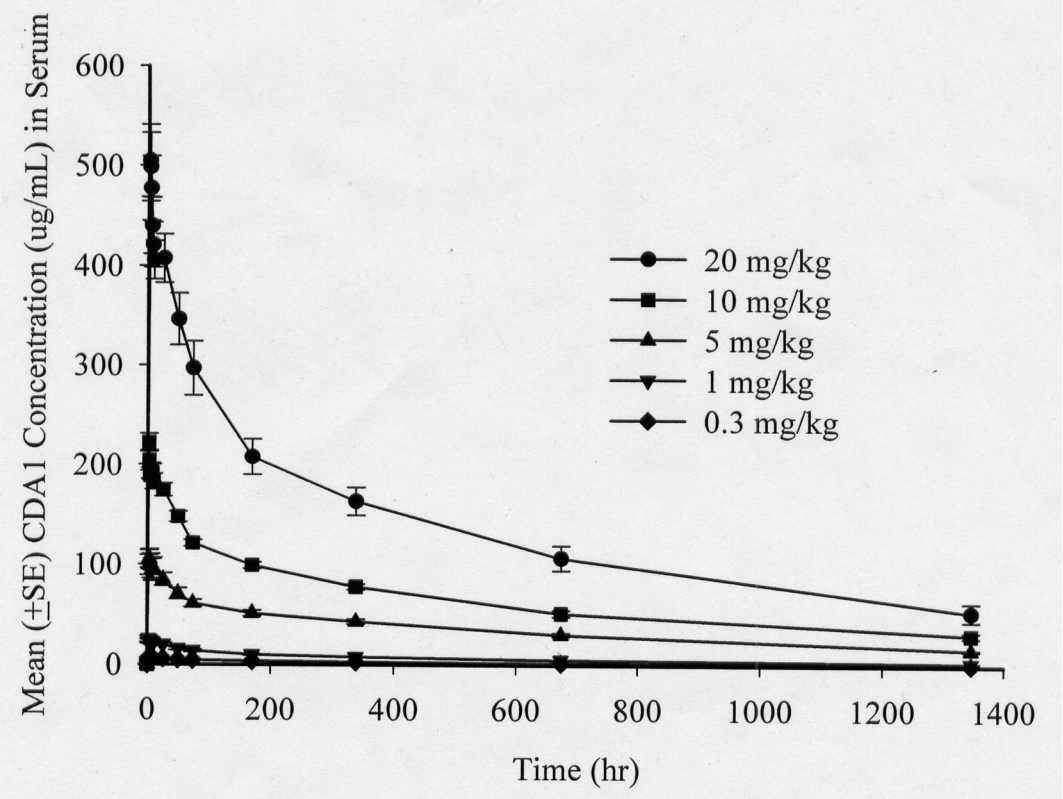

Figure.
Mean CDA1 concentration(μg/ml) after the start of a single intravenous infusion of 5 different dose levels to healthy adults

Table 1

Adverse events

Number	Severity	Relationship to CDA1	Description (number of subjects)
1	Serious	Unrelated	Alcohol and drug overdose requiring hospitalization, recovered fully (1)
3	Moderate	Possibly	• Transient drop in systolic blood pressure during 8-hour post-infusion period; no treatment required (2) • Diarrhea lasting 24 hours, required loperamide (1)
18	Mild	Possibly	• headache (2) • nasal congestion (1) • abdominal discomfort (1) • nausea, loose stools (1) • transient blood pressure changes (13)
27	Mild to Moderate	Unlikely or Uunrelated	• headache (4) • low grade oral temperature while subject was eating hot soup (1) • respiratory symptoms (8) • flu-like symptoms (2) • viral syndrome (1) • transient focal erythema at intravenous catheter site (2) • rash over left hip that resolved after topical corticosteroids (1) • transient bilateral axillary erythema, no treatment required (1) • intermittent abdominal cramps (1) • urinary tract infection (1) • intermittent knee pain, subsided with analgesics (1) • intermittent finger pain (1) • transient postural symptoms (1) • alcohol intoxication requiring medical treatment, fully recovered (1) • excision of digital tumor that was known to exist prior to study enrollment (1)

Table 2

Summary of pharmacokinetic parameters of healthy subjects given a single intravenous infusion of CDA1 by dose level

Dose, mg/kg	T_{max}, h	C_{max} µg/ml	AUC, h*µg/ml	V_d, L/kg	MRT, h	$T_{1/2}$, h	CL, ml/min/kg
0.3	5.0 (2.4, 25.9)	7.0 (5.9, 7.4)	3042 (2516, 4022)	0.070 (0.064, 0.081)	926 (843, 1873)	684 (628, 1350)	0.0012 (0.0006, 0.0015)
1.0	2.6 (2.3, 3.0)	24.7 (18.8, 35.4)	8902 (6457, 10804)	0.081 (0.062, 0.097)	917 (724, 1258)	684 (548, 953)	0.0014 (0.0010, 0.0019)
5.0	3.0 (2.5, 4.7)	112.7 (77.9, 134.4)	47530 (41162, 52660)	0.071 (0.067, 0.093)	981 (812, 1155)	728 (537, 824)	0.0013 (0.011, 0.0015)
10.0	2.6 (2.5, 10.0)	227.7 (194.5, 245.0)	84357 (72193, 86444)	0.079 (0.076, 0.097)	913 (663, 1365)	660 (481, 1015)	0.0014 (0.0012, 0.0019)
20.0	3.9 (3.0, 5.0)	479.1 (430.1, 638.0)	174632 (144521, 242411)	0.076 (0.048, 0.085)	704 (615, 1136)	550 (472, 846)	0.0014 (0.0011, 0.0020)

NOTE. Data are median (range). T_{max}, time to maximum serum drug concentration after start of infusion; C_{max}, maximum serum concentration; AUC, area under the serum concentration time curve to the last collection time; V_d, volume of distribution; MRT, mean residence time; $T_{1/2}$, terminal elimination phase half-life; CL, drug clearance.