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MONOGRAPH

RAXIBACUMAB

Treatment of Anthrax Infection

Rec INN; USAN

PAmAb
ABthrax™

Immunoglobulin G_1, anti-(anthrax protective antigen) (human monoclonal PA heavy chain), disulfide with human monoclonal PA λ-chain, dimer

Human monoclonal antibody to *Bacillus anthracis* protective antigen

CAS: 565451-13-0
EN: 336061

SUMMARY

Raxibacumab, a human monoclonal $IgG_{1\lambda}$ antibody, is effective in the treatment of Bacillus anthracis infection or anthrax toxin exposure in animal studies due to its ability to inhibit the binding of B. anthracis protective antigen to its receptor, thereby preventing the entry and activity of anthrax toxin in cells. Raxibacumab was well tolerated and safe, with no dose-limiting adverse events when administered to healthy human volunteers. Results from the animal studies and phase I clinical studies in humans support the development and use of raxibacumab as a novel therapeutic agent for inhalational anthrax.

BACKGROUND

Anthrax is caused by infection with *Bacillus anthracis*, a Gram-positive bacterium found worldwide in the environment as a highly stable, dormant spore in the soil (1, 2). Although the host range of *B. anthracis* includes all mammals, anthrax disease mainly affects wild and domesticated herbivores (2). Anthrax in humans is caused either through local infection of the skin (cutaneous anthrax), infection through the gastrointestinal tract, or by inhalation. Inhalational and gastrointestinal anthrax are the most severe forms of the disease, with high lethality (3). Inhalational anthrax exposure can rapidly progress to bacteremia and toxemia, with mortality ranging from 45% to 80% (4-6). Increased awareness of anthrax disease arose in 2001, when *B. anthracis* spores were spread in letters transported by the U.S. postal service, leading to the death of 5 people and infection in 17 others (6). Clinically, inhalational anthrax presents in a biphasic pattern with initial nonspecific flu-like symptoms, nausea and vomiting 1-4 days after exposure, followed by severe illness with dyspnea, high fever and circulatory shock. The latter symptoms represent a terminal stage and treatment is often ineffective when started at that time (7).

Anthrax toxins play a critical role in anthrax infection (8). The genes that encode for the anthrax toxin components –protective antigen (PA), lethal factor (LF) and edema factor (EF)– are present on a plasmid (pXO1) (9). The toxin is a tripartite protein, composed of PA (83 kDa), LF (90 kDa) and EF (89 kDa). These toxins conform to the AB model of bacterial exotoxins, where PA is the binding subunit and both LF and EF are catalytic subunits (10). Lethal toxin (LT) is assembled from PA and LF and is believed to be primarily responsible for the acute effects observed in anthrax, while edema toxin (ET) is assembled from PA and EF and is believed to be responsible for the edematous lesions at the site of infection. Separately, LF and EF have no known biological activity. After binding to cell-surface receptors, the full-length PA is cleaved by cellular furin family proteases, and a 20-kDa amino (*N*)-terminal fragment of PA dissociates into the extracellular medium and subsequently plays no further known role in the toxin action. The remaining 63-kDa carboxyl (*C*)-terminal fragment (PA63) then assembles into a homo-heptameric ring structure accompanied by the clustering of the receptors into lipid raft domains of the plasma membrane. Proteolytic release of the *N*-terminal fragment of PA exposes a high-affinity binding site for the EF and LF enzymatic moieties on the surface of PA63. Once assembled, the toxin complex is internalized by receptor-mediated endocytosis, followed by toxin translocation into the cytosol, leading to toxic responses catalyzed by EF and LF (11). LF is thought to be the major virulence factor responsible for impaired immunity, septic shock and death. LT inactivates mitogen-activated protein (MAP) kinase kinase (MEK) and stimulates the release of the sepsis-related cytokines TNF-α and IL-1β. EF is a calmodulin (CaM)-dependent adenylate cyclase, which increases the level of cyclic AMP (cAMP), causing impaired neutrophil function and disruption of water balance, which ultimately results in massive tissue edema (12). The toxins contribute to virulence by suppressing major signaling pathways of the innate and adaptive immune systems, while the capsule is responsible for resistance of the bacillus to phagocytosis (13).

Two different α-integrin-like cellular receptors have been identified to mediate anthrax toxin entry to the cells: anthrax toxin receptor 1,

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also called tumor endothelial marker 8, and anthrax toxin receptor 2, also known as capillary morphogenesis gene 2 protein (CMG-2). The long and medium isoforms of tumor endothelial marker 8 are transmembrane proteins and function as PA receptors. Internalization of anthrax toxin requires the presence of tumor endothelial marker 8 or CMG-2 receptors on the cell surface (7, 14, 15). The extracellular region of both receptors contains a von Willebrand factor type A domain. A metal ion-dependent adhesion site within the von Willebrand factor type A domain has been shown to be necessary for interaction with protective antigen (16).

Vaccines and antibiotics have been the two major approaches used for the treatment of anthrax and to prevent or minimize cellular damage resulting from anthrax toxicity. Several antibiotics that kill the bacteria are approved by the U.S. Food and Drug Administration (FDA) for the treatment of anthrax infection. However, antibiotics do not neutralize or prevent the biological effects of the toxins already released into the body (7). Furthermore, the need for antibiotic therapy for up to 60 days tends to decrease compliance (1). Two vaccines are currently licensed for human use. In the U.S., the Anthrax Vaccine Adsorbed (AVA, BioThrax™; Emergent BioSolutions) is used and consists mainly of PA from cultures of the unencapsulated, toxin-producing *B. anthracis* V770-NP1-R strain adsorbed onto aluminium hydroxide and is administered by the s.c. route. In the U.K., the Anthrax Vaccine Precipitated (AVP), which is an alum-precipitated filtrate of a *B. anthracis* Sterne strain culture, is used and is administered by i.m. injection. However, the two vaccines have a number of disadvantages, including minor reactions at the injection site with the U.S. vaccine and transient reactions associated with the U.K. vaccine. Also, both vaccines require frequent boosting over an 18-month period for maintaining immunity, and mortality rates are high when treatment is initiated after the onset of symptoms (1, 13). Because PA in AVA and AVP is derived from supernatants of modified *B. anthracis* cultures, concentrations of PA vary among lots. AVA and AVP contain LF and EF and other proteins whose contribution toward protective immunity is unknown (13).

Due to the drawbacks of current vaccines, an enormous amount of effort is currently being put into the development of more effective vaccines and other treatment options, such as antibodies and inhibitors (17). Various approaches have been used to develop a next-generation anthrax vaccine to replace the current existing vaccines. A recombinant anthrax vaccine candidate, rPA102 (VaxGen), has been shown to be protective in rabbits and nonhuman primates and well tolerated in a human phase I clinical trial. Passive administration of polyclonal antibodies raised against recombinant PA has also been shown to be protective in mice (18). However, the vaccine may not be rapidly protective therapeutically since it requires multiple doses to be effective (7).

PRECLINICAL PHARMACOLOGY

Raxibacumab (PAmAb, ABthrax™) is a fully recombinant human monoclonal $IgG_{1\lambda}$ antibody directed against the *B. anthracis* PA (1, 19). Neutralization of PA by raxibacumab prevents heptamerization and association with the other anthrax toxins –EF and LF– and inhibits internalization, thus preventing cell death (17). Initially, Choi et al. used in vitro assays to select human monoclonal antibodies to PA from phage display libraries by panning with recombinant PA protein. Antibodies were screened for PA-neutralizing activities in functional screening assays including cell-based assays, and selected antibodies were evaluated in a Fischer 344 rat toxin model for their ability to protect animals from lethality. When administered i.v., i.m. or s.c., a single injection of the antibodies could completely protect rats from lethal toxin-induced lethality. This protective effect conferred by a single administration of the antibodies could last for at least a minimum of 3 weeks (8).

Beebe et al. showed that one of the antibodies to the PA identified in the initial screen, PAmAb, later renamed raxibacumab, when administered therapeutically immediately after or at 12 h postexposure, completely protected New Zealand White (NZW) rabbits against lethality (20). In another study, the efficacy of the neutralizing human monoclonal antibody was evaluated in rabbit and monkey models of inhalational anthrax. PAmAb was administered 48 h prior to spore challenge (180 times LD_{50}) or within 1 h after challenge at 5 different dose levels 48 h prior to spore challenge in rabbits (12 per treatment group) and at 3 dose levels 48 h prior to spore challenge in cynomolgus monkeys (10 per treatment group) as a single dose. Survival at day 14 (rabbits) and day 28 (monkeys) and time to death were evaluated as primary study objectives. PAmAb significantly improved survival in both rabbits ($P < 0.0001$) and monkeys ($P < 0.011$) compared to control groups. Similarly, at all evaluated dose levels, PAmAb significantly prolonged time to death in rabbits ($P \leq 0.0002$) and monkeys ($P \leq 0.0005$) compared to vehicle control. A single dose at the highest level, administered after spore challenge in rabbits, provided 100% protection against lethality. Most of the PAmAb-treated survivors remained negative for bacteremia (21).

In a conventional Fischer 344 rat model of anthrax sepsis employing an i.v. bolus of LT, administration of PAmAb prior to or at the time of LT injection increased survival. When PAmAb or placebo (nonspecific mAb) was administered to rats (N = 185) at 0 h or 3, 6, 9 or 12 h after the start of a 24-h infusion of lethal toxin, survival rates were greater with PAmAb compared to placebo. In rats that received a 24-h lethal toxin infusion in which lethality was high but later than in conventional anthrax models, PAmAb given up to 6 h after initial lethal toxin exposure was very protective, and when given 9 and 12 h after toxin exposure caused beneficial trends in outcome. The study concluded that PAmAb may reduce morbidity or mortality due to lethal toxin release even if administered after patients present with anthrax sepsis (22).

Results from another study by Zmuda et al. indicate that monkeys treated with PAmAb prior to spore challenge produce a robust de novo immune response against PA, and that the majority of animals also generate significant levels of antibodies that specifically neutralize PA activity. Of the 22 surviving PAmAb-treated cynomolgus monkeys, 16 showed detectable levels of neutralizing antibodies against PA postchallenge. All monkeys had total PA titers > 5-fold compared to prechallenge values, with an average 24-fold increase (23).

The use of raxibacumab has also been demonstrated to protect against anthrax-induced death in both prophylactic and postexposure animal models challenged with aerosolized spores (24). Migone et al. conducted randomized, placebo-controlled studies in rabbit and primate models of inhalational anthrax to assess the effi-

cacy of raxibacumab when administered as a prophylactic agent and after the onset of systemic disease (19). Raxibacumab inhibited protective antigen binding to the anthrax toxin receptor with an IC_{50} of 0.5 nM, or 50% of the maximal inhibition of receptor binding. Raxibacumab specifically blocked the binding of PA to its receptor, thus preventing anthrax toxin-mediated damage. Raxibacumab improved survival among rabbits and monkeys after a lethal exposure to inhaled *B. anthracis* spores of approximately 200 times the median lethal dose. After monitoring at frequent intervals of 4-6 h for detection of PA antigen in serum and a significant temperature rise (in rabbits), animals with symptomatic disease received a single infusion of placebo or raxibacumab. The primary endpoint was survival at day 14 (rabbits) or day 28 (monkeys). In both rabbits and monkeys, raxibacumab significantly increased the overall survival rate and the time to death. Data indicated that early intervention before the logarithmic increase in levels of PA was associated with significantly better survival in animals. In therapeutic intervention studies, the survival rate was significantly higher among rabbits that received raxibacumab at a dose of 40 mg/kg (44%, 8 of 18) than among rabbits that received placebo (0%, 0 of 18; $P = 0.003$). Survival in monkeys was also significantly increased in the treatment group (64%, 9 of 14) compared with placebo (0%, 0 of 12; $P < 0.001$). Exposures attained for raxibacumab were sufficient for complete binding of PA throughout the duration of the studies. All surviving animals were asymptomatic at the end of the study and the resolution of bacteremia was coincident with a decrease in serum PA levels (19, 25).

In vitro raxibacumab was shown to directly bind wild-type PA with high affinity by BIAcore analysis, thereby competing for PA complex formation (mean K_D pH 7.4 = 8.5 nM). Raxibacumab protected against in vitro macrophage cell culture killing as effectively as the human anti-PA reference standard used by the CDC. In a cAMP screen chemiluminescent immunoassay using CHO-K1 cells, addition of raxibacumab at 100 µg/mL in the presence of PA/EF prevented the induction of cAMP (22).

CLINICAL STUDIES

When administered alone or in combination with ciprofloxacin, raxibacumab was well tolerated in healthy human volunteers. Raxibacumab had a half-life of 20-22 days and a C_{max} (988 µg/mL) in excess of drug levels that can provide protection in animal models of inhalational anthrax. Repeated administration of raxibacumab was safe and did not result in the development of an immune response (26).

In another phase I study conducted to evaluate the safety and pharmacokinetics of raxibacumab, healthy subjects were given a single i.m. injection (11 subjects per cohort, doses of 0.3, 1 and 3 mg/kg) or i.v. infusion (10 subjects per cohort, doses of 1, 3, 10, 20 and 40 mg/kg) of raxibacumab or placebo. Raxibacumab was well tolerated, with no dose-limiting adverse events. A total of 105 healthy male (n = 70) and female (n = 35) subjects were enrolled in the study, 25 of whom received placebo. The most common adverse events were headache (16%), arthralgia (5%) and nausea (5%). Preliminary pharmacokinetic analysis showed dose-proportional increases in C_{max} and AUC parameters. The mean half-life was 18 days in the four cohorts (27).

Migone et al. showed that a dose of 40 mg/kg i.v. of raxibacumab in healthy volunteers had a half-life of 20-22 days and provided a maximum concentration of the drug in excess of levels that were protective in animals (19).

CONCLUSIONS

The inability to perform *B. anthracis* infection trials in human volunteers makes it obligatory to use surrogate rabbit and nonhuman primate models of infection to predict treatment efficacy and survival in humans. Raxibacumab could become one of the first biological interventions licensed under the FDA's animal rule. Combined with antibiotic therapy, raxibacumab could be invaluable in the treatment of anthrax after the onset of symptoms.

SOURCE

Human Genome Sciences, Inc. (US).

DISCLOSURES

The author states no conflicts of interest.

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