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The DNA-Based Drug BC 007 neutralizes agonistically acting autoantibodies directed against G protein–coupled receptors

Successful mode of action demonstrated in clinical phase 1 trial

KEYWORDS: Aptamer,autoantibodies,DNA-baseddrug,Gprotein-coupledreceptors,neutralization,heartfailure,oligonucleotides.

ABSTRACT

Autoimmunity has been shown to be the pathogenic driver in a variety of diseases whose causes were previously unknown. When agonistic autoantibodies which activate G protein–coupled receptors (GPCR-AAb) are present, they damage primarily the heart and the vascular system. For example, autoantibodies that activate □1-adrenoceptors can cause dilated cardiomyopathy, leading to heart failure. Removal of GPCR-AAbs by immunoadsorption has already demonstrated a very beneficial effect. However, despite its therapeutic success, immunoadsorption has not become a widely accepted therapeutic option. BC 007, a nonmodified DNA aptamer that can neutralize GPCR-AAbs after infusion, is the first applicable alternative for treating the cause of GPCR-AAb–induced diseases. Here we report the successful neutralization of various GPCR-AAbs in autoantibody-positive, healthy elderly volunteers in the framework of phase 1 safety and tolerability testing.

INTRODUCTION

Autoimmunity has increasingly been shown to be involved in the pathogenesis of various diseases whose causes were previously unknown (1,2). When autoantibodies which activate G protein–coupled receptors (GPCR-AAb) are present, they damage primarily the heart and the vascular system. For example, autoantibodies that activate □$_1$-adrenoceptors can cause dilated cardiomyopathy, Chagas cardiomyopathy, or peripartum cardiomyopathy, all of which lead to heart failure (2). Other such autoantibodies target the β_2- or α_1-adrenoceptors (β_2-AAb, α_1-AAb), the endothelin (ETA-AAb) or angiotensin (AT_1-AAb) receptors, and protease-activated receptors (PAR-AAb) or muscarinic (M-AAb) receptors, which are essential factors in the pathogenesis of asthma, glaucoma, pulmonary arterial hypertension, transplant allograft rejection, and other diseases (3). The removal of GPCR-AAbs by immunoadsorption has already demonstrated a very beneficial effect on e.g. cardiac function: mortality rates have been reduced significantly when β_1-AAb is targeted in patients with dilated cardiomyopathy (4); or reduced intraocular pressure at therapy-refractory glaucoma patients (5). However, immunoadsorption has not been widely accepted for a variety of reasons, including its treatment burden on patients and its costs.

With the discovery of aptamers and their transfer into the therapeutic field, a new tool is now available that could be suitable for the *in vivo* neutralization of GPCR-AAbs, as demonstrated by Hwang and colleagues in 2003 (6). Using a model of passively transferred experimental autoimmune myasthenia gravis, Hwang and colleagues neutralized the autoantibody with an *in vitro* selected RNA aptamer (6). Aptamers can abolish function when functional proteins are their target (6-9). The DNA-based aptamer BC 007, which neutralizes a variety of pathogenic GPCR-AAbs (10) *in vivo* after infusion (11), is the first applicable alternative for treating the cause of GPCR-AAb–accompanied diseases. Here we report the first findings of successful neutralization of various GPCR-AAbs in autoantibody-positive, healthy elderly volunteers in the framework of phase 1 safety and tolerability testing.

METHODS

Clinical Study Protocol

The randomized, double-blind, placebo-controlled single ascending dose study involved 3 patient cohorts (8 healthy men aged 18-45 years in each group) who were given 15, 50, or 150 mg of BC 007 or placebo (ratio 3:1) intravenously (over 20 min). An additional 8 elderly healthy subjects of both sexes (55-70 yrs) were given 150 mg BC 007 or placebo (ratio 3:1). Next, in an open-label study, 7 cohorts (each consisting of 6 elderly healthy volunteers aged 50 to 75 years who tested positive for GPCR-AAb, including β_1-*AAb,* β_2-*AAb,* α_1-*AAb, and ETA-AAb*) were given 50, 150, 300, 450, 750, 1350, or 1900 mg BC 007. Infusion lasted for 20 min (50 or 150 mg), 40 min (300, 450, or 750 mg), 75 min (1350 mg), or 105 min (1900 mg). Lower doses were administered as a combination of bolus plus infusion, whereas higher doses were administered by infusion only (NTC02955420).

Subject safety was carefully addressed by monitoring electrocardiograms (ECG) and blood pressure, monitoring various laboratory tests, administering a survey to determine injection site reactions, checking vital signs, performing physical examinations, and monitoring reports of adverse events. Blood samples were collected before the infusion began until 24 h thereafter for analyses of clinical chemistry, hematology, coagulation, and pharmacokinetics. A final follow-up visit for determining GPCR-AAb status took place

approximately one month after the administration of doses of 300 mg or higher. For pharmacokinetic analysis, urine was collected before the infusion and every 4 h for 12 h and then once from 12 to 24 h after infusion.

Autoantibody determination

A bioassay was used to identify autoantibody-positive subjects and to determine the success of treatment (12). For this purpose, we prepared immunoglobulin G (IgG) fractions of the serum samples (13) and added them to spontaneously beating cardiomyocytes from neonatal rats. If functionally active GPCR-AAbs were able to target receptors that were locked to the beating machinery of the cells, the basal beating rate changed. A positive chronotropic response occurred when β_1-, β_2- or α_1-adrenoceptors and/or PAR or AT_1 receptors were targeted by specific autoantibodies. A negative chronotropic response was the result of AAb stimulation of a muscarinic M-receptor and/or the ETA or MAS-receptor.

The subsequent addition of a specific receptor blocker that can abolish the change in chronotropic response enabled us to assign the chronotropic response to a specific receptor. The addition of peptides from a receptor-epitope mapping library enabled us to identify the exact autoantibody epitope involved, as previously described (14).

RESULTS AND DISCUSSION

BC 007 was extremely well tolerated; no clinically relevant adverse events were reported. This finding agrees with those of other studies of this molecule class, which have shown that aptamers are generally well tolerated (9). BC 007 is especially safe because it has a non-modified single-stranded DNA sequence, which is identical to sequences widely found in nature. Hypersensibility, such as that to proteinaceous biologics, has not been seen with this molecule class, especially those members with non-modified nucleic acids. In addition, it has been shown that, when aptamers are referred to as immunogenic, this reputation is the result of modifications in the oligonucleotide substance (e.g., pegylation)(9).

The oligonucleotide sequence of BC 007 was originally selected as a potential short-lasting thrombin inhibitor for transient anticoagulation during coronary bypass graft surgery, but it was not successful because the lack of a persistent effect resulted in a suboptimal dosing profile (therapeutic effect only at too high a dose). Therefore, some subjects, starting with the 300-mg cohort, exhibited slight to moderate elevations in activated partial thromboplastin time (aPTT). This aPTT elevation quickly normalized within minutes after the end of the infusion. Pharmacokinetic data (area under the curve [AUC]; maximum serum concentration [C_{max}]) were analyzed.

BC 007 has a short half-life, increasing from 3 to 11 min with increasing dose. The short half-life of BC 007 is due to very rapid metabolism of the DNA sequence consisting of thymidine and guanosine and even of the nucleotide decay down to □-aminoisobutyric acid (thymidine origin) and uric acid (guanosine origin) (15).

The dose-response relationship of GPCR-AAb neutralization was clearly visible with this method of investigating its action (see Table 1).

Although the absolute numbers of tested volunteers in each specific GPCR-AAb group are small (Table 1), we can nevertheless assume that neutralization of the various GPCR-AAbs may require different doses.

The number of volunteers who tested positive for GPCR-AAb is low because all of the subjects were healthy elderly people. Very few healthy people are carriers of GPCR-AAbs. In addition, agonistically acting GPCR-AAbs are almost undetectable in young subjects when a bioassay is used to detect functionally active autoantibodies (12,13). So far, no solid phase–based assay is appropriate for correctly identifying functionally active autoantibodies, because these assays produce too many false-positive results (16,17) and thus are unacceptable for assisting with therapeutic decisions.

With respect to future dose-finding studies involving patients with various diseases, we must remember that the GPCR-AAbs tested in healthy subjects in this study very likely resulted from a very early immune response. We speculate that, during an early immune response, a multiplicity of low-avidity IgG antibodies normally target a multitude of separate epitopes of a target molecule. Only subsequent clonal selection (beginning pathogenesis) increases the avidity of the antibodies and limits the number of epitopes (18). A change in autoantibody avidity and number might influence the dosing regimen, but this hypothesis must be investigated in more detail in efficacy studies that include patients who test positive for GPCR-AAbs, which are already in progress (phase 2).

The effect of GPCR-AAbs on the pathogenesis of a disease was clearly demonstrated as early as 2000, in a study of β_1-AAbs in patients with dilated cardiomyopathy (19); since then this effect has been confirmed several times (4), also with β_2-AAbs on therapy-refractory glaucoma (5). Arrhythmias are also associated with β_1-AAbs (20). Other autoantibodies of this class have been assigned to various diseases, such as the combination of α_1-AAb and β_2-AAb with forms of dementia (Alzheimer disease) (20) and AT_1-AAb with preeclampsia and transplant allograft rejection (21,22). BC 007 is now an available and excellent tool for effectively neutralizing these autoantibodies *in vivo* and thereby improving the patient's situation.

BC 007	β1-AAb			β2-AAb			α 1-AAb			ETA-AAb		
Dose	1 d	1 week	1 mon	1 d	1 week	1 mon	1 d	1 week	1 mon	1 d	1 week	1 mon
50 mg	n.a.	n.a.	n.a.	0/3	0/3	n.d.	0/5	0/5	n.d.	0/2	0/2	n.d.
150 mg	1/3	0/3	n.d.	0/2	0/2	n.d.	0/2	0/2	n.d.	0/1	0/1	n.d.
300 mg	n.a.	n.a.	n.a.	0/1	0/1	0/1	2/5	1/5	2/5	n.a.	n.a.	n.a.
450 mg	1/1	1/1	1/1	1/4	1/4	2/4	0/2	0/2	1/2	n.a.	n.a.	n.a.
750 mg	1/1	1/1	1/1	2/2	1/2	0/2	2/3	0/3	0/3	n.a.	n.a.	n.a.
1350 mg	1/1	1/1	1/1	1/3	1/3	2/3	1/2	2/2	2/2	n.a.	n.a.	n.a.
1900 mg	1/1	1/1	1/1	3/4	3/4	4/4	3/3	3/3	3/3	n.a.	n.a.	n.a.

Table 1. GPCR-AAb neutralization rate (number of subjects exhibiting neutralization/number of subjects treated) at various time points after infusion. d, day; ETA, endothelin; GPCR-AAb, agonistic autoantibodies activating G protein–coupled receptors; mon, month; n.a., not available; n.d., not determined. Some subjects tested positive for 2 or 3 functionally active GPCR-AAbs.

CONCLUSION

BC 007 is a powerful new drug for neutralizing GPCR-AAbs associated with various pathological constellations. It also exhibits a favourable adverse-effect profile. The ability to neutralize various GPCR-AAbs provides an opportunity for future use with other diseases. BC 007 is currently undergoing an efficacy trial (phase 2) as the first causative agent for patients with β_1-AAb–associated heart failure.

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ABOUT THE AUTHOR

Johannes Müller *(MD, MEng), an accomplished physician, researcher, innovator and entrepreneur with over 20 years of clinical (cardiac surgery, advanced heart failure) and executive leadership experience in biotechnology and medical device companies (CEO Berlin Heart) pioneered the successful development of immunoadsorption as a heart failure treatment.*
Today, at Berlin Cures he leads the development of BC 007, an aptamer compound designed to neutralize autoantibodies in vivo.