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Aptamer BC 007 – A broad spectrum neutralizer of pathogenic autoantibodies against G-protein-coupled receptors

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ABSTRACT

The effect of autoantibodies on G-protein coupled receptors in the pathogenesis of diseases, especially of the heart and vascular system, is an increasingly accepted fact today.

Dilated cardiomyopathy (DCM) is the most intensively investigated pathological situation of these. With DCM, autoantibodies against the β_1-adrenoceptor and the muscarinic M_2-receptor have been found in high percentage of investigated patients. Immunoadsorption for autoantibody removal has already shown a long-term beneficial therapeutic effect, but has remained limited in its application because of the complexity of this method.

A new easy applicable treatment strategy has, therefore, been discovered. Because of intra- and inter-loop epitope variability of the β_1-adrenoceptor specific autoantibodies and also the occurrence of further autoantibodies of this class such as the ones against the β_2- and α_1-adrenoceptor, the ET_A-, proteinase activated-, and the AT_1-receptors in different pathological situations, this newly discovered broad-spectrum neutralizer of all these autoantibodies - aptamer BC 007 - is under development.

The binding and neutralizing effect was investigated applying a bioassay of spontaneously beating neonatal rat cardiomyocytes and enzyme-linked immunosorbent assay (ELISA) - technology. The usefulness of aptamer BC 007 to specify column technology for the removal of serum autoantibodies was also demonstrated. The presented data suggest that aptamer BC 007 might be an appropriate molecule candidate to support future research about the meaning of G-protein-coupled receptor autoantibodies.

1. Introduction

Autoimmune processes are increasingly identified as the origin or amplifier of a lot of very different diseases. Besides autoantibodies (AABs) which induce regular immune responses leading to the destruction of the targeted cells and tissues, there is an additional class of AABs showing functional activity. These AABs target receptors, agonistically influencing their function (Wallukat et al., 2003a).

In diseases of the heart and circulatory system such as dilated cardiomyopathy (DCM), Chagas cardiomyopathy and peripartum cardiomyopathy, reviewed by Bornholz et al. (2014) and Schulze et al. (2005), AABs have been detected which target the β_1-adrenoceptor (beta1-AAB). Other AABs such as the ones against the AT_1-receptor (AT1-AAB), found in kidney allograft rejection (Dragun et al., 2005), AABs against the β_2-adrenoceptor (beta2-AAB), the ET_A-receptor (ETA-AAB), the α_1-adrenoceptor (alpha1-AAB), the muscarinic M_2-receptor (M2-AAB), the proteinase activated receptors, found under different pathologic constellations have been discovered and described, partially summarized by Xia and Kellems (2011) and Wallukat and Schimke (2014). The impact of e.g. AABs against the β_1-adrenoceptor (beta1-AAB) on the

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organism was demonstrated in animal experiments inducing AABs by immunizing rabbits (Matsui et al., 1999) and rats (Jahns et al., 2004) with the receptor epitop, causing heart failure. A subsequent transfer of the AABs to healthy animals induced the symptom also in the recipients (Jahns et al., 2004) which had also been seen before by Matsui et al. while transferring IgG fractions and/or peripheral blood lymphocytes from epitope-immunized rabbits to immunodeficient mice, also inducing early stages of myocardial damage in the recipients (Matsui et al., 2003). Similar observations have already been made at this time with the transfer of lymphocytes of DCM patients into immunodeficient mice (Omerovic et al., 2000).

The significance of beta1-AABs in chronic heart failure has indirectly been shown by Nagatomo et al. in a substudy of the Japanese Chronic Heart Failure study, showing that patients who were tested positive for the beta1-AAB showed a "favourable response" to the beta-blocker carvedilol (Nagatomo et al., 2015). The elimination of beta1-AABs from the blood of end-stage DCM patients by immunoadsorption technology showed a beneficial effect for the first time in 2000 (Müller et al., 2000) and its comparability in its long term outcome to heart transplantation (Dandel et al., 2012). But, a simple drug able to neutralize the AABs has to be the therapeutic goal, since apheresis technology and heart transplantation are complicated technologies, limited in their applicability and being a burden for the patient. The recently discovered aptamers, and their ability to bind to their targets with high affinity and even neutralize functional activity might be an appropriate molecule class for a drug development. This molecule class had already been discovered before as a new option for the possible development of drug candidates hopefully appropriate for future treatment of autoimmune disorders. Li and Lan (2015) summarized existing endeavours for the development of therapeutic aptamers mainly focussing on specific developments for the autoimmune component of diabetes mellitus, multiple sclerosis, rheumatoid arthritis, myasthenia gravis, and systemic lupus erythematosus. While Vorobyeva et al. in their recent review about "aptamers against immunologic targets, diagnostic and therapeutic prospects" describe in a more chronological order besides concrete developments also more general aspects about aptamers (Vorobyeva et al., 2016). We have already selected and described the first DNA-aptamer which specifically targeted and neutralized beta1-AABs under *in vitro* and *in vivo* conditions (Haberland et al., 2011', 2014). This already reported "aptamer 110", a randomized 21 nucleotide region, flanked by 21mer primer binding sequences (Haberland et al., 2011) or its truncated (12mer) version (Wallukat et al., 2012) is, however, specifically directed to AABs targeting the second extracellular loop of the β_1-adrenoceptor (beta1-AAB 2nd loop). It is unable to bind and neutralize 1st loop-specific beta1-AABs which are also significantly relevant for DCM (Wallukat et al., 1995) or AABs against other G-protein-coupled receptors.

In this current paper we describe the first aptamer which showed the highest affinity to all tested AABs of the class of AABs against G-protein-coupled receptors (G-protein coupled receptor-AABs). In our view, it might open up completely new possibilities for treating patients who suffer from diseases associated with the occurrence of G-protein-coupled receptor-AABs and especially the ones carrying a pool of these AABs such as pulmonary hypertension and Chagas' cardiomyopathy patients. Chagas cardiomyopathy is often characterized by the parallel occurrence of AABs against the β_1- and β_2-adrenoceptors and the M_2-receptor (Wallukat et al., 2010b) and pulmonary hypertension presents with AABs against the ET_A-receptor and the α_1-adrenoceptor (Dandel et al., 2009).

2. Materials and methods

2.1. Materials

2.1.1. Consumables

Goat anti-human-adrenergic beta1-antibody against the 2nd extracellular loop of the β_1-adrenoceptor (ADRB1, EB07133) was purchased by Everest Biotech Ltd. UK, polyclonal affinity purified anti-endothelin A receptor antibody (2nd extracellular domain), (ETA-AB, SP4122P) by Acris Antibodies GmbH, Germany. Human IgGs (IgG1 – IgG4) were supplied by AbD serotec (IgG1: 5219-3004, IgG2: 5225-3004, IgG3: 5248-3004, IgG4: 5254-3004). Peroxidase-conjugated AffiniPure goat anti-rabbit IgG (H+L) (111-035-003), peroxidase-conjugated (HRP) AffiPure $F(ab)_2$ fragment goat anti-human IgG, $F(ab')_2$ (min X Bov, Hrs, Ms, 109-036-097), and anti-rabbit IgG (H+L) antibody adsorbed against human IgG (111-035-144) were supplied by dianova, Germany. NeutrAvidin™ (31000) and NeutrAvidin-HRP™ (31001B) were purchased by ThermoScientific, Pierce Biotechnology, USA, Streptavidin coated magnetic beads by Roche, Germany (11641 786 001). IMTEC ANALIA (ITC92000; antigens: dsDNA, histones, nucleosomes SmD1, SS-A/Ro60, SS-A/Ro52, SS-B/La, snRNP, Scl70, CENP-B, Jo-1, RPP/P0) and IMTEC CENP-B Antibodies ELISA (ITC60005) were from Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden.

2.1.2. Human autoantibodies against G-protein-coupled receptors

Serum or IgG-fractions prepared from beta1-, beta2-, alpha1-, AT1-, ETA- or M2-AAB containing sera were obtained from patients treated at the Deutsches Herzzentrum Berlin (German Heart Institute Berlin), or from Chagas' patients recruited from Santa Bárbara Hospital, Sucre, Bolivia. The use of these IgGs for *in vitro* experiments was approved by appropriate authorities at the respective hospitals. All patients signed an informed consent form.

2.1.3. IgG preparation

IgG fractions were prepared from neutralized immunoadsorber eluate material (regeneration waste) by stepwise ammonium sulfate precipitation. In a first step a saturated ammonium-sulfate solution was slowly added to reach a final concentration of 40% ammonium-sulfate. After incubation for 18 h at 4 °C the mixture was centrifuged at 6000*g* for 15 min and the resulting pellet was re-suspended in 75% of the initial sample volume phosphate buffered saline (PBS). A second ammonium-sulfate precipitation at 50% saturation followed, including the centrifugation step. The resulting pellets were re-suspended in 70% of their initial sample volume in PBS and dialyzed against a 100fold volume PBS for 3 days at 4 °C.

2.1.4. Preparation of Fab- fragments

Fab-fragments were obtained cleaving affinity purified patient AABs (ETA-AAB and beta1-AAB) using a commercially available Fab preparation kit (Pierce™ Fab preparation kit) followed by a protein A spin column purification.

2.1.5. Aptamer synthesis

Aptamer BC 007: 5′-GGT TGG TGT GGT TGG-3′, also known as ARC183 and the scrambled control sequence 5′-GGT GGT GGT TGT GGT-3′ were synthetized by BioTez Berlin-Buch GmbH, Germany. Either the aptamers or the 5′-biotinylated or 5′-aminohexyl-modified versions were synthesized using a solid phase method followed by high-performance liquid chromatography (HPLC) purification.