

G-protein coupled receptor auto-antibodies in thromboangiitis obliterans (Buerger's disease) and their removal by immunoadsorption

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Summary

Background: Immunohistopathological and serological data favors an immunopathogenesis of thromboangiitis obliterans (TAO, Buerger's disease). Autoantibodies seem to play a major role. Immunoadsorption (IA) proved to be therapeutically effective. We focused on agonistic autoantibodies (agAAB) directed against G-protein coupled receptors (GPCR) and proved the hypothesis, that these agAAB might be present in TAO and that a five day course of IA might be able to eliminate these agAAB effectively.

Patients and methods: Between December 2012 and May 2014 11 TAO-patients were treated by IA in a five day course. AgAAB-analysis was performed using specific ELISA techniques.

Results: AgAAB were detected in 9 out of 11 patients (81.8%). Multiple agAAB were present in 7 patients (63.6%). A clustering of agAAB directed against loop1 of the adrenergic α 1-receptor and the endothelin-A-(ETA)receptor was identified, representing 72.7 % resp. 54.5% of the patients. AgAAB directed against the angiotensin-1 (AT-1) epitope 1 or 2 were detected in 3 patients and agAAB directed against protease-activated receptor (PAR) loop1/2 were seen in 2 patients. AgAAB directed against ETA-receptor loop1 never appeared without agAAB directed against α 1-receptor loop1. Immediately after a five day-course of IA agAAB were absent in 81.8% of the total study group and in 77.8% of all cases tested positive for agAAB before IA.

Conclusions: AgAAB directed against GPCR were identified in TAO patients with a clustering of agAAB directed against α 1-adrenergic receptor loop1 and ETA-receptor loop1. AgAA were eliminated by IA in the majority of cases. We suggest that these agAA play an important role in the pathogenesis of TAO and that their elimination might be responsible for the positive therapeutic effects reported in patients treated with IA.

Key words: Thromboangiitis obliterans, buerger's disease, immunoadsorption, autoantibodies, g-protein coupled receptor

Zusammenfassung

Agonistische Autoantikörper, die gegen G-Protein gekoppelte Rezeptoren (GPCR) gerichtet sind, bei Patienten mit Thromboangiitis obliterans und ihre Entfernung durch Immunadsorption

Hintergrund: Histopathologische und serologische Befunde sprechen für eine Immunpathogenese der Thromboangiitis obliterans (TAO, Buerger'sche Erkrankung). Autoantikörper scheinen eine wichtige Rolle im Krankheitsgeschehen zu spielen. Es wurden wiederholt positive therapeutische Effekte durch eine Immunadsorption (IA) gezeigt. Wir analysierten agonistische Autoantikörper (agAAK), die gegen G-Protein gekoppelte Rezeptoren (GPCR) gerichtet sind und analysierten, ob diese durch eine IA effektiv beseitigt werden können.

Patienten und Methoden: Zwischen Dezember 2012 und Mai 2014 wurden 11 Patienten mittels IA über 5 konsekutive Tage behandelt. Die agAAK wurden unter Verwendung spezifischer ELISA-Techniken bestimmt.

Ergebnisse: AgAAK wurden bei 9 von 11 Patienten (81.8%) gefunden. Multiple agAAK waren bei 7 Patienten (63.6%) nachweisbar. Darüber hinaus wurde ein agAAK-Cluster entdeckt, das sich gegen Loop1 des adrenergen α 1-Rezeptors und Loop1 des Endothelin A (ETA)-Rezeptors richtet, und sich bei 72.7% respektive 54.5% der TAO-Patienten nachweisen ließ. AgAAK gegen Epitop 1 oder 2 des Angiotensin-1-Rezeptors wurden bei 3 Patienten, agAAK gegen Loop1/2 des Proteinase-aktivierten Rezeptor (PAR) wurden bei 2 Patienten nachgewiesen. AgAAK gegen den ETA-Rezeptor traten niemals ohne gleichzeitigen Nachweis von agAAK gegen den α 1-adrenergen Rezeptor auf und waren immer gegen Loop1 gerichtet. Unmittelbar nach Beendigung der IA ließen sich bei 81.8% aller Patienten und bei 77.8% aller Fälle mit positiven agAAK vor der IA keine agAAK mehr nachgewiesen.

Schlussfolgerungen: Wir konnten bei Patienten mit aktiver TAO agAAK gegen GPCR und darüber hinaus ein Antikörper-Cluster nachweisen, das sich bevorzugt gegen Loop1 des α 1-adrenergen Rezeptors und Loop1 des ETA-Rezeptors richtet. Die agAAK wurden mehrheitlich erfolgreich durch die IA eliminiert. Wir sind der Auffassung, dass gegen GPCR-gerichtete agAAK eine wichtige Rolle in der Pathogenese der TAO spielen und ihre Elimination für die publizierten positiven Effekte der IA verantwortlich sein könnten.

Introduction

Thromboangiitis obliterans (TAO; Buerger's disease) is a inflammatory disease of unknown origin affecting small and medium sized arteries and veins of young tobacco users threatening limbs by vessel occlusions with mononuclear cell rich thrombi [1–4]. Endothelial activation and proliferation as well as the presence of immunocompetent cells together with deposition of immunoglobulins and complement factors alongside the internal elastic membrane of the vessel wall argue for an immunopathogenesis of TAO [5–11]. Furthermore, in the sera of TAO-patients various kinds of autoantibodies have been described including antiendothelial antibodies, antibodies directed against elastin and collagen, anticardiolipin-antibodies, ANCA, and ANA [12–15].

Agonistic autoantibodies (agAAB) reacting with epitopes localized at the extracellular loops of G-protein coupled receptors (GPCR) are increasingly recognized as modulators of various cardiovascular pathologies [16–19]. In this context, agAAB directed against α 1-adrenergic receptor [20–26], β 1-adrenergic receptor [27–29], β 2-adrenergic receptor [23, 26] endothelin A receptor [20] and angiotensin-1 receptor [22, 26, 30, 31] have been described. Binding of agAAB to the receptor leads to activation of downstream signaling cascades [32]. Deviant to physiological GPCR-agonists activation of receptor pathways by agAAB may result in a prolonged and unphysiological activation of postreceptor action [28]. The pathogenic potential of circulating agAAB has already been demonstrated in animal models as well as in clinical studies [25, 33–36].

With this study we tested the hypothesis, that agAAB directed against GPCR are present in the sera of TAO patients and that they might be removed sufficiently by a five day

course of immunoabsorption (IA). Subsequently, we present preliminary results of an ongoing project.

Patients and methods

From December 2012 to May 2014 thirteen patients presumably suffering from TAO were treated with IA in our institution. Diagnosis of TAO was made according to the criteria published by Olin et al. and Shionoya [37, 38]. Diagnosis of TAO was corrected retrospectively in two patients due to the finding of high concentrations of Lp(a) in one case and the detection of atherosclerotic coronary artery disease in another, leaving eleven patients for final analysis.

Patients' characteristics are shown in Table I. All were cigarette smokers; two had recently stopped smoking, while two admitted additional cannabis abuse.

All patients had received best medical treatment before IA including intravenous iloprost-infusions. Four had undergone minor amputations, whereas one patient was already majorly amputated.

IA was performed during a five day course as described elsewhere [39, 40]. Alternatively, Globaffin^R-adsorbers with a synthetic Peptid-GAM^R-ligand coupled on a sepharose matrix (Fresenius Medical Care AG & Co. KGaA, Bad Homburg v.d. Höhe, Germany) were used in all treatment sessions in this series.

Blood samples were drawn each day before and after IA from a central venous line placed immediately before the first treatment. Autoantibody-analysis was performed using peptides corresponding to the first and/or second extracellular loop of following GPCR: α 1, Endothelin A, Angiotensin II Type 1, protease activated receptor (PAR) 1/2. Peptides were coupled to pre-blocked streptavidin-coated 96-well plates (Perbio Science,

Bonn, Germany). Patient serum was added in a 1:100 dilution and incubated for 60 min. As detection antibody a horseradish peroxidase conjugated anti-human IgG antibody was used (Dianova, Hamburg, Germany). Antibody binding was visualized by the 1-Step Ultra TMB ELISA (Perbio Science, Bonn, Germany). The absorbance was measured at 450 nm with a SLT Spectra multiplate reader (TECAN, Crailsheim, Germany).

Results

Results of agAAB-testing before and after IA are summarized in table II. AgAAB were identified in 9 out of 11 patients (81.8%), while multiple agAAB were present in 7 patients (63.6%). Three agAAB were present in 3 patients (27.3%), while 2 agAAB were detected in 5 patients (45.5%). A clustering of α 1-adrenergic receptor and endothelin A (ETA)-receptor agAAB was identified, representing 72.7% resp. 54.5% of the TAO-patients. Six patients (54.5%) were positive for both of these two agAAB. AgAAB directed against the ETA-receptor never appeared without agAAB directed against the α 1-receptor. Both were exclusively directed to the extracellular receptor-loop1. AgAAB directed against the angiotensin-1 (AT-1) epitope 1 or 2 were positive in 3 patients and agAAB directed against protease-activated receptor (PAR) loop1/2 were seen in 2 patients. Immediately after the end of the five-day course agAAB were negative in 9 patients (81.8%). Focusing exclusively on those cases with positive agAAB before IA (n=9) the elimination rate at the end of the last treatment session was 77.8%. Majoritarianly, agAAB were eliminated after the first three sessions. However, persistence of at least one agAAB was observed in 2 patients, while reoccurrence of one agAAB from day-

Table 1: Clinical characteristics of the study group

Patient, initials	Age (y)	Sex	Duration of disease (y)	Smoking status (cigarettes)	Ischemic rest pain, visual analog pain scale result	Acral cyanosis	Active ulcer/gangrene/wounds	Former amputation	Thrombophlebitis	Raynaud's phenomenon	Other diseases
1 NA	40	male	2	active	yes, VAS 7	left forefoot	D ped I, II left	no	no	yes	pulmonary embolism 2008
2 LH	53	male	3	active	yes, VAS 6	fingers right hand	D II right	no	no	yes	acute gastritis and duodenitis, extirpated basalioma, mild hypercholesterolemia
3 MM	48	female	4	active	yes, VAS 9	left forefoot	left forefoot	D IV and V left as well as partial amputations D I, II ped left	no	no	–
4 NC	39	male	2	former	yes, VAS 7	–	D I right	D I ped left	no	no	–
5 RT	42	male	2	former	yes, VAS 9	right forefoot	dehiscent amputation wounds	Dig V transmetatarsal left, dig ped II left	no	no	acute wound infection, drug-induced mild leucopenia and thrombopenia
6 RD	20	male	1,5	active	yes, VAS 8	bilateral forefoot	left forefoot, toes right	no	no	no	additional cannabis abuse
7 VK	52	male	4	active	yes, VAS 7	bilateral forefeet	toes right foot	no	no	yes	Occluded distal arterial bypass
8 BS	26	male	1	active	yes, VAS 8	bilateral forefeet	D I ped left	no	no	yes	additional cannabis abuse, penicillin allergy, seronegative spondylarthritis
9 FB	50	male	2	active	yes, VAS 7	–	D II, V ped left	no	yes	no	acute forefoot-infection; alcoholism, mild hypertension
10 BR	27	male	1	active	yes, VAS 8	right forefoot	DI ped right	no	no	no	–
11 HG	51	male	3	former	yes VAS 9	–	amputation wound left forefoot	right lower leg, recently left forefoot	no	no	–
Σ	40.7	n:f = 10:1	2.3	8 active, 3 former smokers	rest pain 11/11; VAS (Pain-scale) 7.7	8/11 (73%)	11/11	4/11 (36%) minor 1/11 (9%) major	1/11 (9%)	4/11 (36%)	–

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to-day was observed in another case. Thus, effective total elimination rate obtained with our protocol presumably adds up to 66.6 %.

Discussion

AgAA directed against GPCR are increasingly recognized as modulators of various cardiovascular pathologies [16–19] including systemic and pulmonary arterial hypertension [20, 24, 25, 41], idiopathic dilated cardiomyopathy [27, 29, 33, 42], type 2 diabetes [22], dementia [23], pre-eclampsia [19, 31], vascular necrotic kidney graft rejection [30, 43] as well as in scleroderma angiopathy [44]. The prevalence of agAAB in cardiovascular diseases and diabetes varies grossly from 30 to 60 %.

Analyzing preliminary data of an ongoing project we detected agAAB against GPCR in nine out of eleven patients (81.8 %) with active Buerger's disease and multiple agAAB against GPCR in 7 of them (63.6 %). Furthermore, we were able to identify clustering of agAAB binding to the extracellular receptor loop1 of the α 1-adrenergic and the ETA-receptor, while agAAB directed against loop2 of these two receptors were not detectable. To our knowledge, this is the first report addressing this topic and identifying this agAAB-pattern in patients with active TAO.

Notably, after a five-day course of IA agAAB were eliminated in 77.8 % of all cases tested positive for agAAB before the first treatment session. However, effective total agAAB elimination rate might have been lower (66.6 %) as re-occurrence of agAAB from day to day throughout the treatment course was observed in another patient. These results might suggest agAAB-surveillance and modifications of the current IA-protocol in cases where agAAB persist or re-occur.

We assume that the elimination of the agAAB identified by us ameliorates vasospasm and improves microcirculation. The effects might explain published clinical effects of IA in TAO that cover marked improvement of pain, improvement of digital pulse curves, steep incline of tcpO₂-levels, decrease of tcCO₂-levels, and ulcer healing [39, 40]. Nevertheless, the exact pathophysiologic role of GPCR-AAB in TAO has yet to be defined experimentally. Further studies will also have to re-produce our results in larger cohorts and address the time course as well as clinical relevance of re-occurrences of agAAB after IA.

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Conflicts of interest

Autoantibody analysis was supported by Fresenius Medical Care AG & Co. KGaA, Bad Homburg v.d. Höhe, Germany.

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