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Effects of different serotonin receptor subtype antagonists on the development of cardiac allograft vasculopathy in murine aortic allografts

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ABSTRACT

Background: Cardiac allograft vasculopathy (CAV) is the main obstacle for long-term survival after heart transplantation. Alloimmune mediated chronic vascular rejection results in several mechanisms like platelet activation, immigration of inflammatory cells through the endothelial layer and proliferation and migration of smooth muscle cells (SMCs). Serotonin (5-HT) promotes these processes via activation of 5-HT_2 receptors. We hypothesized that inhibiting 5-HT_2 receptors ameliorates the development of CAV.

Methods: CBA/JRj mice recieved aortic grafts from C57BL/6 mice. After transplantation until recovery of organs, recipients were treated with serotonin receptor antagonists: sarpogrelate (5-HT_{2A}), SB 204741 (5-HT_{2B}) or terguride (5-HT_{2A+B}). Mice were sacrificed after 14 days for qRT-PCR analysis or after 30 days for histological evaluation. Serum serotonin ELISA was done at both time points.

Results: Elevated serum serotonin levels were significantly reduced after 5-HT_{2A} antagonist treatment as was 5-HT_{2A} receptor expression. This went along with reduced inflammation characterized by significantly fewer infiltrating macrophages and pro-inflammatory intragraft cytokines and with reduced tissue remodeling evident as significantly less neointima formation.

Conclusion: Inhibition of the 5HT/5-HT_{2A} receptor axis leads to significantly reduced neointima proliferation after aortic transplantation associated with reduced transendothelial migration of macrophages and decreased expression of inflammatory cytokines. These findings have translational implications as inhibitors of 5HT_{2A} like sarpogrelate are already approved for clinical use.

1. Introduction

Cardiac allograft vasculopathy (CAV) as the clinical manifestation of chronic rejection is one of the main leading causes of late mortality after heart transplantation. CAV affects the coronary vessels and is characterized by a thickened vessel wall consisting mainly of smooth muscle cells (SMCs) intermixed with an infiltrate of inflammatory cells like macrophages and lymphocytes [1,2] that induces a diffuse and concentric narrowing of the vessel lumen and ultimately complete occlusion and graft failure [2,3].

CAV gains importance, because about half of the recipients who survive the first year after transplantation develop CAV within 10 years after successful heart transplantation [4]. Despite advantages in immunosuppressive regimens that have decreased acute rejection episodes no effective prevention or treatment is known for CAV until today. The only option for end-stage CAV is re-transplantation which is accompanied by a higher operation risk and a poorer prognosis [4] and additionally poses an ethical problem because of the shortage of donor organs nowadays.

Vascular remodeling during CAV is caused by a chronic alloimmune response that results in a changed tissue milieu characterized by continued expression of cytokines and growth factors finally leading to SMC migration and proliferation [5]. Besides SMCs, macrophages are also found in the developing neointima and promote SMC proliferation by producing several chemokines and growth factors [6]. As already demonstrated in previous studies of our group [7–9], platelets considerably contribute to the development of CAV, too [10]. Activated platelets release a variety of mediators which lead to the attraction of inflammatory cells into the transplant vessels and thereby cause chronic inflammation and rejection. In addition to growth factors and cytokines, platelets also contain serotonin (5-hydroxytryptamine; 5-HT) [11,12] that is produced by enterochromaffin cells in the gut, taken up

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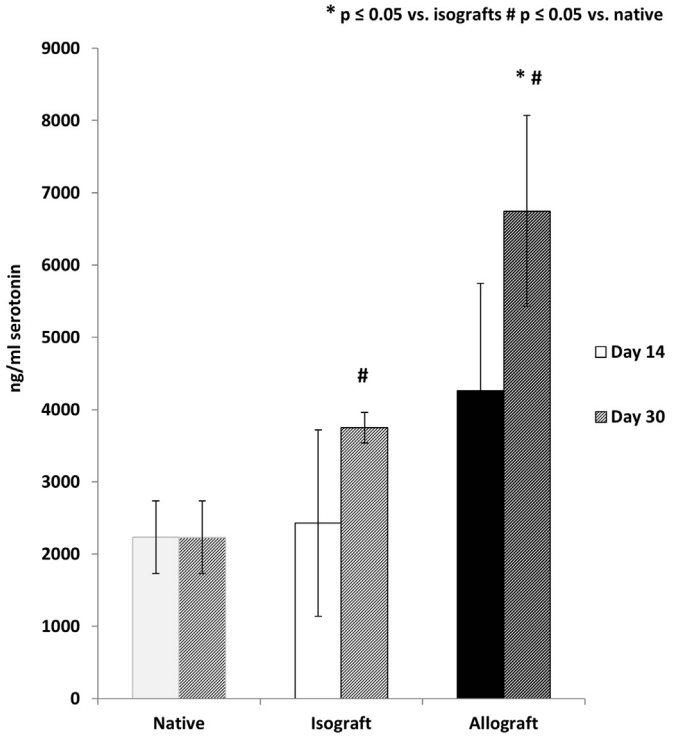

Fig. 1. Serum serotonin levels at day 14 and day 30 in the three control groups: native mice (non-transplanted; white columns with light framing), isografts (white columns with black framing) and allografts (black columns). Values are given as nanogramm Serotonin per milliliter serum. For each group two points of time are depicted: values 14 days after transplantation (uniformly filled columns) and values 30 days after transplantation (diagonally striped columns). Because native mice had no transplantation, serum was taken once from age matched control mice and therefore both columns of this group represent the same value and are interpreted as normal basal serotonin levels of CBA mice. (n = 7 for all groups).

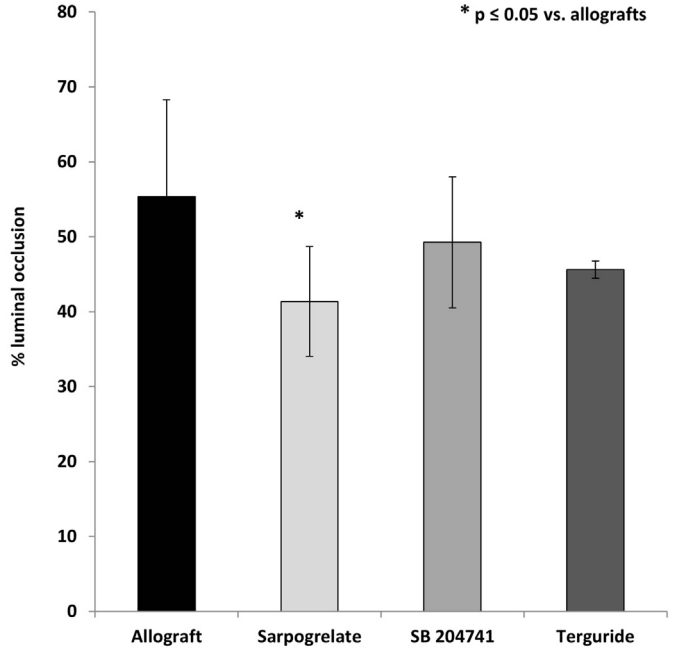

Fig. 2. Percentage of luminal occlusion in allogeneic aortic grafts after treatment with serotonin receptor antagonists. Luminal occlusion was measured as the percentage of the vessel lumen (area within the inner elastic lamina) occupied by neointima (area between inner elastic lamina and endothelium). Values are shown for untreated control allografts (black column) and the three groups of allografts treated with sarpogrelate (light grey column)
SB 204741 (middle grey column) or terguride (dark grey column). (n = 8 per group)

from the blood by platelets and stored within their dense granules [13]. 5-HT affects multiple cells including platelets themselves [14] as well as SMCs [15–17]. Serotonin has been shown to orchestrate vascular remodeling by affecting the functions of VSMCs and ECs such as proliferation, migration and mediator release [18]. Its actions are mediated via special receptors: up to date 7 families of serotonin receptors (5-HTRs) with diverse subspecies are known [14,19].

2. Objective

The fact that serotonin released from activated platelets can promote leukocyte adhesion and recruitment in inflammatory response as well as proliferation and migration of SMCs [15–17,20], which are the main features relevant for developing CAV, combined with the finding that antagonists of the 5-HT_2 receptor family can inhibit these effects [21,22], led us to the hypothesis that 5-HTR antagonists might be efficient in reducing the development of CAV. To investigate this hypothesis we conducted a study using specific clinical approved 5-HT_{2A} or 5-HT_{2B} antagonists as well as a combined 5-HT_{2A+2B} antagonist in a mouse model of aortic transplantation.

3. Materials and methods

3.1. Animals

C57BL/6 ($H2^b$) and CBA/JRj ($H2^k$) mice were originally purchased from Janvier (Saint Berthevin, France). For the experiments male and female mice were used at 1:1 ratio. C57BL/6 mice were used as donors and CBA/JRj mice as recipients for aortic allograft transplantation (male donors for male recipients and female donors for female recipients). All mice were aged between 8 and 12 weeks at the time of experimental use, were maintained at the Preclinical Experimental Animal Center (PETZ) at the University of Erlangen-Nürnberg under specific pathogen-free conditions and treated in accordance with the "Principles of Laboratory animal care" as well as with institutional and state guidelines (animal experiment 55.2–2532.1-62/14 authorized by the government of Unterfranken).

3.2. Abdominal aortic transplantation

The aortic transplantation model already used in earlier studies [7,23,24] is an accepted model for CAV-related research, because developing lesions closely resemble those found in human chronically rejected cardiac grafts [25,26]. Among its advantages are the possibility for orthotopic graft implantation, graft survival without the use of immunosuppression and a more standardized histological analysis [27,28]. The procedure was performed with a surgical microscope (OPMI® 1 FC; Zeiss) using a modified technique initially described by Koulack et al. [29] and explained in previous own studies [7,23,24,30].

3.3. Treatment protocol

Sarpogrelate hydrochloride (Sigma-Aldrich, Germany) was dissolved in sterile water and diluted with 0.9% NaCl. Applied dose was 10 mg/kg/day intraperitoneal (i.p.). SB 204741 (Sigma-Aldrich, Germany) was dissolved in DMSO, diluted with 0.9% NaCl and given 10 mg/kg/day i.p.. Terguride tablets were ground and dissolved in NaCl and 0.6 mg/kg was given orally (p.o.) twice a day. Terguride as the pure substance (Abcam, Cambridge, UK) was dissolved in 99.8% ethanol and diluted with 0.9% NaCl. Application dose was 0.6 mg/kg twice daily intraperitoneally. Used dosages have proved to be effective and