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Imatinib treatment attenuates growth and inflammation of angiotensin II induced abdominal aortic aneurysm

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ABSTRACT

Background: Abdominal aortic aneurysm (AAA) is characterized by vascular remodeling with increased infiltration of inflammatory cells and apoptosis/modulation of vascular smooth muscle cells (SMCs). Imatinib is a selective inhibitor of several tyrosine kinases, including PDGF receptors, Abl, and c-kit. The objective of this study was to characterize the potential protective role of imatinib on AAA development and the molecular mechanisms involved.

Methods: Male *ApoE*$^{-/-}$ mice were infused with angiotensin (Ang) II (1000 ng/kg/min) for 4 weeks to induce AAA or saline as controls. Daily treatment with 10 mg/kg imatinib, or tap water as control, was provided via gavage for 4 weeks.

Results: Treatment with imatinib was found to decrease the aortic diameter and vessel wall thickness, mediated by multiple effects. Imatinib treatment in AngII infused mice resulted in a reduced cellular infiltration of CD3ε positive T lymphocytes by 86% and reduced gene expression of *mast cell chymase* by 50% compared with AngII infused mice lacking imatinib. Gene expression analysis of SMC marker *SM22α* demonstrated an increase by 48% together with a more intact medial layer after treatment with imatinib as evaluated with SM22α immunostaining.

Conclusion: Present findings highlight the importance of tyrosine kinase pathways in the development of AAA. Our results show, that imatinib treatment inhibits essential mast cell, T lymphocyte and SMC mediated processes in experimental AAA. Thus, our results support the idea that tyrosine kinase inhibitors may be useful in the treatment of pathological vascular inflammation and remodeling in conditions like AAA.

1. Introduction

Abdominal aortic aneurysm (AAA) is a chronic inflammatory disease resulting in vascular remodeling. AAA is associated with infiltration of leukocytes, such as T lymphocytes, mast cells, neutrophils and macrophages, which produce various matrix-degrading proteases. Phenotypic modulation and increased apoptosis of smooth muscle cells (SMCs) and degradation of the extracellular matrix (ECM) also occurs [1–4]. In normal blood vessels, the predominant vascular SMC phenotype is the contractile phenotype that regulates vessel diameter and blood flow [5]. Phenotypic modulation of SMCs is critical in regulating vascular function in health and disease. The switch from the contractile to the migratory and proliferative phenotype – known as the dedifferentiated synthetic phenotype – takes place during tissue repair in response to vascular injury. In this phenotype, SMCs increase their rate of cell proliferation and migration in response to growth factors and/or chemoattractant and this process plays a critical role in maintaining the integrity of the vessel [6]. SMCs are one of the

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major producers of medial matrix collagen, providing tensile strength to the aortic wall. Collagen synthesis increases during the early stages of aneurysm formation suggesting a repair process while in later stages of AAA collagen degradation, due to protease produced by infiltrated leukocytes, exceeds its synthesis which may eventually lead to rupture of the aortic wall [7].

Imatinib is a small molecule with potent and selective inhibitory activity against several tyrosine kinases, including; Abl, c-Kit and platelet-derived growth factor receptor (PDGFR) -α and -β [8,9]. Imatinib has good oral bioavailability and has become the standard of care for patients with chronic myelogenous leukemia and gastrointestinal stromal tumors, because it inhibits the growth of malignant cells [8]. However, the mechanism of action of imatinib may be broader than originally expected, and might also be targeting non-malignant disorders such as pulmonary arterial hypertension, hyperlipidemia, hypercholesterolemia and atherosclerosis [10–12]. Furthermore, imatinib may inhibit T lymphocytes proliferation and activation [13] as well as inhibit mast cell activation by inducing apoptosis [14]. Both cells play an evident role in the development and progression of AAA [15,16].

Given the fact that imatinib may inhibit key pathways in formation of AAA, i.e. T lymphocytes, mast cells, SMC proliferation and PDGF signaling, the objectives of this study was to characterize the potential protective role of imatinib on AAA development and the molecular mechanisms involved.

2. Material and methods

2.1. Human subjects

This study included 12 patients in whom preoperative computed tomography had demonstrated an eccentric intraluminal thrombus and who were scheduled for elective surgery for infrarenal AAA at Karolinska University Hospital, Stockholm, Sweden. During surgery the intraluminal thrombus was removed from the aortic vessel wall. The intima/media and adventitia of AAA were separated by adventicectomy. Patients were included after informed, written, and signed consent, and the studies were performed with approval from the local ethical committee at Karolinska Institutet in Stockholm, Sweden.

Control ascending aorta samples (layers not separated) for RNA studies were obtained from 8 organ donors, and infrarenal control aortas for histology were collected from 14 medicolegal autopsies without clinical or macroscopic signs of aortic atherosclerosis or aneurysm. Autopsies of control aorta samples were performed in the Department of Forensic Medicine, University of Helsinki. The sections were immediately fixed in 4% formaldehyde for light microscopy or snap frozen in liquid nitrogen for RNA isolation. The use of organ donor and autopsy tissues was approved by The National Authority for Medicolegal Affairs of Finland.

2.2. AngII-induced AAA in mice

AngII-induced aneurysm is an inflammation-driven model that is frequently used to experimentally induce AAA [17]. Male hypercholesterolemic *ApoE*$^{-/-}$ mice were obtained from Taconic (Bomholt, Denmark). At eight weeks of age, AAA was induced by chronic infusion of 1000 ng/kg/min AngII (Cat.no.9525, Sigma Aldrich, St. Louis, USA) via mini-osmotic pumps (Model 1004, Alzet, CA, USA) as described previously [18]. A group of *ApoE*$^{-/-}$ mice were infused with 0.9% NaCl and were used as control mice. In order to study the significance of tyrosine kinase signaling in AAA development, imatinib, a tyrosine kinase inhibitor was administrated. Oral gavage treatment with 10 mg/kg imatinib mesylate (STI571, Novartis, Stockholm, Sweden) was given daily throughout the study starting two days before implantation of the mini-osmotic pumps. Administration of tap water was given orally to *ApoE*$^{-/-}$ mice which served as control mice. Mice were divided into four groups, two groups obtained mini-osmotic pumps releasing AngII and two groups obtained mini-osmotic pumps releasing 0.9% NaCl. From each exposure one group either obtained 10 mg/kg imatinib or tap water orally. Groups were divided as follow; NaCl and tap water (n = 7), AngII and tap water (n = 9), NaCl and imatinib (n = 9) and AngII and imatinib (n = 8). Standard Chow diet and water was allowed *ad libitum* throughout the whole study and mice were monitored daily for signs of discomfort. After 28 days, mice were sacrificed. The aorta was removed and fixated in RNA-later for 24 h thereafter frozen in −70 °C for gene expression analysis and plasma was taken for cholesterol measurements which were performed using Total cholesterol + HDL + Glucose Panel, CardioChec (Medistore, Stockholm, Sweden). The study was approved by the local ethical committee 2014-09-04, 68-14 in Linköping, Sweden.

2.3. Quantitative real-time PCR

Human and mouse aortas were homogenized with trizol and chloroform in Lysing Matrix D tubes (MP Biomedicals, Illkirch, France) using FastPrep. Total RNA from human and mice aortas were isolated with RNeasy mini kit (Qiagen, Hilden, Germany) and reversely transcribed with random primers and Superscript II (Invitrogen, Carlsbad, USA). cDNA (human 0.5 μg; mouse 0.21 μg) was amplified by RT-PCR with 1× TaqMan Universal PCR Mastermix (Applied Biosystems, Foster City, USA) on a ABI 7700 Real-time PCR Sequence Detector, run in duplicates as previously described [18]. All probes were obtained from Applied Biosystems (Supplementary table I), and the results were normalized to expression levels of human *RPLP0* or mouse *Gapdh*.

2.4. Immunohistochemistry

Paraffin-embedded human and mouse abdominal aortas were sectioned (5 μm) and rehydrated in several changes of ethanol and Tissue-Clear® (Sakura Finetek, Leiden, The Netherlands). Endogenous peroxidase activity was quenched by treatment with 3% hydrogen peroxide for 5 min followed by incubation in 5% blocking bovine serum albumin solution. Sections were then incubated with primary antibodies against mouse CD3ε (1 μg/ml, Cat. no. BS3476, Bioworld Technology, St Louis Park, USA), SM22α (0.6 μg/ml, Cat. no. ab14106, Abcam, Cambridge, UK) and PDGFR-β (phospho-Tyr751, 10 μg/ml, Cat. no. LS-C178098, LSBio, Seattle, USA) or human PDGF-D (0.5 μg/ml, Cat. no. AF1159) and PDGFR-β (1 μg/ml, Cat. no. AF385) (RnD Systems, Minneapolis, USA), Von Willebrand factor (0.2 μg/ml, Cat. no. A0082, Dako, Glostrup, Denmark), CD68 (0.1 μg/ml, Cat. no.NCL-CD68-KP1, Leica Microsystems, Newcastle, UK) and α-actin (0.6 μg/ml, Cat. no. A5228, clone 1A4, Sigma-Aldrich) at 4 °C overnight followed by secondary biotinylated goat anti-rabbit IgG or goat anti-mouse IgG (Dako) antibody. Isotype-matched immunoglobulin was used in the same concentration as corresponding primary antibody in order to assess the level of non-specific binding (Cat. no. ab27472, Abcam). Avidin-biotin peroxidase complexes (Dako) were added followed by visualization with 3,3′-diaminobenzidine tetrahydrochloride (Dako). All sections were counterstained with Mayer's hematoxylin (Histolab Products, Göteborg, Sweden). Sections were studied under light microscopy (Zeiss, Jena, Germany). Quantification of CD3ε positive cells in aortas was defined as number per mm^2. A scoring system from 1 to 4 was used for quantification of phosphor-specific PDGFR-β expression, with 1 defined as no or low expression, 2 as expression in aortic media, 3 as expression in aortic media and