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Evidence of deregulated cholesterol efflux in abdominal aortic aneurysm

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ABSTRACT

Previous studies indicated that lipids may be associated with abdominal aortic aneurysm (AAA); however the molecular mechanism involved is unclear. Our study aimed to investigate the expression pattern of cholesterol efflux related proteins in AAA. Liver X receptors (LXRα and LXRβ), ATP-binding-cassette transporter A1 (ABCA1), Apolipoprotein AI (ApoAI), smooth muscle α-actin (α-SM) and vimentin expression levels were evaluated in human AAA, atherosclerotic (ATH) and normal abdominal aortic tissues. We found significant differences in LXRα, LXRβ and ABCA1 mRNA expression levels between AAA, ATH and normal whole aortic tissues and also within the AAA, ATH and normal "intima-media" layers. Specifically, LXRα, LXRβ and ABCA1 mRNA levels were decreased in AAA compared to ATH-whole tissues, as well as in AAA "intima-media" compared to ATH and normal "intima-media" layers. Moreover, immunohistochemical evaluation revealed that LXRα and ABCA1 immunoreactivities (IR) were reduced in the AAA media compared to the normal and ATH media layers and that they were also reduced in the intima layer of AAA and ATH tissues, whereas ApoAI-IR was increased in the AAA and ATH aortic walls compared to normal pointing to possible deregulation of the cholesterol efflux mechanism in AAA. Furthermore, double staining for vimentin and α-SM showed vimentin expression in the intima and inner media layer of AAA with sparse vimentin positive SMCs designating possible SMCs phenotype switch from contractile to synthetic form. In addition, histochemical analysis showed excessive lipid accumulation in the AAA wall, while co-staining using Oil Red O with α-SM or CD68 revealed lipid accumulation in SMCs and macrophages, respectively. Our study provides novel evidence for impaired cholesterol efflux in AAA associated with lipid accumulation in SMCs and macrophages, as well as switch of SMCs phenotype from contractile to synthetic form.

1. Introduction

Abdominal Aortic Aneurysm (AAA) is a degenerative disease characterized by weakening of the aortic wall and progressive dilation of the aorta. It represents an asymptomatic condition that primarily affects up to 8% of men above 65 years of age and is associated with nearly 90% mortality in the event of rupture (Kent, 2014; Roger et al., 2012). AAA is considered to be a multifactorial disorder, as environmental and genetic factors contribute to its onset (Cornuz et al., 2004; Larsson et al., 2009). Hallmarks of AAA pathology are the extensive infiltration of inflammatory cells and the extracellular matrix degeneration with upregulation of proteolytic pathways and apoptosis of smooth muscle cells (SMCs), the major cell type populating the media aortic layer (Hellenthal et al., 2009; Henderson et al., 1999; Shimizu et al., 2004).

A number of studies have indicated that atherosclerosis is a common finding in the wall of aneurysm (Johnsen et al., 2010; Zarins et al., 2001). Due to the significant role of cholesterol transport and lipid metabolism in the pathophysiology of atherosclerosis (Ohashi et al., 2005), genes involved in these pathways are also

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of interest in AAA pathology. Even though several studies point to the direction that lipids may be associated with AAA presence, they have led to controversial results. A limited number of studies revealed an association between low serum high-density lipoprotein (HDL) and high serum low-density lipoprotein (LDL) with AAA presence, suggesting that HDL may be important in AAA development (Forsdahl et al., 2009; Golledge et al., 2010; Hobbs et al., 2003; Simoni et al., 1996), while other studies failed to reproduce these findings (Golledge et al., 2010; Lindholt et al., 2001). In addition, in a recent metabolomics analysis, increased concentrations of sphingolipids in plasma were associated with AAA pathogenesis (Ciborowski et al., 2012), while Tanaka et al. (2013) demonstrated the presence of phosphatidylcholine (PC) and lyso-PC (LPC) lipid molecules on AAA tissue. In addition, a report in a mouse model of AAA demonstrated that loss of peroxisome proliferator activated receptor γ (PPARγ), a gene involved in lipid metabolism, in vascular smooth muscle cells (SMCs) promoted AAA development (Hamblin et al., 2010). It has also been suggested that lipid-lowering drugs might have an impact on AAA progression, as their routine use in AAA patients appears to be associated with beneficial effects (Kalyanasundaram et al., 2006). Further evidence for the involvement of lipid metabolism in AAA development, comes from histological findings, which demonstrate that the thickening of the aortic wall is correlated with the presence of fibrous tissue with cholesterol clefts in the intima layer of the abdominal aorta (Hellenthal et al., 2009; Kramer et al., 2004). All above findings point toward the involvement of cholesterol efflux and lipid metabolism in AAA initiation. However the molecular mechanism involved is still unclear.

Key element in the cholesterol efflux pathway is the ligand activated transcription factor LXR (liver X receptor), which forms an heterodimer with retinoid X receptor (RXR) and binds to a promoter sequence of ATP-binding-cassette transporter A1 (ABCA1) gene (Edwards et al., 2002). Several studies have reported deregulation of cholesterol efflux in atherosclerosis (Ohashi et al., 2005), as well as intimal SMCs- and macrophage-foam cell formation (Allahverdian and Francis, 2010; Cuchel and Rader, 2006; Stary et al., 1994; Yu et al., 2013). Furthermore, a recent study, showed that a subset of foam cells in the intima layer of human atherosclerotic coronary arteries is derived from SMCs (Allahverdian et al., 2014), which exhibit reduced ABCA1 expression levels and therefore have impaired ability to efflux excess cholesterol (Choi et al., 2009). Moreover, it has been documented that lipid-loaded SMCs are associated with switch in their phenotype from contractile to synthetic form (Doran et al., 2008). Synthetic SMCs populate the intima aortic layer of atherosclerotic tissues and exhibit altered expression of cytoskeleton marker proteins, such as decreased expression of contractile proteins, as α-SM and increased expression of vimentin, a synthetic SMCs marker (Rensen et al., 2007; Vukovic et al., 2006).

In the present study, we tested the hypothesis whether cholesterol efflux is impaired in AAA and possibly related to SMCs- and macrophage-foam cell formation contributing thus to AAA pathology.

2. Materials and methods

2.1. Human aortic samples

Full thickness AAA segments were collected from patients undergoing open repair operation for AAA in the Vascular Surgery Department of University Hospital of Larissa, Greece. AAA repair surgery was based on aneurysm size exceeding 5.0 cm in diameter estimated by CTA (Computed Tomography Angiography). A total of 11 AAA aortic tissue specimens (all male; mean age 73.7 ± 7.7 years, range from 60 to 87 years) were included in the study. Patients' tissue samples were obtained upon individuals' written informed consent. Normal abdominal aortic specimens free of atherosclerotic disease were collected post-mortem from 5 male individuals (mean age 62 ± 5.3 years, range from 56 to 70 years) in the Department of Forensic Sciences of University of Crete, Heraklion, Greece. Five non-aneurysmal atherosclerotic abdominal aortic tissues (all male; mean age 64 ± 5.3 years, range from 60 to 70 years) were collected post-mortem in the Department of Forensic Medicine, Democritus University of Thrace, Alexandroupolis, Greece and were used as atherosclerotic controls (ATH). All post-mortem specimens were collected within 12 h from death without any chemical treatment. The study has been approved by the Local Ethical Committee of the University Hospital of Larissa and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2. Samples preparation

Each AAA, ATH and normal aortic tissue was immediately rinsed several times in Phosphate Buffer Saline (PBS). All three types of samples (normal, ATH and AAA) were divided into two portions. In the first portion, the entire aortic wall was homogenized and was used for evaluation of mRNA expression levels. In the second portion, the adventitia layer was mechanically removed from all tissues and the remaining tissue named "intima-media" was kept as a whole for mRNA and protein quantification taking into consideration the fact that in the diseased tissues (ATH and AAA) the "intima-media" layers could not be separated due to the presence of atherosclerosis.

2.3. RNA extraction and quantification of mRNA expression

Total RNA was extracted from whole AAA, ATH and normal aortic tissues, as well as from the "intima-media" layers of all tissues. LXRα, LXRβ and ABCA1 mRNA expression levels were evaluated using real-time PCR, as previously described (Papathanasiou et al., 2012). All primers used are shown in Table 1.

2.4. Protein extraction and Western blot analysis

Proteins were extracted from the "intima-media" layers of AAA, ATH and normal tissues and western blot analysis was performed, as previously described (Papathanasiou et al., 2012). The membrane was probed with antibodies against LXRα (mouse monoclonal; 1:200 dilution; ab41902; Abcam, Cambridge, UK) and LXRβ (mouse monoclonal; 1:200 dilution; ab76983; Abcam, Cambridge, UK) and the results were normalized using anti-GAPDH mouse monoclonal antibody (1:1.000 dilution; CB1001; Calbiochem, Darmstadt, Germany). Western blot bands were quantified using the Image J programme (1.47r, Wayne Rasband National Institutes of Health, USA, http://imagej.nih.gov/ij).

2.5. Tissue preparation

For the histochemical experiments, normal ($n = 4$), AAA ($n = 3$) and ATH ($n = 3$) aortic tissue specimens (length: 0.5–1.0 cm) were

Table 1
Oligonucleotide primers used in real-time PCR assay.

Gene	Forward primer sequence	Reverse primer sequence
LXRα	CCGCCTGAAGAAACTGAA	CGAAGCCGGTCAGAAAAG
LXRβ	CGCTACAACCACGAGACAGA	GTGGAAGTCGTCCTTGCTGT
ABCA1	GGAGGCAATGGCACTGAGGAA	CCTGCCTTGTGGCTGGAGTGT
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG