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Research Paper

Elastin aging and lipid oxidation products in human aorta

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ABSTRACT

Vascular aging is associated with structural and functional modifications of the arteries, and by an increase in arterial wall thickening in the intima and the media, mainly resulting from structural modifications of the extracellular matrix (ECM) components. Among the factors known to accumulate with aging, advanced lipid peroxidation end products (ALEs) are a hallmark of oxidative stress-associated diseases such as atherosclerosis. Aldehydes generated from the peroxidation of polyunsaturated fatty acids (PUFA), (4-hydroxynonenal, malondialdehyde, acrolein), form adducts on cellular proteins, leading to a progressive protein dysfunction with consequences in the pathophysiology of vascular aging. The contribution of these aldehydes to ECM modification is not known. This study was carried out to investigate whether aldehyde-adducts are detected in the intima and media in human aorta, whether their level is increased in vascular aging, and whether elastin fibers are a target of aldehyde-adduct formation. Immunohistological and confocal immunofluorescence studies indicate that 4-HNE-histidine-adducts accumulate in an age-related manner in the intima, media and adventitia layers of human aortas, and are mainly expressed in smooth muscle cells. In contrast, even if the structure of elastin fiber is strongly altered in the aged vessels, our results show that elastin is not or very poorly modified by 4-HNE. These data indicate a complex role for lipid peroxidation and in particular for 4-HNE in elastin homeostasis, in the vascular wall remodeling during aging and atherosclerosis development.

Introduction

Aging is the largest known risk factor for most human diseases [1–4]. The aging process generates a progressive loss of biological functions and abilities to manage metabolic changes, with particular impact on the development of cardiovascular diseases [5,6], which represent the major cause of morbidity and mortality in aged people [7,8]. It is very important to understand the physiological mechanisms involved in the natural process of aging, to develop new prevention, diagnosis and treatment approaches, allowing to slow down the onset of aging consequences.

In normal arteries, the proteins of the extracellular matrix (ECM) (collagen, elastin, fibrillin, glycoproteins and proteoglycans) produced by smooth muscle cells (SMC) ensure the stability, resilience, and compliance of arteries [9]. Collagen and elastin, two major scaffolding ECM proteins provide structural integrity and elasticity to the vessels, allowing them to stretch while retaining their ability to return to their original shape when the pressure is over. Vascular aging is most of the time associated with structural and functional modifications of the arteries, even in healthy elderly, and particularly by an increase in arterial wall thickening in the intima and the media, mainly resulting from the accumulation and structural modification of ECM components and a disorganization of SMC [10,11]. Increased expression of matrix metalloproteases, (MMP-2, MMP-1, MMP-9), as well as the decreased expression of tissue inhibitors of MMPs (TIMPs) contribute to the fragmentation of elastic fibers [12,13]. Increased collagen deposition and reduction of elastin content due to elastin fiber degradation, often associated with vascular calcifications, contribute

Abbreviations: **4**-HNE, 4-hydroxynonenal; ALEs, advanced lipoxidation end products; AGEs, advanced glycation End Products; ECM, extracellular matrix; MMPs, matrix metalloproteases; SMC, smooth muscle cells

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to the development of arterial stiffening [5].

Arterial stiffness is characterized by structural and functional alterations of the intrinsic elastic properties of the arteries and an increased resistance to vessel deformation, resulting from a decrease in artery elasticity (compliance) and an increase in pulse wave velocity (pwv) [5,14], generating an increased systolic pressure, with deleterious consequences on the heart, generating cardiac hypertrophy and increased ventricular oxygen consumption. Arterial stiffening is a hallmark of vascular aging, and a major risk factor for the development of cardiovascular diseases, that can be exacerbated by diabetes, hypertension or atherosclerosis. It is a direct cause of ventricular hypertrophy, renal dysfunction and stroke, independently of the other causes of vascular aging [15]. It is an independent risk factor for cardiovascular diseases, which may predispose to atherosclerosis, and *vice-versa*. The mechanisms linking these two risk factors are not known [16] .

Arterial stiffness is aggravated by the presence of advanced glycation-end products (AGEs), formed during glucose oxidation, which slowly accumulate in normal aging, and are strongly increased in diabetes [17,18]. AGEs form cross-links on ECM proteins (on collagens; but also on elastin) [19,20], by reacting with their lysine residues, which decreases their turnover and promotes arterial stiffness and intima-media thickness [21]. These modifications are implicated in the loss of vascular elasticity.

Though electrophilic carbonyl compounds derived from lipid peroxidation play a major involvement in the modulation of life-longevity [22], and the development of oxidative stress-associated diseases such as atherosclerosis or neurodegenerative diseases [23,24], it is not known so far whether and how these agents are involved in the onset of vascular aging. Moreover, since arterial stiffness and atherosclerosis are independent cardiovascular risk factors, and are characteristic of vascular aging, it is difficult to link atherosclerosis and oxidized lipids to arterial stiffness development [8,16]. Lipid peroxidation products accumulate with aging, due to a reduction in antioxidant defenses and increased oxidative stress [25–27]). Aldehydes generated from the peroxidation of polyunsaturated fatty acids (PUFA), particularly 4-hydroxynonenal (4-HNE), malondialdehyde (MDA), acrolein, share several properties with AGEs, as they form adducts on cellular proteins, particularly on histidine and cystein (4-HNE) or lysine (acrolein), leading to a progressive protein dysfunction with consequences in the pathophysiology of vascular aging [24]. As a consequence, detoxification mechanisms, including the removal of electrophiles by glutathione transferase-catalyzed conjugation, are considered as major longevity assurance mechanisms [22]. It is not known whether aldehydes accumulate on ECM proteins (elastin or collagen), and contribute to elastin aging and arterial stiffness. However the formation of 4-HNE adducts on elastin has been reported in the dermis in human patients affected with actinic elastosis, suggesting that 4-HNE generated by UVA and UVB from solar radiations, is able to modify elastin in the skin [28].

The objectives of this study were to investigate whether aldehyde-adducts are detected in the intima, media and adventitia in human aorta, whether their level is increased as function of aging, and whether elastin fibers are a target of aldehyde-adduct formation. Immunohistological and confocal immunofluorescence studies indicate that 4-HNE-adducts accumulate in aged aortas, mainly on SMC. In contrast, even if the structure of elastin fiber is strongly altered in aged vessels, our results show that elastin is not or very poorly modified by 4-HNE. These data do not rule out a role for lipid peroxidation in the metabolism (degradation, lack of renewal) of elastin in the vascular wall.

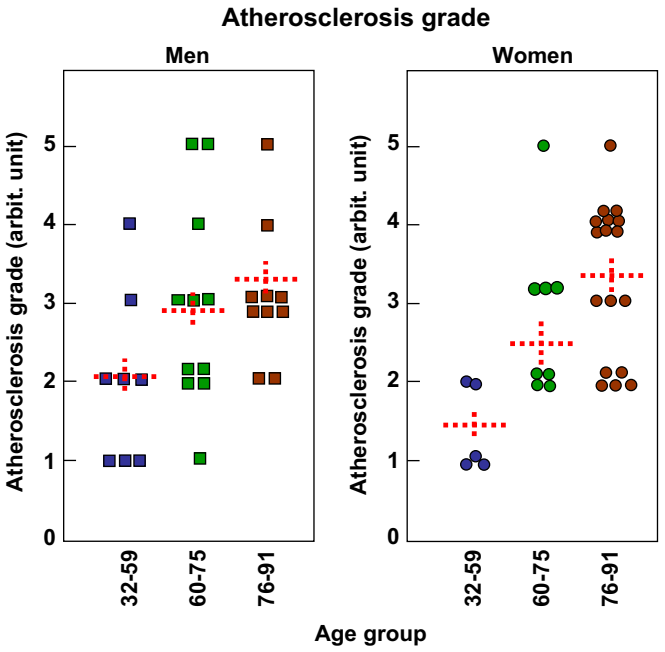

Fig. 1. Age- and sex-related atherosclerosis grade of the different subjects. 59 subjects were analyzed, 29 men and 30 women, each divided in three groups, group I (32–59 year-old), group II (60–75 year-old), group III (76–91 year-old). The results are presented as function of the atherosclerotic grade, classified from 1 to 5 as described in the Materials and Methods section. Statistical analysis was performed using one-way ANOVA followed by Holm-Sidak test, group I being compared to group II and III (SigmaStat software). Comparison group II to group III was not significant. Mean ± SEM are indicated by the doted line. * $p < 0.05$.

Material and methods

Aortas recovery

The material for the study consisted of 59 human abdominal aorta specimens removed by autopsy from patients who died at the age comprised between 32 and 91 years (average 71 year-old), out of which 30 were women and 29 men. None of the deceased persons suffered from any type of connective tissue disorder, 30 were hypertensive, 31 diabetic and 17 smokers. Specimen of aorta of the 3 year-old child was taken as a healthy control sample in the study.

The autopsies were performed within 6–10 h after death in the Department of Pathology, Clinical Hospital, Zagreb. Aorta tissue was grossly inspected, cut by consecutive sections into slabs of about 5 mm thickness and fixed in 10% formalin. Upon fixation the aorta tissues were dehydrated in graded ethanol and embedded in paraffin.

Immunohistochemistry

Formaldehyde-fixed aorta segments embedded in paraffin were cut into 10 μm sections. Monoclonal antibodies that detect 4-HNE-modified proteins were obtained from culture medium of clone "HNE g4" which was derived from a fusion of S2-Ag8 myeloma cells with B-cells of a BALBc mouse immunized with 4-HNE modified keyhole limpet hemocyanin [29]. The antibody is specific for the HNE-histidine epitope in HNE-protein conjugate. 4-HNE-lysine and 4-HNE-cysteine give 5% and 4% cross-reactivity with HNE Ig4. Immunohistochemical detection of 4-HNE adducts was done using the Envision System (Dako, Denmark), with contrast staining using hematoxylin and Masson's trichrome. Positive reaction to HNE was stained by 3, 3-diaminobenzidine tetrachloride (DAB, Dako, Denmark), and pale red positive elastin staining with