Journal of the mechanical behavior of biomedical materials 65 (2017) 881–889

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Age-related changes of wall composition and collagen cross-linking in the rat carotid artery – In relation with arterial mechanics

Kozaburo Hayashi[a,b,*], Emiko Hirayama[c]

[a] Department of Mechanical Science and Bioengineering, Graduate School of Engineering Science, Osaka University, Toyonaka, Osaka 560-0043, Japan
[b] Medical Device Innovation Center, National Cheng Kung University, Tainan 704 03, Taiwan
[c] Market Planning, Japan Shave Care, Proctor & Gamble Japan K.K., Tokyo Opera City Tower, Shinjuku, Tokyo 163-1472, Japan

ARTICLE INFO

Keywords:
Stiffness
Elastic modulus
Connective tissue
Smooth muscle cell
Collagen cross-linking
Hydrothermal isometric tension method

ABSTRACT

In association with age-related changes in arterial wall mechanics, the composition of connective tissues, the fraction and size of vascular smooth muscle cells (SMCs), and the degree of collagen cross-linking were studied with common carotid arteries harvested from 8, 16, 32, and 64 week-old Wistar rats. For histomorphometric studies, each arterial segment was fixed under in vivo operating force condition, and then sequentially sliced into thin specimens, followed by selective staining for the observation of collagen, elastin, and SMCs. Then, the fraction of each component, and the number and size of SMCs were determined with an image analyzer. The content of collagen and elastin, their ratio, and the number and the area fraction of SMCs showed no significant correlations with age, while the density and the size of SMCs were significantly smaller and larger, respectively, in 64 week-old animals than in the others. The results of collagen and elastin cannot explain the biomechanical data obtained in our previous study using the same animal model, which showed that the elastic modulus and wall stiffness were significantly larger in 64 week-old animals compared to younger ones. To investigate the reason for the discrepancy between the histological and the biomechanical results, a hydrothermal isometric tension method was applied to the analysis of the cross-linking of collagen, and we found that the amount of cross-links was significantly greater in 64 week-old arteries than in the others. This result corresponded well with the biomechanical results, and therefore the higher wall stiffness and elastic modulus in older arteries might be ascribed to their larger amount of collagen cross-links.

1. Introduction

The elasticity of arterial wall is commonly expressed by two different indices: 1) stiffness or compliance, where compliance is equivalent to the reciprocal of stiffness, and 2) such an elastic modulus as Young's modulus (ex. Hayashi and Naiki, 2009). The stiffness is a structural property of a material body, and is obtained from the force applied to the body and the deformation of the body produced by the force. On the other hand, the elastic modulus is a property inherent to a material, which is basically determined from the stress applied to the material and the strain induced in the material by the stress. Roughly speaking, therefore, the stiffness of a body is a function of the elastic modulus of the material constituting the body and the dimensions of the body.

Arterial stiffness, which is quantitatively represented, for example, by pulse-wave velocity (PWV) and stiffness parameter β (Hayashi et al., 2015), is an effective or measured stiffness, and it depends not only on such a material property as the elastic modulus of arterial wall but also on the thickness of the wall relative to its lumen diameter. As arterial stiffness or compliance represents the relation between blood pressure and diameter, its clinical measurements are very important and useful for the diagnosis and prognosis of cardiovascular diseases. Many clinical studies have shown that arterial stiffness increases with age. For example, Kawasaki et al. (1987) measured the stiffness of the abdominal aorta and the common carotid, femoral, and brachial arteries in healthy, normotensive subjects who had no symptoms of cardiovascular diseases, and observed significant correlations between stiffness parameter β and age between 6 and 81 years. In clinical cases, such cardiovascular diseases as atherosclerosis, arteriosclerosis, and hypertension mask the net effect of aging on arterial stiffness, because these diseases also change the mechanical properties and dimensions of arterial wall (ex. Hayashi et al., 1994; Hayashi and Sugimoto, 2007). However, aging itself induces both functional and morphological changes in the vasculature, which occur even in the absence of cardiovascular diseases, and therefore the mechanical properties of arterial wall may be changed by aging.

* Correspondence to: 2-3-45-602 Kamokogahara, Higashinada-ku, Kobe 658-0064, Japan.
E-mail address: kozaburohayashi@gmail.com (K. Hayashi).

http://dx.doi.org/10.1016/j.jmbbm.2016.10.007
Received 22 July 2016; Received in revised form 12 October 2016; Accepted 17 October 2016
Available online 19 October 2016
1751-6161/

On the other hand, the elastic modulus of arterial wall represents the elasticity of wall material itself, and it expresses an intrinsic property of the material. To obtain the elastic modulus, we need to measure wall thickness. However, it has been difficult to precisely determine wall thickness in vivo in human subjects until recently, although the recent great progress of ultrasonic technologies has made this possible (ex. Astrand et al., 2011). As the elastic modulus of arterial wall is inherent to the wall material, it is directly related to the structural composition. The major portion of arterial wall is the media, which is primarily responsible for arterial elasticity, stiffness, and strength. The main structural components of the media are elastin, collagen, vascular smooth muscle cells (SMCs), and ground substance in the form of a mucopolysaccharide gel. Therefore, we need to have the correct knowledge of age-related changes of their content, to link it with the data of mechanical properties, and to understand the underlying mechanisms that govern the relationship between them. However, there are not so many studies on the aging effects on the relation between the property (elastic modulus) and the structure (composition) of arterial wall; in particular, there is a shortage of systematic studies using the same animal model. Moreover, the results obtained are not always consistent.

For example, Cantini et al. (2001) studied aortic elasticity and composition in 3–30 months old normotensive rats, and found that the aortic PWV significantly increased with aging, while the elastic modulus calculated from the PWV using Moens–Korteweg equation showed no significant alterations with age. Moreover, they observed no age-related changes in the content of total protein, i.e. elastin and collagen, and the content ratio of collagen to elastin which is believed to correlate with the elastic modulus. We also observed that the elastic modulus and the stiffness of the rat common carotid artery (CCA) did not significantly change with age until maturation (8–32 weeks), although both of these increased between 32 and 64 weeks (middle age) (Sugimoto et al., 2003). However, no histological studies have been performed.

On the other hand, Bruel and Oxlund (1996) studied the stiffness, elastic modulus, and connective tissue compositions of the thoracic aorta obtained from rats aged 4.5 (young), 14 (adult), and 27 (old) months. The stiffness and elastic modulus were significantly greater but the fractions of collagen and elastin were smaller in the old group compared with the young and adult ones, which implies a negative correlation between the wall elastic modulus and the collagen fraction. From their data of collagen and elastin fractions, it is estimated that there were no differences in the content ratio of collagen to elastin among the 3 age groups. If this is true, there was no correspondence in age-related changes between the wall elasticity and the composition.

These studies investigated the effects of aging on the biomechanical properties and the histology of arterial wall. As stated above, however, there have been only a limited number of systematic studies on the property-structure relationship of arterial wall using same models and arteries, and the results and conclusions obtained are not always consistent. The mechanisms of age-related changes in the elastic properties of arteries are complex, and multi factors rather than a single mechanism including variations in the supramolecular organization of connective tissue components may induce the alterations (Spina et al., 1983). For example, age-related changes of cross-linking in connective tissues may be one of the keys for the progressive increase in the elastic modulus of arterial wall observed during aging (Tsamis et al., 2013). In fact, Fujimoto (1982) chromatographically indicated that the content of a cross-linking amino acid increased markedly with age in the human aorta, and supposed that this is formed between acidic structural proteins and collagen, and also between those and elastin. Moreover, it was reported by Aronson (2003) that advanced glycosylation end-products (AGEs), the formation of which causes cross-linking of collagen molecules, accumulate in vascular wall with aging, and that the inhibition of AGEs formation and cross-linking delays or prevents age-related arterial stiffening. Although these studies are concerned with correlations between alterations in biochemically measured crosslinking and mechanical properties, they do not directly link between these factors (Naimark et al., 1998). Contrastively, such a thermomechanical approach as the hydrothermal isometric tension (HIT) method may make possible to directly analyze the relationship between mechanical behavior and degree of collagen crosslinking. Actually, Wells et al. (1998) applied the technique to the ovine thoracic aorta, and observed the increase of collagen cross-linking during postnatal development.

As mentioned above, we have already reported the studies on age-related changes of the wall stiffness and elastic properties of the rat CCA (Sugimoto et al., 2003). In the present study, first we investigated histomorphometric changes of arterial wall during growth and aging using the same animal model as that used for the previous study, and determined the content of elastin and collagen, and the size and number of SMCs. Moreover, we applied the HIT method to the analysis of collagen cross-linking. Thus obtained histological and microstructural results are discussed in relation with the previous biomechanical results. Our hypothesis is that collagen cross-linking is responsible for increase in the elastic modulus of arterial wall with age.

2. Materials and methods

We used the same animal model as that used for our previous studies on the effects of aging on the biomechanical properties of arterial wall (Sugimoto et al., 2003). All animal experiments and procedures were carried out within the Animal Welfare Regulations and Guidelines for Animal Experiments, Graduate School of Engineering Science, Osaka University, by the approval of the Committee for Animal Experimentation in the School.

2.1. Measurement of wall composition

Twenty four male Wistar rats aged 8 weeks (CLEA, Japan, Tokyo) were used for the present histomorphometric studies. Six rats were euthanized at this age; the remaining 18 animals were kept in cages for the following 8, 24, and 56 weeks (6 animals for each), being given a standard rat chow and tap water ad libitum. Thus, we prepared 4 age groups (8, 16, 32, and 64 weeks). The life span of the rat is approximately 2 years. Assuming that human life is 80 years, these 4 ages in the rat are equivalent to 6, 12, 24, and 48 years in human, respectively, which correspond to growing (6 and 12 years in human, or 8 and 16 weeks in rats), matured (24 years or 32 weeks), and middle (48 years or 64 weeks) ages.

Immediately before euthanization, systolic blood pressure was measured with tail plethysmography. Then, the left CCA was carefully exposed under anesthesia with pentobarbital sodium, and marked with gentian violet dots on the surface along the axial direction at the interval of 2.5 mm. After the measurement of the external diameter with a caliper, an approximately 10 mm long arterial segment was excised, and then the rat was euthanized by a gradual and continuous administration of pentobarbital sodium. Following the measurements of the external diameter and the distance between the above-mentioned gentian violet markers with the caliper under no load condition, each arterial segment was attached to the pressure-diameter tester used for our previous biomechanical studies (Sugimoto et al., 2003). Then, the axial strain determined referring to the above-mentioned markers and the internal pressure that is similar to the in vivo systolic blood pressure measured prior to euthanization were applied to the segment. After the outer diameter was measured with a video dimension analyzer installed in the tester, the segment was fixed with buffered 10% formalin under the in vivo load condition, and then embedded in paraffin.

We used the procedures and methods for the preparation of histological specimens and histomorphometric analysis similar to those reported elsewhere (Hayashi and Shimizu, 2016). Briefly, 4 ring