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Short communication

Composition of connective tissues and morphometry of vascular smooth muscle in arterial wall of DOCA-salt hypertensive rats – In relation with arterial remodeling

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ABSTRACT

Hypertension (HT) was induced in Wistar rats aged 16 and 48 weeks by a deoxycortico-sterone acetate (DOCA)-salt procedure. Common carotid arteries were resected 16 weeks after, and their histological specimens were selectively stained for observations of collagen, elastin, and vascular smooth muscle (VSM) cells. Then, the fractions of collagen and elastin and their radial distributions, and the size and number of VSM cells were determined with an image analyzer. These results were compared with the results from age-matched, non-treated, normotensive (NT) animals and also with those from our previous biomechanical studies.

In both age groups, there were no significant differences in the fractions of collagen and elastin, and the ratio of collagen to elastin content between HT and NT arteries. These results correspond well with our previous biomechanical results, which showed no significant difference in wall elasticity between HT and NT vessels. Moreover, in the innermost layer out of 4 layers bordered with thick elastic lamellae, the fraction of collagen was significantly greater in HT arteries than in NT ones, which is attributable to HT-related stress concentration in the layer. VSM cells were significantly hypertrophied and their content was increased by HT, although their total number in the media remained unchanged. The increased size and content of cells correspond to the enhancement of vascular tone and contractility in HT arteries.

1. Introduction

Structure and property of living organs, tissues and cells change in response to mechanical stress acting on them. This phenomenon is called the functional adaptation and remodeling of living systems (Hayashi et al., 1996). For example, the chronic elevation of blood pressure, i.e. hypertension (HT), changes arterial wall dimensions and properties (Hayashi and Naiki, 2009). One of the specific biomechanical phenomena occurring in response to HT is arterial wall hypertrophy, which maintains wall hoop stress and stiffness/elasticity at physiologically normal level (Matsumoto and Hayashi, 1994; Laurent, 1995). Moreover, vascular tone and contractility are enhanced by HT (Fridez et al., 2001). To understand the underlying structure-function relationship of such arterial wall responses to HT, we performed histological studies on arterial specimens obtained from DOCA (deoxycortico-sterone acetate)-salt HT rats, and quantified the composition of collagen and elastin, their distributions across the media, and the size, number and content of VSM cells.

2. Methods

2.1. Animals and induction of hypertension

We used male Wistar rats aged 16 weeks (young group) and 48 weeks (middle-aged group) at the beginning of experiments; 6 animals were used for each group. We applied a DOCA-salt method to these animals for the induction of HT; its detailed procedures have been reported previously (Hayashi and Sugimoto, 2007). In DOCA-salt animals, Na^+ and water are absorbed in the kidney, which increases circulating blood volume and results in HT. This process is analogous to that in human essential HT (Zuckerman and Yin, 1989). Moreover, the mechanism for HT in this model is not directly associated with arterial wall itself. Therefore, DOCA-salt model is suitable for the study of arterial remodeling observed in human essential HT (Watts et al., 2007). To obtain control data, we used age-matched, normotensive (NT) animals (6 animals for each age group).

These animal procedures were carried out within the Animal Welfare Regulations and Guidelines for Animal Experiments, Graduate School of Engineering Science, Osaka University.

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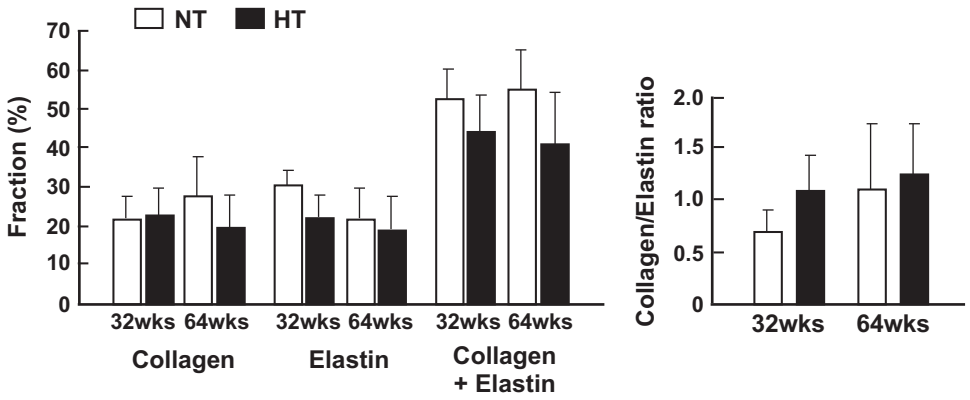

Fig. 1. Effects of hypertension on the fractions of collagen, elastin, and the total of collagen and elastin, and the ratio of collagen to elastin content. NT=Normotension; HT=Hypertension. Each data are expressed as mean+standard deviation.

2.2. Arterial specimens and histological analysis

Immediately before euthanization, systolic blood pressure was measured with tail plethysmography. The left common carotid artery was exposed under anesthesia with pentobarbital sodium, and marked with gentian violet on the surface at the interval of 2.5 mm along the axial direction. After a 10 mm long arterial segment was excised, each rat was euthanized by a gradual and continuous administration of pentobarbital sodium. Each segment was attached to an apparatus for pressure–diameter tests (Sugimoto et al., 2003), and fixed with 10% formalin under the application of the load similar to in vivo blood pressure and the axial extension determined referring to the above-mentioned intervals of gentian violet markers. Four ring specimens having the thickness of 4 μm were cut out from each segment. These thin specimens were then individually and selectively stained with Picro-Sirius red for the observation of collagen (Montorzi et al., 2004), Weigert's resorcin fuchsin for elastin (Miki et al., 2007), Picro-Sirius red after Weigert's resorcin fuchsin for the double staining of collagen and elastin, and Ehrlich's haematoxylin for VSM cells (Montorzi et al., 2004), respectively.

Histologic images at 4 locations arbitrarily selected from each stained specimen were photographed with a color digital camera (DP12, Olympus, Tokyo), fed into computers, and analyzed with software for image analysis (SigmaScan Pro5, Informer Tech., San Jose, CA). Specifically, the distributions of "Intensity (0–100)" and "Hue (0–255)" were obtained from each image, and the mean value of the maximum and the minimum of each was used as the threshold for each structural component. The quality of thus obtained binary images was confirmed by visual comparison with their original images (Ng et al., 2011). Assuming homogeneous staining, the percentage of the area corresponding to each component in each media (area fraction) was calculated and evaluated. To determine the radial distributions of collagen and elastin, each media was zoned into 4 layers from the innermost layer to the outermost one (L1–L4); each layer was clearly bordered by relatively thick elastic lamellae. The content of each tissue in each layer was also expressed by its area fraction in each layer. On the other hand, the total number of VSM cells in the media was obtained from the image of each haematoxylin-stained specimen, and the number of cells per unit medial area, i.e. cell density, was calculated. The fraction of VSM was determined from the area that was not double-stained with Picro-Sirius red and Weigert's resorcin fuchsin, assuming that VSM existed in the non-stained area. Then, the area of a single VSM cell was calculated from dividing this area fraction by the above stated cell density. The mean value of the data obtained from 4 locations in each selectively stained specimen was used as the data for the specimen.

2.3. Statistical analysis

Means and standard deviations were calculated for each data set. Statistical comparisons between two groups were performed with paired student's t-tests. Differences were considered to be significant, if the probability level (p) is below 0.05 (5%).

3. Results

Final systolic blood pressures in DOCA-salt rats (164.0 ± 18.0 and 164.8 ± 11.2 mm Hg in young and middle-aged groups, respectively) were significantly higher than those in age-matched, non-treated animals (124.9 ± 7.2 and 129.2 ± 7.2 mm Hg, respectively).

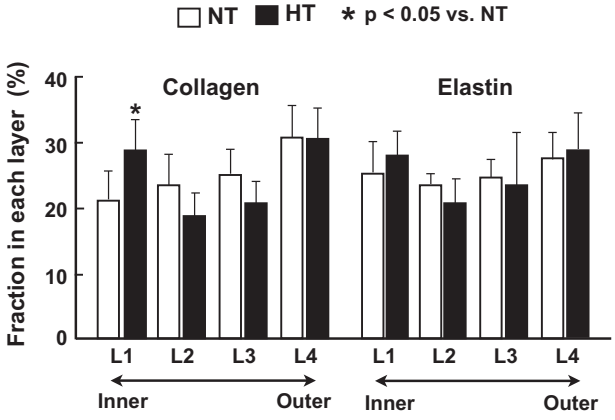

Fig. 2. Distributions of the fraction of collagen and elastin through the medial thickness in 32-week old animals. NT=Normotension; HT=Hypertension. Each data are expressed as mean+standard deviation.

No significant differences were observed in the fractions of collagen and elastin, and the fraction of total content of collagen and elastin between HT and NT arteries (Fig. 1). HT-related changes in the ratio of collagen content to elastin content, that is commonly regarded as an index of arterial stiffness, were also insignificant. As stated above, each media was zoned into 4 layers bordered with thick elastic lamellae. HT significantly increased the fraction of collagen in the innermost layer compared with NT, although this was not the case for elastin (Fig. 2). The results similar to Figs. 1 and 2 were also obtained from middle-aged arteries (data not shown).

Examples of the photographs of histological specimens stained for the observation of VSM cells are demonstrated in Fig. 3. There were no significant differences in the number of VSM cells in the media between HT and NT arteries in both age groups (Fig. 4). As the medial thickness was significantly increased by HT (data not shown; see Hayashi and Sugimoto, 2007), the relative number (density) of medial VSM cells, namely cell number in unit medial area, was significantly smaller in HT arteries in both groups (data not shown). However, HT significantly increased the area of cell and the fraction of VSM in the media, although the change of the fraction was not significant in the young group. In other words, HT hypertrophied VSM cells but did not proliferate them.