Effects of Atherosclerosis on Mean and Daily Variation of Arterial Pressure in Conscious WHHL Rabbits

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ABSTRACT. Effects of atherosclerosis on the mean value and daily variation of arterial pressure were studied in 12 Watanabe-heritable hyperlipidemic (WHHL) rabbits aged 12 to 35 months and 25 normal Japanese white rabbits aged 6 to 30 months. A pressure catheter was inserted through the left subclavian artery under pentobarbital anesthesia. A few days after the catheterization, the mean arterial pressure (MAP) of the rabbits, which were active and in a good state of appetite, was recorded by an analogue-to-digital converter every second for about 6 hrs and stored in a computer. The mean (M) and standard deviation (SD) in the WHHL rabbit, calculated from each successive MAP record, ranged widely from 85.8 to 131.4 mmHg and 5.6 to 12.6 mmHg, respectively. There was no significant correlation between M and SD in the WHHL rabbit. M and variance (V) of MAP in the WHHL rabbit were significantly higher than those in the normal rabbit. M did not show any significant change with increasing ages, whereas SD increased significantly with aging in the WHHL rabbit. Concentrations of serum total cholesterol and triglyceride in the WHHL rabbit were 475 and 328 mg/dl, which were about nine and seven times as high as those in the normal rabbit, respectively. Macroscopic and histopathological examinations of the aorta revealed development and spread of sclerotic lesions with aging in the WHHL rabbit. We can conclude that development of atherosclerosis with aging in the WHHL rabbit causes malfunction of the baroreceptors, which contributes to hypertension and liability of arterial pressure.—KEY WORDS: aging, atherosclerosis, hypertension, rabbit, WHHL rabbit.


The Watanabe-heritable hyperlipidemic (WHHL) rabbit is the strain carrying spontaneous hypercholesterolemia produced by inbreeding from a mutant discovered in 1973 [21, 25, 27, 28]. Since the WHHL rabbit is deficient in low density lipoprotein receptors, hypercholesterolemia develops from birth [7, 20, 24, 25]. Atherosclerosis develops from the ascending aorta, carotid arteries and coronary arteries with aging [27–29]. The WHHL rabbit is an ideal animal model for studying cardiovascular hemodynamics as well as biochemical and pathological characteristics of atherosclerosis and hypercholesterolemia. It is expected that atherosclerosis alters vascular properties and characteristics of baroreflex control of arterial pressure (AP). Baroreceptor activity is closely related to vasoelastic properties [2–5]. Baroreflex function is therefore susceptible to atherosclerotic lesions, which could be responsible for elevation of AP and liability of AP.

AP is one of the important variables in the circulatory system. In conscious animals, AP fluctuates at all times [8, 16–19]. AP could be changed by behavioral, environmental and emotional conditions. Successive or repetitive AP recording in an individual animal could reveal some cardiovascular properties peculiar to the individual animal, which contributes to analysis of cardiovascular hemodynamics under normal or pathological conditions. Variance (V) or standard deviation (SD) of successive mean arterial pressure (MAP) record has often been used as an index of stability of AP or baroreflex function [8, 16–18].

In the present study, we investigated effects of atherosclerosis on the mean value and variability of AP in the conscious WHHL rabbit. MAP was recorded continuously for about 6 hrs in the daytime. The mean value and variability of MAP, concentrations of serum total cholesterol (S-Ch) and triglyceride (S-TG), and histopathological findings of the aorta in the WHHL rabbit were compared with those in the normal rabbit.

MATERIALS AND METHODS

Animals: Twelve WHHL rabbits (age: 12 to 35 months, weight: 3.0±0.1 kg (mean ± SE)) and 25 normal Japanese white rabbits (age: 6 to 30 months, weight: 3.8±0.1 kg (mean ± SE)) were used in the present study. Twenty-five normal rabbits, selected
at random from a large colony, were divided into 5 age-groups, i.e., 6, 12, 18, 24 and 30 month-old groups. Each rabbit was kept individually in a metal cage in the Experimental Animal Laboratory at room temperature of 20-24°C and supplied with commercial rabbit diet (Type ORC-4, Oriental Yeast) at 100 g a day.

**Experimental procedure:** The rabbits were anesthetized by an intravenous administration of pentobarbital sodium in a dose of 20.0 mg/kg. One tip of a large bore catheter (1.2 mm, I.D.) was implanted into the aortic arch through the left subclavian artery and the other tip was connected to a stopcock placed on the rabbit’s back. The catheter was filled with heparin (1000 U/kg). A few days after the catheterization, each animal was transferred from the previous cage in the Experimental Animal Laboratory to a metal cage (30(W)×45(D)×35(H) cm), where free access to food and water was provided. They were acclimatized to the experimental environment for about 2 hrs and were confirmed to be active and in a good state of appetite before MAP recording. AP was monitored with a catheter-transducer system (MPU-0.5A, Nihon Kohden). MAP derived from AP by a low-pass filter with a time constant of 2 sec was recorded on a strip-chart and fed into a computer (JEC-7E, Nihon Denshi) through an analogue-to-digital converter every second for about 6 hrs. During MAP measurement, one investigator watched over the rabbit. External disturbances to the rabbit, i.e., noise, flash etc., were prevented. MAP recording from each rabbit was performed between 10 a.m. and 4 p.m. on one to three separate days in an air-conditioned room at the temperature of 19-22°C and humidity of 44-52%.

**Data analysis:** Each successive MAP record for 6 hrs was analysed for the mean (M), variance (V) and standard deviation (SD). M and SD were plotted against age. A correlation diagram between M and SD of MAP was obtained. When MAP recording was repeated twice or three times on different days in the same rabbit, M, V and SD values were averaged from the separate measurements.

**Serum lipid assay:** The blood was sampled in the morning 1-2 days before the MAP recording. S-Ch was determined by the Zurkowski method with an automatic blood analyser (RABA, Kyoto Daiichi Kagaku). S-TG was measured with Wako TG-test reagent (Wako Pure Chemical Industries).

**Pathology:** After the MAP recording, the rabbits were subjected to autopsy. The aorta was fixed in 10% Formalin-saline solution and embedded in paraffin. The histological sections of the aorta were stained with Elastica-Van Gieson.

**RESULTS**

Table 1 shows averaged values of M, V, S-Ch and S-TG calculated for 12 WHHL rabbits compared with those for 25 normal rabbits. The values of M and V were significantly higher in the WHHL rabbit than in the normal rabbit (p<0.05). The values of S-Ch and S-TG in the WHHL rabbit were approximately nine and seven times as high as those in the normal rabbit, respectively. There were significant differences in these values between the normal and WHHL rabbits (p<0.001).

Figure 1 is a correlation diagram between M and SD. In the WHHL rabbit, SD scattered widely from 5.6 to 12.6 mmHg and tended to rise with elevation of M, but not significantly (p>0.05). In the normal rabbit, SD decreased significantly with an elevation of M around 100 mmHg (p<0.01). Figures 2 and 3 showed relations of M and SD to age, respectively. There was no significant correlation between M and

<table>
<thead>
<tr>
<th>M(mmHg)</th>
<th>V</th>
<th>S-Ch (mg/dl)</th>
<th>S-TG(mg/dl)</th>
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<tbody>
<tr>
<td><strong>Normal</strong> (n=25)</td>
<td>94.7±1.1</td>
<td>22.8±1.9</td>
<td>51.2±3.0</td>
</tr>
<tr>
<td><strong>WHHL</strong> (n=12)</td>
<td>111.9±3.1</td>
<td>85.5±9.3</td>
<td>475.1±36.5</td>
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p <0.05<sup>a</sup>  <0.05<sup>b</sup>  <0.001<sup>a</sup>  <0.001<sup>a</sup>

<sup>a</sup> Student's t-test, <sup>b</sup> Fisher's F-test.
Fig. 1. Correlation diagram between the mean value and standard deviation of MAP for 6 hrs in the normal and WHHL rabbits. Open circle: normal rabbit, closed circle: WHHL rabbit. SD decreased significantly with an increase in M to the normotensive range in the normal rabbit (r=−0.74, p<0.01). There is no significant correlation between M and SD in the WHHL rabbit (r=0.46, p>0.05).

Fig. 3. Effects of aging on variability of arterial pressure in the normal and WHHL rabbits. Open circle: Normal rabbit, closed circle: WHHL rabbit. Each data point in the normal rabbit represents the mean and standard error of 5 observations. SD did not significantly correlate with age in the normal rabbit (r=−0.17, p>0.05), whereas it increased significantly with aging in the WHHL rabbit (r=0.61, p<0.01).

Fig. 2. Effects of aging on the mean arterial pressure in the normal and WHHL rabbits. Open circle: normal rabbit, closed circle: WHHL rabbit. Each data point in the normal rabbit represents the mean and standard error of 5 observations. There is no significant correlation between MAP and age in both normal and WHHL rabbits (r=0.38 and r=0.11, respectively, p>0.05).

Fig. 4. Histopathological findings of the thoracic aorta (proximal region) in the WHHL rabbit. A: Development of fatty streaks in the intima in the 12 months old WHHL rabbit. Elastica-Van Gieson × 70. B: Atheroma with abundance of cholesterol clefts in the intima in the 18 months old WHHL rabbit. Elastica-Van Gieson × 50. C: Formation of fibrous plaque in the intima in the 24 months old WHHL rabbit. Elastica-Van Gieson × 50.
age in both normal and WHHL rabbits (p<0.05). SD increased significantly with aging in the WHHL rabbit (p<0.01), while it did not show any significant change with aging in the normal rabbit (p>0.05).

Macroscopic examination of the arteries of the WHHL rabbit revealed growth of plaque and spread of sclerotic lesions from the ascending aorta to the descending aorta, coronary arteries and common carotid arteries with aging. Figure 4 shows histopathological changes in the thoracic aorta (proximal region) in the WHHL rabbit. At the age of 12 months old, atherosclerosis was relatively in an early stage. Development of fatty streaks was observed in the intima (Fig. 4A). The intimal thickening was progressively advanced to the age around 18 months old due to development of atheroma with abundance of cholesterol clefts. The elastic lamella was split and duplicated (Fig. 4B). At the age of 24 months old, formation of fibrous plaque was observed in the intima. The thickness of the intima was not progressive in comparison with that of the 18 month-old rabbit (Fig. 4C). In the normal rabbit as control, however, there was no remarkable alteration of the aorta at any ages.

DISCUSSION

The mean value of MAP was significantly higher in the WHHL rabbit than in the normal rabbit. It did not show any significant change with aging in both WHHL and normal rabbits. These indicate that MAP was not affected by aging over the age range investigated in the present study. One possible reason for the hypertension seen in the WHHL rabbit is morphological or physiological disorders in the vascular wall or AP control system caused by atherosclerosis. Randall et al. [23] showed that the MAP was not significantly changed in spite of a decrease in vascular compliance. The distensibility of the vascular wall, however, decreased in the rabbit given a high cholesterol diet [6] and in the WHHL rabbit [12]. Angell-James [3, 4] reported that in the rabbit with experimental atherosclerosis, i.e., dietary induced atherosclerosis or vitamin D sclerosis, a decrease in baroreceptor activity was closely related to a reduction in distensibility of the arterial walls due to histological lesions in the blood vessels including baroreceptor regions. Although hormonal and humoral effects on hypertension were not examined in the present study, it could be considered that high blood pressure in the WHHL rabbit was partly caused by a decrease in sensitivity of the carotid sinus and aortic arch baroreceptors due to growth of plaque and spread of sclerotic lesions to the baroreceptor regions with aging. In addition, abnormally high concentrations of S-Ch and S-TG may induce an increase in viscosity of the blood, leading to the increase in the MAP level.

The variability of MAP was significantly larger in the WHHL rabbit than in the normal rabbit. SD did not correlate significantly with M in the WHHL rabbit, while SD decreased significantly with a change in MAP from the subnormal to normal level in the normal rabbit. These facts seem to be related to baroreceptor function. SD has been considered as one of the useful indexes to represent the variability of MAP [8, 16–18]. An increase in SD reflects a decrease in baroreflex control of AP. This is in good accordance with a decrease in the overall open-loop gain of the baroreflex system after sectioning the carotid sinus, aortic arch and cardiopulmonary depressor nerves [15]. The baroreceptors respond to stretching of the vessel wall in which they lie. They exert a more powerful buffering action on the circulatory system in the normotensive range and minimize variation of AP [1, 10, 11, 22]. Baroreceptor activity is susceptible to any alteration in the structure of the arterial wall [2–5]. A reduction in sensitivity of the baroreceptors could lead to an increase in lability of AP, since a change in AP produces a smaller change in individual baroreceptor activity. In the WHHL rabbit, an increase in variability of MAP over the pressure range observed in this experiment could be explained by a reduction in transducer sensitivity of the baroreceptors and distensibility of the arterial wall due to development and spread of atherosclerosis.

Hosomi et al. [14] previously demonstrated that the overall open-loop gain of the baroreflex system in the anesthetized WHHL rabbit was progressively diminished with aging, while it was not changed in the normal rabbit [13]. This means a progressive decrease in capacity of baroreflex control of AP with aging. In the present study, the increase in variability of MAP with aging in the WHHL rabbit could be caused by a decrease in responsiveness of the baroreceptors and a decrease in vascular compliance due to growth of plaque and spread of sclerotic lesions with aging.

Anesthetics could affect cardiovascular function in animals [9, 26, 30]. In the elderly and those with cardiovascular disease, anesthetics and vasoactive
drugs may exert a variety of extraordinary pharmacological and pathological actions on the cardiovascular system. Prior to the analysis of cardiovascular responses to a given stimuli such as drug, movement, hemorrhage in the conscious animal, it is important to obtain daily values of blood pressure and its variation particular to the individual animal under normal or pathophysiological conditions. This procedure could be one of the useful aids for the later analysis of cardiovascular hemodynamics and control ability of the baroreflex system.

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REFERENCES