Relationship between Permeability and Endothelial Cell Morphology in Rat Aortae*

Noriyuki KATAOKA**, Yoshinobu OGAWA***, Kouichiro TAKEDA**** and Masaaki SATO*****

The permeability and endothelial cell morphology in rat aortae were measured. Using FITC-albumin as a tracer, the permeability was measured on the lateral sides of ascending aorta (immediately before the brachiocephalic artery, A), aortic arch (immediately after the left subclavian artery, C) and thoracic aorta (E), and on their medial sides, B, D and F, respectively. By silver staining endothelial cell morphology was observed in the same portions where the permeability was measured. The permeabilities in A and B of the ascending aorta were higher than those of the aortic arch and thoracic aorta. The cell morphology in A and B was almost the same as in other portions. However, the deviation values of the cell orientation to the axis of the blood vessel in A and B were higher than those in other portions. These results indicate that a complex blood flow in the ascending aorta affected the orientation of endothelial cells and might induce the changes in the permeability through endothelial cells.

Key Words: Biological Engineering, Permeability, Endothelial Cell, Blood Flow, Cell Morphology, Atherosclerosis, Secondary Flow

1. Introduction

It has been known that atherosclerotic lesions are prone to localize at arterial branch and curved sites(1). Those regions would have experienced localized com-

** Graduate School of Mechanical Engineering, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai, Miyagi 980-8579, Japan (Present address: Kawasaki College of Allied Health Professions, Matsushima 316, Kurashiki, Okayama 701-0194, Japan). E-mail: kataoka@me.kawasaki-m.ac.jp
*** Graduate School of Mechanical Engineering, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai, Miyagi 980-8579, Japan (Present address: Kubota Co., Ltd.)
**** Graduate School of Mechanical Engineering, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai, Miyagi 980-8579, Japan (Present address: Tokyo Gas Co., Ltd.)
***** Graduate School of Mechanical Engineering, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai, Miyagi 980-8579, Japan

plex blood flows including elevated or reduced wall shear stress, boundary layer separation and secondary flows(2)(3). In in vivo conditions, it is well known that endothelial cell morphology reflects the local blood flow pattern(4)(5)(6). Cultured endothelial cells change their shape and orientation when exposed to shear stress(6). Some in vitro studies have focused on the flow conditions, such as turbulent(7) and pulsatile flows(8). Turbulent shear stress stimulated endothelial DNA synthesis substantially without cell alignment. In the oscillatory flow condition (mean flow = 0), endothelial cells remained polygonal shape as in static culture, and no developed stress fibers as bundles of actin filaments were observed.

One of the important endothelial cell functions, i.e., endothelial permeability, also reflects the blood flow conditions and is related to endothelial cell shape(9). The cell shape has been reported to be polygonal in the regions of high permeability in in vivo studies. Since the endothelial cell layer is constantly exposed to shear stress induced by blood flow, the complex blood flow pattern at arterial branch and curved sites are considered to affect directly cell morphology and functions.

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In the present study, we quantitatively measured the FITC-labeled albumin permeability and endothelial cell morphology of the Wistar rat aorta, and the relationship between blood flow pattern and permeability was discussed.

2. Materials and Methods

2.1 Permeability measurement in rat aorta

Male Wistar rats (body weight = 365 ± 38 g, mean ± SD, n = 6) were anesthetized with Nembutal (Abot Laboratories; 50 mg/kg B.W.) and placed supine on an operating table. Then polyethylene tubes (3 Fr) filled with heparin sodium (Novo Nordisk A/S; 100 units/ml) were inserted to the common carotid artery and internal jugular vein. After blood sampling from common carotid artery for autofluorescence measurement, FITC (Fluorescein Isothiocyanate) labeled albumin (Sigma) dissolved in a Hanks balanced salt solution (Gibco; 45 mg/ml) was injected via the internal jugular vein. After the blood was sampled for measurement of the tracer concentration in the blood, the abdomen was opened and the abdominal aorta was cut for the blood draining, when perfusion was terminated. Perfusion time was set 10 - 30 min. The breast was opened, and the ascending and descending aortic segments were excised. The surrounding tissues were then removed in 10% formalin (Kanto Chemical), and aortic segments immersed in a cryoembedding agent (Tissue-Tek O.C.T. Compound, Miles). The frozen aortic segments were sliced to 10 μm-thick ring specimens by a cryostat (Histostat STAT Microtome 855, American Optical). The fluorescence image of the aortic ring specimen was observed with a microscope equipped with epifluorescence optics (IMT2, Olympus) and transferred to a personal computer (Power Macintosh 7100/80 AV, Apple Computer) through an SIT camera (C2400, Hamamatsu Photonics). The fluorescence intensity was measured with NIH Image (version 1.60, written by Wayne Rasband at the U.S. National Institutes of Health).

The average tracer concentration in the vessel wall, $\bar{C}_r$ [mg/ml], was determined by subtracting the autofluorescence intensity of the vessel wall from the fluorescence intensity of the perfused aortic segments. A calibration of FITC-albumin was constructed using a solution containing of FITC-labeled albumin. The tracer concentration in the blood, $C_w$, was determined from the blood sampled at the end of perfusion by the same method for intensity measurement that in the vessel wall. The permeability of FITC-albumin was estimated as follows. The macromolecular transport from the blood to the vessel wall was described as the Fick's first law.

$$J = P \cdot \Delta C$$  \hspace{2cm} (1)

where $J$ is flux [mg/cm² s], $P$ is permeability [cm/s] and $\Delta C$ is the concentration difference between the blood and the vessel wall [mg/ml]. Flux $J$ is described as follows:

$$J = \frac{\bar{C}_r \cdot V}{A \cdot t} = \frac{\bar{C}_r \cdot r}{t}$$  \hspace{2cm} (2)

where $t$ is perfusion time [sec], $V$ is volume of vessel wall [cm³], $A$ is surface area of endothelial cell layer [cm²] and $r$ is the thickness of vessel wall [cm]. The initial tracer concentration in the vessel wall is zero, and the tracer concentration on the luminal surface is considered to be very low compared with that in the blood. Therefore, the driving force, i.e., concentration gradient is considered to be the same as the tracer concentration in the blood, $C_w$, throughout the perfusion time.

$$\Delta C = C_w$$  \hspace{2cm} (3)

From Eq.(1)-(3), $P$ is described as follows:

$$P = \frac{\bar{C}_r \cdot r \cdot \frac{1}{t}}{C_w}$$  \hspace{2cm} (4)

The FITC-albumin permeability was measured quantitatively on the lateral and medial sides of ascending aorta (immediately before the brachiocephalic artery; A-B), aortic arch (immediately after the left subclavian artery; C-D) and thoracic aorta (25 cm downstream from the branching of the left subclavian artery; E-F) as shown in Fig. 1. In each position, 5 ring specimens were sampled, and permeability was then estimated.
2.2 Endothelial cell morphology measurement in rat aorta

For measurements endothelial cell morphology, male Wistar rats (body weight = 340±24 g, mean ± SD, n=4) were anesthetized with Nembutal (Abot Laboratories; 50 mg/kg B.W.) and placed supine on an operating table. Rats were heparinized (500 units) and injected with sodium nitroprusside (Wako Pure Chemical) in 75 mg/4 ml saline solution via the internal jugular vein. A cannula (4 Fr) was introduced to the abdominal aorta in the opposite direction to blood flow, and 5% glucose solution was perfused for washing out the luminal surface of the vessel with draining from the renal vein. For staining the boundary of the endothelial cells, 0.25% silver nitrate solution (Wako Pure Chemical) prefiltered with a 0.22-μm pore filter (Millex-GS, Millipore) was perfused for 2 min, and 10% formalin was then perfused at 100 mmHg for 15 min. After excision, the aorta was again immersed in a 0.25% silver nitrate solution for 5 min. All perfused solutions were kept at 37°C. The luminal surface of each aortic segment where the measurement of permeability was performed were photographed with a microscope (FS60, Mitsutoyo), and the images were transferred to a personal computer (Macintosh Quadra 950, Apple Computer) through an image scanner (HP Scan Jet II cx, Hewlett Packard). The morphological analysis was performed using the NIH Image 1.60. The geometrical parameters measured were the angle of cell orientation, defined as the deviation of the longest diagonal of the cell from the axial direction of the vessel (Fig. 2), and shape index, defined as $4\pi A/P^2$ ($A$ : cell area, $P$ : cell perimeter). In this study, additional morphological analysis was performed in the entire circumference of the ascending aorta immediately before the branching of the brachiocephalic artery. The lateral position was defined as 0 degree in view from the heart and 20 cells were sampled in the every 10 degrees clockwise.

Animal experiments were performed according to "the Guideline for Animal Experiments at Tohoku University".

Student’s t-test was performed to determine the significance of differences in comparison of the permeability and shape index mean values in each portion. F-test was performed in comparison of S.D. values of the cell orientation. All data are expressed as mean ± S.D.

3. Results

A typical tracer profile in the perfused vessel wall is shown in Fig. 3. In the fluorescence image, a high fluorescent region caused by transport from the vasa vasorum was observed in the adventitial side (the right side of photomicrograph). In this study, we focused on the mass transport from the luminal side of aorta, and thus neglected the fluorescent intensity observed in the adventitial side.

Measured FITC-albumin permeabilities in the each aortic segment are shown in Fig. 4. The permeability was measured on the lateral side of the ascending aorta immediately before the branching of the brachiocephalic artery (location A) was significantly higher than those on the aortic arch (locations, C and...
Fig. 4 Permeabilities measured in the rat aorta. A: \( P = 8.94 \pm 0.36 \times 10^{-7} \) [cm/sec], B: \( P = 6.84 \pm 0.65 \times 10^{-7} \), C: \( P = 1.84 \pm 0.83 \times 10^{-7} \), D: \( P = 3.52 \pm 1.33 \times 10^{-7} \), E: \( P = 2.91 \pm 1.11 \times 10^{-7} \), F: \( P = 2.53 \pm 1.11 \times 10^{-7} \). mean ± S.D., *p < 0.05

D) and the thoracic aorta (locations, E and F). There was no statistical significant difference; however, the permeability on the medial side of the ascending aorta (location B) was higher than those at locations, C, D, E, and F. In the medial side of the aortic arch (location D), immediately after the branching of the left subclavian artery, permeability was higher than other three locations, C, E, and F.

Typical photomicrographs of silver-stained endothelial cells in location A, high permeability region, and E, low permeability region, are shown in Fig. 5. In both locations, elongated endothelial cells were observed.

Measured shape index values in each location of aorta are shown in Fig. 6. There was no statistical significant difference among them; however, shape index values in locations B and D were slightly larger than the other location.

Distributions of angle of cell orientation are summarized for each aortic segment in Fig. 7. After the branching of the left subclavian artery, endothelial cells were well aligned with the axis of blood vessel. However, the deviation values of the cell orientation to the axis of the blood vessel in A and B were larger than those of other locations. The values of the angle of cell orientation for location, A and B, are 28.6 ± 12.6 and 34.1 ± 18.8, respectively. These values were obtained from the en face preparation of the aortic segments. Observation in the cylindrical segments would give us cell orientation in the same direction.

In addition, we observed the endothelial cell morphology for the entire circumference of the ascending aorta immediately before the branching of the brachiocephalic artery including locations, A and

Fig. 5 Typical photomicrograph of silver-staining endothelial cells. (a) the lateral side of ascending aorta (A), (b) the lateral side of thoracic aorta (E)

Fig. 6 Shape index values in the rat aorta. A: \( SI = 0.44 \pm 0.12 \), B: \( SI = 0.56 \pm 0.12 \), C: \( SI = 0.39 \pm 0.09 \), D: \( SI = 0.55 \pm 0.11 \), E: \( SI = 0.47 \pm 0.11 \), F: \( SI = 0.48 \pm 0.10 \). mean ± S.D.

B. Shape index and angle of cell orientation are shown in Fig. 8 and Fig. 9, respectively. The shape index values were 0.4 to 0.5 except for the positions of
30 degree and 170 degree. In the positions of 30 degree and 170 degree (Fig. 8), endothelial cells were relatively rounded than the other positions. The values of angle of cells orientation were 0 degree around the positions of 170 degree and 320 degree. In the ascending aorta, cells were showing symmetrical orientation as bounded in 170 degree and 320 degree and a pair of spiral configuration.

4. Discussion

In the present study, we measured the permeability in the curved and branching aortic segments where the blood flow patterns are complex, and in straight segments where the blood flow pattern is relatively simple. The permeabilities in the segments before the branching of the brachiocephalic artery were higher than those of the aortic arch and thoracic aorta. Although the clear relationship between the permeability and the endothelial cell morphology was not observed, it was found that the deviation of the cell orientation to the axis of the blood vessel in locations, A and B, were higher than those of the other portions. Further, endothelial cell orientation in the ascending segment showed a pair of spiral configuration.

The endothelial cell morphology in the ascending aorta and aortic arch obtained in this study was similar to the results in the rabbit aorta reported by Ohyama et al. It can be assumed that the blood flow patterns are similar both in rats and rabbits, although there is some difference in branching pattern in the aortic arch between them, i.e., three vessel branches in rats and two in rabbits.

In this aspect, there are many researches who have been interested in the macromolecular transport of endothelial cells since atherogenesis is initiated from the lipid accumulation in the vessel wall.

Barakat et al. investigated the spatial distribution of HRP (horseradish peroxidase) in the rabbit aorta. They reported that the density of HRP spots was highest in the aortic arch, decreased distally, reached a minimum in the lower descending thoracic aorta, and then increased again in the abdominal aorta. Gerrity et al. reported the uptake of the protein-binding azo dye Evans blue and endothelial cell morphology in the aorta of pig. From their
results, areas of enhanced permeability were observed in the aortic arch and endothelial cells were cuboidal in these areas. In contrast, endothelial cells in areas of devoid of dye accumulation were flat and elongated. Okano and Yoshida(19) studied the lipid deposition and endothelial cell morphology at bifurcations of the brachiocephalic and left subclavian arteries in cholesterol fed rabbits. They reported that in the stagnation point of flow and leading edges of flow dividers, round and long fusiform endothelial cells were observed respectively, and lipid deposition was not observed. The hips of flow dividers of both branchings, proven to be relatively low shear stress regions, were covered by ellipsoidal cells and lipid deposition was observed. Their results indicated the correlation between the blood flow condition and lipid deposition. However, there was no clear correlation between the endothelial cell morphology and lipid deposition.

It is difficult to measure the detailed blood velocity profile and wall shear stress distribution in the aortic arch in vivo. By the model study on the curved tube and the cast of animal aortic arch, blood flow pattern was elucidated(12). In the aortic arch, the axial flow superposed on the secondary flow results in biehlal flow. An unsteady flow is the particular feature in an in vivo blood flow pattern. By considering the secondary flow and unsteadiness of flow, the wall shear stress is expected to alter its magnitude and direction every moment. Ku et al.(13) reported the precise correlation between the fibrous plaque localization, the initial stage of atherosclerosis, and the appearance of reversed wall shear stress (OSI, oscillatory shear index) in the human internal carotid artery. Naruse et al.(14) also reported the same correlation from the pulsatile flow measurement in the model curved tube. When we applied the alternately orthogonal flow with a 30 min interval, endothelial cells oriented to the medial between two flow directions(15). Therefore, it is expected that the endothelial cell orientation at the ascending aorta reflect the secondary flow at that site. The present study aimed at elucidating the relationship among blood flow pattern, endothelial cell function and morphology. From the results, it is suggested that the wall shear stress fluctuation in magnitude and direction affect the endothelial cell function, resulting in increased macromolecular permeability leading to atherogenesis.

5. Conclusion

The results in this study indicate that a complex blood flow in the ascending aorta affected the orientation of endothelial cells and might induce the changes in the permeability through endothelial cells. The clear correlation between endothelial cell morphology and permeability was not observed. We need further studies to clearly understand the flow effects on the endothelial cell function and mechano-sensing mechanisms.

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