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Association of variance in anatomical elements of myocardial bridge with coronary atherosclerosis

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Abstract

Objectives: The myocardial bridge (MB) is an anatomical structure consisting of myocardium covering a part of the left anterior descending coronary artery (LAD). The extent and spatial distribution of atherosclerosis in the LAD with an MB is influenced by the anatomical properties of the MB. In this study, the relationship between the overall anatomical framework of the MB including the periarterial adipose tissue as well as fibrosis of the MB itself and coronary atherosclerosis was histomorphometrically examined.

Methods: Full-length LADs with an MB from 180 autopsied hearts were cross-sectioned at 5-mm intervals. Together with measurements of MB-length, thickness, and location, proportional decrease of the atherosclerosis ratio of LAD segments beneath MB for that of LAD segments proximal to MB was defined as the atherosclerosis suppression ratio. The area ratio of adipose tissue in the periarterial area beneath MB and area ratio of fibrosis in the MB muscle were also measured.

Results: The atherosclerosis suppression ratio was significantly proportional to MB length and thickness. Periarterial adipose tissue beneath MB was detected in all cases (100%), and fibrosis within MB muscle for 136 cases (75.6%). The amount of adipose tissue beneath MB and MB fibrosis did not statistically affect the atherosclerosis suppression ratio. Multivariate analysis revealed MB length and thickness were the independent factors affecting the atherosclerosis suppression ratio.

Conclusions: The anatomical properties of an MB, especially of its length and thickness, play decisive roles as regulators of atherosclerosis in the LAD regardless of the amount of adipose tissue around LAD and MB fibrosis.

Key Words: Myocardial bridge, Atherosclerosis, Coronary artery, Adipose tissue, Anatomy

1. Introduction

A myocardial bridge (MB) is an anatomical structure consisting of myocardial tissue that covers a part of the coronary artery in the epicardial adipose tissue [1]. It presents almost exclusively in the left anterior descending coronary artery (LAD) and is detected angiographically by a finding of coronary stenosis during cardiac systole, which is caused by contraction of the bridging myocardium [2]. Its frequency in the LAD varies from 0.5% to 60% as assessed by coronary angiography, multidetector computed tomography, or autopsy [3]. As for clinical significance, MBs have been considered benign anomalies of the coronary arteries [4-7], but are sometimes associated with various coronary heart diseases [2, 8]. Histopathological studies have determined that the LAD segment beneath MB is always free from atherosclerosis, but the LAD segment proximal to MB is susceptible to it. The extent of atherosclerosis suppression in the LAD segment beneath MB is dependent on the anatomical properties of MB, such as its length and thickness [9]. This implies that compressive force from the MB to the LAD segment beneath the MB is closely related to atherosclerosis suppression in this LAD segment.

It has recently been suggested that perivascular tissue beneath MB is histopathologically notable in the longitudinal sections of the LAD [10]. This perivascular space contains adipose tissue which may practically function as a ñcoronary cushionò against compression by the MB during systole [10]. Furthermore, a perivascular adipose tissue surrounding the coronary artery may be involved in local stimulation of atherosclerotic evolution through active functions of proinflammatory cytokines and adipokines secreted from the periarterial adipose tissue [11-13]. The significance of the presence of periarterial adipose tissue on atherosclerosis suppression in the LAD segment beneath MB has not yet been examined. In addition, when examining the MB histopathologically, fibrotic changes

in the bridging myocardium are sometimes apparent. Fibrotic change of varying degrees may reduce the compressive force of the MB influencing on atherosclerosis progression.

In this study, using a histomorphometric approach, we attempted to clarify the significance of the anatomical framework of the MB - including its thickness, length, and location, the perivascular adipose tissue, and the extent of fibrosis in the MB muscle itself - to the development of atherosclerosis in the LAD.

2. Materials and Methods

2.1. Materials

A total of 180 autopsied hearts having an MB over the LAD were collected at Ohmori Hospital, Toho University Medical Center from 2006 to 2010 (Table 1). None of the patients had a history of cardiovascular disease and none of the autopsied hearts had histopathological evidence of significant lesions. The study protocol was approved by the Ethics Committee of Toho University (No. 22001).

2.2. Tissue preparation

The LAD from the left coronary ostium to the cardiac apex was removed from the heart together with the surrounding adipose and myocardial tissue at autopsy. They were fixed in 10% neutral buffered formalin, and the full-length LAD was cross-sectioned at 5-mm intervals. Each tissue section was embedded in paraffin, which was then thin-sectioned at 4-5 μm. Thin-sectioned specimens were stained with hematoxylin and eosin and elastic van Gieson (EVG). After microscopic observation, the sections showing the LAD covered by the MB were then stained with Azan-Mallory [14] for an estimation of myocardial fibrosis comprising the MB. In addition, on the purpose of discriminating adipocytes and identifying

collagen fibers, immunohistochemistry using antibodies against S-100 protein (rabbit polyclonal; Dako Japan, Tokyo), collagen type I (mouse monoclonal; Abcam, Tokyo, Japan), collagen type III (rabbit polyclonal; Abcam, Tokyo, Japan), collagen type IV (rabbit polyclonal; Abcam, Tokyo, Japan) was performed by the labeled streptavidin-biotin complex method (Dako, Carpinteria, CA, USA). The immunostaining was visualized by treating the slides with 3,3ô-diaminobenzidine tetrahydrochloride and then counterstained with hematoxylin.

In cases having multiple MBs in one LAD, only the most proximal MB was assessed in this study. In each anatomical element, the cases were divided into three groups by almost trisection of the largest value in each categorical element.

2.3. Atherosclerosis ratio and atherosclerosis suppression ratio

To evaluate the extent of atherosclerosis, we measured the area of intima and media in each LAD section with EVG staining using automated image analysis system of Visual Measure 32 (Rise System, Sendai, Japan). The ratio of the cross-sectional area of the intima to media was defined as atherosclerosis ratio as follows:

$$\text{Atherosclerosis ratio} = \frac{\text{Cross sectional area of the intima}}{\text{Cross sectional area of the media}}$$

In addition, atherosclerosis suppression ratio was defined as the percentage as follows:

$$\text{Atherosclerosis suppression ratio} = 100\text{X}\left(100 - \frac{\text{Mean atherosclerosis ratio of the bridged segment}}{\text{Mean atherosclerosis ratio of the three sections proximal to MB}}\right)$$

As mean MB length was 1.5 cm which corresponded to the length of three paraffin blocks, we used mean atherosclerosis ratio of three sections proximal to MB.

2.4. MB location

Distance from the left coronary ostium to the first segment of the LAD covered by the MB was defined as MB location. Cases were classified into three categories according to MB location: proximal ($\leq$ 3.5 cm from the left coronary ostium), common (4.0-5.0 cm), and distal ($\geq$ 5.5 cm).

2.5. MB length

The number of sections covered by the MB multiplied by 5 mm was defined as MB length. Cases were classified into three categories according to MB length: short ($\leq$ 1.0 cm), common (1.5-2.0 cm), and long ($\geq$ 2.5 cm).

2.6. MB thickness

The thickness of myocardial tissue covering the LAD was measured microscopically, and the largest value was defined as MB thickness. Cases were classified into three categories according to MB thickness: thin (< 500 μm), common (500-1000 μm), and thick (> 1000 μm).

2.7. Adipose tissue density beneath MB

The area except myocardial tissue beneath MB was measured in the LAD section with the thickest MB by the same method as for the atherosclerosis ratio, using a section stained with Azan-Mallory. The periarterial space area beneath MB was also measured. The periarterial space mostly consists of adipose tissue, including nerve fibers and small veins, but in this study all of the periarterial area was treated adipose tissue area. The ratio of adipose tissue area to all tissue area beneath MB was defined as adipose tissue density (%). All cases were classified into three groups according to adipose tissue density; small (< 30 %) , common (30-60 %), and large (> 60 %).

2.8. MB fibrosis rate

The cross-sectional area of the thickest MB in one LAD section was measured by the same method as for the atherosclerosis ratio in a section stained with Azan-Mallory, and the area of MB fibrosis stained blue was also measured. The ratio of fibrosis area to MB area was defined as the MB fibrosis rate (expressed as a percentage). Cases were categorized into three groups according to the MB fibrosis rate: small (< 1.0 %), common (1.0-10.0 %) , and large (> 10.0 %).

2.9. Statistical analysis

Quantitative data were expressed as mean ± SD and, according to a normality test, were normally distributed. For the analysis of unpaired samples, we used one-way ANOVA with Scheffeôs *F*-test for variance and multiple comparison analysis. For paired samples, we used repeated measure ANOVA with Scheffeôs *F* test. We performed multivariate logistic regression analysis, to confirm whether MB location, length, thickness, fibrosis rate, and

adipose tissue density were independent factors related to the atherosclerosis suppression ratio.

In all statistical tests, *P* values < 0.05 were considered significant.

3. Results

3.1. Histopathological structure of MB and perivascular space beneath MB

The thickness and length of myocardial tissue varied. Fibrosis in the MB muscle was recognized to varying extents in 136 cases (75.6%) and stained blue by Azan-Mallory method. Immunohistochemistry demonstrated that these fibrous regions were strongly positive for collagen type I but weakly positive for collagens type III and type IV (supplementary data; Figs. A-D). From such results, fibrosis lesion in the MB was mainly composed of collagen type I.

Perivascular space around the LAD beneath MB consisted mainly of adipose tissue with small amounts of nerve fibers and small veins (Fig. 1). Adipocytes are generally spherical, and its cytoplasm is compressed at the perimeter of the cell and displaced by a single lipid vacuole in sections stained with hematoxylin- eosin and Azan-Mallory[15]. By immunohistochemistry using S-100 protein antibody, S-100 reactivity was seen both in the nuclei and in the cytoplasm surrounding the lipid vacuole. These staining patterns of S-100 protein were recognized in both the adipocytes around the LAD under the MB and the adipocytes in the epicardial space (supplementary data; Figs. E-F). In addition, adipocytes confirmed by immunostaining with S-100 protein antibody corresponded with adipocytes confirmed by Azan-Mallory staining. In this perivascular space under the MB, adipose tissue was present without exception (100%).

3.2. Atherosclerosis ratio in MB segments

The mean atherosclerosis ratio was 1.72±1.04 in the segments proximal to MB, 0.83±0.47 in the segments under MB, and 0.93±0.51 in the segments distal to MB. The atherosclerosis ratio of MB segments was significantly (p = 0.00001) lower than that in the segments proximal to MB (Fig. 2a).

3.3. Association of MB location with atherosclerosis ratio

Mean MB location was 4.43±1.21 cm (range 1.5-10.0 cm) from the coronary ostium. There were 58 proximal cases ($\leq$ 3.5 cm), 56 common cases (4.0-5.0 cm), and 66 distal cases ($\geq$ 5.5cm). Mean age and sex ratio were not statistically different among three groups. The atherosclerosis ratio in the segment under MB was significantly lower than that in the segments proximal to MB in all groups (p = 0.00001) (Fig. 2b).

3.4. Association of atherosclerosis suppression ratio with MB location, length, and thickness

The atherosclerosis suppression ratio showed no significant difference (p = 0.5584) among the three groups (Fig. 3a).

Mean MB length was 1.50±0.97 cm (range 0.5-5.0 cm). There were 95 short cases ($\leq$ 1.0 cm), 53 common cases (1.5-2.0 cm), and 32 long cases ($\geq$ 2.5 cm). The atherosclerosis suppression ratio significantly increased with MB length (p = 0.0016) (Fig. 3b).

Mean MB thickness was 853.5±654.3 μm (range 156-4940 μm). There were 65 thin cases (< 500 μm), 61 common cases (500-1000μm), and 54 thick cases (>1000 μm). The atherosclerosis suppression ratio increased with MB thickness (p =0.0013) (Fig. 3c).

3.5. Association between adipose tissue density of MB and atherosclerosis suppression ratio

Mean adipose tissue density was 43.5±13.2 % (range 3.2-83.1%). There were 27 small cases (< 30%), 131 common cases (30-60%), and 22 large cases (> 60%). The atherosclerosis suppression ratio showed no significant differences among the three groups (Fig. 4a).

3.6. Association between MB fibrosis rate and atherosclerosis suppression ratio

Mean fibrosis rate of the MB was 5.6±9.5% (range 0-70.7%), and there were 44 small cases (<1.0%), 112 common cases (1.0-10.0%), and 24 large cases (>10%). The atherosclerosis suppression ratio showed no significant difference among these groups (Fig. 4b).

3.7. Multivariate logistic regression analysis for atherosclerosis suppression ratio

Multivariate analysis indicated that only two variables-MB length and MB thickness-were the independent factors influencing the atherosclerosis suppression ratio (Table 2).

4. Discussion

The existence of an MB contributes to atherosclerosis suppression in the LAD intima beneath MB, and MB length and thickness are strongly associated with the atherosclerosis suppression ratio [9]. In the present study, we further validated the association of anatomical properties of the MB with atherosclerosis development, taking into account the anatomical circumstances around the LAD beneath MB. The adipose tissue density around the LAD beneath MB and the extent of fibrosis of the MB muscle did not affect atherosclerosis development. Multivariate analysis yet demonstrated that MB length thickness were the

only independent factors for atherosclerosis development. These results indicate that MB length and thickness consistently regulate atherosclerosis development in the LAD regardless of the amount of adipose tissue in the periarterial space beneath MB and myocardial fibrosis in the MB itself.

As for a relationship between hemodynamics of blood flow and atherosclerotic evolution, low shear stress blood flow promotes atherosclerosis by an increase of lipids transfer into the arterial intima [16]. Scanning electron microscopy of the intimal surface has revealed that endothelial cells are flat and polygonal proximal to the MB, indicating that this area is affected by low shear stress [8]. Conversely, endothelial cells beneath MB are influenced by high shear stress blood flow and are thus spindle-shaped and engorged [8]. Considering such changes in endothelial shape as well as the close association of MB length and thickness with the atherosclerosis suppression ratio, the compressive force of the MB on the LAD directly contributes to an alteration of atherosclerosis development across the MB.

In recent studies, various kinds of adipokines released from adipose tissue surrounding the coronary arteries have been show to affect the local advance of atherosclerosis [11-13]. However, it is still controversial whether adipokines derived from the blood within the coronary arteries or adipokines derived from local adipose tissue function as a promoter of atherosclerosis. In addition, an absence of atherosclerosis in the coronary segment beneath MB is caused by a lack of adipose tissue around this segment [11,12]. However, in a study using multi-detector computed tomography, the extent of calcium deposition in the LAD wall covered with an MB is lower than that in an arterial segment without an MB, which is dependent on periarterial adipose tissue thickness [17]. In the present study, adipose tissue around the LAD beneath an MB was detectable in all cases (100%). Furthermore, there was no significant association between the adipose tissue ratio of perivascular space beneath an

MB with atherosclerosis suppression ratio. From our present results, it is conceivable that adipose tissue surrounding the LAD beneath an MB neither functions as a coronary cushion against MB compression nor is associated with atherosclerosis development in the LAD. Apart from the matter of periarterial adipose tissue, in this study, 136 cases (75.6%) had fibrosis in the myocardial tissue composing the MB. It is plausible that a large amount of fibrosis in the MB muscle may reduce the compressive force on the LAD, but we were unable to find a significant association between the MB fibrosis rate and the atherosclerosis suppression ratio.

Our analysis revealed that only MB length and thickness are the independent factors affecting the atherosclerosis suppression ratio. Previous reports indicate that anatomical properties, such as length and thickness, are closely related to the contractile force of the MB, which is associated with the magnitude of retrograde blood flow during systole [18-20]. In addition, the location of the MB along the LAD correlates with the location of the arterial segment exhibiting the greatest intimal lesion in the LAD proximal to MB [21]. In conclusion, taking into account a more overall anatomical element of the MB consistently indicates that the anatomical properties of an MB, especially its length and thickness, play a decisive role as regulators of atherosclerotic evolution in the LAD segment proximal to MB.

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References

[1] Geiringer E. The mural coronary. Am Heart J 1951;41:359-68.

[2] Möhlenkamp S, Hort W, Ge J, et al. Update on myocardial bridging. Circulation 2002 ;106:2616-22.

[3] Ishikawa Y, Kawawa Y, Kohda E, et al. Significance of the anatomical properties of a myocardial bridge in coronary heart disease. Circ J 2011;75:1559-66.

[4] Kramer JR, Kitazume H, Proudfit WL, et al. Clinical significance of isolated coronary bridges: benign and frequent condition involving the left anterior descending artery. Am Heart J 1982;103:283-8.

[5] Juilliére Y, Berder V, Suty-Selton C, et al. Isolated myocardial bridges with angiographic milking of the left anterior descending coronary artery: a long-term follow-up study. Am Heart J 1995;129:663-5.

[6] Ural E, Bildirici U, Celikyurt U, et al. Long-term prognosis of non-interventionally followed patients with isolated myocardial bridge and severe systolic compression of the left anterior descending coronary artery. Clin Cardiol 2009;32:454-7.

[7] Kim SS, Jeong MH, Kim HK, et al. Long-term clinical course of patients with isolated myocardial bridge. Circ J 2010 ;74:538-43.

[8] Ishii T, Asuwa N, Masuda S, et al. The effects of a myocardial bridge on coronary atherosclerosis and ischaemia. J Pathol 1998;185:4-9.

[9] Ishikawa Y, Akasaka Y, Ito K, et al. Significance of anatomical properties of myocardial bridge on atherosclerosis evolution in the left anterior descending coronary artery. Atherosclerosis 2006 ;186:380-9.

[10] Saidi H, Ongeti WK, Ogeng'o J. Morphology of human myocardial bridges and association with coronary artery disease. Afr Health Sci 2010;10:242-7.

[11] Verhagen SN, Visseren FL. Perivascular adipose tissue as a cause of atherosclerosis. Atherosclerosis 2011;214:3-10.

[12] Payne GA, Kohr MC, Tune JD. Epicardial perivascular adipose tissue as a therapeutic target in obesity-related coronary artery disease. Br J Pharmacol 2012;165:659-69.

[13] Silaghi AC, Pais R, Vallea A, et al. Epicardial adipose tissue and relationship with coronary artery disease. Cent Eur J Med 2011; 6:251-62.

[14] Bloom W, Fawcett DW. Connective tissue proper. In: Bloom W and Fawcett DW, editors, A Text Book of Histopathology, Tenth Edition, Tokyo, Japan. Igaku Shoin, 1976:158-195.

[15] Brooks JSJ, Perosio PM. Adipose tissue. In; Mills SE, editor, Histopathology for Pathologists, Fourth Edition, Philadelphia, USA. Lippincott Williams and Wilkins. 2012:179-210.

[16] Caro CG, Fitz-Gerald JM, Schroter RC. Atheroma and arterial wall shear. Observation, correlation and proposal of a shear dependent mass transfer mechanism for atherogenesis. Proc R Soc Lond B Biol Sci 1971;177:109-59.

[17] Verhagen SN, Rutten A, Meijs MF, et al. Relationship between myocardial bridges and reduced coronary atherosclerosis in patients with angina pectoris. Int J Cardiol 2012 Mar 3. [PMID: 22386701]

[18] Tsujita K, Maehara A, Mintz GS, et al. Comparison of angiographic and intravascular ultrasonic detection of myocardial bridging of the left anterior descending coronary artery. Am J Cardiol 2008;102:1608-13.

[19] Liu SH, Yang Q, Chen JH, et al. Myocardial bridging on dual-source computed tomography: degree of systolic compression of mural coronary artery correlating with length and depth of the myocardial bridge. Clin Imaging 2010;34:83-8.

[20] Elmali M, Soylu K, Gulel O, et al. Correlation between depth of myocardial bridging and

coronary angiography findings. Acta Radiol 2008;49:883-8.

[21] Ishikawa Y, Akasaka Y, Suzuki K, et al. Anatomic properties of myocardial bridge predisposing to myocardial infarction. Circulation 2009;120:376-83.

Table 1. Patient background

Number of cases	180 cases
Sex (male/female)	131/49
Mean age (mean ± SD)	67.9 ± 11.9 years (range 19-92)
Main disease	
Malignant tumor	106 cases (58.9 %)
Pneumonia	38 cases (21.1 %)
Liver failure	19 cases (10.6 %)
Renal failure	6 cases (3.3 %)
Others	11 cases (6.1 %)

SD, standard deviation; MB, myocardial bridge

Table 2. Multivariate logistic regression analysis for atherosclerosis suppression ratio

Variable	95% CI	Odd's ratio	*p*
Fibrosis rate	0.00187-0.00586	1.03641	0.31007
Adipose tissue density	−0.00266-0.00283	0.00368	0.95173
MB thickness	0.0000-0.00010	7.69383	0.00614
MB length	0.00246-0.00845	4.37660	0.03789
MB location	−0.01110-0.05103	1.60858	0.20639

CI: confidence interval; MB: myocardial bridge

Figure Legends

Fig. 1. Variation of myocardial bridge (MB) with Azan-Malloryôs staining. In each figure, black bars indicate the confines of the MB covering the artery and periarterial space.

a. A thick MB is shown above the left anterior descending coronary artery (LAD). Evidently small amount of adipose tissue (asterisks) is evident in the periarterial space, and mild fibrosis stained with blue (arrow) is found within the MB myocardium.
b. The periarterial adipose tissue (asterisks) is more abundant than in the case of Fig.1a. A small amount of fibrosis (arrow) is seen within the MB muscle.
c. A thin MB with a small amount of adipose tissue (asterisk) around the periarterial space is shown. Fibrous change (arrow) in the MB muscle is very mild. Nerve fibers are also evident in the periarterial space.
d. A thin MB with a large amount of periarterial adipose tissue (asterisks) is found. Fibrosis within the MB muscle is relatively abundant. Nerve fibers are also evident in the periarterial space.

Fig. 2. Atherosclerosis ratio of the LAD

a. The atherosclerosis ratio in the segments under MB is significantly lower than that in the segments proximal to MB. (p = 0.00001, repeated measure ANOVA).

b. Association of MB location with atherosclerosis ratio. Atherosclerosis ratio in the segment under MB is significantly lower than that in the segments proximal to MB in each location group. (p = 0.00001, repeated measure ANOVA).

Fig. 3. Atherosclerosis suppression ratio

The atherosclerosis suppression ratio shows (a) no significant difference between the three location groups (p=0.5584, one-way ANOVA), (b) significant increase with MB length (p = 0.0016, one-way ANOVA), and (c) significant increase with MB thickness (p =0.0013, one-way ANOVA).

Fig. 4. Association of atherosclerosis suppression ratio with adipose tissue density ratio in the periarterial space and MB fibrosis rate.

No significant differences are seen in atherosclerosis suppression ratio between the three groups by (a) adipose tissue density ratio (p=0.9987) or (b) MB fibrosis rate (p=0.3958, one-way ANOVA).

Legends of Supplementary Data

Fig. A is a LAD tissue section stained with Azan-Mallory. In a myocardial bridge (MB), fibrosis stained blue is recognized. Figs. B-D are the serial sections obtained from the same paraffin-block as Fig. A, and they are immunostained using antibodies against collagen type I, type III, and IV, respectively. Collagen type I (B) is evidently detected in the fibrosis lesion, but immunostainings against collagen type III and type IV are weakly positive in the lesion. Immunohistochemistry against S-100 protein demonstrates a positivity in adipose tissue of the epicardial region (Fig. E) and the peri-LAD space under an MB (Fig. F).