Functional medial thickening and folding of the internal elastic lamina in coronary spasm

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Coronary spasm is the cause of vasospastic angina. Coronary spasm was induced by brushing the coronary adventitia in nine anesthetized beagles, and structural changes in the spastic coronary segments were examined by light and electron microscopy, making comparisons with the adjacent nonspastic segments. The % diameter stenosis of the spastic segments as measured angiographically was 79.4 ± 12% (mean ± SD). Light microscopic changes in the spastic and nonspastic segments were as follows: medial thickness 1.512 vs. 392 μm (P < 0.0001) and % diameter and % area stenoses of spastic segment 81.0% and 96.5%, respectively, indicating that spasm was induced by medial thickening. Circular smooth muscle cells (SMCs) in the media were arranged in parallel with the internal (IEL) and external (EEL) elastic lamina in nonspastic segments but radially rearranged in spastic segments. SMCs were classified by their patterns of connection to IEL into six types by electron microscopy. Of these, three contracted and pulled the IEL toward the EEL, causing folding of the IEL and waving of EEL resulting in thickening of the media and narrowing of the lumen. We conclude that coronary spasm was elicited by radial rearrangement of the medial SMCs due to their own contraction and resultant medial thickening and folding of IEL, creating a piston effect to narrow the lumen, i.e., spasm.

smooth muscle cell rearrangement

CORONARY SPASM IS THE CAUSE of vasospastic angina. Coronary spasm is thought to be provoked by excessive contraction of circular smooth muscle cells (SMCs) present in the media of the coronary artery.

Since medial SMCs can shorten their length by contraction by 30% at most, to attain a luminal narrowing of ≥75% is theoretically difficult without the existence of a thickened intima if their circular arrangement is not altered during contraction. Two concepts have been proposed as to the structural (morphological) mechanisms underlying coronary spasm: concentric narrowing of the coronary lumen in the presence of diffuse intimal thickening (6, 9, 12) and eccentric narrowing of the lumen in the presence of eccentric intimal thickening (2). However, no definitive evidence has emerged to support whether either of these mechanisms actually occurs clinically.

There are a number of studies on humoral factors that participate in coronary spasm (7, 15). Structural changes at the cellular level that occur in the coronary media during spasm, however, have not been clarified, probably because of the difficulty in analyzing coronary arterial architecture during spasm in patients and the lack of appropriate animal models of coronary spasm.

Coronary spasm occurs not only in the large epicardial coronary arteries, often the site of atherosclerotic intimal thickening, but also in arterioles in which intimal thickening infrequently occurs (13). It is therefore necessary to elucidate the mechanisms of coronary spasm that occurs without intimal thickening.

Morikawa et al. (10) demonstrated with the use of optical coherence tomography (OCT) that in patients with vasospastic angina the luminal surface exhibits a markedly wavy configuration (folding) during spasm. Mortensen et al. (11) observed folding of the coronary arterial internal elastic lamina (IEL) in patients dying suddenly because of coronary spasm. Joris and Majno (3) observed folding of the IEL in rat mesenteric artery during norepinephrine infusion. Van Citters et al. (14) observed folding of IEL and medial thickening in canine mesenteric artery during coronary spasm. However, they did not clarify the mechanism(s) for IEL folding and medial thickening. Nevertheless, these findings may provide important morphological positive proof of spasm and important clues for clarifying the underlying structural mechanisms of coronary spasm.

The authors of the present study developed an experimental canine coronary spasm model and examined the kind of structural changes at the cellular level that occur in the spastic coronary arterial wall.

METHODS

Coronary Spasm Provocation

We conducted animal experiments at the Jikei University School of Medicine Institute for Animal Experiments. The experiment protocol was approved by the University Administrative Panel on Laboratory Animal Care.

For the experiment, nine beagles were anesthetized with pentobarbital sodium (30 mg/kg iv). The left upper second to fifth ribs were removed, and the heart was exposed by pericardiotomy. A middle segment 2–2.5 cm in length of either the left anterior descending artery or the circumflex artery was carefully dissected free of the surrounding tissues with two pairs of microscopic...
dissection forceps so as not to damage the segment. An 8-F catheter was introduced via the right common carotid artery into the left coronary ostium, and contrast material was injected for coronary angiography. A 5-cm × 5-mm filter paper was carefully inserted between the segment and the surrounding tissues so as not to touch the segment. The adventitial surface of the segment was gently brushed by the filter paper to induce spasm. Subsequently, 1 ml of 20% glutaraldehyde solution was applied to the surface of the segment for vital fixation. No further obvious changes in external diameter of the vessel were induced by topical application of glutaraldehyde solution. A mixture of contrast material and glutaraldehyde solution was injected into the artery for simultaneous angiography and vital fixation. The % diameter stenosis of the coronary arteries was measured by quantitative coronary angiography using TCS Symphony 2.02 (McKesson, North Charleston, SC).

The animals were killed with intravenous pentobarbital sodium (100 mg/kg) and potassium chloride (10 mg/kg) 10–15 min later. The spastic coronary segment and an adjacent nonspastic segment were excised and stored in 5% glutaraldehyde solution. The spastic coronary segment was fixed similarly 1 h after spasm provocation for examination of the changes inside SMCs in an additional two beagles.

**Histology**

The center of each spastic segment was transected and sliced at 10-μm thickness successively along the shorter axis. The slices were stained with the azan stain, which stains SMCs red or yellow. A portion of both the spastic and nonspastic segments was excised for electron microscopic examination.

**Light microscopic examination.** The following parameters were measured and compared between nonspastic and spastic coronary segments: 1) thickness (μm) of the IEL was obtained with the following formula: maximum IEL thickness + minimum IEL thickness/2; 2) thickness (μm) of the media was obtained with the following formula: maximum medial thickness + minimum medial thickness/2; 3) luminal diameter was obtained with the following formula: maximum diameter + minimum diameter/2; 4) external elastic lamina (EEL) diameter was obtained with the following formula: maximum distance from 1 portion of the EEL to the opposite EEL + minimum distance from 1 portion of EEL to the opposite EEL/2; 5) medial area (μm²) and luminal area (μm²) were measured by planimetry; 6) % diameter stenosis was calculated with the following formula: (luminal diameter of nonspastic segment − luminal diameter of spastic segment) × 100/luminal
diameter of nonspastic segment (% diameter stenosis ≥75% was defined as spasm); 7) % area stenosis was calculated with the following formula: (luminal area of nonspastic segment − luminal area of spastic segment) × 100/luminal area of nonspastic segment.

Electron microscopic examination. Morphological changes in the IEL and SMCs of the nonspastic and spastic coronary segments were examined by electron microscopy.

Statistical Analysis

Data are expressed as means ± SD and were tested by Student’s t-test. A P < 0.05 was considered significant.

RESULTS

Angiographic Changes

On brushing of the adventitial surface, the coronary segment became thin, and luminal narrowing was seen at angiography (Fig. 1). The % diameter stenosis ranged from 69.0% to 91.2% (79.4 ± 12%).

Light Microscopic Changes

Figure 2 shows the cut surfaces of nonspastic and spastic segments. The thickness of spastic segments was markedly increased compared with the nonspastic adjacent segments.

Internal elastic lamina. The IEL was flat and smooth in nonspastic segments (Fig. 3, A and E). Contrarily, in spastic segments multiple folds were observed, resembling the bellows of an old-fashioned camera (Fig. 3B). Folds existed singly, or multiple folds together produced a larger fold, dragging into the media as if pulled by SMCs (Fig. 3C).

Media. Mediinal SMCs were arranged in parallel with the IEL in nonspastic segments (Fig. 3, A and E). In spastic segments, the SMCs connecting to the bottom of the fold were arranged radially, extending toward the EEL (Fig. 3D).

SMCs adjacent to the EEL ran parallel to the EEL in nonspastic segments. In spastic segments, SMCs connecting to the EEL were arranged radially, extending toward the IEL (Fig. 3, F and G).

External elastic lamina. The EEL was flat and smooth in nonspastic segments. In contrast, the EEL was wavy in spastic segments (Fig. 3G).

Assessment of structural changes. Folds were observed in all spastic segments. The depth of the folds ranged from 80 to 300 μm. The number of folds/100 μm of IEL length ranged from 1 to 3 (Table 1).

IEL thickness was not different between nonspastic and spastic segments. In contrast, the thickness of the media was markedly increased in spastic segments, although no difference was seen in the medial area between nonspastic and spastic segments (Table 1).

The lumen was markedly narrowed; % diameter stenosis in spastic segments was 81.0% on average, indicating that the narrowing was spasm (Table 1).

Electron Microscopic Changes

Luminal surface. In nonspastic segments, endothelial cells (ECs) were arranged in parallel and longitudinally. In spastic segments, multiple folds were observed, as with light microscopy (Fig. 4).

Folds in IEL. In nonspastic segments, IELs were arranged side by side and were connected by a junction, and SMCs were arranged in parallel with the IEL. In spastic segments, folds were observed, as with light microscopy. Junctions between IELs sank into the media, forming a fold.

Changes in SMCs. Dense areas and filaments are the skeleton of SMCs. They were wavy in nonspastic coronary segments (Fig. 5A). In contrast, in spastic segments, dense areas and filaments became straight (Fig. 5B). Vacuoles, which are the characteristic change of spasm in SMCs, were observed in the SMCs of the spastic segments fixed and excised 1 h after provocation of spasm but not in those excised 10–15 min after spasm provocation or in nonspastic segments (Fig. 5C).

Fig. 2. Light microscopic appearance of nonspastic and spastic coronary segments. A: transected surface of a nonspastic segment. L, lumen; M, media. B: transected surface of a spastic segment; markedly thickened media. Arrow, folding of internal elastic lamina (IEL). Scale bars: 100 μm.
Connecting pattern between SMCs and IEL. In both non-spastic and spastic segments, connections between SMCs and the IEL were observed. SMCs were classified by their pattern of connection to the IEL into six types: type 1, one end connected to the junction and the other end extended toward the EEL (Figs. 6A and 7A, c); type 2, one end connected to the edges of two neighboring IELs and the other end extended toward the external elastic lamina (EEL). C: spastic segment. Several folds together have formed a larger fold (arrows). D: spastic segment. Connection of an SMC (arrowhead) to a fold (arrow). E: nonspastic segment. SMCs (arrowhead) are arranged in parallel with EEL (arrow). F: spastic segment. SMCs (arrowhead) connecting to the wavy EEL (arrow) are directed toward the lumen. G: higher magnification of F. SMCs (arrowhead) connected to the wavy EEL (arrow) are directed toward the lumen. Scale bars: 20 μm (A–C, E, and F), 10 μm (D and G). Arrowhead in A, B, and G refers to lumen. A in E–G refers to adventitia.

Table 1. Changes in coronary arterial structure during spasm

<table>
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<tr>
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<th>Nonspastic Segment</th>
<th>Spastic Segment</th>
<th>( P ) Value</th>
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<tr>
<td>IEL thickness, μm</td>
<td>27.6 ± 4</td>
<td>31.4 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>Medial thickness, μm</td>
<td>151.6 ± 19</td>
<td>406.1 ± 65</td>
<td>&lt;0.0001</td>
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<tr>
<td>Medial area, ( \times 100 ) μm²</td>
<td>941.6 ± 291</td>
<td>953.6 ± 291</td>
<td>NS</td>
</tr>
<tr>
<td>Laminal diameter, μm</td>
<td>1,141.7 ± 395</td>
<td>192.2 ± 85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Laminal area, ( \times 100 ) μm²</td>
<td>18,454 ± 10,851</td>
<td>327 ± 312</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>EEL diameter, μm</td>
<td>1,466 ± 357</td>
<td>1,103 ± 214</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>% Diameter stenosis</td>
<td>81.0 ± 10.0</td>
<td></td>
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<tr>
<td>% Area stenosis</td>
<td>96.5 ± 4.0</td>
<td></td>
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<tr>
<td>Depth of folds, μm</td>
<td>180.0 ± 32</td>
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<tr>
<td>No. of folds/100 μm</td>
<td>1.7 ± 0.5</td>
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Values are means ± SD for \( n = 9 \) coronary segments/group. IEL, internal elastic lamina; EEL, external elastic lamina; NS, not significant.

DISCUSSION

There are a number of studies on the substances that may participate in coronary spasm and on the roles of ECs in the genesis of coronary spasm (1, 5, 8). Morphological studies on the behavior of medial SMCs that play an important role in spasm, however, have been ignored.
In this study, coronary luminal narrowing (defined as \( \% \) diameter stenosis \( \geq 75\% \)) was provoked by brushing the coronary adventitia. This indicates that coronary spasm was induced in the absence of intimal thickening.

Clinically, coronary spasm is frequently induced mechanically by the catheter that is introduced into the targeted coronary artery during coronary angiography or intervention. The mechanism by which spasm is induced is not well understood, however. Since spasm induced by adventitial brushing was prevented by an \( \alpha \)-adrenergic blocking agent in a preliminary study, it is likely that \( \alpha \)-adrenergic receptors played a part in spasm induction in this study.

Compared with nonspastic adjacent coronary segments, the most outstanding microscopic changes in spastic coronary segments were increased medial thickness with no change in the medial area, folding of the IEL, and luminal narrowing.

Since the medial area was the same in spastic and nonspastic segments, some mechanisms other than increased medial volume must have contributed to the increased medial diameter.

The most outstanding structural change in the media during spasm was radial rearrangement of SMCs connecting the IEL and EEL (Fig. 7A, a–c). It appears that these SMCs contracted vigorously and, as a consequence, were rearranged radially (Fig. 7D; standing up phenomenon) and that they pulled the IEL toward the EEL, causing folding of the IEL and waves in the EEL, with resultant decreases in luminal and medial thickening. These structural changes together may have created a piston effect that caused severe luminal narrowing, i.e., spasm. This radial mechanism would cause medial and enhanced intimal thickening in the presence of plaque that is squeezed into a small diameter because of medial thickening, thereby causing an even greater piston effect than in the absence of intimal plaque.

**Fig. 4.** Scanning electron microscopic appearance of the endothelial surface of nonspastic and spastic coronary segments. *A*; nonspastic segment. Endothelial cells (arrow) are arranged in parallel. *B*; spastic segment. Folds can be seen (arrow). Scale bars: 20 \( \mu \)m.

**Fig. 5.** Electron microscopic changes in SMCs during spasm. *A*; a SMC in a nonspastic coronary segment. Dense areas (arrow) and filaments (arrowhead) were wavy. *B*; a SMC in a spastic coronary segment. Dense areas (arrow) and filaments (arrowhead) were thick and straight. *C*; vacuoles in a SMC of a segment excised 1 h after spasm provocation (arrows). Scale bars: 20 \( \mu \)m.
Van Citters et al. (14) were the first to observe folding of IEL and medial thickening of canine mesenteric artery during spasm. However, they did not observe radial rearrangement of SMCs during spasm (14).

In spastic segments, dense areas and filaments that maintain configuration of SMCs became straight and caveolae were protruded outwardly, indicating contraction. In addition, vacuoles, which are the characteristic change of spasm, were observed in SMCs of the spastic segments that were excised in a later phase. Similar vacuoles were observed by Joris and Majno (4).

In addition to three types of SMCs that possibly connect the IEL and EEL, four types of SMCs that connected IEL junctions to the IEL, or connected between IELs, were observed. These may act to prevent dislocation of the IEL during spasm (Fig. 7D).

In the clinical situation, luminal folding has been observed by ultrasound cardiography (UCG) or OCT during coronary spasm (10). Folding of the IEL has been observed in patients dying from coronary spasm (11). These clinical findings strongly suggest that the structural mechanisms observed in this study also occur in the coronary artery in patients with vasospastic angina.

Fig. 6. Electron microscopic appearance of the cut surface of coronary segments, showing connections between SMCs and the IEL. A: nonspastic segment. An SMC is connected to the junction (j) of 2 IELs by a threadlike structure. The other end extends toward the deeper layer of the media. c, Connection. B: nonspastic segment. An SMC is connected to the edges (c) of the neighboring 2 IELs. The other end of the SMC extends toward the deeper layer of the media. EC, endothelial cell. C: nonspastic segment. An SMC (arrow 1) connected to the IEL extends toward the deeper layer and connects in series to another SMC (arrow 2). D: nonspastic segment. An end of an SMC connects to the junction (j) and the edges (c) of two neighboring IELs and the other end to the middle portion of one IEL (c). E: spastic segment. One end of an SMC connects to 1 IEL (c) and the other end to the neighboring IEL (c). F: threadlike structure that connects an SMC to the IEL (c). Scale bars: 20 μm (A–E), 10 μm (F).
It remains to be elucidated whether and how vasodilating agents act on radial rearrangement of SMCs. There are no eradicative treatments of vasospastic angina, and patients must take vasodilating agents every day. Prevention of radial rearrangement of SMCs by any means is one possible approach for eradicative treatment of vasospastic angina.

**Study Limitations**

This coronary spasm study was performed in nonsclerotic coronary arteries with normal intima. Therefore, it remains to be clarified whether radial rearrangement of SMCs in the media, and consequent medial thickening and folding of the IEL, occur in atherosclerotic coronary arteries with a thickened intima.

**Conclusions**

We investigated the structural mechanism of coronary spasm in animals. During spasm, the SMCs connecting between the IEL and EEL contracted and were radially rearranged, causing medial thickening and folding of the IEL, creating a piston effect to narrow the lumen, i.e., spasm. To the best of our knowledge, such radial rearrangement of medial SMCs due to their own contraction and resultant coronary spasm has not been described in the literature.

**DISCLOSURES**

The authors have no conflicts of interest to declare. The authors have no relationships with industry. No external funding was received for this study.
REFERENCES


