Animal Models of Coronary Spasm and the Pathophysiological Events in Regional Vascular Hypercontraction

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An animal model of coronary spasm was designed 1) to reproduce coronary spasm similar to that seen in patients with variant angina, 2) to determine whether hypercontraction of the vascular smooth muscle occurs at the site of the spasm, 3) to document the relationship between functional and structural changes of the vascular wall and 4) to characterize the pathophysiological features of coronary spasm.

After balloon de-endothelialization and feeding of a high cholesterol diet in mongrel dogs and Göttingen miniature pigs, there was evidence of vascular hypercontraction associated with arteriosclerotic changes. Coronary spasm of more than 75% narrowing of the artery was provoked with ischemic signs in miniature swine. These events could be repeatedly provoked by an intra-coronary injection of histamine following pretreatment with cimetidine. The site of hypercontraction corresponded well with the site of the de-endothelialization, an area where the basal vascular tone was increased and was related to histamine activity. Thus, the present animal model will shed light on mechanism involved in vasoactive angina pectoris and aid in clarifying the pathophysiology of vascular smooth muscle.

PATHOPHYSIOLOGICAL features in patients with coronary artery spasm are 1) an angiographic demonstration of transient coronary vasoconstriction at the site of previously normal or stenotic portion of the epicardial coronary artery and 2) primary reduction of coronary flow resulting in regional myocardial ischemia. However, clinical evidence such as subtotal occlusion of the angiographically normal coronary artery during spontaneous or evoked attacks has not heretofore been reproduced in an experimental setting. The large coronary artery is a conduit vessel and its physiological role in regulation of coronary blood flow is estimated as less than 5%. With regards to coronary spasm related events, several hypotheses have been proposed: 1) physiological contraction of the coronary vascular wall at the site of organic coronary stenosis, which results in myocardial ischemia. This hypothesis is often called the "geometric theory"2; 2) transient platelet aggregation and resulting coronary occlusion3; 3) a passive wall collapse in an elastic coronary stenosis secondary to a fall in distending pressure4; 4) hypercontraction of the regional epicardial coronary artery which was documented angiographically as a coronary stenosis5 Cyclic ST-changes6 and variable exercise tolerance7 strongly suggest the contribution of vascular hypercontraction to the triggering of mechanisms related to ischemia in patients with variant angina. Therefore, we set out to design an animal model of coronary spasm to obtain data

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as to whether vascular hypercontraction plays an important role in the provocation of transient myocardial ischemia and to clarify the mechanism and pathophysiology of regional vascular hypercontraction. Since atherosclerotic changes are often noted along the loci of coronary spasm, the approaches we used were balloon endothelial denudation in combination with a high cholesterol diet.

Provocation of Coronary Spasm in Experimental Animal Model

1. Animal model
Mongrel dogs and Göttingen miniature swine were used. Under pentobarbital anesthesia, selective coronary catheterization was performed via the carotid artery. Using cineangiographic techniques, the left coronary artery was photographed before and after the administration of vasoactive agents and the endothelium was denuded by a 2F Fogarty embolectomy catheter. The efficacy of de-endothelialization was confirmed by an increased permeability of the denuded site with Evans blue. After the endothelial denudation, animals were fed a 2% cholesterol diet for up to 6 months.

2. Documentation of coronary spasm
Figure 1 shows a representative coronary arteriogram before and during the coronary spasm induced by intracoronary injection of histamine after pretreatment with cimetidine, an H$_2$-blocker, in miniature swine with 3 months of coronary denudation and a high cholesterol diet. Marked vasoconstrictions were noted at two locations of the left circumflex coronary artery along with ischemic ST-elevation (Fig. 1 right). These loci of coronary spasm were angiographically intact before the provocation, despite the balloon de-endothelialization performed 3 months before. We observed severe transient vasoconstriction (>75% stenosis) in 19 of 29 consecutive pigs after one month of denudation. There were five sudden deaths due to an intractable coronary spasm.

3. Determination of the locus of primary vasoconstriction and quantitative evaluation of geometric theory
To determine the primary locus of the histamine-induced vasoconstriction, histamine was infused intracoronarily at sites proximal and distal to the denuded area, while regional segmental shortenings, peripheral coronary pressure and epicardial ECG were continuously monitored. When histamine was infused into the left circumflex coronary artery distal to the denuded site, regional dysfunction or reduction in the periph-

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Hypercontraction or Increased Narrowing due to Geometry

![Swine Control and Serotonin 60μg ic](image)

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*: observed narrowing. :: predicted narrowing

Fig. 2. The extent of vasoconstriction determined by an angiographic method was compared with the predicted coronary stenosis calculated from the histological coronary stenosis and the degree of vasoconstriction of the normal coronary artery (Geometric theory). As illustrated in the insert, the observed stenosis was more severe than the calculated one, and this suggests a vascular hypercontraction.

eral coronary pressure did not occur. However, marked dysfunction following reduction of peripheral coronary pressure was noted after the proximal injection of histamine. This evidence strongly suggested that the primary foci of the histamine-induced coronary stenosis was limited to the site between the proximal and distal injection sites.

The contributions of intimal thickening on an enhanced coronary stenosis as estimated by a geometric theory was tested by comparing the observed degree of vasoconstriction and the magnitude of stenosis predicted from the histologically defined intimal thickening and the degree of luminal narrowing of the control branches after the administration of a vasoactive agent (Fig. 2). The level of coronary stenosis was more severe than the level predicted from the geometric theory alone. Thus, vascular hypercontraction seemed to be a valid explanation for the excess vasoconstriction.

Pathophysiology of Coronary Spasm

1. Vascular hypercontraction specific to agonists

The degree of vasoconstriction due to agonists was compared before and 3 months after denudation in the miniature swine. As shown in Fig. 3, effects of agonists were categorized to specifically enhanced and non-specific vasoconstrictions. The former included histamine, before or after pretreatment with cimetidine, serotonin and ergonovine. The latter included thromboxane A2 (STA2) and phenylephrine. The specifically enhanced vasoconstriction was also noted in in-vitro tension development in a canine model in which the denuded site responded excessively to ergonovine or serotonin yet the response to phenylephrine was similar between the intact and denuded sites.

2. Protection of histamine induced vasospasm

The degree of angiographical vasoconstriction due to histamine was tested before and after indomethacin 2 mg/kg, iv, diphenhydramine 1
Fig. 3. Vasoconstrictive response before and 3 months after de-endothelialization in a swine model. Note that the denuded site responded at an enhanced level to histamine 200 µg ic after pretreatment with cimetidine 60 mg/kg iv and serotonin 60 µg ic. Data are presented as the mean ± SE.

Fig. 4. Effects of pretreatments for histamine-induced coronary spasm. Data are presented as the mean ± SE. Abbreviations: DPH = diphenhydramine; PGJ₂ = prostacyclin. p < 0.01 vs non-pretreatment by a student t-test.
mg/kg iv, diltiazem 100 μg/kg iv or prostacyclin 50 ng/kg/min iv. As shown in Fig. 4, diphenhydramine and diltiazem but not indomethacin and prostacyclin prevented the histamine-induced vasoconstriction. These results suggest that the denuded site constricts excessively with H₁ receptor stimulation and this response does not relate to the exogenous or endogenous levels of prostacyclin. ST₄₂ showed no augmented response specific to the denuded site. This evidence indicates the non-significant role of thromboxane A₂ during histamine-induced coronary spasm.

3. Enhancement of basal vascular tone peculiar to the denuded site of the coronary artery

The extent of coronary vasodilation by nitroglycerin was examined as an index of basal coronary tone. It was observed chronologically, for one to six months in miniature swine, using an angiographic technique. There was no difference in the extent of coronary vasodilatory response to nitroglycerin between the intact and denuded sites, before balloon de-endothelialization. After one month of denudation, the degree of vasodilatation due to nitroglycerin increased along the denuded site, and corresponded well with the occurrence of histamine-induced coronary spasm. In cases (n = 7) in which coronary artery spasm was provoked by histamine, the effects of diphenhydramine 1 mg/kg on coronary diameter change were examined angiographically. After administration of diphenhydramine, the denuded site dilated by 12 ± 3 (mean ± SE) %, such being larger than that of the intact portion, 3 ± 1% (p < 0.01). Thus, the basal tone of the denuded portions of the epicardial coronary artery uniquely increased with concomitant gain to not only H₁ receptor stimulation as a trigger of coronary spasm but also the H₁ receptor related coronary tone.

4. Structural change of the coronary artery after balloon-de-endothelialization

Three to six months after denudation in 12 dogs and before and after 3 months in 12 miniature swine, the heart was excised and fixed in 20% formaldehyde. The major trunks of the left circumflex and the left anterior descending coronary arteries were stained with hematoxylin-eosin and Weigert-van Gieson. The maximum thickness of the intima and the media was measured microscopically. In a canine model, intimal thickness of the denuded site was 135 ±
28 μm and that of the contralateral intact site was 6 ± 1 μm. Despite such an enormous difference (p < 0.01) in intimal thickness between the denuded and intact sites, the difference in medial thickness between these locations was not significant, as shown in Fig. 5.

In a swine model, we started the experiments using young miniature pigs 3–4 aged months. Intimal thickness as well as medial thickness of the intact site increased significantly with age, as shown in the right of Fig. 5. However, the intimal thickness at the denuded site increased enormously during the three months, as compared to findings at the intact site. Despite such a difference in intimal thickness, medial thickness was much the same between the left circumflex and the left anterior descending coronary arteries, three months after as well as before denudation.

There was a close correlation between the site of vascular hypercontraction and the site of intimal thickening, both in canine and miniature swine models.

**PERSPECTIVE**

Clinical observations of vasospastic angina revealed a variety of symptoms, and certain cause-effect relationships were found to explain the mechanism of so-called “primary angina”. There seems to be no single event which will explain the variety of factors seen in case of ischemic heart disease. However, whether or not vascular smooth muscle plays a role in provocation of abnormal hypercontraction can be studied in an animal model. In 1976, Chahine and Luchi noted that efforts should be made to design an animal model in which coronary spasm can be provoked either by pharmacologic or mechanical stimulation.

In our present series of experiments, we noted an acquired property of vascular hypercontraction to ergonovine and serotonin in a canine model, and histamine, serotonin and ergonovine in a swine model after balloon-de-endothelialization followed by feeding of a high cholesterol diet. In a swine model, the angiographically normal coronary artery, previously denuded, constricts subtotally together with a concomitant regional myocardial ischemia. These findings have not been reproduced in a normal coronary circulation where the role of coronary flow regulation inherent to the epicardial coronary artery is less than 5%. As our current animal model reveals findings which are phenomenologically similar to the coronary spasm seen clinically, not only detailed pathophysiological analysis of the coronary circulation but also basic mechanisms regulating the smooth muscle properties, can be elucidated.

In addition, the topological correlation between the site of angiographical coronary spasm and intimal thickening observed by a histological technique will shed light on the background of coronary spasm. An increase in basal vascular tone correlating with the prevalence of histamine-induced coronary spasm provide a clue as to basic mechanisms involved in the control of vascular reactions as well as a tool for detecting the site of coronary spasm, without undue intervention.

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