Spontaneous and Diet-Induced Coronary Atherosclerosis in Normal Swine and Swine with von Willebrand Disease

Valentin Fuster, J. T. Lie, Lina Badimon, James A. Rosemark, Juan-Jose Badimon, and E.J. Walter Bowie

We have observed that pigs with impaired platelet function in the form of severe von Willebrand's disease (vWd) are resistant to spontaneous and to diet-induced aortic atherosclerosis. However, it has been reported that vWd pigs are susceptible to coronary atherosclerosis produced by balloon-induced injury of coronary arteries combined with an atherogenic diet. We have evaluated the development of coronary atherosclerosis in normal control (NC) and homozygous vWd pigs in two prospective studies: 1) as a spontaneous process in five NC and vWd pigs receiving a regular diet from the age of 3 months to 4 years; and 2) in nine NC and five vWd receiving a high-fat and high-cholesterol (2%) diet from the age of 3 to 9 months. All of the coronary arteries were analyzed postmortem in 5-mm sections. None of the NC nor the vWd pigs in the spontaneous study showed coronary atherosclerosis or myocardial lesions. In the study of diet-induced atherosclerosis, only one NC and one vWd pig had discrete stenoses; the stenoses affected the three coronary arteries and were significant (50% to 80%) in the NC and mild (>25%) in the vWd pigs; no pigs showed myocardial lesions. Pigs with vWd are resistant to atherosclerosis of the aorta. To assess the resistance or susceptibility to coronary disease in these pigs, a longer follow-up study would be necessary.

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For several years we have maintained a colony of pigs with homozygous von Willebrand (vWd) disease. These animals share all of the hemostatic abnormalities of the severe form of the disease usually observed in humans. They have a serious autosomal transmitted bleeding tendency, a long bleeding time, reduced retention of platelets in a glass beads column, reduced levels of factor VIII coagulant activity (VIII:C), very low levels (0.02% of normal) of factor VIII-related antigen (VIII-AG), and a lack of ristocetin cofactor in the plasma (VIII:WF).\(^1,3\) We have postulated that the absence of VIII:AG or VIII:WF may be responsible for the impairment of platelet-arterial wall interaction and resistance to atherosclerosis. Indeed, these pigs have been less susceptible both to spontaneous aortic atherosclerosis\(^4,5\) and to arteriosclerosis induced by a high-fat, high-cholesterol diet.\(^6,7\) It has been reported,\(^6,7\) however, that vWd pigs are susceptible to atherosclerosis of the coronary arteries produced by balloon catheter-induced injury of coronary arteries combined with an atherogenic diet. This paper presents the results of studying the development of coronary atherosclerosis in normal and vWd pigs:

1) as a spontaneous process in a long-term prospective study up to 4 years; and 2) as an induced process by an atherogenic diet fed up to 6 months.

Methods

Swine

The original Poland-China pigs with vWd\(^1\) were crossed with Yorkshire-Hampshire pigs to establish our present colony. Our control pigs were also a
mixture of Poland-China and Yorkshire-Hampshire. All pigs were housed at the Mayo Institute Hills Farm and all studies were carried out concurrently. The study and procedures followed in these pigs were approved by the institutional guidelines on Animal and Human Research.

Spontaneous Coronary Atherosclerosis Study
Fifteen newborn pigs, five control and 10 with homozygous vWd, were fed maternal milk supplemented with cow’s milk until 3 months of age, at which time they began to receive a normal diet (low in fat and cholesterol), approximately 500 g/40 kg body weight, that was continued for up to 4 years (Table 1). Five pigs with vWd bled to death at 5 and 8 months of age, and three at 12 months of age and were excluded from the study. Thus, there remained five normal control pigs (three males and two females), and five vWd (four males and one female), which were under surveillance for more than 1 year. Thereafter, in each instance that a vWd pig bled to death, the control animal closest in age was killed. The mean age of the control pigs was 37 months (range 20–47) and of the vWd pigs, 36 months (range 23–52).

Diet-Induced Atherosclerosis Study
Fourteen additional pigs were subjected to an atherogenic, high-fat, high-cholesterol diet (Table 1), approximately 500 g/40 kg body weight, for 6 months, beginning at the age of 3 months. Nine were normal control pigs (five males and four females) and five suffered from homozygous vWd (two males and three females). Two vWd pigs bled to death at 3 and 4 months, and in each instance a control animal was killed.

Hemostatic and Lipid Laboratory Data
The hemostatic data (Table 2) during the life of normal and vWd pigs in the low-fat, low-cholesterol diet and in the high-fat, high-cholesterol diet were obtained by previously described methods. Serum lipids, cholesterol, triglycerides, and lipoproteins were also determined by previously described methods.

Table 1. Experimental Diets

<table>
<thead>
<tr>
<th>Diet components</th>
<th>Low-fat and low-cholesterol diet*</th>
<th>High-fat and high-cholesterol diet†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed</td>
<td>532</td>
<td>532</td>
</tr>
<tr>
<td>Soybean oil meal</td>
<td>190</td>
<td>190</td>
</tr>
<tr>
<td>Dextrose</td>
<td>240</td>
<td>10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>Tallow</td>
<td>—</td>
<td>200</td>
</tr>
<tr>
<td>Hog bile extract (hog gall, 75% solids)</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Salt, iodized</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Vitamins, trace minerals, and antibiotics‡</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

*Of the total calories, fat contributed to 6% of the calories and essential fatty acids to 3.5%. (Data from Spontaneous Atherosclerosis Study.)
†Of the total calories, fat contributed to 48% of the calories and essential fatty acids to 7%. (Data from Diet-Induced Atherosclerosis Study.)
‡Vitamins in both diets included choline, thiamine, pantothenate, riboflavin, niacin, pyridoxine, folacin, B12, K, A, D3, and E. Trace elements in the practical diet included Zn, Fe, Mn, I and Co. The antibiotic Anero SP250 (Allerd Mills, Inc., Waynes Division, Chicago, Illinois), which contains chlortetracycline, sulfamethazine, and penicillin, was given.

Table 2. Hemostatic Data during Life

<table>
<thead>
<tr>
<th>Test</th>
<th>Low-fat and low-cholesterol diet*</th>
<th>High-fat and high-cholesterol diet†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>vWd</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Platelet count (µl)</td>
<td>271,000</td>
<td>457,000</td>
</tr>
<tr>
<td></td>
<td>±95,000</td>
<td>±93,000</td>
</tr>
<tr>
<td>Partial thromboplastin time (sec)</td>
<td>27 ± 2</td>
<td>34 ± 6</td>
</tr>
<tr>
<td>Bleeding time (min)</td>
<td>4.6 ± 2.1</td>
<td>&lt;15</td>
</tr>
<tr>
<td>VIII:C (% of normal)</td>
<td>124 ± 15</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>VII:RAG (% of normal)</td>
<td>124 ± 34</td>
<td>&lt;3</td>
</tr>
<tr>
<td>VII:RWF (% of normal)</td>
<td>111 ± 34</td>
<td>0</td>
</tr>
</tbody>
</table>

VIII:C = procoagulant; VII:RAG = related antigen; VII:RWF = ristostin cofactor; vWd = von Willebrand disease. Values are means ± sd.
*Data from Spontaneous Atherosclerosis Study.
†Data from Diet-Induced Atherosclerosis Study.
Morphologic Studies

After the removal of the aorta, the entire heart of each pig was preserved in 10% formaline solution. All of the hearts and coronary arteries were analyzed blindly and at the same time. The four major coronary arteries (left main, left anterior descending, left circumflex, and right coronary arteries) and their largest branches were examined by a series of cross-sectional cuts, and proximal and distal segments (5 mm lengths) were processed for light microscopy. These histologic sections of all arteries were stained with hematoxylin-eosin and elastic-Van Gieson stain. Coronary artery sections prepared by the elastic stain were evaluated by light microscopy and assigned a grade from 0 to 4 based on the percentage of reduction of the cross-sectional lumen area: 0 = no reduction; 1 = <25%; 2 = 25% to 50%; 3 = 51% to 75%; and 4 = >75%. Normal or the maximally available arterial lumen (grade 0) referred to the area bound by the internal elastic lamella. It has been shown that there was an excellent agreement between the percentage of reduction of the luminal area assessed visually by microscopy and that determined by video planimetry. Any fixation artifact such as tissue shrinkage was minimal and would apply to all sections. Such artifacts are common to all morphologic studies and do not invalidate the quantitation of arterial disease.

The cardiac valves were examined grossly. The myocardium was studied grossly, and tissue samples from the right ventricle free wall, left ventricle free wall, and anterior papillary muscle were stained with hematoxylin-eosin and PAS and studied by light microscopy.

Results

Spontaneous Coronary Atherosclerosis Study

The heart showed minor variations in size; the thickness of the ventricular wall of the left ventricle was 20 to 30 mm (average 26 mm) and of the right ventricle, 6 to 12 mm (average 9 mm). The coronary arteries showed a right dominant pattern in all ten hearts, and the vessels were of large caliber. Gross examination revealed no stenosing disease in any vessel examined, although in most hearts the vessel walls appeared uniformly thick. The left main artery and proximal 1 to 2 cm of the right coronary artery were elastic arteries; the remainder of the vessels were typical muscular arteries, with a prominent tunica media. Microscopic examination of the coronary arteries revealed that small myointimal cushions were present at most branching points of the vessels, but diffuse intimal proliferation (Grade 1) was absent or minimal in all arteries. There were some arteries, particularly the distal left main, that showed a sphincter-like focal proliferation of the inner media.

Cardiac valves, when intact, were examined grossly. There was no evidence of disease, although the mitral valve leaflets were somewhat redundant in all hearts. The tricuspid valve was less frequently affected. The myocardium showed no gross evidence of scarring or acute lesions. Fat infiltration was evident, particularly in the papillary muscles and right ventricle wall. From microscopic observation, there was no evidence of disease or ischemic injury to the heart. A thin, subendocardial fat layer was present in sections from the left ventricle. Scattered "microfoci" of fat were present throughout the myocardium, more numerous and larger in the right ventricle. Arterioles and intramural arteries were uniformly thick-walled (mostly due to a thick muscular media) with some variability in degree between hearts and within a section.

We conclude that none of the pigs' hearts, control or vWd, showed atherosclerosis of the coronary arteries or myocardial lesions (Table 4, Figure 1).
Diet-Induced Coronary Atherosclerosis Study

The hearts in this group had a wall thickness of 13 to 22 mm (average 17 mm) in the left ventricle, and 4 to 9 mm (average 6 mm) in the right. As in the spontaneous atherosclerosis study, the coronary arteries showed a right dominant pattern in all 14 hearts. In some hearts the vessel wall appeared uniformly thick. In two hearts (one control and one vWd) there was evidence of stenosing disease present as soft, fatty, yellow plaques along the inner surface of the coronary vessels. Microscopically, the myointimal cushions were larger and more frequently seen than in the group with spontaneous disease. Only in the two hearts mentioned above, one control (Figure 2) and one vWd (Figure 3), was intimal thickening com-

Figure 1. Study of spontaneous disease. Completely normal left circumflex coronary artery in a normal control pig. Hematoxylin-eosin stain. × 40.

Figure 2. Study of diet-induced disease. Left circumflex coronary artery showing severe atherosclerosis in a normal control pig. Hematoxylin-eosin stain. × 40.
posed of smooth muscle cells and foam cells large enough to cause localized reduction in the cross-sectional lumen of the artery. However, the stenoses became moderate or severe (Grade 3 or 4, 50% to 80% stenosis) only in the control pig, being present in the left circumflex, left anterior descending, and acute marginal and posterior descending branches of the right coronary artery, with a corresponding thinning of the underlying tunica media (Table 5). It is of interest that the two pigs with coronary atherosclerotic disease were the animals that developed the highest degree of abdominal aortic lesions within their respective groups; both had normal hemostatic and lipidic patterns within their groups.

Table 5. Reduction in Luminal Area in Diet-Induced Coronary Atherosclerosis

<table>
<thead>
<tr>
<th>Normal (n = 9)</th>
<th>vWd (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD</td>
<td>LCX</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(19%)</td>
<td>(17%)</td>
</tr>
</tbody>
</table>

See Table 4 for explanation of data.

The gross analysis of cardiac valves was unremarkable, as in the previous study group. Similarly, the myocardium was grossly and microscopically unremarkable as in the previous study group.

We concluded that only one vWd pig showed mild coronary atherosclerosis and one normal pig had moderate-to-severe coronary atherosclerosis, both without myocardial lesions. The hearts of all the remaining pigs were free of coronary atherosclerosis.

**Discussion**

There is increasing evidence that interaction between platelet and vessel wall may play a role in the development of atherosclerosis. In vWd, the absence of Willebrand factor may cause an impairment of interaction between platelet and vessel wall resulting in resistance to atherosclerosis. Indeed, we have previously reported that pigs with homozygous vWd that were fed a high-fat, high-cholesterol diet for up to 6 months were less susceptible to the development of aortic atherosclerosis than were normal pigs; such a finding has been confirmed by Griggs et al. We have also observed that vWd pigs fed a low-fat, low-cholesterol diet for up to 4 years have a pronounced resistance to the development of spontaneous aortic atherosclerosis.

In view of the reduced development of aortic atherosclerosis in vWd pigs when compared with normal pigs, we expected to find reduced incidence and extent of coronary atherosclerosis in the vWd animals, both in the long-term perspective study and in the short-term, diet-induced atherosclerosis study. However, as shown in the present study, we ob-
served: 1) when normal control and vWd pigs were given a low-fat, low-cholesterol diet for up to 4 years, there were no signs of localized or stenotic coronary atherosclerosis in either group; and, 2) when normal control and vWd pigs were given a high-fat, high-cholesterol diet for up to 6 months, only two pigs showed mild (von Willebrand pig) and moderate to severe (normal pig) coronary atherosclerosis, but without myocardial lesions; the hearts of all the remaining pigs were free of stenotic coronary atherosclerosis.

According to our prospective studies, it is of interest that the normal control pigs, despite developing significant atherosclerotic disease of the distal abdominal aorta, developed very little or no atherosclerotic disease of the coronary arteries. The most likely explanation for this finding is that coronary athero-sclerotic disease develops at a later stage than atherosclerotic disease of the abdominal aorta. Indeed, this time sequence in the development of atherosclerotic disease of the aorta and coronary arteries has been well documented in humans.\(^2,26\) Therefore, for evaluation of both groups of arteries in the pig model, a longer follow-up study would be necessary. Since hemodynamic factors appear to influence the development of atherosclerosis in diffuse lesions, it may be of importance in such a time sequence; that is, hemodynamic factors may be responsible for the earlier development of atherosclerosis in the large distal abdominal aorta in contrast to the later development of atherosclerosis in the smaller arterial regions, such as the coronary arteries.

In a recent study, Griggs et al.\(^6\) reported that pigs with vWD are susceptible to intimal hyperplasia and atherosclerosis of the coronary arteries produced by balloon catheter-induced injury combined with an atherogenic diet. The experimental design of their study differed significantly from our study. As they have indicated, in their model the degree of intimal hyperplasia and atherosclerosis was probably the result of the degree of the acute injury; thus, the balloon procedure causes both superficial denudation of endothelium and, at other areas, deeper medial injury.\(^27\) In contrast, in our long-term study no acute arterial injury was produced. Most likely the mechanisms leading to vascular disease are quite different in either study model, the advantage of ours being that it is probably closer to the human model since no acute vascular injury is produced.

In summary, pigs with homozygous vWD are resistant to atherosclerosis of the aorta. To assess the resistance or susceptibility of coronary disease in these pigs, a longer follow-up would be necessary.

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