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.

(Arteriosclerosis 5:67-73, January/February 1985)

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.1 They have a serious autosomally transmitted bleeding tendency,2 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 atherosclerosis4,5 and to arteriosclerosis induced by a high-fat, high-cholesterol diet.4,6 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 vWd1 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 3 compares the results preceding and during the high-fat, high-cholesterol diet. During this diet, serum cholesterol was increased about fivefold, and the levels were not significantly different between controls and vWd pigs. Serum triglycerides and the corresponding lipoprotein fractions were also about the same in the controls and vWd pigs. In both control and vWd pigs, 70% of the cholesterol was in the LDL and 70% of the triglycerides in the VLDL.

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>150</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, B₃₂, K, A, D₃, and E. Trace elements in the practical diet included Zn, Fe, Mn, I and Co. The antibiotic Anero S250 (Allied 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>(μl)</td>
<td>Normal (n = 5)</td>
<td>vWd (n = 5)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>271,000 ± 95,000</td>
<td>457,000 ± 93,000</td>
</tr>
<tr>
<td>Partial thromboplastin time</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>VIII:RWF (% of normal)</td>
<td>111 ± 34</td>
<td>0</td>
</tr>
</tbody>
</table>

VIII:C = procoagulant; VII:RAG = related antigen; VIII:RWF = ristocetin cofactor; vWd = von Willebrand disease. Values are means ± so.
*Data from Spontaneous Atherosclerosis Study.
†Data from Diet-Induced Atherosclerosis Study.
CORONARY ATHEROSCLEROSIS IN PIGS  Fuster et al. 69

Table 3. Average Serum Cholesterol and Serum Triglycerides in the Diet-Induced Atherosclerosis Study

<table>
<thead>
<tr>
<th>Serum lipids</th>
<th>Normal</th>
<th>vWd</th>
<th>Normal</th>
<th>vWd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>109±13</td>
<td>88±15</td>
<td>631±150</td>
<td>481±59</td>
</tr>
<tr>
<td>VLDL</td>
<td>6±3</td>
<td>7±3</td>
<td>47±38</td>
<td>17±8</td>
</tr>
<tr>
<td>LDL</td>
<td>69±9</td>
<td>48±7</td>
<td>495±109</td>
<td>406±33</td>
</tr>
<tr>
<td>HDL</td>
<td>34±8</td>
<td>33±8</td>
<td>89±22</td>
<td>59±19</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>50±19</td>
<td>46±25</td>
<td>66±17</td>
<td>53±38</td>
</tr>
<tr>
<td>VLDL</td>
<td>30±20</td>
<td>31±19</td>
<td>39±13</td>
<td>38±34</td>
</tr>
<tr>
<td>LDL</td>
<td>11±4</td>
<td>7±3</td>
<td>8±2</td>
<td>8±4</td>
</tr>
<tr>
<td>HDL</td>
<td>9±4</td>
<td>8±3</td>
<td>19±7</td>
<td>7±2</td>
</tr>
</tbody>
</table>

Values are means ± sd. VLDL = very low density; LDL = low density lipoprotein; HDL = high density lipoprotein.

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 PAS and studied by light microscopy. These histologic sections of all arteries were stained with hematoxylin-eosin and PAS and studied by light microscopy.

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).

Table 4. Reduction in Luminal Area in Spontaneous Development of Coronary Atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>LAD</th>
<th>LCX</th>
<th>RCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 5)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>vWd (n = 5)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Values are expressed as percentage of reduction (see in text, Grade 0 to 4). LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RCA = right coronary artery; NS = no statistically significant difference between groups after applying the nonparametric sum rank test (Mann-Whitney U Test).

# = Arbitrary index of the summation of the observed luminal area reduction (Grades 0 to 4) over the maximum possible grade of lesion (Grade 4).
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.
Figure 3. Study of diet-induced disease. Left circumflex coronary artery showing mild intimal thickening in a von Willebrand 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;4 both had normal hemostatic and lipidic patterns within their groups.

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.19-21 In vWd, the absence of Willebrand factor may cause an impairment of interaction between platelet and vessel wall22-24 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;4 such a finding has been confirmed by Griggs et al.6 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.5

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 atherosclerotic 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 human.

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 diath-, they 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.

References


18. Inser JM, Wu M, Virmani R, Jones AA, Roberts WC. Comparison of degrees of coronary arterial luminal narrowing determined by visual inspection of histologic sections under magnification among three independent observers and comparison to that obtained by video planimetry: an analysis of 559 five-millimeter segments of 61 coronary arteries from eleven patients. Lab Invest 1980;42:566-570


21. Fuster V. Platelets and atherosclerosis: Role of platelets in the development of atherosclerotic disease and possible interference with platelet inhibitor drugs. Scand J Haemost,


Index Terms: coronary atherosclerosis • pig • von Willebrand disease
Spontaneous and diet-induced coronary atherosclerosis in normal swine and swine with von Willebrand disease.
V Fuster, J T Lie, L Badimon, J A Rosemark, J J Badimon and E J Bowie

doi: 10.1161/01.ATV.5.1.67

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/5/1/67

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/