Comparison of pathologic and angiographic findings in a porcine preparation of coronary atherosclerosis

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ABSTRACT  Coronary atherosclerosis was induced in Yorkshire swine by diet-induced hyperlipidemia and balloon intimal abrasion of a coronary artery. Severe stenoses pathologically similar to the lesions of human atherosclerosis were seen after 8 months of the atherogenic regimen. To examine the relationship between the angiogram and pathology in the assessment of the extent and location of coronary atherosclerosis, antemortem angiographic results were compared with results of pathologic examination. Vernier caliper measurements of the coronary angiogram were compared with results of morphometric evaluation of perfusion-fixed coronary arteries. Isolated focal stenoses were correctly localized and quantified, as were focal lesions within vessels diffusely diseased. Both overestimation and underestimation of lesions occurred at bifurcation sites. Diffuse disease without focal stenoses was not well demonstrated angiographically. Vessels that were angiographically thought to be normal or only minimally diseased demonstrated significantly larger lumens angiographically than pathologically. This is believed to be due to fixation and paraffin-processing artifact, even though fixation was performed by perfusion at physiologic pressure. The demonstration of an excellent correlation between the luminal size as determined angiographically and morphometrically at sites of focal obstruction confirms the value of quantitation of coronary angiograms in vivo as a diagnostic tool in coronary atherosclerosis.


CORONARY ANGIOGRAPHY has become the most widely used tool for determining the status of the coronary arteries in man. Controversy exists, however, over the relationship between the angiographic and the histopathologic assessments of the extent of atherosclerosis.1–6 Arnett et al.,1 in studies comparing meticulous pathologic examinations of nonperfusion-fixed hearts to antemortem angiograms obtained at various times relative to death of the patient, has stated that angiography underestimates the severity of coronary atherosclerosis. More recently, Marcus et al.7 studied a primate model of atherosclerosis and suggested that nondistended pathologic arterial specimens do not accurately reflect the physiologic status of these vessels.

The current study uses a model of coronary atherosclerosis in swine to address the relationship between the degree of atherosclerosis as assessed by antemor-

tem coronary angiographic examination and that seen in pathologic examination of the coronary arteries fixed by perfusion at physiologic pressure.8–13

Methods

Seven 12 to 15-week-old male Yorkshire pigs from a stable breeding stock were studied. After initial determination of plasma lipid levels, the animals were placed on a high-cholesterol, high-fat diet (table 1), which was continued for the duration of the study. At the end of 2 weeks, to allow for the development of hyperlipidemia, determination of lipid levels, including levels of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and triglycerides, was performed with a Beckman airfuge ultracentrifuge and the technique of Bonzert and Brewer.14

The animals then underwent cardiac catheterization and balloon abrasion of the proximal left anterior descending coronary artery (LAD) by the following technique: Each animal was sedated with 35 mg/kg ketamine hydrochloride. After intubation, anesthesia was maintained with a Bird respirator (Palm Springs, CA) with 1% halothane, 30% nitrous oxide, and 70%
TABLE 1
Atherogenic diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>g/day</th>
<th>kcal/day</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut oil</td>
<td>15.4</td>
<td>135.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Sodium cholate</td>
<td>10.3</td>
<td>91.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>15.4</td>
<td>144.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Lard</td>
<td>190.4</td>
<td>1711.8</td>
<td>48.5</td>
</tr>
<tr>
<td>Milk powder</td>
<td>51.4</td>
<td>283.2</td>
<td>8.0</td>
</tr>
<tr>
<td>Vitamin mix (Teklad Mills)</td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt mix (U.S. Biochemical Co.)</td>
<td>15.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mash (Agway)</td>
<td>341.8</td>
<td>1162.1</td>
<td>32.9</td>
</tr>
<tr>
<td>Total</td>
<td>650</td>
<td>3529.1</td>
<td></td>
</tr>
</tbody>
</table>

The animals were kept at our institution for observation for 1 to 2 days after the procedure. Long-term housing was provided at the Tufts University School of Veterinary Medicine farm facility (Grafton, MA). At 4 months intervals, the animals underwent repeat lipid and coronary arteriographic studies. After completion of these studies, the animals were killed (two at 4 months and five at 8 months) by perfusion of 2.5% gluteraldehyde (two animals) or 10% formalin (five animals) into the aortic root at mean arterial pressure for 20 min following anesthetization. The aorta was cross-clamped distal to the arch vessels before perfusion was initiated. The hearts were removed intact and were maintained in fixative until processing.

The coronary arteries were dissected free from the myocardium and were sectioned at 3.0 mm intervals. Careful maps of the blocks with definitions of branch points were made for comparison to the angiograms. The sections were then paraffin embedded. For quantitation of the size and components of lesions, a representative hematoxylin and eosin-stained section from each block was projected onto a vertical surface, using a 4 × objective in a Leitz Tele-Promar 500 projecting microscope, 3.5 feet from the vertical surface.

Elements of the lesion, including the ideal and the residual lumen, the area occupied by lesions, and areas of necrosis and calcification within the lesion, as projected, were traced and the tracings were subjected to quantitative analysis using a Zeiss MOP-3 digital image analyzer system.

Ideal luminal area was calculated from the perimeter of the internal elastic membrane, assuming that the true vessel shape was circular. The intimal area within this membrane was considered to be the lesion area and was planed directly. The difference between these values was residual luminal area, from which luminal diameter could be calculated. Areas of calcification or necrosis were determined directly by digital planimetry. Comparison with adjacent areas was not performed for the purpose of calculation of percent diameter reduction.

The antemortem angiograms obtained within 24 hr of the animals being killed were independently evaluated both quantitatively and qualitatively by two experienced angiographers (B. H. W., I. S. O.). For each angiogram, the epicardial coronary arteries and their branches were reviewed for the presence or absence of disease and six areas of focal narrowing were indentified. The films were projected on a screen with a Vanguard XR-35 projector, resulting in a total magnification of 5 ×. The magnification correction factor was calculated with a standard 1 cm × 1 cm grid.
positioned at the level of the left ventricle. The areas of focal narrowing were measured with a Vernier caliper with 0.1 mm divisions and their locations were identified relative to vessel branch points. Segments proximal or distal to lesions were measured in a similar manner. Sites were chosen that could be easily identified to facilitate comparison between the angiographic and pathologic data. Percent stenosis, based on these values, was not compared. If no areas of focal disease were identified, locations that could be clearly defined on the basis of proximity to a branch point were measured by the same technique for comparison to the pathologic specimens.

To further evaluate the angiographic-pathologic discrepancy found in the study animals, three normal animals were studied and 12 angiographically identified sites were examined. The animals were sedated, anesthetized, and prepared and catheters were inserted as described above. An initial angiogram was obtained. The chest and pericardium were then opened and a second angiogram was obtained. A segment of the LAD was then dissected free and the external dimension was measured with a Vernier caliper. Coronary angiography was again performed. After perfusion fixation by the same procedure described above, similar measurements and postmortem angiographic examinations were performed. The coronary arteries were then subjected to the same morphometric evaluation described above.

Results

The plasma lipid levels in the study animals are listed in table 2. Their baseline lipids were distributed among the lipoprotein classes in much the same way as in humans. The absolute values, however, were lower. With the antherogenic diet, a marked increase in total cholesterol, LDL, HDL, and VLDL occurred. Although HDL₃ was not measured, the elevation in HDL is likely to be in that fraction, as has been shown in other lipid-fed animal preparations.¹⁷¹⁸ These elevations persisted throughout the study. There was no significant change in triglyceride levels.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tr>
<td>Plasma lipoprotein levels (mean ± SD)</td>
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</table>

<table>
<thead>
<tr>
<th>Total cholesterol (mg%)</th>
<th>LDL (mg%)</th>
<th>HDL (mg%)</th>
<th>VLDL (mg%)</th>
<th>Triglyceride (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>116 ± 59</td>
<td>68 ± 28</td>
<td>52 ± 14</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>2 weeks</td>
<td>568 ± 287</td>
<td>322 ± 285</td>
<td>118 ± 78</td>
<td>128 ± 125</td>
</tr>
<tr>
<td>4 months</td>
<td>559 ± 256</td>
<td>359 ± 249</td>
<td>116 ± 44</td>
<td>60 ± 55</td>
</tr>
<tr>
<td>8 months</td>
<td>589 ± 261</td>
<td>283 ± 94</td>
<td>186 ± 120</td>
<td>124 ± 80</td>
</tr>
</tbody>
</table>

Two animals were studied 4 months after the abrasion procedure. Ninety-five coronary arterial sections were examined morphometrically. Forty-five sections had no disease. The other 50 sections (53%) had pathologic evidence of disease, with a mean lesion area of 0.25 ± 0.35 mm² (range 0.003 to 1.5 mm²). This represents a mean luminal encroachment of 7.8% in these segments (range 0.3% to 42.1%). Eleven sections (12%) had intimal calcification (mean 7.7% of the lesion area in those segments). No necrosis was seen in any of these lesions.

Five animals were studies 8 months after the abrasion procedure. Two hundred and six sections were examined morphometrically. One hundred and eighty-two (88%) of the sections had lesions. The mean lesion area in these sections was 2.75 ± 2.68 mm² (range 0.004 to 14.8 mm²). The mean luminal encroachment was 48.5 ± 35% (range 0.02% to 100%). Of the 182 segments with disease, 102 had calcification (56%) (9.5 ± 11.5% of the lesion area, range 0.2% to 54.2%). Similarly, 122 (67%) sections had areas of necrosis (mean 43.5 ± 21.3% of the lesion area).

Figure 1 is a representative section from one of the animals studied at 8 months. It demonstrates the major features of complex atherosclerotic disease in man. There is a fibrous cap, intimal necrosis, calcification, and cholesterol clefts. Variable amounts of foam cells are seen in these animals. The two animals studied at 4 months had more focal disease, less overall involvement, and less complex disease, with some calcification but without necrosis.

No evidence of myocardial infarction was found on gross examination of any heart. No electrocardiographic evidence of myocardial infarction was indentified on serial tracings.

Comparison of angiographic and pathologic results

Atherosclerotic animals. Three focal areas of stenosis could be identified in the two animals studied at 4 months and comparable angiograms were available for two of them. The third was in the right coronary artery of an animal in which, for technical reasons, no angiogram of that vessel was obtained. One of the focal lesions was correctly identified both as to significance and location. The other lesion occurred at a bifurcation and was not identified by angiography.

Ten of 12 vessels from the five animals studied at 8 months were described angiographically as having some or multiple areas of irregularity (figure 2). Pathologically, nine of these 10 vessels had severe, diffuse atherosclerosis, defined as greater than 75% of segments being involved with severe atherosclerosis similar to that shown in figure 1. The remaining vessel had...
mild disease but was diffusely involved. The other two vessels were pathologically normal. Two focal lesions within diffusely diseased vessels were correctly identified angiographically. An additional lesion, at a bifurcation, was thought to be present angiographically, but was not demonstrated pathologically. In one animal, an area of ostial tapering was commented on as unusual, but the readers could not decide if this represented disease or normal variation. On pathologic evaluation, a significant lesion was found. Two vessels that were interpreted as angiographically normal were “normal” pathologically. No lesion, focal or otherwise, resulting in less than 20% reduction in luminal area as determined pathologically was detected by angiography. Similarly, no area of greater focal stenosis was missed in these animals.

When the luminal areas and diameters of the vessels determined angiographically and morphologically were compared (tables 3 and 4), there was near identity in the values for focal lesions defined angiographically. In areas without focal lesions, however, whether or not the vessel was normal or diffusely diseased, the antemortem angiogram demonstrated significantly greater luminal diameter and area as compared with the pathologic specimens (p <.001 for both diameter and area).

Normal animals. There were no significant differences among the angiographically determined luminal sizes at the first three determinations (baseline, open chest/pericardium, and after isolation of a segment of the LAD). There was, however, a mean 35.7 ± 5.2% reduction in luminal diameter after fixation. There was an additional 12.0 ± 4.5% diminution of measured luminal size when the pathologic specimens were compared before and after paraffin imbedding. This would completely account for the 44% difference in angiographic and pathologic assessments of the diffusely diseased and normal segments described in table 4. Although the time sequence of fixation and the use of perfusion for fixation differs from the method routinely used in humans, it seems likely that similar (or greater) fixation artifacts occur in the examination of human pathologic specimens.

Discussion

Arnett et al.1 studied 10 patients who had undergone coronary angiography and bypass graft surgery within 69 days of their death. Although the hearts were not perfused, the pathologic specimens were quantitated in a manner similar to that used in our study. Only per-
cent reductions in area or diameter were compared; residual luminal size was not. As seen in our pigs, diffuse disease was present. Arnett et al. conclude that comparing clearly abnormal segments to less but still abnormal adjacent segments does not accurately reflect the status of the artery. On the other hand, since absolute vessel size is not reported, only inaccuracies of the angiograms are discussed, and no comment as to potential errors of pathologic assessment is made.

Vladover et al.\textsuperscript{5} reached similar conclusions after study of a comparable group of patients. Although a statement was made that some plaques may be intramural during life, no evidence was supplied to support this concept. This study also used nonperfused specimens, and eccentric luminal contours (slit-like lumen) were the major explanation offered for the observed discrepancies.

Hutchins et al.\textsuperscript{3} demonstrated reasonable correlations between antemortem and postmortem coronary angiograms in determining site and percent stenosis of lesions. Luminal diameters or areas were not reported. They also compared postmortem angiograms and pathologic specimens. The hearts were fixed at 20 to 40 cm H\textsubscript{2}O; the coronary arteries were not specifically perfused. The major errors in detection of lesions occurred at ostia and branch points. These authors believed that the major sources of error in the antemortem angiograms were radiographic overlap and catheter-induced arterial spasm.

To evaluate the relationship between angiographic and pathologic assessment of atherosclerosis, techniques of similar quantitative ability must be used. Our technique for angiographic evaluation is similar to that described by Gensini et al.\textsuperscript{19} This type of evaluation allows for direct comparison of luminal size between angiograms and pathologic specimens while obviating the inaccuracies inherent in discussing percent diam-

ter stenosis, although human pathologic specimens are not routinely perfusion fixed.

In the present study, errors in the evaluation of focal lesions occurred only at sites of bifurcation or at the coronary ostia; no other focal lesions were misinterpreted. This is in accord with the findings of Hutchins et al.\textsuperscript{3} that the overlap of vessels makes bifurcation sites particularly difficult to interpret. Furthermore, the normal tapering of the porcine coronary ostium makes evaluation of that segment more difficult. However, pathologically proven diffuse lesions of major severity and extent were missed completely. This presumably results from the visual interpretation of stenosis in the angiogram as the difference between markedly narrowed and adjacent less narrowed sites without taking into account the absolute luminal size. In addition, there is evidence that atherosclerosis is associated with vessel dilatation, and therefore, even in diffusely diseased arteries the lumen may appear angiographically normal.\textsuperscript{20}

Our data demonstrate that at sites of focal narrowing, there is excellent agreement between the luminal size determined angiographically and that observed pathologically. This finding validates coronary angiography as a measure of clinical disease in humans, in whom hemodynamically significant disease is often segmental in nature.

In contrast, there was considerable discrepancy between angiographic and pathologic assessment of segments that were read as either normal or mildly diseased angiographically. This discrepancy was uniformly in the direction of smaller luminal size as assessed pathologically. In a study of left main coronary

\begin{table}[h]
\centering
\caption{Luminal area and diameter of angiographically defined focal lesions}
\begin{tabular}{ccc}
\hline
\textbf{Angio} & \textbf{Path} & \textbf{Angio} & \textbf{Path} \\
\textbf{diameter} & \textbf{diameter} & \textbf{area} & \textbf{area} \\
\textbf{(mm)} & \textbf{(mm)} & \textbf{(mm\textsuperscript{2})} & \textbf{(mm\textsuperscript{2})} \\
\hline
1.4 & 1.3 & 1.5 & 1.4 \\
1.0 & 0.9 & 0.8 & 0.7 \\
2.3 & 2.1 & 4.1 & 3.6 \\
2.0 & 2.0 & 3.2 & 3.0 \\
1.6 & 2.0 & 1.5 & 1.8 \\
2.0 & 2.0 & 3.1 & 3.0 \\
\textit{r} = 0.91 & \textit{r} = 0.99 \\
y = 0.95x + 0.1 & y = 0.86x + 0.21 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Luminal diameter and area of sites defined angiographically as normal or irregular}
\begin{tabular}{cccc}
\hline
\textbf{Angio} & \textbf{Path} & \textbf{Angio} & \textbf{Path} \\
\textbf{diameter} & \textbf{diameter} & \textbf{area} & \textbf{area} \\
\textbf{(mm)} & \textbf{(mm)} & \textbf{(mm\textsuperscript{2})} & \textbf{(mm\textsuperscript{2})} \\
\hline
\textbf{Diseased vessels} & & & \\
2.4 & 1.6 & 4.5 & 2.0 \\
2.7 & 1.6 & 5.7 & 2.1 \\
3.0 & 1.2 & 7.1 & 1.1 \\
2.2 & 0.6 & 3.8 & 0.3 \\
2.0 & 1.1 & 3.1 & 0.9 \\
\textbf{Normal vessels} & & & \\
4.2 & 2.3 & 13.9 & 4.2 \\
2.1 & 1.4 & 3.5 & 1.5 \\
3.2 & 2.3 & 8.3 & 4.3 \\
4.0 & 2.3 & 12.6 & 4.1 \\
\textit{p} < .001 & \textit{p} < .001 \\
y = 0.6x - 0.14 & y = 0.33x + 0.005 \\
\hline
\end{tabular}
\end{table}
artery disease, Isern et al.21 found a discrepancy between angiographic and morphologic results in 71% of cases. Underestimation by angiography was found chiefly in markedly narrowed segments, whereas overestimation was found in the less narrowed segments. Zarins et al.22 demonstrated a 20% to 31% reduction in luminal diameter when antemortem femoral and aortic angiograms were compared with pathologic specimens.22 The major shrinkage occurred with fixation (22%), with an additional 11% decrease related to paraffin processing. Similarly, Bahr et al.23 showed 30% to 40% reduction in aortic size related to the preparation of pathologic specimens. The coronary vessels, however, may react differently than the large muscular arteries.

Siegel et al.24 studied 12 human, nonperfused hearts. In vessels with minimal disease there was a 35% reduction in luminal area, the majority of which was related to paraffin processing, not fixation (25% vs 10%). There was only 7.7% total shrinkage in vessels that were moderately to severely diseased, suggesting that more severely diseased segments of the coronary arteries react less to fixation and processing. Our data in normal animals demonstrate a 44% reduction in luminal size with perfusion fixation and processing. The major change resulted from the fixation process in these vessels. The difference between our data and Siegel’s may be related to use of perfusion fixation rather than immersion as well as to the fact that our initial measurements were made antemortem. In our diseased animals, focal lesions did not show this reduction in measurement as a result of fixation and processing, suggesting that because of extensive fibrosis and calcification these areas could no longer react to the fixation process.

We believe that angiography more accurately reflects luminal caliber in vivo than does pathologic examination and that the decrease in the size of the lumen in the histologic section is due to processing artifact. It is likely that focal lesions are less capable of reacting to the fixation and embedding process.

References

Comparison of pathologic and angiographic findings in a porcine preparation of coronary atherosclerosis.
B H Weiner, I S Ockene, J Jarmolych, K E Fritz and A S Daoud

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