Development of the PUCA pump
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Chapter 6:
Development of an Animal Model of Selective Coronary Atherosclerosis

Abstract

An animal model of selective coronary atherosclerosis was developed by combining a guide-wire induced endothelial injury and cholesterol-enriched diet. Twelve pigs were subjects of a guide-wire induced endothelial injury of LAD. Six animals (control group A) were fed a standard pig food; the remaining six animals (cholesterol group B) were fed a 6% cholesterol-enriched diet. Three animals from group A were terminated immediately after the endothelial injury (acute control group A0). The other three animals from the control group (chronic control group A4) and all animals from the cholesterol group were terminated four weeks after the injury.

The endothelial surface and the media of the LCX were intact in all animals. Long eccentric areas of endothelial injury were found in the LAD in the acute control group. Numerous fibrous atherosclerotic plaques in LAD were found in the chronic control group as well as in the cholesterol group, but were highly pronounced present in the last group. Lipid accumulation was not found in the plaques of both groups. We concluded that administration of 6% cholesterol diet for a period of six weeks as such is not sufficient to develop coronary atherosclerosis in pigs. Selective coronary atherosclerosis can be induced within four weeks with the same diet when the blood vessel has been injured with a guide-wire.

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Chapter 6

Introduction

Atherosclerosis is the most prevalent disease in the industrialized world, causing over 40% of all deaths in the United States and Western Europe. Each atherosclerotic lesion contains significant elements of three cellular phenomena: smooth muscle proliferation, formation by the proliferated cells of large amounts of connective tissue matrix, and accumulation of intracellular and extracellular lipid. The lesions of atherosclerosis occur primarily in the intima. They include the fatty streak, the fibrous plaque and the so-called complicated lesions: calcification, ulceration, thrombosis, hemorrhage, etc. Subsequent changes occur in the media of the artery underlying the lesion. Although several hypotheses have been proposed, the etiology and pathogenesis of the atherosclerosis are still unknown.

The goal of the present study was to develop a model of selective coronary atherosclerosis in pigs.

Methods

The animal experiments were carried out according to the rules of the Ethical Committee for Animal Experiments of the Faculty of Medical Sciences, University of Groningen.

Experimental protocol

Twelve clinically healthy pigs with a body weight $24.1 \pm 4.2$ kg were premedicated with 30 mg Diazepam IM (Dumex, Hilversum, Holland). Anesthesia was induced with 10 mg/kg Ketamine IM (A.U.V., Cuijk, Holland) and maintained after endotracheal intubation with Isoflurane (ABBOTT, Queenborough, Kent, UK) and oxygen. After heparinization with 5000 IU Heparin IV (Leo, Weesp, Holland), a 9 F sheath-introducer (Cordis, Holland) was inserted into the left main carotid artery. An 8 F Judkins right coronary guiding catheter (Cordis, Roden, Holland) was introduced via the sheath in the left coronary artery ostium under fluoroscopic control. A 0.014" guide-wire (Biotronik, Germany) was introduced into the Left Anterior Descending (LAD) coronary artery via the guiding catheter. The correct position of the guide-wire was controlled by X-ray using the branches of LAD as reference points. The guide-wire was pulled back and introduced again 3 times. The endothelium of the Left Circumflex (LCX) coronary artery was kept intact as a control. The sheath-introducer, the guiding catheter and the guide-wire were removed, and the carotid artery was ligated.

Six of the animals (control group A) were fed a standard pig’s food (Hope Farms, Woerden, Holland). The remaining six animals (cholesterol group B) were fed a 6% cholesterol-enriched diet (Hope Farms, Holland) two weeks before and four weeks
after the injury. Three animals from the control group (acute control group A₀) were terminated immediately after the surgery to analyze the LAD endothelial injury due to the guide-wire introduction. The remaining three animals from the control group (chronic control group A₄) were kept alive as a control. Four weeks after the surgery all animals from the chronic control group and cholesterol group were terminated, the hearts were removed and fixed for histological examination.

**Histological examination**

LAD and LCX were perfusion-fixed with 2% Glutaraldehyde in 0.1 M Na-cacodylate buffer for histological examination with light microscopy (LM), transmission (TEM) and scanning (SEM) electron microscopy. For LM the coronary arteries were impregnated and embedded in Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany), cut at three-micron sections, and stained with toluidin blue. For TEM the materials were cut in pieces 3x3 mm, post-fixed in 1% OsO₄, 1.5% K₄Fe(CN)₆ in PBS, dehydrated in graded alcohol, and embedded in EPON 812 (Serva Feinbiochemica, Heidelberg, Germany). Ultra-thin sections were stained with uranyl acetate and lead citrate, and were examined with Philips EM 201. For SEM the materials were treated with 2% Tannic acid, 2% Guanidine-HCL in water, post-fixed in 1% OsO₄, all at 4°C. After dehydration in alcoholic gradients till 100%, critical point drying was performed in CO₂. The specimens, after a longitudinal cutting, were sputter-coated with 10 nm gold and examined in a JEOL FEG-SEM (Brussels, Belgium).

**Plasma cholesterol measurement**

Total plasma cholesterol level was measured (Mega, Merck, Darmstadt, Germany) in the chronic control group at the day of surgery and before termination of the animal. In the cholesterol group the cholesterol level was measured before the diet was started, at the day of surgery, and before termination of the animal. The results were compared with paired Student’s t-test and are presented in the text as mean ± Standard Deviation (SD). The differences were considered significant at $P<0.05$.

**Results**

The endothelial surface and the media of LCX, used as a control, were intact in both control and cholesterol groups (Table 1). The histological examination of LAD with SEM, LM, and TEM in the acute control group A₀ showed long eccentric areas of endothelial injury (Fig. 1-A, B, C) in all animals. Numerous eccentric fibrous atherosclerotic plaques in LAD were found with LM in the chronic control group A₄ (Fig. 1-E) as well as in the cholesterol group B (Fig. 1-H), but were highly
pronounced present in the last group. Plaques are difficult to judge by SEM, but in group B irregular endothelial layers and cellular aggregates were obviously present (Fig.1-G). The chronic control group A₄ induced little aggregates (Fig. 1-D). The histological examination with TEM in group A₄ (Fig. 1-F) and group B (Fig. 1-I) showed depolarization of large parts of the smooth muscle cells; the internal elastic lamina was partly disrupted and smooth muscle cells were proliferating through it. The atherosclerotic plaques contained smooth muscle cells exposing actin and myosin filaments, as well as some degenerated cells (Fig. 1-I). Collagen fibers were formed between the cells and a thin granular elastic layer was partially covered by newly formed endothelial cells (Fig. 1-I). Thrombus formation or lipid accumulation (e.g., foamy cells) was not observed in the plaques.

**Results from histological examination (Fig. 1):**

A. Disrupted endothelial layer covered by aggregates of platelets and some erythrocytes (arrows) - SEM, acute control group A₀

B. Overview of the artery wall with intact media (M); endothelial cells are not present - LM, acute control group A₀

C. Some smooth muscle cells, the lamina elastica (L), part of a poorly adhered endothelial cell (E), and some platelets (arrow) - TEM, acute control group A₀

D. Disrupted endothelial layer and underlying matrix components (M), some erythrocytes are present - SEM, chronic control group A₄

E. Proliferating muscle cells at the top of the media, and some endothelial cells (arrows) present on the disrupted lamina elastica (E) - LM, chronic control group A₄

F. Poorly attached degenerating endothelial cells - TEM, chronic control group A₄

G. Irregular covering with some re-endothelialization (arrows), platelets and erythrocytes - SEM, cholesterol group B

H. Depolarization of smooth muscle cells (D), lamina elastica (arrows), and arteriosclerotic plaque (A), partly covered with endothelial cells (arrowheads) - LM, cholesterol group B

I. Atherosclerotic plaque containing smooth muscle cells (S), collagen formation (C), and thin elastica lamina (arrowheads) covered by a platelet aggregate. Note a part of the original lamina elastica at the left top site - TEM, cholesterol group B.
Fig. 1. Results from histological examination with Scanning Electron Microscopy (SEM), Light Microscopy (LM), and Transmission Electron Microscopy (TEM) of the acute control group A₀ (A,B,C), the chronic control group A₄ (D,E,F), and the cholesterol group B (G,H,I).
Table 1. Animal groups and results from histological examination (LAD = Left Anterior Descending cor. artery; LCX = Left Circumflex cor. artery)

<table>
<thead>
<tr>
<th>Experiment type</th>
<th>Control group A (n=6)</th>
<th>Cholesterol group B (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>Acute</td>
<td>Chronic</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>LAD</td>
<td>LAD</td>
</tr>
<tr>
<td>Guide-wire endothelial injury</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Smooth muscle cell proliferation</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Formation of connective tissue</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Lipid accumulation</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

The cholesterol values in the animals from the chronic control group A remained almost constant (1.77±0.09 mmol/L basic value versus 1.84±0.07 mmol/L after 4 weeks). The difference in the cholesterol values in the cholesterol group B become significant after six weeks diet (3.07±0.29 mmol/L basic value versus 8.52±3.19 mmol/L after 6 weeks, \( P<0.002 \)).

Discussion

Pigs have proven to be one of the best animal models for studying atherosclerosis. Their cardiovascular system, including the coronary distribution and behavior, is very similar to that in man; the manner in which dietary lipids are adsorbed, transported and metabolized is quite similar as well. Moreover, according to Gal et al., the atherosclerotic plaque in swine appeared histologically similar to the so-called "complex" lesion that is typical of human atherosclerosis, which consists predominantly of collagen and a minor lipid component.

Atherosclerosis can be developed using a high-fat diet or by combining a high-fat diet with an endothelial injury. Thorpe et al. reported development of coronary atherosclerosis in Yucatan microswine induced only by atherogenic diet for up to 310 days. Such a long development period increases significantly the cost of the study, especially when fast-growing farm pigs are used. In the present study the endothelial surface and the media of LCX were intact in all animals from the cholesterol group despite 6 weeks cholesterol-enriched diet. However, fibrous atherosclerotic plaques were found in the LAD in the same animals. The last
proved that that a cholesterol-enriched diet administered for a period of 6 weeks is insufficient to develop atherosclerotic lesions and that the endothelial injury is essential. On the other hand, the obvious difference between the stages of atherosclerotic lesion development in the LAD in the cholesterol group and the chronic control group proved the necessity of cholesterol-enriched diet for the acceleration of the atherosclerotic lesions after an endothelial injury. The last is in line with the results reported by others as well.

The most common method of mechanical endothelial injury uses a PTCA or a Fogarty catheter (so-called balloon endothelial denudation): the catheter is advanced beyond the desired region, the balloon is inflated and the catheter is withdrawn a predetermined distance keeping the balloon inflated. The procedure could be performed several times to ensure the endothelial denudation. In that case the balloon denudation develops large circular endothelial injury and interrupts, even temporarily, the coronary blood flow. The guide-wire endothelial injury, a method used during the present experiments, keeps the coronary blood flow running and prevents development of acute myocardial ischemia, often followed by fibrillation. Furthermore, the present study proved that the introduction of guide-wire causes an eccentric endothelial injury (acute control group), which led later to development of eccentric atherosclerotic lesions (cholesterol group) as well.

In the present study no lipid accumulation was observed in the developed fibrous plaques. It is generally accepted that the lipid is present in the lesions at certain stages of their development. According to Haust, “pure and young” fibrous plaques are largely free of intra- and extra-cellular fat. The accumulation of lipid in lesions after an endothelial injury appears late, when compared with the lipid accumulation in lesions after an intimal injury. According to the recommended histological classification of atherosclerotic lesions we classified the developed lesions as type Vc or fibrotic lesions.

The present model could be used to study atherogenesis as well as for investigations of mechanical properties of the coronary arteries. It should be kept in mind that the plaques developed during the present study were fibrous. Therefore this preparation cannot be used to evaluate the effect of specific interventions on the development of vulnerable plaques. As known, the vulnerable plaques have a large lipid core with a thin fibrous cap, and are the most frequent cause of acute coronary syndromes. Long-term experiments should be performed to study the progress of the developed lesions (lipid accumulation, calcification, etc.). In addition, the present study emphasized the need of a cholesterol-reducing treatment for patients with hypercholesterolemia prior to invasive diagnostic or
therapeutic procedures in the arteries (PTCA, introduction of catheters, guidewires, etc.). This will prevent the development of “iatrogenic atherosclerosis” in patients with hypercholesterolemia.

References
