Swine as a Model of Experimental Atherosclerosis

Świnia jako eksperymentalny model do badań arteriosklerozy

Abstract

In Western countries, lifestyle changes in the past century are considered to be a major factor in obesity and related diseases such as atherosclerosis and diabetes. To gain a better understanding of the relationship between disorders of lipid metabolism and atherogenesis, many animal species have been tested. The most commonly used are laboratory animals such as mice, rats, rabbits, guinea pigs and pigeons. However, the pig is considered a very good model of human atherosclerosis, because it is similar to humans in terms of body size and other physiological features, including its tendency to overeat. Even though there is no perfect animal model that completely replicates human atherosclerosis, the pig seems to be a promising subject for exploring the etiopathogenesis of atherosclerosis (Adv Clin Exp Med 2011, 20, 2, 211–215).

Key words: swine model, experimental atherosclerosis.

In Western countries, lifestyle changes in the past century are considered to be a major factor in obesity and related diseases such as atherosclerosis and diabetes. Excessive consumption of carbohydrates and fat has a profound effect on the strictly regulated energy balance and thereby provoke metabolic disorders, resulting in atherosclerosis and cardiovascular diseases. Atherosclerosis is defined as “a chronic immunoinflammatory, fibroproliferative disease of large and medium-sized arteries fuelled by lipids” [1–3] and is, as Falk expressed it: “by far the most frequent underlying cause of coronary artery disease, carotid artery disease, and peripheral arterial disease” [4]. To gain a better understanding of the relationship between disorders of lipid metabolism and atherogenesis, many animal species have been tested. Commonly used laboratory animals such as mice, rats, rabbits, guinea pigs and pigeons have a lot of advantages as test subjects: low cost, ease of housing, small size, speed of breeding and a well-defined genetic background. Unfortunately wild-type rodents are highly resistant to atherogenesis and have no similarity to human lipid and lipoprotein metabolism; further, they do not develop cardiovascular diseases. Moreover, as Cos et al. pointed out, in guinea pigs “gender and hormonal status affect the extent of the atherosclerotic plaque” [5].
As compared with rats, guinea pigs and pigs, the localization of atherogenic lesions in rabbits is less similar to the localization in humans; and rabbits have no spontaneous atherosclerosis. On the other hand, genetically altered rabbits, such as the Watanabe Heritable Hyperlipidemic, Houston RT and St. Thomas’ Hospital strains, develop massive fulminant atherosclerosis, which is similar to the human genetic defect familial hypercholesterolemia [6]. Pigeons have no Apolipoprotein E (ApoE) B_{48} or chylomicron formation [7]. The pig, however, is a considered a very good model of human atherosclerosis, because it is similar to humans in terms of body size and other physiological features, including its tendency to overeat [8]. Atherosclerosis occurs naturally even on a normal porcine diet and can be exacerbated by modification of the diet [9]. The changes in plasma lipoproteins in response to changes in diet closely resemble those occurring in humans and, like humans, swine transport most cholesterol in low-density lipoprotein (LDL) [8]. As Stender and Zilversmit noted, “It has been well documented that free cholesterol from plasma enters the arterial wall in excess of estriified cholesterol relative to their concentrations in plasma, in both natural and experimental conditions” [10]. In contrast to rodents, swine atherosclerosis, like the human illness, progresses to advanced stages. Diet-induced atherosclerosis alone is rarely fatal; it is only simultaneous erosion or rupture of atherosclerotic plaque superimposed by thrombosis (which occurs at advanced stages) that leads to life-threatening clinical events such as coronary syndromes and stroke [11]. The maintenance of pigs is expensive and requires special facilities that are beyond the capabilities of most laboratories. This has led to a tendency to conduct research on smaller-size strains of this species: various mini-pig breeds or young (sometimes very young) domestic pigs. Studies on atherosclerosis in pigs have varied markedly in terms of the breed, sex and age of the pigs, the hyperlipidemic diet composition (from 1% to 6% cholesterol) and the feeding schedule. Some examples are presented in Table 1.

In all these experiments, regardless of the composition of the high-fat diet, it was relatively easy to induce distinct elevation of the plasma cholesterol level; however, the majority of atherosclerotic lesions develop only at certain vessels sites – mostly in the distal abdominal aorta [20]. Histological examination of a cross-section of this aortic plaque showed marked intimal thickening containing smooth muscle cells, foam cells, monocytes and connective tissue [13, 18]. The localization and features of these plaques are similar to human atherosclerosis. Reitman et al. speculated that the young age of the mini-pigs used in their study might explain the lack of significant coronary artery atherosclerosis they observed [13]. A second possibility is that atherosclerotic pathologies develop in a particular sequence, and shorter research studies allow the formation of changes only in the abdominal part of the aorta. In the 18-month study conducted by Jacobsson et al., mini-pigs fed lipid-enriched food showed fatty streaks not only in the abdominal aorta but also in the thoracic aorta and coronary arteries. Diet cholesterol concentration also affects the extension and exacerbation of atherosclerotic changes, as the results of the research studies presented in Table 1 show. It is thought that the regional differences in the extent of atherosclerotic lesions in the same breed of animal are due to a difference in the susceptibility of specific arterial sites to the development of atherosclerosis [13]. One disadvantage of the mini-pig is that a mature mini-pig fed ad libitum becomes obese so quickly that it is difficult to conduct control tests [21]. However, domestic pigs are considered unsuitable for chronic studies because of their rapid growth.

Recently genetically modified mice and pigs have been developed to serve as models for studying atherosclerosis. Gene deletion technology has allowed the creation of a variety of transgenic animal models. The most popular knock-out (KO) mice provide an excellent opportunity to study gene interactions in atherosclerosis. Successfully created gene knock-out mice include (among others) Apo E-KO mice [22], LDL receptor KO mice [23], hepatic lipase KO mice [24], and mice expressing human Apo-B_{100} [25] and human cholesterol transfer protein [26]. However, it is important to bear in mind that these mice are models of atherogenesis, not advanced atherosclerosis, and they do not exhibit the single most important event in humans: plaque rupture leading to vessel occlusion.

Gene modifications in domestic mammals are difficult and most animals used for experiments are obtained through genetic selection. Genetically modified pig models display one or more phenotypes associated with atherosclerosis. In Poland-China pigs with von Willebrand’s disease, Fuster et al. documented impairment of platelet-arterial wall interaction and resistance to arteriosclerosis in absence of the von Willebrand factor [14]. Harris et al. worked with low and high serum cholesterol swine and showed that although serum cholesterol concentration differed between the high and low cholesterol lines, “there were no interactions between diet and genetic background on cholesterol accretion or on the […] fat in the tissues” [27]. A modified pig has also been developed “compris-
Table 1. Examples of experimental protocols using a swine model

<table>
<thead>
<tr>
<th>Author (Autor)</th>
<th>Breed of pig (Rasa świńi)</th>
<th>Age of swine (Wiek świńi)</th>
<th>Animal Body weight (Masa ciała zwierzęcia)</th>
<th>Length of study (Czas trwania doświadczenia)</th>
<th>Diet (Dieta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samochowiec et al. 1981 [12]</td>
<td>domestic cross-breed pigs</td>
<td>lack of data</td>
<td>17 kg</td>
<td>6 months</td>
<td>standard pig diet + 15% coconut oil + 5% cholesterol + 0.5% cholic acid</td>
</tr>
<tr>
<td>Reitman et al. 1982 [13]</td>
<td>Yucatan miniature swine</td>
<td>8–12 weeks</td>
<td>15–20 kg</td>
<td>12 months</td>
<td>conventional pig diet + 1.5% cholesterol + 15% lard</td>
</tr>
<tr>
<td>Fuster et al. 1985 [14]</td>
<td>crossbred Poland-china and Yorkshire-Hampshire pig</td>
<td>3 months</td>
<td>lack of data</td>
<td>6 months</td>
<td>modified pig diet (soybean oil meal 190 g/kg, dextrose 10 g/kg, dicalcium phosphate 24 g/kg, vitamins, trace minerals and antibiotics 9 g/kg) + 5 g/kg iodized salt + 20 g/kg cholesterol + 200 g/kg tallow + 10 g/kg hog bile extract</td>
</tr>
<tr>
<td>Kim et al. 1989 [15]</td>
<td>Yorkshire swine</td>
<td>lack of data</td>
<td>11 kg</td>
<td>4 months</td>
<td>in g/700 g mash: corn starch 200, corn oil 10, casein (90% protein) 130, alphacel (cellulose type fiber) 64, salt mix 4, vitamin mix 4, butter 90, cholesterol 10</td>
</tr>
<tr>
<td>Kobari et al. 1991 [16]</td>
<td>Gottingen miniature swine (Vietnamese pig+miniature pig+German Landrace)</td>
<td>7–12 months</td>
<td>25–36 kg</td>
<td>15 months</td>
<td>conventional pig diet (11.5% water, 15.1% protein, 2.9% fat, 5.2% roughage, 7.4% mineral salts, 57.9% carbohydrate) + 15% beef tallow + 1.5% cholesterol</td>
</tr>
<tr>
<td>Jacobsson et al. 1994 [17]</td>
<td>Gottingen miniature swine</td>
<td>10–13 weeks</td>
<td>15 kg</td>
<td>18 months</td>
<td>conventional pig diet (carbohydrates 610 g/kg, protein 148 g/kg, water 130 g/kg, crude fiber 51 g/kg, fat 33 g/kg, essential amino acids 22 g/kg, vitamins and minerals 6 g/kg) + 11.7% freeze-dried egg yolk + 1% cholesterol</td>
</tr>
<tr>
<td>Holvoet et al. 1998 [18]</td>
<td>miniature pig</td>
<td>3 months</td>
<td>28 kg</td>
<td>9 months</td>
<td>conventional pig diet + 14% beef tallow + 4% cholesterol + 1% hog bile extract</td>
</tr>
<tr>
<td>Johnson et al. 2005 [19]</td>
<td>domestic cross-breed pigs</td>
<td>lack of data</td>
<td>20–30 kg</td>
<td>2 months</td>
<td>conventional pig diet + 6% cholesterol</td>
</tr>
<tr>
<td>Artinger et al. 2009 [20]</td>
<td>domestic cross-breed pigs</td>
<td>2–2.5 months</td>
<td>30.7 kg</td>
<td>13 weeks</td>
<td>conventional pig diet (11.5% water, 15.1% protein, 2.9% fat, 5.2% roughage, 7.4% mineral salts, 57.9% carbohydrate) + 17% coconut + 2 or 4% cholesterol</td>
</tr>
</tbody>
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ing a mutation in the endogenous ApoE gene or part thereof and/or LDL gene or part thereof, and/or LDL receptor gene, and/or transcriptional and/or translational product or part thereof" [28].

Even though there is no single perfect animal model that completely replicates human atherosclerosis, pigs seem to be the most similar to humans in terms of anatomy, pathophysiology and their tendencies for overconsuming, obesity and natural artery wall pathologies. Pig models allow for the observation of all stages of atherosclerosis, including the more advanced stages. Despite some disadvantages swine seems to be a promising subject for exploring atherosclerosis.

References


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