We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Experiment and Animal Models of AAA

Karel Houdek

Additional information is available at the end of the chapter

Abstract

Introduction: The incidence of abdominal aortic aneurysms has been increasing throughout the world. The etiology and pathophysiology of this disease are very complicated and complex and include biomechanical aspects as well as biological processes. The effect of these mechanisms is the degradation of the aortic wall, which leads to its dilation and rupture. The possibilities for studying such complex pathophysiology in humans are very limited. That is why we use various mathematical models and a number of different animal models of aneurysm. Methods: A summary of the basic characteristics, findings and examples of using the most widely used animal models of abdominal aortic aneurysm. Information has been obtained from our own experience with laboratory animals and from studies published and available on the Pubmed Internet database. The following search terms were used: aneurysm, aorta, animal model and experiment. Conclusion: Animal models of aortic aneurysms are a usable and useful tool in the study of AAA etiopathogenesis. They also serve as a means to find novel therapeutic pathways. Each model, like any animal species, is different and has its own limitations, advantages and disadvantages, which we should always consider during their use and while interpreting the results.

Keywords: experiment, aneurysm, aorta, animal, model

1. Introduction

1.1. Introduction

Infrarenal aortic aneurysm is a disease, which puts patients at risk primarily due to its long, asymptomatic course, often resulting in abrupt pain caused by rupture as the first sign of the disease [1]. Aneurysmal rupture often has a fatal outcome. Infrarenal aortic aneurysm is not a single group of diseases. The etiology is different in patients with congenital connective tissue

disorders in Marfan syndrome and Ehlers-Danlos syndrome [2], different in infectious aneurysms with bacterial agents clearly confirmed by culture [3] and different in aneurysms classified as degenerative [4], which represent the most common ones. These are diseases with etiology that has not been completely elucidated. The pathophysiology of aneurysmal development is a very complex process with complicated interrelated and interconnected physical and biological mechanisms that lead to the degradation of the molecular and cellular structure of the vessel wall [4]. It is exactly this complicated and not yet fully elucidated etiopathogenesis that makes aneurysms the subject of continued interest across scientific disciplines. One of the options to study the individual processes at different levels are animal models and experimental animal research. Animal models, unlike aneurysm samples obtained from surgery or autopsy, are used to study the individual mechanisms from the early stages of aneurysmal development. Studies in humans are conducted to examine the changes at an advanced stage.

Experimental work with animals requires strict adherence to the rules, careful planning and a lot of effort. The advantage is the possibility to see the individual processes and mechanisms in the context of the whole body, including all interactions.

1.2. Materials and methods

This chapter gives a summary of basic characteristics of most commonly used animal models of aortic aneurysms. By giving few examples of each model, it also points out the advantages and disadvantages and their practical application. The authors gain the information from published studies that are available on the Pubmed Internet database. For searching in the database, the words experiment, aneurysm, aorta, animal and model were used. Only those papers were read and accepted, if the full text was written in English. Another source of knowledge presented in this chapter is a long-time experience and practice with laboratory animals of different species in various models and studies on the authors' place of work. Due to the nature of this chapter and many variables, no statistical analysis is presented.

2. Experimental work and models of AAA

2.1. General conditions for working with laboratory animals

The current issues of experimental work with animals are subject to European and global conventions on the protection of animal rights, which may be further regulated and specified by national legislations. Several fundamental rules apply in this field. In general, there have been attempts to reduce the total number of animals used for experimental purposes. The interests of researchers may be in conflict with those of animal rights defenders and a reasonable compromise should be sought. The conditions in which the animals are kept, how they are treated during transportation and throughout the experiments, including the killing and subsequent handling of the remains, have been constantly improved. The basic principles and rules of working with experimental animals, which are valid still today, were defined by

William Russell and Rex Burch in the mid-twentieth century in the book "The Principles of Human Experimental Technique" [5]. They can be summarized in three points or rules known as "3R"—Replacement, Reduction, Refinement [6].

Replacement: an effort to find other, alternative methods of conducting research without the use of laboratory animals. When considering the initiation of research, we should first ask and answer the question of whether it is possible to obtain the result without using laboratory animals. The current level of knowledge allows the use of a variety of mathematical or computer models. Cell or tissue cultures alone can often be used to verify hypotheses. If this is not possible, we should always try to use animals from lower evolutionary groups. If work with animals is a part of teaching programs, it can often be replaced by video recordings.

Reduction: an effort to reduce the total number of laboratory animals used. This rule is closely related to the previous one. The already mentioned use of nonanimal models and cell and tissue cultures should include the careful planning of experimental work so that we do not duplicate experiments that have already been carried out unnecessarily or do not verify hypotheses that have already been adequately verified. The total number of laboratory animals used can be reduced by appropriate selection of the animal species, choice of appropriate sex and age. Careful consultation with the statistician (appropriately chosen model, number of animals in each group, length and ways of monitoring) should be an integral part of the planning.

Refinement: includes measures to improve the living conditions and the environment of laboratory animals. Working with laboratory animals requires the possession of authorizations that can be obtained based on professional education and experience. The Federation of European laboratories animal science associations (FELASA) determines four categories of authorization (A-D) according to the level of education and length of practice. Correct or wrong animal handling can significantly affect the results of the experiment. Any handling of animals, including transportation and environmental changes, is stressful for animals. In addition to stress, transportation also poses the risk of the transmission of infections not only to the animal but also to the transporter, and it is therefore necessary to choose suitable transport boxes (air conditioning, protection). Acclimatization to the new environment is always necessary between transportation and the beginning of the experiment. The acclimatization time varies according to the type of animal chosen and also serves to normalize changes caused by stress during transportation (weight loss, change in heart rate). The environment in which the animal is kept (box size, number of animals in the box, temperature, humidity, observation of circadian rhythm, appropriate feeding) and how the animal is treated is very important. Smaller laboratory animals are less expensive, and handling them is not so physically demanding and does not require much space. On the other hand, greater size of the animals, such as a rabbit or a pig, may be an advantage when handling organs and tissues. Any painful handling, investigations, and procedures should be performed under anesthesia, and suitable analgesia should be provided, including in the postoperative period. The method of anesthesia should be selected according to the type of animal chosen and plays an important role in the successful completion of the experiment and achievement of the necessary results.

The anesthesiologist should be sufficiently experienced and knowledgeable about the specific differences of the chosen animal species.

Strict adherence to the established rules and standardized conditions is an inherent part of any experimental work so that the results of the work are reproducible, repeatable and the statistical analysis is valid.

At present, multiple animal species are used in animal experiments. The same is true for experimental works related to aneurysms. Wild-type (WT) animals, whose genome is not modified, can be used for each animal species. Interindividual differences, for example, in enzymatic activity are a certain disadvantage when studying such populations [7]. This is one of the reasons why genetically modified strains of animals are often used in studies in which a population of similar or virtually identical animals is being studied [7]. Another advantage of using modified strains is a specific modification that allows for the monitoring of, for example, the involvement of a particular enzyme and its activity in the studied process. Especially in mice, a large variety of different genetically modified strains are available. With a properly chosen animal, we are able to model very specific situations. A properly chosen animal type and methodology can significantly influence the results of the work in both positive and negative terms, as documented below in the text. When choosing an animal model, it is necessary to answer the question of whether it will be possible to compare the model with the real situation in human medicine and to what extent the conditions studied will be similar (enzymatic equipment etc.) or different from the reality.

2.2. Animal models of AAA

Animal aneurysm models help clarify the complex etiopathogenesis, can be used to develop new treatment methods or to improve endovascular and surgical procedures. The first animal aneurysm models were published in the 1960s, and many other methods and models have been developed since then and have been variously upgraded and improved [8–11]. In principle, the methods of inducing an aneurysm in animals can be divided into those using different chemicals and those using physical laws and their various combinations. Papers that are presenting research with different models of aneurysm and different animal species are summarized in **Table 1**.

2.2.1. Elastase model

Perhaps the most important changes that can be observed in the aneurysm wall in humans are degeneration of extracellular matrix—degradation of elastin in the presence of matrix metalloproteinases 1, 2, 3 and 9 (MMPs) and the inflammatory infiltration. The first attempts to develop an experimental aneurysm used proteolytic enzymes to cause the degradation of elastin fibers. Wills et al. [8] used porcine aortic tissue to demonstrate the effect of exogenous elastase in the development of degenerative changes of the extracellular matrix. He confirmed the results and observations attained by Anidjar et al. [21]. Anidjar repeatedly demonstrated the possibility of establishing an aortic aneurysm model in rats by applying porcine pancreatic elastase (PPE). Anidjar's model represents the basis for a various PPE model modifications. In

Model	Animal	Study	Additional information
PPE	Mice	Pyo et al. [12], Moore et al. [13], Bigatel et al. [14], Curci et al. [15], Boyle et al. [16]	
		Bhamidipati et al. [17]	Periadventitial apply
		Zhou et al. [18], Johnston et al. [19], Parodi et al. [20]	Genetically manipulated
	Rat	Holmes et al. [21], Anidjar et al. [22], Carsten et al. [23], Dobrin [24], Azuma et al. [25], Yamaguchi et al. [26]	
	Dog	Strindberg et al. [27], Economou et al. [28]	
	Yucatan miniature swine	Marinov et al. [29]	
	Rabbit	Nie et al. [30], Bi et al. [31], Kobayashi et al. [32]	
CaCl$_2$	Rabbit	Gertz et al. [9], Freestone et al. [33]	
	Mice	Chiou et al. [11], Watanabe et al. [34]	
		Basalyga et al. [35]	Genetically manipulated
	Rat	Gacchina et al. [36]	
Angiotensin II	Apolipoprotein E deficient mice	Daugherty et al. [10], Wang et al. [37], Saraff et al. [38], Inoue et al. [39], Rateri et al. [40], Briones et al. [41]	
	Zebrafish	Folkesson et al. [42]	
Stenosing cuff	Rat	Mata et al. [43]	
Patch	Minipig	Lin et al. [44]	
Tissue transplantation	Rat/Hartley guinea pig	Allaire et al. [45], Schneider et al. [46]	
Combined	Rat	Tanaka et al. [47], Morimoto et al. [48]	PPE + CaCl$_2$
	Pig	Moláček et al. [49]	PPE/stenosis/ patch
		Houdek et al. [50]	PPE + stenosis
		Turnbull et al. [51]	PPE + balloon dilatation

Table 1. Animals and models of experimental aneurysm used in presented studies.

this model, a segment of infrarenal aorta is perfused with a PPE solution through a directly inserted tube or needle. The authors and models can differ in the concentration of PPE, method of perfusion (pump, single or repeated applications, or application with increased pressure), duration of perfusion and the laboratory animal [24–26, 28, 52]. Anidjar perfused a 1 cm long segment of aorta of rat with a porcine elastase solution. Other proteases (papain, trypsin, and collagenase) can lead to the development of an aneurysm as well. Carsten et al. [23] studied several batches of elastase and confirmed the need for inflammatory infiltration with activated macrophages to achieve the necessary extracellular matrix degradation and

aneurysmal development in rats. PPE model was widely used to study the pathophysiology and possible treatment options of AAA. For this purpose, genetically modified mice were used [12] and many anti-inflammatory acting drugs and agents were studied (TIMPs, doxycycline, indomethacin) [13–16, 21]. Periadventitial application of elastase in mice may cause similar changes and lead to development of AAA as well [17]. Nie et al. [30] induced an aortic aneurysm using PPE in the New Zealand White Rabbit within 14 day. Despite mild differences in the method of perfusion, similar conclusions were made by Bi et al. [31] and Kobayashi et al. [32]. Both used higher pressure for the perfusion. In elastase-induced models small animals are commonly used. In large animals, such as different species of pigs or in dogs, the results are not so unambiguous. Marinov et al. [29] observed elastin fiber destruction, inflammatory infiltration, a change in wall thickness and changes in smooth muscle cells, and even calcium depositions after aortic perfusion with PPE in Yucatan miniature swine, but he did not observe the development of aneurysmal dilation after 3 weeks. Strindberg et al. [27] wanted to use the elastase-induced aneurysm model in a dog for control and development of stent grafts. He compared the changes while using different elastase concentrations, different perfusion times, and the combined use of elastase with collagenase and/or an inflated intraluminal balloon catheter. By extending the perfusion time to 2 h and using elastase alone or in combination with collagenase, aortic dilation of $65.6 \pm 20.8\%$ was present, which was not enough for his need. Degradation of elastin fibers, a reduced number of smooth muscle cells and an intimal thickening were present during the examination of the aorta samples. Many modifications of the elastase-induced aneurysm model employ differently genetically modified and specified animal clones [18–20].

2.2.2. Calcium chloride model (CaCl$_2$)

Inflammatory infiltration is another significant contributor to the development of aneurysms. This reaction can also be induced by an external insult to the adventitia. The use of calcium chloride to induce an aneurysm was first described in the carotid arteries of rabbits [9] more or less as a secondary observation. However, the histological structure of these aneurysms somewhat differs from findings in human aneurysms. In both cases, we can see changes in the media with wall thickening and inflammatory reaction, but in carotid artery aneurysms in rabbits, the wall thickening is more pronounced with marked intimal hyperplasia and marked calcification of elastin fibers in the media. For the abdominal aorta, this methodology and experience was described by Freestone et al. [33]. He studied the effects of different concentrations of calcium chloride and sodium chloride solutions applied to the surface of the infrarenal aorta for 15 min. He also examined the possibility of influencing the effect of calcium chloride by added sodium thioglycolate and a high-cholesterol diet. Histological changes (intimal hyperplasia, media injury, calcification of the media) increased with the increasing calcium chloride concentrations. The leading symptom was infiltration of the media and adventitia by macrophages and increased activity of MMP2 and 9. Aneurysms developed at a concentration of 0.25 mol/L. High cholesterol and/or thioglycolate levels did not significantly affect the development of aneurysms. The effect of sodium chloride has not been demonstrated as well. Chiou et al. [11] provided a similar comparable study but he used mice as a laboratory animal. Calcification of the vascular wall is a common denominator of a number of vascular diseases.

We can find calcification in the aortic and aneurysm walls. Basalyga et al. [35] used the application of calcium salts at various concentrations in unmodified and genetically engineered mice to verify the association of elastin degradation caused by the action of MMP and the resulting calcification. Watanabe et al. [34] used genetically engineered mouse clones with calcium chloride-induced aneurysm for studying the role of phospholipase A2 (PLA2) and inflammation in the pathogenesis of AAA. Other study confirmed a protective effect of PLA2 inhibitor. Using the calcium chloride-induced aneurysm model in mice, Gacchina et al. [36] referred the role of vascular smooth muscle cells (VSMC) for the AAA growth.

2.2.3. Angiotensin II model

Like previous aneurysm models, another model that uses the effect of angiotensin II has several common characteristics with human aneurysms. It is an association with hyperlipidemia, wall remodeling, inflammation and thrombosis and also a higher incidence in males [53]. The model is more animal-specific and uses apolipoprotein E deficient mice (ApoE $-/-$). Daugherty et al. [54] examined the effect of Angiotensin II on the development of atherosclerosis in relation to hyperlipidemia. He administered angiotensin II to ApoE $-/-$ clones of mice for 1 month using a minipump. In addition to the development of atherosclerotic changes, both by the action of higher blood pressure and independent of elevated blood pressure on the basis of activation of the monocyte-macrophage system and oxidative stress, Daugherty observed development of aneurysm as a secondary effect. This phenomenon was not dependent on blood pressure or lipid levels or their distribution in the blood. The mice thus treated were found to have a number of macrophages and lymphocytes, that is, inflammatory infiltration in the external elastic lamina and adventitial hypertrophy. In contrast to human aneurysm, the effects of angiotensin II result in dilation and development of aneurysm in the suprarenal segment [10]. This is explained by a higher proportion of fat cells in the adventitia region in the suprarenal segment of the aorta. Dissection and rupture have been reported to occur more frequently [38, 39]. In animals, rupture of the media occurs with thrombus formation and further stimulation and activation of macrophages with elastin disintegration and matrix remodeling. The described changes and the rate of aortic dilation are not the same in identical animals even under the same experimental conditions [10, 37]. Based on these differences, four subtypes of angiotensin II-induced aneurysm models can be distinguished. This heterogeneity can also be observed when comparing samples from different levels of aneurysm in one animal [40]. In this model, further growth of the aneurysm occurs for several weeks after the last angiotensin infusion [39]. Another animal that was used as an angiotensin-induced aneurysm model was the zebrafish. This is primarily due to similar vasculogenesis with humans [42]. This model was used primarily to investigate the effects of smoking tobacco.

2.2.4. Combined and other newer models

Very often, experimental aneurysm models combining the effects of calcium chloride and pancreatic porcine elastase are used. These models are often associated with rats. As an example, we can mention the Tanaka group [47], who achieved aneurysmatic dilation in almost 93% of animals by using the combined approach, but only in 25% and 0% of animals when using PPE alone or $CaCl_2$ alone, respectively. Even histological changes copied this

trend: less elastin, more pronounced infiltration by inflammatory cells, and higher activity of cytokines and MMPs 2 and 9 were recorded in the group combining the effects of PPE and calcium chloride. Morimoto et al. [48] used this combined model in rats to study the effects of free oxygen radicals. Molacek et al. [49] compared different AAA animal models in pigs. He compared the PPE model, stenosing cuff model, Dacron patch model and their combinations. He observed best results in combination of PPE model with hemodynamical changes caused by a stenosing cuff placed around the subrenal aorta (p < 0.0156) and the same group used this knowledge to influence the growth of experimentally created aneurysm in rats and pigs with atorvastatin [50]. They observed no thrombus, lipid deposition, media necrosis, intramural hematoma, dissection, or rupture in this combined model. **Figures 1–3** show the combination of placed stenosing cuff and PPE infusion and the aortic dilatation after 4 weeks in pig. **Figures 4** and **5** are images from ultrasound, showing dilatation of porcine infrarenal aorta after 2 weeks.
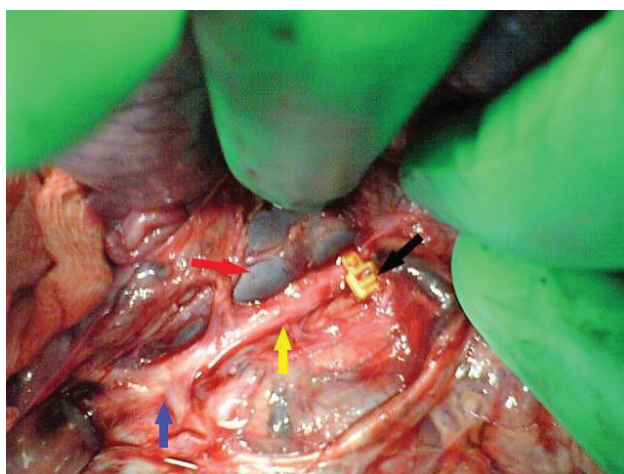

Figure 1. Porcine infrarenal aorta with stenosing cuff day 0. Black arrow—stenosing cuff; yellow arrow—infrarenal aorta; blue arrow—aortic bifurcation; red arrow—inferior caval vein.
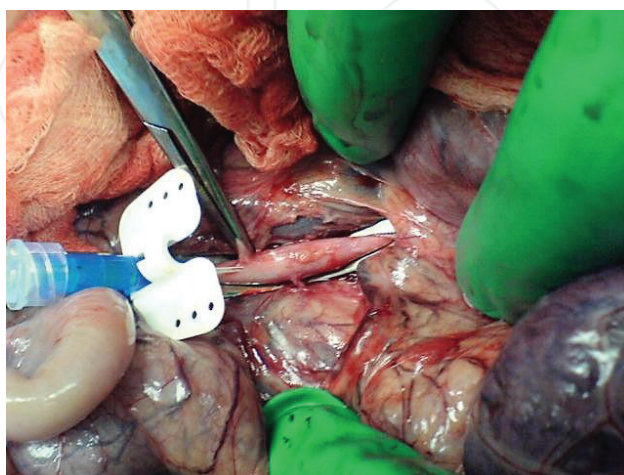

Figure 2. Infusion of clamped infrarenal aorta with porcine pancreatic elastase day 0.
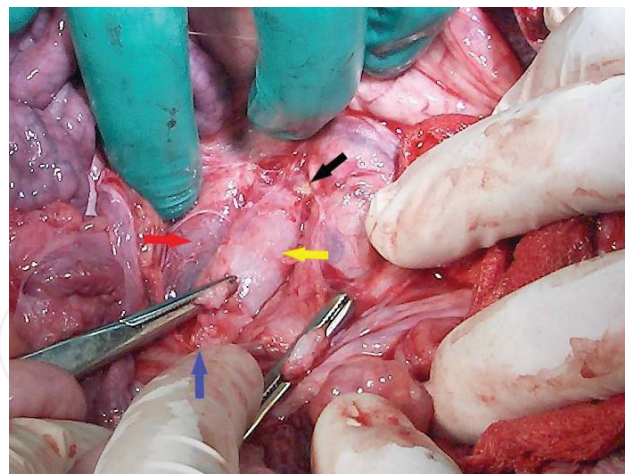
Figure 3. Dilatation of porcine infrarenal aorta. Combined model—stenosing cuff + PPE. Day 28. Black arrow—stenosing cuff; yellow arrow—dilated infrarenal aorta; blue arrow—aortic bifurcation; red arrow—inferior caval vein.
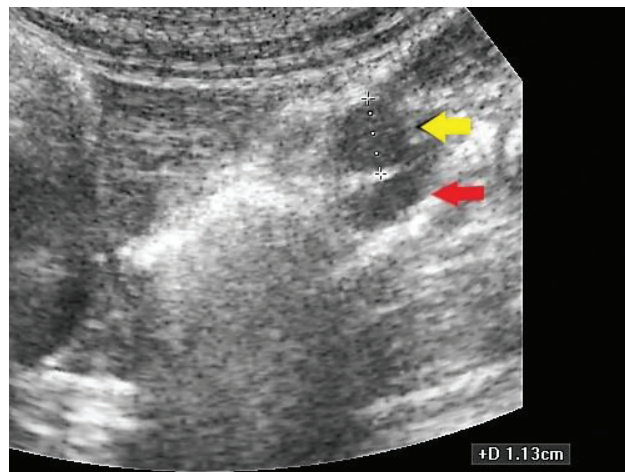

Figure 4. Ultrasound image of dilated porcine infrarenal aorta. Translumbal approach. Transverse view. Day 14. Combined model—stenosing cuff + PPE. Yellow arrow—dilated infrarenal aorta; red arrow—inferior caval vein.

Another models that combine the use of PPE, $CaCl_2$ or Angiotensin II in mice, rats, rabbits or pigs were used to explain the effects of various statins and other drugs [41, 55–60].

The possibilities of using stem cells to influence the growth and rupture of aneurysms have been increasingly studied in recent years. This topic is studied by many authors and no consensus has been achieved as to the optimal experimental model or laboratory animal. Mesenchymal stem cells (MSCs) have been used in studies to treat a number of cardiovascular diseases, such as critical limb ischemia, cerebral ischemia or myocardial infarction. It is believed that mesenchymal stem cells (MSCs) could help to inhibit degenerative changes in the AAA wall and promote its regeneration. Turnbull et al. [51] attempted to demonstrate the uptake and the presence of stem cells in the aortic wall after insult. She used an experimental pig model, where she combined physical (balloon dilation) and chemical (the effect of PPE and collagenase) methods, and administered stem cells to the pigs. Her methods have led to the
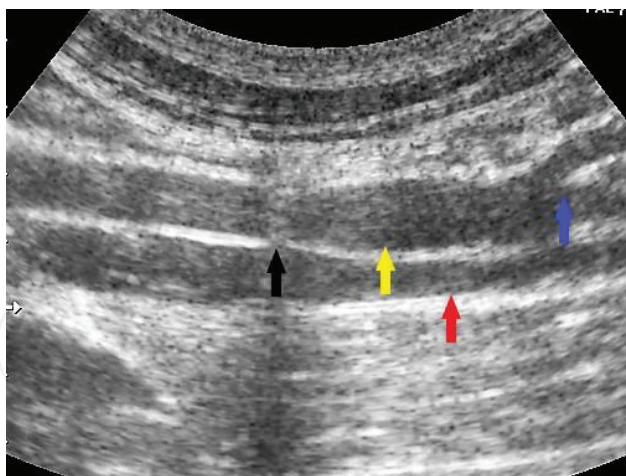
Figure 5. Ultrasound image of dilated porcine infrarenal aorta. Translumbal approach. Longitudinal view. Day 14. Combined model—stenosing cuff + PPE. Black arrow—stenosing cuff; yellow arrow—dilated infrarenal aorta; blue arrow—aortic bifurcation; red arrow—inferior caval vein.

development of aneurysms with characteristics close to human ones, such as expression of MMP2 and 9. By proving the presence of stem cells in the affected aortic wall, she verified her hypothesis and provided the basis for further research. Regeneration of the damaged aortic wall largely depends on the capabilities and presence of VSMC. Schneider et al. [46] was able to improve the regeneration of the aortic wall and thereby influence the progression of aortic dilation in the negative sense using mesenchymal stem cells with a wide differentiation capacity. The effect of MSC was greater than that of VSMC alone. In his work, he induced aneurysms in rats by implanting an aortic graft from guinea pigs. Before the implantation, the xenografts were perfused with a solution containing VSMC or MSC or with a cell-free solution in a control group. The development of aneurysms occurred 14 days after. Grafts colonized by MSC showed significantly less dilation after 1 and 4 weeks compared to those colonized by VSMC and to the controls, where further dilation occurred (p = 0.006). The presence of MSC led to a reduction in inflammatory cell infiltration, a decrease in activity of MMPs, increased TIMP-1 activity, and triggered regeneration of the damaged aortic wall.

3. Discussion

Experimental studies have an irreplaceable role in a research of etiopathogenesis and possible treatment options of AAA. Experimental works with animals and aneurysm models, in contrast to human aneurysms, allow us to monitor the development of aneurysms over time and take samples for analysis at any time during the development. Exploitation of experimental animal models provides, beyond the research of etiopathogenesis, a wide range of possibilities for studying therapeutic interventions, influencing growth or preventing aneurysmal development and rupture. Pharmacotherapy used in experimental models is strongly influencing the initial changes and triggers, and in some models, even a pretreatment is used. To better understand the etiopathogenesis of infrarenal aortic aneurysm, especially how to prevent the

growth and rupture, comprehensive studies are needed. Triggers and initial steps leading to the development of aneurysms in animals under experimental conditions are known. Studies with animal AAA models have promising results, but if they are repeated in humans, the results are inferior. The models are representing "acute" aneurysms. Aneurysms in humans are growing slowly usually with degenerative changes. Degenerative aneurysms usually develop in humans over many years. For animal models, this time is significantly shorter, ranging from days to weeks. There are differences not only between animal AAA model and AAA in human, but also various changes in the results, if a different animal species or different AAA model is used. **Table 2** summarizes the advantages and disadvantages of each model. Not all animal aneurysm models are capable of achieving sustained growth and dilation, and ruptures of already existing aneurysms cannot be observed in all models. Specifically, no ruptures were observed in models with calcium chloride alone. The presence of thrombus in the aneurysm is common in the human aorta, but thrombus formation does not occur in most animal models. Common for majority of animal AAA models is the degradation of extracellular matrix and elastin fibers, increased MMP activity and inflammatory infiltration of aortic wall.

The angiotensin II model is, to a certain extent, very specific not only due to the choice of animal (apolipoprotein deficient mouse clone), but in contrast to other models, the aortic dilation occurs predilectively, in the suprarenal region, and more than other models encounters dissection and rupture, and the development of dilation may be less predictable.

The use of PPE alone to induce aneurysm model is effective in small animals (mouse, rat), can be used in large animals (rabbit, pig, dog) as well, but in large animals, this model is less effective. With respect to the proven and dominant changes in the wall of such aneurysms (inflammatory infiltration, degradation of elastin fibers, increased MMP activity), which are more or less consistent with the changes that can be observed in human aneurysms, such model can be considered to be appropriate. It has been used extensively to study possible

Model	Advantage	Disadvantage
PPE	Possible in majority of animal species	Surgery
	Good results in small animals	AAA development within 2–4 weeks
		Less effective in big animals
CaCl$_2$	Possible in majority of animal species	Surgery
	Common in combined models	No rupture has been observed
	Widely used with knockout mice	AAA development within 2–4 weeks
Angiotensin II	Shorter time for development	Apolipoprotein E deficient mice
	Common rupture	Dilatation of suprarenal aorta
Stenosing cuff/patch	Shorter time for development	Difficult in small animals
Tissue transplantation	Common thrombus	Difficult surgery

Table 2. Advantages and disadvantages of different models of experimental aneurysm.

prophylactic and therapeutic methods and to explore the individual pathogenetic mechanisms of aneurysmal development. PPE can also be used to study isolated aortic tissue.

Small animal—mice is commonly used for the calcium chloride-induced aneurysm model as well. Changes and characteristics are comparable to human. It is most often used in the infrarenal region; the aneurysmatic wall contains calcifications with inflammatory cellular infiltration. Oxidative stress, degradation of elastin fibers and changes in SMC play role in this model. In addition, the mechanisms involved in the induction of aneurysms in this model appear to be involved in the pathogenesis of aneurysm in humans, for example, sPLA2 and plasminogen. Unlike human aneurysms, no rupture, intraluminal thrombus or atherosclerotic changes other than calcification have been observed in this model. Studies have confirmed that this model can be used in both WT animals as well as in genetically modified animals. This aneurysm model is perhaps more often used in combination with other techniques of aneurysm modeling in different animal species.

Most of the models described herein were used in more than one animal species. The advantage of larger laboratory animals, such as pigs or rabbits, is their size and hence the size of the aorta, which improves tissue handling. On the other hand, the size itself may also be a disadvantage in terms of spatial capacity and handling of the animal itself. The pig has an anatomy and physiology generally similar to humans, which is undoubtedly important for interpreting the results and possible use in human medicine. If we select a mouse as a laboratory animal, we have the option of choosing wild species or a variety of genetically modified strains. Lower financial burden is certainly a great advantage of small laboratory animals. In any case, adequate methods of application and administration of pharmaceutical doses should be observed for the selected laboratory animal and aneurysm model. We have mentioned contrast between animal models and the real human aneurysm.

Examples were included for all the abovementioned animal models of AAA, where the possibilities of positive pharmacological effects on aneurysm growth and potential rupture were studied. The effects of drugs should be first verified in laboratory animals or in tissue culture and afterwards in a clinical trial.

4. Conclusion

Animal models of AAA are still essential in searching for novel treatment options. Successful aneurysm induction depends on the choice of the right laboratory animal in each method. In general, small laboratory animals are preferred in experimental studies. Small animals are cheaper, handling with them is easier and they require less space. This enables to design trials with more individuals. There are different genetically modified mouse clone available on the market and that makes mouse a widely used laboratory animal. Regarding current experiences, no universal animal AAA model can be recommended. The aim of the study, advantages and disadvantages of each model should be taken into consideration when preparing the design of a new study. The most commonly observed features of various animal models and human aneurysms are the presence of cellular inflammatory infiltration in the aortic wall,

degradation of the elastin fiber network, increased activity of MMP2 and 9, and a lower number of smooth muscle cells, but many differences and contrasts are observed as well. Because of these contrasts, each observation and result of animal study have to be confirmed in clinical study before they can be implanted into daily medical practice. Unfortunately, ideal model similar to human's AAA remains undeveloped.

Acknowledgements

Conflict of interest

None of the authors are aware of facts that will represent conflict of interest.

Acronyms and abbreviations

AAA	abdominal aortic aneurysm
FELASA	the Federation of European laboratories animal science associations
WT	wild-type
MMP	matrix metalloproteinase
PPE	porcine pancreatic elastase
TIMP	metalloproteinase tissue inhibitor
SMC	smooth muscle cell
PLA2	phospholipase A2
MSC	mesenchymal stem cell

Author details

Karel Houdek

Address all correspondence to: houdekkarel7@gmail.com

University Hospital and Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

References

[1] Moll FL, Powell JT, Fraedrich G, Verzini F, Haulon S, Waltham M, et al. Management of abdominal aortic aneurysms clinical practice guidelines of the European Society for Vascular Surgery. European Journal of Vascular and Endovascular Surgery. 2011;**41**(Suppl 1):S1-S58

[2] Pyeritz RE. Etiology and pathogenesis of the Marfan syndrome: Current understanding. Annals of Cardiothoracic Surgery. 2017;**6**(6):595-598

[3] Molacek J, Treska V, Baxa J, Certik B, Houdek K. Acute conditions caused by infectious aortitis. Aorta (Stamford). 2014;**2**(3):93-99

[4] Lindholt JS, Shi G-P. Chronic inflammation, immune response, and infection in abdominal aortic aneurysms. European Journal of Vascular and Endovascular Surgery. 2006;**31**(5): 453-463

[5] Russell WMS, Burch RL. The Principles of Humane Experimental Technique. London: UFAW; 1959

[6] Russell WMS. The development of the three RS concept. Alternatives to Laboratory Animals: ATLA. 1995;**23**(3):298-304

[7] Liška V. Experimental Surgery. NAVA; 2016

[8] Wills A, Thompson MM, Crowther M, Brindle NP, Nasim A, Sayers RD, et al. Elastase-induced matrix degradation in arterial organ cultures: An in vitro model of aneurysmal disease. Journal of Vascular Surgery. 1996;**24**(4):667-679

[9] Gertz SD, Kurgan A, Eisenberg D. Aneurysm of the rabbit common carotid artery induced by periarterial application of calcium chloride in vivo. The Journal of Clinical Investigation. 1988;**81**(3):649

[10] Daugherty A, Cassis LA, Lu H. Complex pathologies of angiotensin II-induced abdominal aortic aneurysms. Journal of Zhejiang University, Science B. 2011;**12**(8):624-628

[11] Chiou AC, Chiu B, Pearce WH. Murine aortic aneurysm produced by periarterial application of calcium chloride. The Journal of Surgical Research. 2001;**99**(2):371-376

[12] Pyo R, Lee JK, Shipley JM, Curci JA, Mao D, Ziporin SJ, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. The Journal of Clinical Investigation. 2000;**105**(11):1641-1649

[13] Moore G, Liao S, Curci JA, Starcher BC, Martin RL, Hendricks RT, et al. Suppression of experimental abdominal aortic aneurysms by systemic treatment with a hydroxamate-based matrix metalloproteinase inhibitor (RS 132908). Journal of Vascular Surgery. 1999;**29** (3):522-532

[14] Bigatel DA, Elmore JR, Carey DJ, Cizmeci-Smith G, Franklin DP, Youkey JR. The matrix metalloproteinase inhibitor BB-94 limits expansion of experimental abdominal aortic aneurysms. Journal of Vascular Surgery. 1999;**29**(1):130-138 discussion 138-139

[15] Curci JA, Petrinec D, Liao S, Golub LM, Thompson RW. Pharmacologic suppression of experimental abdominal aortic aneurysms: A comparison of doxycycline and four chemically modified tetracyclines. Journal of Vascular Surgery. 1998;**28**(6):1082-1093

[16] Boyle JR, McDermott E, Crowther M, Wills AD, Bell PR, Thompson MM. Doxycycline inhibits elastin degradation and reduces metalloproteinase activity in a model of aneurysmal disease. Journal of Vascular Surgery. 1998;**27**(2):354-361

[17] Bhamidipati CM, Mehta GS, Lu G, Moehle CW, Barbery C, DiMusto PD, et al. Development of a novel murine model of aortic aneurysms using peri-adventitial elastase. Surgery. 2012;**152**(2):238-246

[18] Zhou H, Yan H, Cannon JL, Springer LE, Green JM, Pham CTN. CD43-mediated IFN-γ production by CD8+ T cells promotes abdominal aortic aneurysm in mice. Journal of Immunology (Baltimore, Md.: 1950). 2013;**190**(10):5078-5085

[19] Johnston WF, Salmon M, Su G, Lu G, Stone ML, Zhao Y, et al. Genetic and pharmacologic disruption of interleukin-1β signaling inhibits experimental aortic aneurysm formation. Arteriosclerosis. Thrombosis, and Vascular Biology. 2013;**33**(2):294-304

[20] Parodi FE, Mao D, Ennis TL, Bartoli MA, Thompson RW. Suppression of experimental abdominal aortic aneurysms in mice by treatment with pyrrolidine dithiocarbamate, an antioxidant inhibitor of nuclear factor-kappaB. Journal of Vascular Surgery. 2005;**41**(3): 479-489

[21] Holmes DR, Petrinec D, Wester W, Thompson RW, Reilly JM. Indomethacin prevents elastase-induced abdominal aortic aneurysms in the rat. The Journal of Surgical Research. 1996;**63**(1):305-309

[22] Anidjar S, Salzmann JL, Gentric D, Lagneau P, Camilleri JP, Michel JB. Elastase-induced experimental aneurysms in rats. Circulation. 1990;**82**(3):973-981

[23] Carsten CG, Calton WC, Johanning JM, Armstrong PJ, Franklin DP, Carey DJ, et al. Elastase is not sufficient to induce experimental abdominal aortic aneurysms. Journal of Vascular Surgery. 2001;**33**(6):1255-1262

[24] Dobrin PB. Animal models of aneurysms. Annals of Vascular Surgery. 1999;**13**(6):641-648

[25] Azuma J, Asagami T, Dalman R, Tsao PS. Creation of murine experimental abdominal aortic aneurysms with elastase. Journal of Visualized Experiments: JoVE. 2009;**29**:1280

[26] Yamaguchi T, Yokokawa M, Suzuki M, Higashide S, Katoh Y, Sugiyama S, et al. The time course of elastin fiber degeneration in a rat aneurysm model. Surgery Today. 2000;**30**(8): 727-731

[27] Strindberg G, Nichols P, Ricci MA, Marinov G, Marois Y, Roby P, et al. Experimental modifications to a canine infrarenal aortic aneurysm model for the validation of endovascular stent-grafts: An exploratory study. Journal of Investigative Surgery. 1998;**11** (3):185-197

[28] Economou SG, Taylor CB, Beattie EJ, Davis CB. Persistent experimental aortic aneurysms in dogs. Surgery. 1960;**47**:21-28

[29] Marinov GR, Marois Y, Pâris E, Roby P, Formichi M, Douville Y, et al. Can the infusion of elastase in the abdominal aorta of the Yucatán miniature swine consistently produce experimental aneurysms? Journal of Investigative Surgery. 1997;**10**(3):129-150

[30] Nie M, Yan Y, Li X, Feng T, Zhao X, Zhang M, et al. Effect of low-pressurized perfusion with different concentration of elastase on the aneurysm formation rate in the abdominal aortic aneurysm model in rabbits [Internet]. BioMed Research International. 2016. Available: https://www.hindawi.com/journals/bmri/2016/6875731/ [Cited: November 2017]

[31] Bi Y, Zhong H, Xu K, Ni Y, Qi X, Zhang Z, et al. Performance of a modified rabbit model of abdominal aortic aneurysm induced by topical application of porcine elastase: 5-month follow-up study. European Journal of Vascular and Endovascular Surgery. 2013;**45**(2):145-152

[32] Kobayashi H, Matsushita M, Oda K, Nishikimi N, Sakurai T, Komori K. Effects of atherosclerotic plaque on the enlargement of an experimental model of abdominal aortic aneurysm in rabbits. European Journal of Vascular and Endovascular Surgery. 2004;**28**(1):71-78

[33] Freestone T, Turner RJ, Higman DJ, Lever MJ, Powell JT. Influence of hypercholesterolemia and adventitial inflammation on the development of aortic aneurysm in rabbits. Arteriosclerosis, Thrombosis, and Vascular Biology. 1997;**17**(1):10-17

[34] Watanabe K, Fujioka D, Saito Y, Nakamura T, Obata J, Kawabata K, et al. Group X secretory PLA2 in neutrophils plays a pathogenic role in abdominal aortic aneurysms in mice. American Journal of Physiology—Heart and Circulatory Physiology. 2012;**302**(1): H95-H104

[35] Basalyga DM, Simionescu DT, Xiong W, Baxter BT, Starcher BC, Vyavahare NR. Elastin degradation and calcification in an abdominal aorta injury model: Role of matrix metalloproteinases. Circulation. 2004;**110**(22):3480-3487

[36] Gacchina C, Brothers T, Ramamurthi A. Evaluating smooth muscle cells from CaCl2-induced rat aortal expansions as a surrogate culture model for study of elastogenic induction of human aneurysmal cells. Tissue Engineering. Part A. 2011;**17**(15–16):1945-1958

[37] Wang J, Chen W, Wang Y, Zhang S, Bi H, Hong B, et al. Statins exert differential effects on angiotensin II-induced atherosclerosis, but no benefit for abdominal aortic aneurysms. Atherosclerosis. 2011;**217**(1):90-96

[38] Saraff K, Babamusta F, Cassis LA, Daugherty A. Aortic dissection precedes formation of aneurysms and atherosclerosis in angiotensin II-infused, apolipoprotein E-deficient mice. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;**23**(9):1621-1626

[39] Inoue N, Muramatsu M, Jin D, Takai S, Hayashi T, Katayama H, et al. Involvement of vascular angiotensin II-forming enzymes in the progression of aortic abdominal aneurysms in angiotensin II-infused ApoE-deficient mice. Journal of Atherosclerosis and Thrombosis. 2009;**16**(3):164-171

[40] Rateri DL, Howatt DA, Moorleghen JJ, Charnigo R, Cassis LA, Daugherty A. Prolonged infusion of angiotensin II in apoE_/_ mice promotes macrophage recruitment with continued expansion of abdominal aortic aneurysm. The American Journal of Pathology. 2011; **179**(3):1542-1548

[41] Briones AM, Rodríguez-Criado N, Hernanz R, García-Redondo AB, Rodrigues-Díez RR, Alonso MJ, et al. Atoervastatin prevents angiotensin II-induced vascular remodeling and oxidative stress. Hypertension (Dallas, Tex.: 1979). 2009;**54**(1):142-149

[42] Folkesson M, Sadowska N, Vikingsson S, Karlsson M, Carlhäll C-J, Länne T, et al. Differences in cardiovascular toxicities associated with cigarette smoking and snuff use revealed using novel zebrafish models. Biology Open. 2016;**5**(7):970-978

[43] Mata KM, Prudente PS, Rocha FS, Prado CM, Floriano EM, Elias J, et al. Combining two potential causes of metalloproteinase secretion causes abdominal aortic aneurysms in rats: a new experimental model. International Journal of Experimental Pathology. 2011;**92**(1): 26-39

[44] Lin P-Y, Wu Y-T, Lin G-C, Shih YH, Sampilvanjil A, Chen L-R, et al. Coarctation-induced degenerative abdominal aortic aneurysm in a porcine model. Journal of Vascular Surgery. 2013;**57**(3):806e.1-815.e1

[45] Allaire E, Muscatelli-Groux B, Guinault A-M, Pages C, Goussard A, Mandet C, et al. Vascular smooth muscle cell endovascular therapy stabilizes already developed aneurysms in a model of aortic injury elicited by inflammation and proteolysis. Annals of Surgery. 2004;**239**(3):417-427

[46] Schneider F, Saucy F, de Blic R, Dai J, Mohand F, Rouard H, et al. Bone marrow mesenchymal stem cells stabilize already-formed aortic aneurysms more efficiently than vascular smooth muscle cells in a rat model. European Journal of Vascular and Endovascular Surgery. 2013;**45**(6):666-672

[47] Tanaka A, Hasegawa T, Chen Z, Okita Y, Okada K. A novel rat model of abdominal aortic aneurysm using a combination of intraluminal elastase infusion and extraluminal calcium chloride exposure. Journal of Vascular Surgery. 2009;**50**(6):1423-1432

[48] Morimoto K, Hasegawa T, Tanaka A, Wulan B, Yu J, Morimoto N, et al. Free-radical scavenger edaravone inhibits both formation and development of abdominal aortic aneurysm in rats. Journal of Vascular Surgery. 2012;**55**(6):1749-1758

[49] Moláček J, Treska V, Kobr J, Certík B, Skalický T, Kuntscher V, et al. Optimization of the model of abdominal aortic aneurysm—Experiment in an animal model. Journal of Vascular Research. 2009;**46**(1):1-5

[50] Houdek K, Moláček J, Třeška V, Křížková V, Eberlová L, Boudová L, et al. Focal histopathological progression of porcine experimental abdominal aortic aneurysm is mitigated by atorvastatin. International Angiology: A Journal of the International Union of Angiology. 2013;**32**(3):291-306

[51] Turnbull IC, Hadri L, Rapti K, Sadek M, Liang L, Shin HJ, et al. Aortic implantation of mesenchymal stem cells after aneurysm injury in a porcine model. The Journal of Surgical Research. 2011;**170**(1):e179-e188

[52] Nabseth DC, Martin DE, Rowe MI, Gotlieb LS, Deterling RA. Enzymatic destruction of aortic elastic tissue and possible relationship to experimental atherosclerosis. The Journal of Cardiovascular Surgery. 1963;**4**:11-17

[53] Manning MW, Cassi LA, Huang J, Szilvassy SJ, Daugherty A. Abdominal aortic aneurysms: Fresh insights from a novel animal model of the disease. Vascular Medicine (London, England). 2002;**7**(1):45-54

[54] Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. The Journal of Clinical Investigation. 2000;**105**(11):1605-1612

[55] Shiraya S, Miyake T, Aoki M, Yoshikazu F, Ohgi S, Nishimura M, et al. Inhibition of development of experimental aortic abdominal aneurysm in rat model by atorvastatin through inhibition of macrophage migration. Atherosclerosis. 2009;**202**(1):34-40

[56] Wilson WRW, Evans J, Bell PRF, Thompson MM. HMG-CoA reductase inhibitors (statins) decrease MMP-3 and MMP-9 concentrations in abdominal aortic aneurysms. European Journal of Vascular and Endovascular Surgery. 2005;**30**(3):259-262

[57] Sluijter JPG, de Kleijn DPV, Pasterkamp G. Vascular remodeling and protease inhibition—Bench to bedside. Cardiovascular Research. 2006;**69**(3):595-603

[58] Mastoraki ST, Toumpoulis IK, Anagnostopoulos CE, Tiniakos D, Papalois A, Chamogeorgakis TP, et al. Treatment with simvastatin inhibits the formation of abdominal aortic aneurysms in rabbits. Annals of Vascular Surgery. 2012;**26**(2):250-258

[59] Wang L, Wang B, Li H, Lu H, Qiu F, Xiong L, et al. Quercetin, a flavonoid with anti-inflammatory activity, suppresses the development of abdominal aortic aneurysms in mice. European Journal of Pharmacology. 2012;**690**(1–3):133-141

[60] Wang L, Cheng X, Li H, Qiu F, Yang N, Wang B, et al. Quercetin reduces oxidative stress and inhibits activation of c-Jun N-terminal kinase/activator protein-1 signaling in an experimental mouse model of abdominal aortic aneurysm. Molecular Medicine Reports. 2014;**9**(2):435-442