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Experimental Models in Abdominal Aortic Aneurysm

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<http://dx.doi.org/10.5772/intechopen.79393>

Abstract

Abdominal aortic aneurysm (AAA) is a potentially fatal disease and survival rate is very low when rupture occurs. Experimental models related with abdominal aortic aneurysm are performed on intact and ruptured aneurysm (RAAA) models. By using AAA models; complex mechanisms of aneurysm formation, aneurysm progression, chance of rupture, preventative and treating methods are researched. Most commonly used methods for creating aneurysm are utilization of transgenic or knockout animals; intra/extraluminal pharmacologic treatments such as elastase, calcium chloride or angiotensin II; hyperlipidemic diet application and surgical interventions such as xenograft, stenosis or graft. Pathogenesis of aneurysm is predominantly examined on rodents whereas studies aimed at development of treatment modalities such as surgical or endovascular interventions are predominantly performed on large animals like rabbit, porcine or dog. Experimental studies modeling aneurysm rupture (RAAA) simulate shock (total hypoperfusion) occurred due to rupture and ischemia/reperfusion (I/R) occurred due to surgical treatment; without creating aneurysm. In this model, end organ or distal organ injuries and methods for reducing these injuries or their hemodynamic effects are investigated by creating shock +I/R.

Keywords: abdominal aortic aneurysm, ruptured abdominal aortic aneurysm, experimental models, elastase, rat

1. Introduction

Abdominal aortic aneurysm is a degenerative disease characterized by structural degeneration and progressive dilatation in aorta wall. Progressive increase in arterial diameter results with rupture which is a life-threatening condition. Only 50% of cases with ruptured aneurysm can reach to hospital and 30–50% of these patients dies in hospital. It is an important health problem which has been seen in 5–9% of men and 1–1.3% of women aged 65. It is 10th cause

of death in developed countries and its incidence increases as population ages. However, complex and multifactorial pathophysiology of AAA has not been thoroughly understood [1].

Many animal models have been developed for understanding pathophysiology of AAAs and developing treatment models. It has firstly been incidentally observed by Ponseti IV et al. in 1952 that medial necrosis, dissection and aneurysm formation occurred after a special diet. Abdominal aortic aneurysm has been created with different techniques in many different models, its pathophysiology and drugs for preventing aneurysm formation have been investigated [2].

Among these models, most effective models developed for learning disease progression are elastase-based models performed on small animals. Large animal models have been required for endovascular or current surgical treatment methods, many surgical models like saccular or aortic patch have been developed. Most commonly used models among many different AAA animal models, difference between models and their applications will be explained in this chapter [3].

2. Pathogenesis of abdominal aortic aneurysm

In this section, pathogenesis of aneurysm will be briefly reviewed for clarify the development mechanism of experimental models of abdominal aortic aneurysm (AAA).

2.1. Building stones of aortic wall

2.1.1. *Elastin and collagen*

Two major building stones of aortic wall are elastin and collagen. Elastin is a structural protein produced by fibroblasts. Collagen is a solid, insoluble and fibrous protein which is majorly produced by fibroblasts and also produced by cells like chondroblast and osteoblast. Elastin is the major lifting structure against aneurysm development whereas collagen is the safety barrier which is resistant to high pressure and which provides protection against rupture after aneurysm is occurred. Degeneration of elastin and collagen results with aneurysm and rupture. Some studies revealed that these two proteins are reduced in intima and media layers of aneurysmatic aortas [4].

Presence of various mechanisms in development of AAAs which has been known as progressive dilatation of aortic wall has been well-recognized. Most important one of all factors causing degeneration in aortic wall is altered homeostasis between matrix synthesis and degradation due to inflammation. Adventitial and medial inflammatory cell infiltration, elastin fragmentation and degeneration, medial attenuation are observed during aneurysm development [5].

Collagen synthesis in the media and adventitia layers (especially type I and III) increases in favor of repair during first stages of aneurysm formation, then it becomes excessively degraded like other extracellular matrix macromolecules such as elastin during late stage

and causes aortic rupture. Inflammatory cells like polymorphonuclear neutrophils, T cells, macrophages, mast cells, NK cells are present in all layers of aneurysm wall and intraluminal thrombus. These cells secrete various humoral-inflammatory factors like cytokines, chemokines, leukotrienes, reactive oxygen species (ROS) and immunoglobulins. Inflammatory cells enter to aortic intima and media layers through vasa vasorum vessels. Neovascularization and decreased number of smooth muscle cells in medial layer are typical features of aneurysm. Intraluminal thrombus causes functional hypoxia in luminal intima and media layers, therefore neovascularization and inflammation increase. Also, inflammatory cells in thrombus secrete active proteases like matrix metalloproteinase (MMP-9) and urokinase-type plasminogen activator (u-PA). Therefore, AAAs occur as a complex pathology consisted of many cellular and humoral mechanisms like inflammatory cells, various enzymes and complement system [6].

2.1.2. Cellular and molecular mechanisms

2.1.2.1. Proteases

Elastase is a group of serine endopeptidases which catalyzes degradation of elastin and other proteins to simpler molecules; breaks polypeptide chains in the bonds including carbonyl group of amino acids; and is secreted from neutrophils and macrophages. α -1 antitrypsin is a protease inhibitor and it suppresses elastase activity and protects the tissue from inflammation. Elastase levels have been found to be high in ruptured aneurysms whereas α -1-antitrypsin levels have been found to be low [7].

Matrix metalloproteinases (MMPs) are homolog peptidases which contain zinc in their active region; and can degrade extracellular matrix and basal membrane components. They are enzymes playing role in physiological processes like tissue regeneration, morphogenesis and wound healing.

Tissue inhibitor of metalloproteases (TIMPs) are antiprotease enzymes. MMPs secretion degrades structural proteins of aortic wall. Impaired balance between MMPs and TIMPs plays an important role in development of acute and chronic cardiovascular diseases. Important MMPs which play an important role in development of AAA are MMP-1, -2, -3, -9, -12 and -13. There have been inadequate evidence for other MMPs; however, it has been stated that MMP-3 plays a very important role for AAA [8]. Elastin degradation and extracellular matrix loss in addition to destruction of smooth muscle cells via MMPs cause media layer thinning and aortic dilatation. Especially in enlarged aneurysms, intraluminal thrombus along with local inflammation and proteolysis occurs. Local hemodynamic forces and weakened vessel wall increases aneurysm enlargement. If wall stress exceeds tensile strength; rupture occurs. Inflammation, matrix remodeling and neovascularization reduce tensile strength [9].

Others are serine proteases, tissue plasminogen activators (t-PA, u-PA), plasmin, neutrophil elastase, cysteine protease (cathepsin D, L, K and S); also cysteine and serine proteases have been shown in all AAAs. Concentrations of dipeptidyl peptidase which is a lysosomal cysteine protease, is found normal at aneurysm wall or abundant in stenotic arterial walls when neutrophil elastase and other proteases are activated [6, 10].

2.1.2.2. *Phospholipids*

Phospholipids play an important role in cell membrane structure; also they have been known as very important inflammatory mediators. 5-lipoxygenase (5-LO) and leukotriene C4 synthase levels have been found high in human AAA tissues [11]. Association between AAA and cyclooxygenase (COX) and its sub-component prostaglandin E2 (PGE2) has been demonstrated, indometacin which is a non-selective COX inhibitor has been shown as preventing AAA created with elastase in rat [12]. PGE2 has been shown to activate IL-6 secretion of macrophages in studies performed on human aortic tissue or aortic smooth muscle cells [13].

2.1.2.3. *Inflammatory cells*

Most commonly seen inflammatory cells among many inflammatory cells identified in AAA tissue are macrophages and it has been known that they play an active role in aneurysm formation by many macrophage-mediated inflammatory responses [11, 14]. T and B lymphocytes have also identified in AAA tissues and functional insufficiency of CD25+ T regulator cells in AAAs patients has been reported [15]. Neutrophils have been identified in both human AAAs and animal aneurysm models, also L-selectin which is an adhesion molecule has been shown as an important mediator in AAA formation created with elastase in rats. Neutrophil depletion in mice with aortic perfusion of elastase led to attenuation of AAAs [16]. In addition, mast cells have also been identified in human AAA tissues and animal models. These cells secrete many proteases and inflammatory mediators which play role in inflammation and immunity. It has been shown that mast cell insufficiency in rat and mouse models decreases aneurysm formation [17].

2.1.2.4. *Complement system*

Complement activation is an immune response started with classic antigen-antibody reaction, lectin pathway or alternative C3 hydrolysis pathway. Factor B is the most important component of alternative pathway whereas C4 is the most important component of both classic and alternative pathway. Factor B insufficiency decreases the development of AAA created by elastase in rats [18].

2.1.2.5. *Cytokines and chemokines*

Cytokines regulate expressions of matrix metalloproteases (MMPs), serine proteases and cathepsin.

Without a doubt, tumor necrosis factor (TNF)- α has a very important place among many cytokines and chemokines which are related with inflammatory response. Increased plasma and tissue TNF- α levels have been found in AAA patients. Genetic or pharmacological (with infliximab) TNF- α inhibition has been shown to decrease calcium chloride-induced AAA formation in rats [19].

Another cytokine which plays an important role in inflammatory process is transforming growth factor (TGF)- β . This cytokine acts as a protector from inflammation and cell death. It has been shown that systemic blockage of TGF- β activity causes smooth muscle cell death,

elastin degradation and vascular inflammation in AAAs created with angiotensin II in hypercholesterolemic rats with genetic tendency [20, 21].

2.1.2.6. *MicroRNAs*

MicroRNAs are small and single-stranded RNA molecules which directs genes and complex pathophysiologic events in many diseases; and a few of them have been known to contribute AAA development. It was found that miR-29b which is one of 3 miR-29s (miR-29a, miR-29b and miR-29c) of MicroRNA family is increased in AAA tissues [22]. Another mediator responsible for smooth muscle cell proliferation and apoptosis in aneurysm tissue is miR-21; and its overexpression prevents aneurysm formation whereas its inhibition increases [23].

2.1.2.7. *Gender-dependent mediators*

Male gender is an important risk factor AAAs in humans. In pharmacological aneurysm creation models of animals, it was observed that aneurysm expansion is more in males and protection from aneurysm disappeared when female aortas are transplanted to males whereas aneurysm diameter reduces when estradiol is given to male rats [24].

2.2. Hemodynamic effects

Hemodynamic forces defines kinetic energy applied on arteries and veins by blood flow. Vascular endothelial and smooth muscle cells are constantly exposed to dynamic effect of blood flow during blood circulation. Three important hemodynamic components play role in AAA pathogenesis.

1. Hydrostatic pressure, perpendicular force acting on the vascular wall.
2. Wall shear stress (WSS), tangential force exerted by moving blood along the axis of flow.
3. Tensile hoop stress, the stress in the aortic wall acting circumferentially and produced by the resulting pressure [25].

It is well-known that AAA pathophysiology involves many factors as biological, biochemical and biomechanical processes. Although biochemical and biological factors are well-defined in AAA, role of biomechanical factors in AAA pathology is still poorly understood.

Altered flow types (turbulence etc.) may contribute aneurysm development by injuring arterial endothelium and increasing progression of arterial wall degeneration. Flow oscillation areas and areas with extreme shear stress are correlated with atherosclerosis development in aorta. Flow types in AAA have been demonstrated as smooth and laminar or irregular and turbulent; however, effects of wall shear stress on aneurysm is still poorly known. Geometry of the aneurysm sac and surrounding vasculature (including existence, size and symmetry of branches arising near the aneurysm) as well as position of the aneurysm sac relative to parent vessel affect intraaneurysmal flow [25, 26].

Coarctation increases hemodynamic stress on the aortic wall and alters flow dynamics. In some studies, it was shown that hemodynamic stress facilitates AAA predisposition and flow alterations significantly affect arterial lumen diameter. Also, poststenotic dilatation was detected at the area of oscillatory shear stress distal to the cast in some studies [27].

3. Intact aneurysm models

Various experimental models have been used for creating abdominal aneurysm. Pharmacological methods, xenograft, large animal models are a few of them. Most commonly used pharmacological methods are methods like intraluminal elastase, periaortic calcium chloride application, systemic angiotensin II infusions. Application of these models in rodents will be explained in detail in a different chapter. It is briefly presented in this part.

3.1. Elastase model

Elastase is a member of serine proteases. Its endoluminal infusion alters the normal structure of tunica media by causing elastic fiber destruction. It stimulates receptors which are activated by protease in the smooth muscle cells on the aortic wall; therefore inhibits Ca^{2+} inflow required for vascular contraction. This inhibition of smooth muscle contractions causes aortic dilatation [28].

As the response to acute elastase damage, elastic lamellae become fragmented by the leucocytes invading media layer; then formation of intraluminal thrombus (ILT) starts due to endothelial injury. This process promotes aneurysm growth by causing release of endogenous proteases activated by fibrinolytic system, activation of MMP-2, secretion of urokinase and leucocyte elastase.

This model can be utilized on animal species like rat, mice, hamster and rabbit (**Figure 1**). It can also be applied on larger species like dogs; however, intra-aortic perfusion, aortic balloon angioplasty and simultaneous collagenase infusion may be performed for avoiding problems caused by high dose of elastase infusion [28, 29].

Waiting period is approximately 7–14 days after the elastase infusion in rodents. An increase over 300% in aortic diameter in first week has been observed in this model. If rupture is not occurred in first week, stabilization is occurred at 2–3 weeks due to mesenchymal cells colonized into intraluminal thrombus (ILT) and fibrosis.

Generally, aneurysm development is completed in this time period, also histopathologic changes on aortic wall at the level of cellular and inflammatory levels are arisen along with mechanic dilatation occurred in first week. Relaparotomy is performed at the end of these durations, aortic measurements are performed and aorta tissue is excised for histopathologic examinations [30].

3.2. Calcium chloride model

Local CaCl_2 is applied on adventitial layer without direct intervention to abdominal aorta in this model which has firstly been designed for developing aneurysm on rabbit carotid artery. Therefore, it is technically easier than intraluminal elastase infusion model. In this model which has especially been used in rats, a CaCl_2 -impregnated gauze is directly applied

Figure 1. Intraluminal elastase infusion in rat abdominal aorta.

to infrarenal aorta. Calcium ions have high degree of affinity to elastin. After application, ionized calcium intracellularly turns into calcium phosphate (CaPO_4) due to alkaline phosphatase activity from vascular smooth muscle cells (vSMC) and this compound precipitates as hydroxyapatite crystals in elastin fibers, causing their mechanical degradation. In some models, this process is accelerated with applying local phosphate-buffered saline (PBS) after CaCl_2 application. It has also been known that human aneurysm wall calcification is caused by these CaPO_4 crystals. Aortic dilatation in CaCl_2 model increases with time and it is caused by progressive infiltration of mast cells and T lymphocyte to adventitia layer. Vascular smooth muscle cells disappear due to calcification and fragmentation in elastic fibers and they are replaced by neutrophils [27]. Most important aneurysm formation mechanisms of CaCl_2 application are medial degeneration and leucocyte infiltration. Endoluminal and intramural thrombus are not seen in these type of aneurysms, also rupture chance is very low. Although it is mostly used in mice, it can also be applied on rats and pigs [31].

3.3. Elastase and calcium chloride combined model

It has been developed by Tanaka. In this model, CaCl_2 application is performed around the aorta while intraluminal elastase infusion through femoral artery is being given. CaCl_2 -impregnated gauze is applied around aorta along with 30 U elastase infusion into aorta (**Figure 2**). Total duration is 20 minutes. Elastase infusion duration is decreased from 120 to 20 minutes. In this model, it has been reported that no atherosclerosis and intraluminal thrombus occurs and it can easily be performed [28].

Figure 2. Intraluminal elastase infusion and adventitial CaCl_2 application.

3.4. Angiotensin II (AngII) model

In this model, intravascular infusion and aortic exploration are not performed. It is the most easily performed aneurysm model on mice. It has been firstly described by Daugherty at 2000. It is only used on mice and it is the most frequently used method on this species.

Angiotensin II is a potent vasoconstrictor octapeptide. It is produced from angiotensin I after the removal of two amino acids at the C-terminal by angiotensin converting enzyme. It maintains blood pressure and body fluid/sodium balance by causing constriction of blood vessels.

It has been shown that continuous infusion of angiotensin II causes vascular remodeling; especially causes atherosclerosis in transgenic or knockout animals; therefore it is an important model for researching AAA development and preventative mechanisms [32, 33].

3.5. Spontaneously mutated and transgenic mice models of AAA

Genetically determined types are Blotchy, Lox (lysyl oxidase) deficiency, MMP-3 or TIMP-1 (tissue inhibitor of matrix metalloproteinase) deficiencies, LDL receptor $-/-$, ApoE $-/-$, eNOS $-/-$, C57BL/6 and “transgenic mice overexpressing renin and angiotensin”. Food supplements, different feeding methods or some drugs increase rupture risk of aneurysm. For example, beta-aminopropionitrile, sweet pea, diethylstilbestrol, monoamine oxidase inhibitors and hydralazine are some of them; and they increase rupture risk by reacting collagen in media layer without causing any hemodynamic effect. On the contrary, propranolol and reserpine decrease rupture risk by their hemodynamic effect; propranolol also decreases rupture risk by causing connective tissue with increasing cross-linkage of elastin.

Blotchy Mouse is the mutant with impaired intestinal copper absorption due to X chromosome mutation. It is one of the species with spontaneous aneurysm development. Copper is co-factor of lysyl oxidase (Lox). Lox plays a role in vascular growth and extracellular matrix (ECM) production. Elastin and collagen productions are impaired in Lox-deficient mice, neutrophil infiltration in tunica adventitia occurs and spontaneous aneurysm formation is seen in male rats in 3 weeks. Elastic fiber fragmentation and disintegration of smooth muscle cell layers of aortic wall are seen. Although saccular or fusiform aneurysm formations occur through whole aorta in these mice; thoracic aorta is most commonly involved. However; it has not been primarily preferred model for experimental aneurysm studies because it usually results in spontaneous thoracic aorta rupture [34].

Spontaneous aneurysm development on both abdominal and thoracic aortas is seen in MMP-3 and TIMP-1 deficient mice. The importance of MMPs for AAA formation was further investigated by Eskandari et al. They demonstrated a protective role for tissue inhibitor of metalloproteinase (TIMP)-1 on elastase induced AAA in mice. Compared with wild type mice, TIMP-1 deficient mice developed larger AAAs after AAA induction with elastase [35].

Although LDL receptor and ApoE deficient mice are more commonly used in atherosclerosis studies, suprarenal AAAs may occur when they are fed with high-fat diet for 6 months. Adventitia thickening along with media layer injury in these animals prevent rupture. These aneurysms are very similar to atherosclerotic aneurysms due to presence of elastin degradation medial electrolysis, vascular dilatation and necrotic core. Duration of aneurysm development can be shortened with pharmacologic methods like intraluminal elastase, periaortic CaCl_2 application or subcutaneous AngII infusion in these mice.

It has been demonstrated that suprarenal AAA occurred with a rate of 25% in ApoE+ eNOS deficient mice which had been fed with high-fat diet for 4–6 months. These aneurysms are characterized with perimedial thrombotic and fibrous material accumulation [31, 33, 34].

Chronic hypertension was created in transgenic mice by cross-mating with human renin or human angiotensin genes, then occurrence of aortic rupture was observed when they were fed with water containing 1% sodium chloride. Aortic aneurysm was predominantly occurred in aortic arch or juxtarenal segments in these mice [36].

Role of MMPs in AAA formation in genetically altered mice have been defined. It was shown that after CaCl_2 -mediated aortic injury was created; aneurysm did not occur in MMP2 $-/-$ and MMP9 $-/-$ knockout mice whereas aortic dilatation was decreased in MMP12 $-/-$ mice when compared with wild-types. Studies indicating regulation of matrix metalloproteinases by TIMPs have been conducted [31, 36].

3.6. Large animal models

Aneurysm models performed on large animals have been predominantly developed for pre-clinic research of surgical or endovascular treatment methods. Most important of these methods are Elastase model, xenograft model, graft models (patch, pouch, interposition grafts), coarctation model and balloon dilatation model.

3.6.1. Elastase model

This model which has been mostly used on rodents has also been used on large animals. Aneurysm occurs due to destruction of medial elastic lamellae. Intraaortic elastase infusion may be applied by reaching aorta through femoral artery without laparotomy, alone or with applying methods like balloon angioplasty, collagenase infusion, CaCl_2 application. Aorta aneurysm may be created in swine and dog aortas with this method by creating intimal hyperplasia, medial elastic fiber rupture and matrix degeneration. It is the most similar method to human aneurysm without tendency towards rupture [37].

Complications like livedo, lower limb paraplegia, neurologic bladder and rectal prolapse may occur in this method. Changes in aortic wall and aneurysmatic dilatation, calcification and blood flow are followed with weekly ultrasonographic examination under sedation or anesthesia. After 2 weeks, it is possible to transformation of dilatation to an aneurysm (>50%). Experiment is terminated at the end of third week; then aortic exploration and necessary examinations are performed. It has been reported that chance of rupture is increased in monitoring period which exceeds 4 weeks.

3.6.2. Xenograft model

It is also called “decellularized aortic xenograft model”. In this model, aorta implantation between two different species is performed. This model is based on vascular smooth muscle cell suppression and extracellular matrix immunogenicity between species. In this model, roles of immune system and extracellular matrix proteins in aneurysm development can be researched and pharmacological or immunologic mechanisms preventing aneurysm development can be examined [38].

3.6.2.1. Experimental application

A 1 cm infrarenal aorta segment of Guinea pig (300–350 g) is excised after ligating collateral branches with median laparotomy under general anesthesia; then it is decellularized in sodium dodecyl sulphate (SDS 1%, Sigma, St-Louis, USA) at 37°C for 18 hours. After this procedure, it is washed with Triton X-100 solution (Sigma) and process is completed after washing four times in 24 hours with 0.1% phosphate buffered-saline (PBS) solution. Xenograft prepared with this method is transplanted to Lewis rats in orthotopic position by using 10/0 sutures with microsurgery method [39]. Decellularized aortic xenograft triggers immune reactions without an acute fatal rejection. All cells on the distal part of aorta are removed during donor graft preparation; however, collagen and elastin network of extracellular matrix is preserved. Degraded guinea pig extracellular matrix becomes infiltrated by intimal monocytes and T-lymphocytes, luminal thrombus along with aortic dilatation starts. AAA occurs due to reaction between species in extracellular matrix after 14 days, xenograft destruction may result with aortic rupture. Doubling time of aortic diameter is short as 10 days in this model [31, 40].

3.6.3. Graft models

In these models, aneurysm is created by performing biologic or prosthetic graft interposition to abdominal aorta. Tubular graft or patch application may be performed. Most frequently

used biologic grafts are peritoneum, bovine pericardium, fascia of rectus muscle, jejunum whereas prosthetic materials are dacron or polytetrafluoroethylene (PTFE) grafts. Most common graft application methods are patch model, pouch (saccular aneurysm model), graft interposition and coarctation models.

3.6.3.1. Patch graft model

In this experimental model developed on large animals like swine and dog, developing new endovascular devices for AAA repair by creating aneurysm similar to human anatomy became possible. It is an easily applicable method. Most commonly used one of this method is the an elliptic patch application. Patch materials used in this method which is also named as anterior patch model are materials such as prosthetic grafts, venous grafts (iliac vein or jugular vein), rectus fascia, jejunum treated with glutaraldehyde and gastric serosa.

In this procedure, abdominal aorta is explored between renal artery and iliac bifurcation by performing median laparotomy under general anesthesia. One each silicon loops are placed on renal arteries and above of aortic bifurcation. Aortic segment is occluded with silicon loops after systemic heparin injection (200 U/kg). Inferior mesenteric artery and lumbar arteries are temporary closed with mini hemoclips. If juxtarenal aneurysm is created, renal arteries are also temporary clamped; then segments of 2–3 mm from edge of incision are longitudinally excised by performing 5–10 cm aortotomy. Patch graft is sutured to aortotomy incision with 5/0 prolene suture by using continuous technique. Aorta clamps are opened with well-known air removal techniques and circulation is restored. Incisions are closed. Aneurysm formation is generally seen in first 1 months. Rupture rate varies according to graft types and length of aneurysmatic segment. It has been reported that segments whose length is more than 6 cm have a rupture rate of 70%. Lowest rupture rate has been seen in iliac vein patches (0%) whereas highest rupture rates have been seen in jejunum patches (100% in 42 hours), jejunum patches treated with glutaraldehyde (66% at 11 days) and peritoneal patches (50%, 2 weeks). For preparing peritoneal patches, peritoneal part is isolated and resected with blunt dissection and it is shaped as an ovoid-shaped patch whose length is 5–10 cm and width is 2–3 cm. A double-layered peritoneal patch is created after folding the free end on itself. It is kept in saline solution for 30 minutes before use, and it is anastomosed with continuous suture like other grafts [41].

3.6.3.2. Saccular aneurysm model

It is an another aneurysm model which has been firstly used by Perini. Biologic or prosthetic material used in this model is cut into a material sized 3X6 cm and its both sides are sutured after folding it on itself. It becomes a sac sized approximately 3X3 cm; then opening of the sac is anastomosed to an aortotomy sized 3 cm which is created on the anterior part of aorta. Result is a saccular aneurysm. Bovine pericardium is most frequently used material. Venous graft materials or prosthetic materials may also be used; however, largest dilatation is acquired with biomaterials.

Swines whose weight are approximately 20 kg are used for applying this model. Similar to anterior patch model which uses median laparotomy, aorta between renal arteries and bifurcation is explored. A 3 cm segment is chosen for aneurysm. After administrating IV heparin (with a dose of 100 UI/kg), proximal and distal aorta are clamped and 3 cm longitudinal aortotomy is

performed on the chosen area; then previously prepared saccular bovine pericardium is anastomosed to this area with continuous technique using 6/0 polypropylene. Result is a saccular aneurysm. Retroperitoneum and abdomen are appropriately closed. Stabilization is provided a few weeks after surgery, mortality and morbidity of this procedure are low. Continuity of aneurysm is followed with Doppler ultrasonography with intervals of 15 days, aorta diameter increases by more than 50% and it is most frequently used for evaluating endovascular methods. Terminal branches of aorta and lumbar plexus are preserved, partial thrombus formation is seen in lumen. Endovascular graft applications may easily be performed on aneurysm formation created with this model, it is a good model for endoleak researches due to patency of side branches. Most important disadvantages are lack of characteristic features of human aneurysm like atherosclerosis, medial degeneration, medial or adventitial lymphocyte infiltration. Complications like renal failure, intestinal perforation, sepsis, iliac artery thrombus may be seen in this model. Bovine pericardium is cheaper than synthetic materials because it can be acquired easily [42].

Most frequently used animal in this model due to anatomic and hematologic (coagulation and fibrinolytic system) similarity to humans is swine. In addition, it is an easily manipulable model. Lipid metabolism, lipoprotein profile, thrombocyte aggregation/thrombus formation and fibrin deposits after intimal injury, histologic structure of neointimal are also very similar to human. Disadvantages are rapid growth of animal, its low tolerance to anesthesia, high cost and possible paralysis due to medullary ischemia. Pericardium used in this model is treated with glutaraldehyde for reducing antigenicity and increasing resistance to degeneration. Monitoring is performed with Doppler ultrasonography in this model, other imaging modalities like angiography is not required.

3.6.3.3. *Interposition grafts*

In this model, various types of grafts are interposed to infrarenal area of aorta. Graft whose diameter is twofold of abdominal aorta is replaced to aorta after spinal artery are ligated. Pigs are generally used and endovascular approaches can be used after two weeks. In this model, biologic materials (bovine jugular vein treated with glutaraldehyde), fusiform-shaped dacron grafts or PTFE grafts dilated with balloon are used. For creating aneurysm, a 8 mm PTFE graft is dilated with balloon until its final diameter reaches 30 mm. Graft which becomes fusiform-shaped is anastomosed as it is placed between renal arteries and trifurcation. Caudal paraplegia may occur due to ligated spinal arteries. Two lumbar arteries are re-implanted through posterior of aorta with Carrel patch technique in endoleak researches. Endovascular repair can be done 2 weeks after surgery. Type II endoleak researches can be done after placing intraluminal pressure transducer into sac during surgery [43].

3.6.4. *Stenosing cuff*

Aneurysm development can be maintained due hemodynamic effect by creating stenosis at the infrarenal area of aorta. Stenosis below renal arteries can be created by nylon tape or plastic cuff whose width are generally 5 mm. Dilatation of aortic wall and aneurysm formation are seen due to turbulent flow after stenosis. This model is generally used together with intraluminal elastase infusion and balloon angioplasty.

After performing median laparotomy, aortic exploration and entrance right above aortic trifurcation; balloon plasty and elastase infusion are performed. Amount of administered elastase when a pig weighting approximately 30 kg is 10 ml; and stenosing cuff is placed below renal arteries by performing balloon dilatation after infusion. Presence of palpable thrill on aorta is the indicator of adequate stenosis. Parameters like “pulsatility index” which provides quantitative measurement of degree of stenosis may also be used [44].

Increase in aortic diameter is expected over 50%. Most important advantage of this model is preservation of lumbar arteries. Disadvantages are requirement of laparotomy, occurrence of retroperitoneal fibrosis and aneurysm extension limited at proximal [2]. Turbulent flow in this model provides appropriate hemodynamic effect for damaging intercellular matrix after protective barriers like tunica intima and lamina elastic interna are weakened by elastase and effect of balloon; rather than creating aneurysm alone [45].

3.6.5. Balloon dilatation

Balloon dilatation alone cannot produce enough aneurysmatic dilatation in large animal models. Therefore, it is always used with elastase or collagenase infusion or sometimes both of them. Infra-renal stenosing cuff is also occasionally used with them. High pressure balloons with width of 10–12 cm and length of 4 cm are used. Angioplasty balloons produced for peripheral arteries may be used for this purpose. Applications can be performed with or without stenting. It is percutaneously performed and whole side branches of aorta are preserved. It results with moderate degree of dilatation [2].

4. Ruptured abdominal aortic aneurysm (RAAA) model

Clinical condition occurred in aneurysm rupture is modeled in this experimental model without creating a real aneurysm. In this model which has been firstly described by Thomas Lindsay at 1995, first aneurysm rupture by creating shock, ischemic stage of surgical treatment by placing aortic clamp then revascularization and reperfusion processes after removing clamp are modeled. Lindsay identified factors like degree of pulmonary injury, ideal clamping area and duration by measuring “lung permeability index” and “neutrophil sequestration” levels; he reported that highest damage had been observed on rats with created lower torso ischemia due to 1 hour of shock + supramesenteric clamp. This model has also been used by various investigators [46, 47].

In experimental RAAA model, hemorrhagic shock studies evaluating hemodynamic effects of all kinds of drugs, molecules or resuscitation fluids as well as ischemia/reperfusion researches evaluating their effects on remote or end organ injuries can be conducted [48].

Most important features which differ RAAA model from other aortic ischemia/reperfusion (I/R) studies or hypovolemic shock studies are initial shock creation and placement of aortic clamps on both supramesenteric level and aortic bifurcation level. Total body hypoperfusion due to initial hypovolemic shock, lower torso ischemia with aortic clamp and then reperfusion are done by creating both shock and I/R [49–51].

Therefore an effect stronger than both of them alone is acquired. Besides, most important cause of high mortality in RAAA is comorbidity of these two important pathology [52].

Application: Most commonly used animals are rats; however, large animals may also be used. Right carotid artery for measuring mean arterial pressure (MAP) and jugular vein for venous access are cannulated with cut-down method in anesthetized rats with spontaneous respiration (No 22 cannula) (**Figure 3**).

Heart rate, MAP, rectal temperature and respiratory rate are monitored. Saline infusion with a rate of 3 ml/kg/h is given during whole experiment period for preventing insensible losses. Rectal temperature is kept at 36.5°C by using heat lamp. After stabilization is acquired, shock is created as MAP is set to 50 mmHg for 60 minutes by drawing blood in plastic injector containing standard heparin; aneurysm rupture is simulated and withdrawn blood is kept in room temperature (**Figure 4**).

Blood which will be drawn is calculated as not exceeding 30% of total blood volume. Lower torso ischemia is created at the end of 1 hour by clamping abdominal aorta with microvascular clamps on superior mesenteric level and iliac bifurcation level after performing median laparotomy and systemic heparinization (250 U/kg). Half of the withdrawn blood is slowly reinfused through venous line; therefore surgical x-clamp and resuscitation are simulated (**Figure 5**).

Figure 3. Cannulations of jugular vein and carotid artery and monitorization.

Figure 4. Drawing blood from carotid artery to create shock.

At the end of ischemic period which is 60 minutes long, all of remaining withdrawn blood is re-infused right before opening clamp and the subject is left to reperfusion for 120 minutes after removing clamps and closing abdomen. During reperfusion period, MAP is kept at approximately 100 mmHg and fluid replacement is performed if necessary. Hemodynamic values are recorded in every 10 minutes (**Figure 6**). All given fluids are recorded and most commonly used fluid is Ringer lactate. At the end of the period, rats are sacrificed by drawing blood method; then necessary blood and tissue samples are collected.

Experiment can be modified with different ways.

Figure 5. Aortic clamps on superior mesenteric and iliac bifurcation levels in rat aorta.

Figure 6. Mean arterial blood pressure during the experiment.

5. Conclusion

Until today, many animal experimental models have been developed for investigating development mechanisms, factor affecting expansion and treatment methods of AAA which has been a very common disease with high mortality in community. It is obvious that as the technology advances, larger number of studies which are more sophisticated will be needed for both better understanding etiopathogenesis and developing less invasive methods for treatment.

Conflict of interest

Author declares that there is no conflict of interests regarding the publication of this paper.

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References

- [1] Hoornweg LL, Storm-Versloot MN, Ubbink DT, et al. Meta analysis on mortality of ruptured abdominal aortic aneurysms. *European Journal of Vascular and Endovascular Surgery*. 2008;**35**(5):558-570
- [2] Kloster BO, Lund L, Lindholt JS. Induction of continuous expanding infrarenal aortic aneurysms in a large porcine animal model. *Annals of Medicine and Surgery*. Jan 14, 2015;**4**(1):30-35
- [3] Czerski A, Bujok J, Gnus J, Hauzer W, Ratajczak K, Nowak M, et al. Experimental methods of abdominal aortic aneurysm creation in swine as a large animal model. *Journal of Physiology and Pharmacology*. Apr 2013;**64**(2):185-192
- [4] Dobrin PB. Pathophysiology and pathogenesis of aortic aneurysms. *Current concept. Surgical Clinics of North America*. 1989;**69**:687-703
- [5] Lu H, Rateri DL, Bruemmer D, Cassis LA, Daugherty A. Novel mechanisms of abdominal aortic aneurysms. *Current Atherosclerosis Reports*. Oct 2012;**14**(5):402-412
- [6] Shimizu K, Mitchell RN, Libby P. Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arteriosclerosis, Thrombosis, and Vascular Biology*. May 2006;**26**(5):987-994

- [7] Songhera SS, Hingorani A, Tilson MD. Etiology of abdominal aortic aneurysm. In: Ascher E, editor. Haimovici's Vascular Surgery. 6th ed. USA: Wiley-Blackwell publishing; 2012. pp. 221-232. DOI: 10.1002/9781118481370
- [8] Rabkin SW. The role matrix metalloproteinases in the production of aortic aneurysm. *Progress in Molecular Biology and Translational Science*. 2017;**147**:239-265. DOI: 10.1016/bs.pmbts.2017.02.002 [Epub Mar 15, 2017]
- [9] Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. Pathophysiology and epidemiology of abdominal aortic aneurysms. *Nature Reviews. Cardiology*. 2011;**8**:92-102
- [10] Pagano MB, Bartoli MA, Ennis TL, et al. Critical role of dipeptidyl peptidase I in neutrophil recruitment during the development of experimental abdominal aortic aneurysms. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**:2855-2860
- [11] Di Gennaro A, Wagsater D, Mayranpaa MI, et al. Increased expression of leukotriene C4 synthase and predominant formation of cysteinyl-leukotrienes in human abdominal aortic aneurysm. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**:21093-21097
- [12] Holmes DR, Wester W, Thompson RW, Reilly JM. Prostaglandin E2 synthesis and cyclooxygenase expression in abdominal aortic aneurysms. *Journal of Vascular Surgery*. 1997;**25**:810-815
- [13] Bayston T, Ramessur S, Reise J, et al. Prostaglandin E-2 receptors in abdominal aortic aneurysm and human aortic smooth muscle cells. *Journal of Vascular Surgery*. 2003;**38**:354-359
- [14] Ishibashi M, Egashira K, Zhao Q, et al. Bone marrow-derived monocyte chemoattractant protein-1 receptor CCR2 is critical in angiotensin II-induced acceleration of atherosclerosis and aneurysm formation in hypercholesterolemic mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2004;**24**:174-178
- [15] Yin M, Zhang J, Wang Y, et al. Deficient CD4+CD25+ T regulatory cell function in patients with abdominal aortic aneurysms. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2010;**30**:1825-1831
- [16] Eliason JL, Hannawa KK, Ailawadi G, et al. Neutrophil depletion inhibits experimental abdominal aortic aneurysm formation. *Circulation*. 2005;**112**:232-240
- [17] Sun J, Sukhova GK, Yang M, et al. Mast cells modulate the pathogenesis of elastase-induced abdominal aortic aneurysms in mice. *The Journal of Clinical Investigation*. 2007;**117**:3359-3368
- [18] Pagano MB, Zhou HF, Ennis TL, et al. Complement-dependent neutrophil recruitment is critical for the development of elastase-induced abdominal aortic aneurysm. *Circulation*. 2009;**119**:1805-1813
- [19] Xiong W, MacTaggart J, Knispel R, et al. Blocking TNF-alpha attenuates aneurysm formation in a murine model. *Journal of Immunology*. 2009;**183**:2741-2746

- [20] Wang Y, Ait-Oufella H, Herbin O, et al. TGF-beta activity protects against inflammatory aortic aneurysm progression and complications in angiotensin II-infused mice. *The Journal of Clinical Investigation*. 2010;**120**:422-432
- [21] Dai J, Michineau S, Franck G, et al. Long term stabilization of expanding aortic aneurysms by a short course of cyclosporine a through transforming growth factor-beta induction. *PLoS One*. 2011;**6**:28903
- [22] Maegdefessel L, Azuma J, Toh R, et al. Inhibition of microRNA-29b reduces murine abdominal aortic aneurysm development. *The Journal of Clinical Investigation*. 2012;**122**:497-506
- [23] Milewicz DM. MicroRNAs, fibrotic remodeling, and aortic aneurysms. *The Journal of Clinical Investigation*. 2012;**122**:490-493
- [24] Henriques T, Zhang X, Yiannikouris FB, Daugherty A, Cassis LA. Androgen increases AT1a receptor expression in abdominal aortas to promote angiotensin II-induced AAAs in apolipoprotein E-deficient mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2008;**28**:1251-1256
- [25] Dua MM, Dalman RL. Hemodynamic influences on abdominal aortic aneurysm disease: Application of biomechanics to aneurysm pathophysiology. *Vascular Pharmacology*. Jul-Aug 2010;**53**(1-2):11-21
- [26] Greve JM, Les AS, Tang BT, Draney Bloome MT, Wilson NM, Dalman RL, Pelc NJ, Taylor CA. Allometric scaling of wall shear stress from mice to humans: Quantification using cine phase-contrast MRI and computational fluid dynamics. *American Journal of Physiology. Heart and Circulatory Physiology*. Oct 2006;**291**(4):H1700-H1708
- [27] Patelis N, Moris D, Schizas D, Damaskos C, Perrea D, Bakoyiannis C, Liakakos T, Georgopoulos S. Animal models in the research of abdominal aortic aneurysms development. *Physiological Research*. Dec 20, 2017;**66**(6):899-915
- [28] 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. *Journal of Vascular Surgery*. 2009;**50**:1423-1432
- [29] Senemud J, Caligiuri G, Etienne H, Delbosc S, Michel JB, Coscas R. Translational relevance and recent advances of animal models of abdominal aortic aneurysm. *Arteriosclerosis, Thrombosis, and Vascular Biology*. Mar 2017;**37**(3):401-410
- [30] Holmes DR, Petronec D, Wester W, Thompson RW, Reilly JM. Indomethacin prevents elastase-induced abdominal aortic aneurysm in the rat. *The Journal of Surgical Research*. Jun 1996;**63**(1):305-309
- [31] Lysgaard Poulsen J, Stubbe J, Lindholt JS. Animal models used to explore abdominal aortic aneurysms: A systematic review. *European Journal of Vascular and Endovascular Surgery*. Oct 2016;**52**(4):487-499
- [32] Davis FM, Rateri DL, Daugherty A. Mechanisms of aortic aneurysm formation: Translating preclinical studies into clinical therapies. *Heart*. Oct 2014;**100**(19):1498-1505

- [33] Kujyaniemi H, Ryer EJ, Elmore JR, Tromp G. Understanding the pathogenesis of abdominal aortic aneurysms. *Expert Review of Cardiovascular Therapy*. 2015;**13**(9):975-987
- [34] Daugherty A, Cassis LA. Mouse models of abdominal aortic aneurysms. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2004;**24**:429-434
- [35] Eskandari MK, Vijungco JD, Flores A, Borensztajn J, Shively V, Pearce WH. Enhanced abdominal aortic aneurysm in TIMP-1-deficient mice. *The Journal of Surgical Research*. 2005;**123**(2):289-293
- [36] Tsui JC. Experimental models of abdominal aortic aneurysms. *The Open Cardiovascular Medicine Journal*. 2010;**4**:221-230
- [37] Lederman A, Saliture Neto FT, Ferreira R, de Figueiredo LF, Otoch JP, Aun R, da Silva ES. Endovascular model of abdominal aortic aneurysm induction in swine. *Vascular Medicine*. Jun 2014;**19**(3):167-174
- [38] Allaire E, Bruneval P, Mandet C, Bacquemin JP, Mitchel JB. The immunogenicity of the extracellular matrix in arterial xenografts. *Surgery*. 1997;**122**:73-81
- [39] Schneider F, Saucy F, de Blic R, Dai J, Mohand F, Rouard H, Ricco JB, Becquemin JP, Gervais M, Allaire E. 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*. Jun 2013;**45**(6):666-672
- [40] Alsac JM, Journe C, Loudec L, Dai J, Julia P, Fabiani JN, Michel JB. Downregulation of remodeling enzymatic activity induced by an angiotensin-converting enzyme inhibitor (perindopril) reduces the degeneration of experimental abdominal aortic aneurysms in a rat model. *European Journal of Vascular and Endovascular Surgery*. Apr 2011;**41**(4):474-480
- [41] Maynar M, Qian Z, Hernandez J, Sun F, DeMiguel C, Crisostomo V, Usón J, Pineda LF, Espinoza CG, Castañeda WR. An animal model of abdominal aortic aneurysm created with peritoneal patch: Technique and initial results. *Cardiovascular and Interventional Radiology*. Mar–Apr 2003;**26**(2):168-176
- [42] Aquino M de A, Baeeros SM, Castro AA, Pitta GB, Pereira AH. Experimental model of saccular abdominal aortic aneurysm in Swines with Pericardium Sac. *Brazilian Journal of Cardiovascular Surgery*. Feb 2016;**31**(1):70-73
- [43] Rhee JY, Trocciola SM, Dayal R, Lin S, Chaer R, Kumar N, Mousa A, Bernheim J, Christos P, Prince M, Marin ML, Gordon R, Badimon J, Fuster V, Kent KC, Faries PL. Treatment of type II endoleaks with a novel polyurethane thrombogenic foam: Induction of endoleak thrombosis and elimination of intra-aneurysmal pressure in the canine model. *Journal of Vascular Surgery*. Aug 2005;**42**(2):321-328
- [44] Lin PY, Wu YT, Shih YH, Sampilvanjil A, Chen LR, Yang YJ, Wu HL, Jiang MJ. Coarctation-induced degenerative abdominal aortic aneurysm in a porcine model. *Journal of Vascular Surgery*. Mar 2013;**57**(3):806-815
- [45] Molacek J, Treska V, Kober J, Certik B, Skalicky T, Kuntscher V, Krizkova V. Optimization of the model of abdominal aortic aneurysm-experiment in an animal model. *Journal of Vascular Research*. 2009;**46**:1-5

- [46] Lindsay T, Walker P, Romaschin A. Acute pulmonary injury in a model of ruptured abdominal aortic aneurysm. *Journal of Vascular Surgery*. 1995;**22**:1-8
- [47] Harkin DW, Maron CD, Rother RP, Romaschin A, Rubin BB, Lindsay TF. C5 complement inhibition attenuates shock and acute lung injury in an experimental model of ruptured abdominal aortic aneurysm. *The British Journal of Surgery*. 2005;**92**:1227-1234
- [48] Harkin D, Rubin B, Romaschin A, Lindsay T. Selective inducible nitric oxide (iNOS) inhibition attenuates remote acute lung injury in a model of ruptured abdominal aortic aneurysm. *The Journal of Surgical Research*. 2004;**120**:230-241
- [49] Shahani R, Marshal J, Barry R, Walker PM, Lindsay TF. Role of TNF- α in myocardial dysfunction after hemorrhagic shock and lower-torso ischemia. *American Journal of Physiology. Heart and Circulatory Physiology*. 2000;**278**:942-950
- [50] Lozano FS, Rodriquez JM, Criado FJ, et al. Postoperative evolution of inflammatory response in a model of suprarenal aortic cross-clamping with or without hemorrhagic shock. Systemic and local reactions. *World Journal of Surgery*. 2005;**29**:1248-1258
- [51] Lomas-Niera JL, Perl M, Chung CS, Ayala A. Shock and hemorrhage: An overview of animal models. *Shock*. 2005;**24**(Supp 1):33-30
- [52] Lindsay TF, Luo XP, Lehotay DC, Rubin BB, Anderson M, Walker PM, Romaschin AD. Ruptured abdominal aortic aneurysm, a “two-hit” ischemia/reperfusion injury: Evidence from an analysis of oxidative products. *Journal of Vascular Surgery*. Aug 1999;**30**(2):219-228