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EXPERIMENTAL METHODS OF ABDOMINAL AORTIC ANEURYSM CREATION IN SWINE AS A LARGE ANIMAL MODEL

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Animal models of abdominal aortic aneurysms (AAA) enable preclinical studies on new therapeutic approaches and help to understand pathophysiology of the disease. The aim of this study was to demonstrate the effectiveness of selected methods of experimental induction of abdominal aortic aneurysm in swine and to adapt the EMG examination (electromyography) to record the vessel wall changes. The animals were divided into 3 groups comprising 4 individuals in whom AAA was surgically induced. In the first group the AAA was induced by mechanical stretching of the aortic wall and injection of 500 IU elastase under pressure. The second group received elastase and 6000 IU of collagenase. In the third group 0.5 M $CaCl_2$ solution was introduced additionally. Enlargement of abdominal aorta was monitored for 4 weeks. The first group did not show any aorta dilatation. In the second group the aortic lumen was dilated on average by 71±3.5% ($P \leq 0.001$) as shown at autopsy and by 76.6±9.3% as measured by the ultrasound method. In the third group aorta was dilatated by 104.2±11.3% as obtained by ultrasound and 72±3% at post-mortem examination. Myoelectric activity of VSMC (vascular smooth muscle cell) was demonstrated and it was characterized by the presence of three types of waves closely related to the pressure changes in the vessel lumen. We conclude that collagen fibers damage plays a significant role in the AAA development in swine. The inflammatory process in the vessel's wall also contributes to AAA development. However, myoelectrical activity of VSMC does not significantly change despite histologically confirmed loss of muscular layer.

Key words: *abdominal aortic aneurysm, aneurysm creation, electromyography, myoelectric activity, abdominal aorta, vascular smooth muscle cell*

INTRODUCTION

Scientific research often involves creating experimental animal models for studying human diseases. Those models are mostly used for testing new surgical techniques, drugs or medical supplies in order to confirm their effectiveness and meeting certain safety standards before they are implemented into the clinical practice. One of those models is the swine abdominal aortic aneurysm (AAA) described in this paper. Swine is an animal considered to be most similar to humans in terms of physiology and anatomy and therefore it is often used in scientific research (1-3). The experimental AAA created in pigs involves inducing dilatation of aorta in order to imitate the disease process observed in humans. Experimental model should be reproducible and minimally invasive for the animal. Therefore, during the studies on creating an experimental AAA we decided to develop and implement a new surgical access to the abdominal aorta to demonstrate the best procedure for inducing aorta dilatation and to adapt the EMG (electromyography) signal measurement as a minimally invasive method for monitoring the *in vivo* functional changes in the vessel wall.

MATERIAL AND METHODS

The study was approved by the Local Ethical Committee. The experiments were conducted on 12 piglets weighing between 20 and 30 kg. The animals were divided into 3 groups comprising 4 individuals. The AAA was surgically induced using three different methods.

Aneurysm induction procedures

1) In the first group the aneurysm was induced by mechanical stretching of the abdominal aorta wall with Foley catheter size 6, after previously blocking the aortic blood flow with the vascular clamps placed below the renal arteries and above iliac arteries, additionally lumbar arteries were temporarily clamped. The aortic wall was stretched for 2 minutes. Then, 5 ml of solution containing 500 IU of elastase was administered into the aortic lumen through the catheter under gentle pressure causing visible tension of the aortic wall. Elastase incubation time within the aortic lumen was 20 minutes. The aortic wall was closed with vascular sutures 6.0.

2) The procedure of aneurysm induction in the second group was the same, but the total volume of 5 ml solution administered to the aortic lumen through the catheter contained 500 IU of elastase and 6000 IU of collagenase.

3) In the third group of animals the technique of inducing the aneurysm was also the same, but the total volume of 5 ml solution containing 500 IU of elastase and 6000 IU of collagenase was administered through the catheter to the aortic lumen and a swab soaked in 0.5 M calcium chloride was placed for 20 minutes on the external aortic wall. A sterile cotton gauze measuring 5×5 cm was soaked in a solution prepared immediately prior to surgery by dissolving anhydrous calcium chloride in 10 ml of distilled water. Gauze was placed with surgical forceps on the isolated portion of the aorta after filling the lumen with the enzyme solution (*Fig. 1*).

Surgical access and anaesthesia

The animals were operated on using a retroperitoneal approach. They were immobilized and pretreated with medetomidine (0.1 mg/kg body weight, intramuscularly (i.m.)), ketamine (10 mg/kg body weight, i.m.) and atropinum sulphuricum (0.02 mg/kg body weight, subcutaneously). Anesthesia was maintained with ketamine and diazepam, intravenously titrated according to their effectiveness and deepened with propofol. Skin incision was performed in one third of the lower left part of the lateral abdominal area, 3–4 cm behind the costal arch line until the knee fold. To reach the aorta an incision between the rectus abdominis muscle (m. rectus abdominis) and the abdominal external oblique muscle (m. obliquus externus abdominis) followed by blunt dissection of the muscles from the peritoneum was performed. Before aorta clamping 2000 IU of heparin and 4 mg dexamethasone were given intravenously. The wound was closed in layers.

Pain control was based on meloxicam (0.4 mg/kg body weight i.m.) in a single dose given immediately after surgery. Preventive antibiotic was administered 30 minutes before the operation (amoxicillin 15 mg/kg body weight). Following the surgery the animals received for a week 50 mg of Clexane Forte (Sanofi Aventis) subcutaneously.

Abdominal ultrasound examination

The induced AAA was monitored on the weekly basis using Mindray M5Vet apparatus with color Doppler and 5 to 8 mHz sector microconvex probe. At least 10 transverse and longitudinal measurements of aortic dilatation and normal aorta were performed. Arithmetic mean of the measurements and the percentage of aortic dilatation in comparison to the unchanged aorta were calculated.

Testing the myoelectric activity of the aortic muscular layer (VSMC)

To monitor myoelectric activity, unipolar epicardial electrodes used for temporary pacing, produced by Medtronic Inc., (Medtronic Inc., Minneapolis, MN USA Cat. No. 55 432), were implanted into the aortic muscular layer, just below the renal arteries. Total of 5 electrodes were implanted, in 2 sets of 2 electrodes and one electrode constituting a zero into the surrounding tissue. The distance between the electrodes in the pair was 3-4 mm, so that they formed a bipolar electrode. The electrodes were implanted below the renal arteries into the abdominal aorta wall at a distance of about 2.5–3 cm between the

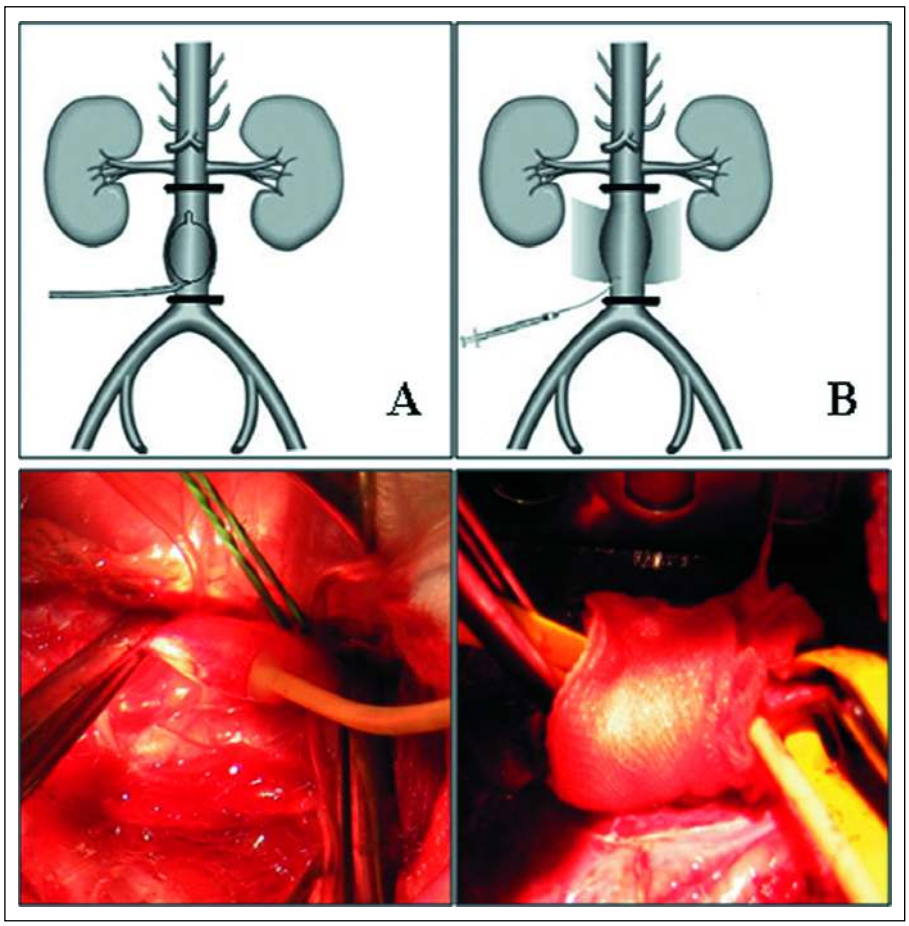

Fig. 1. A diagram of the experimental model of AAA induced with a mechanical stretching, proteolytic enzymes and $CaCl_2$. (*A*) The mechanical widening of the aortic lumen with a Foley catheter. (*B*) Intravascular incubation of elastase (500 IU) and collagenase (6000 IU) dissolved in 5 ml of NaCl with simultaneous treatment of the external surface of the aortic wall with $CaCl_2$ (sterile gauze soaked in 0.5 M calcium chloride solution).

electrode pairs, so that one pair was implanted into the normal aorta and the other one into the dilatated part of the vessel. The electrodes were connected to the DSI implant (Data Science International USA), catalogue No. TL11M3-D70-CCTP. A signal from the implant was transmitted to a recording device *via* telemetry. The implant was placed on the left side of the animal. Additionally, a catheter was introduced into the aortic lumen to record the changes in blood pressure using a direct method. The study data were collected and analyzed using Dataquest ART software version 4.3 Silver. Electrical activity of the abdominal aorta wall (EMG), changes in blood pressure, and body temperature were recorded. The monitoring was carried out once a week continuously for 48 hours.

The obtained data on the electrical activity of the abdominal aorta were analyzed off-line. In order to analyze and compare the records of aortic smooth muscle myoelectric activity, three types of waves were evaluated (waves of I, II and III type, description in the text). The waves were divided into those types experimentally, based on the analysis of the VSMC myoelectric activity recordings. The measurements included the duration, amplitude and wave frequency. The results are presented as mean ± standard error (±S.D.).

Measurements of the aorta and histolopathological examination

The abdominal aortae were posthumously collected for the measurements of normal and dilated sections. After sealing the aorta was filled with compressed air at a pressure of 180 mmHg in order to better visualize the size of the aneurysms, and the measurements of the cross-section and length of the created lesion were taken.

Aortic sections collected for the histopathological examination were fixed for 24 hours in 7% buffered formalin, and then embedded in paraffin and cut into 4 μm thick sections. Histopathological evaluation of the preparations was performed following staining using the hematoxylin-eosin method.

Photomicrographs of all examined vessels were processed using computer-aided image analysis on a computer integrated with the optical microscope Olympus BX53 (Olympus, Japan). This unit can save images and perform their digital analysis. The measurements were made using cell^A software (Olympus Soft Imaging Solutions GmbH, Germany).

RESULTS

Postoperative complications and recovery period

All animals in the study group survived the procedure. Clinical examination showed no postoperative complications. Slight infection of the surgical wound was observed in one subject. The applied surgical approach proved to be very effective and resulted in the lack of postoperative complications and rapid recovery of the animals.

Dimensions of normal and dilated aorta

External diameter of the abdominal aorta below the renal arteries (normal section) in piglets weighing 20–30 kg was on average 10±2 mm as shown by posthumous measurements. Ultrasound examination revealed that the average cross section of the abdominal aorta was 6.6±0.8 mm. Within 4 weeks of the animal life slight increase in the cross section by 0.7±0.3 mm was observed in ultrasound examination.

In the first study group (4 subjects), in which the experimental aorta dilatation was induced by mechanical stretching and incubation of elastase solution in the aortic lumen, no AAA formation was seen. Significant dilatation resulting from mechanical stretching was observed intraoperatively. However, the change gradually regressed and a week after the surgery the ultrasound image confirmed slight lumen dilatation by 1–2 mm and strong reaction around the dilatation spot. During subsequent measurements the changes subsided and a slight dilatation below 50% did not allow to qualify them as aneurysms (*Table 1, Fig. 3B*). The post-mortem examination also revealed mild enlargement of the vessel lumen and tissue reaction mainly in response to the implanted electrodes.

For the second and third experimental group the increase in the aortic lumen cross section significantly exceeded 50%, which clearly qualifies the induced lesion as the abdominal aortic aneurysm.

Post-mortem measurements of the aorta from the second group filled with compressed air showed 71±3.5% ($P \leq 0.001$) increase of the transverse dimension of the aneurysmal section as compared to the normal aorta. The average size of the aneurysmal aorta cross section was 17±2.5 mm (posthumous measurement). The measurements made using ultrasound revealed mean dilatation of the abdominal aorta by 76.6±9.3% ($P \leq 0.001$) as compared to the diameter of the normal aorta (*Figs. 2* and *3C*). The average transverse dimension of the induced AAA determined using ultrasound was 11.0±1.2 mm. The relative enlargement of the aorta did not show significant changes during the follow-up period. The absolute size of AAA increased proportionally to normal aorta (*Table 1*).

Post-mortem examination of the abdominal aorta in the third group showed enlargement of the diameter by 72±3% ($P \leq 0.001$). Measurements made by ultrasonography demonstrated enlargament of lumen transverse dimension by 104.2±11.3% ($P \leq 0.001$). The average diameter of AAA was 13.0±0.9 mm (*Fig. 3D*). The absolute size of induced AAA tended to remain stable

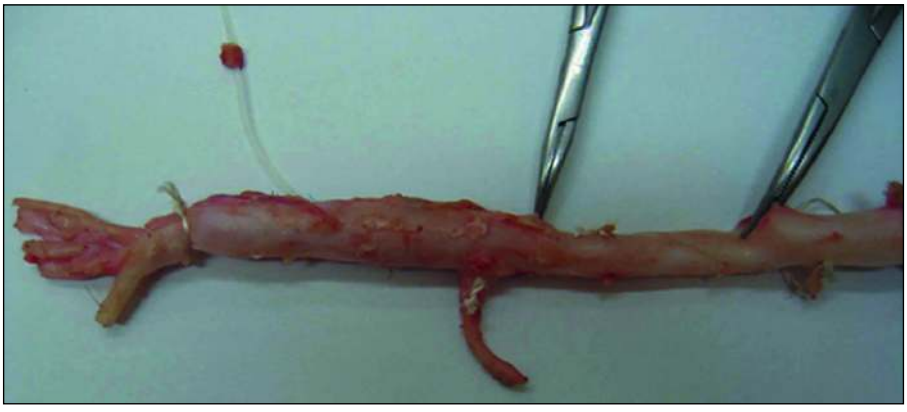

Fig. 2. A section of normal abdominal aorta (right side) and aneurysmal aorta (left side). Aorta filled with compressed air at a pressure of 180 mmHg. Collateral vessels were closed in order to seal the aorta. Significant dilatation of the aortic lumen by over 50% is visible. The catheter used for blood pressure measurements can be seen within the aneurysmal lesion.

over a control period. Slight reduction of the relative diameter by average 8.7% was observed in consecutive measurements which was related to the growth of the body (*Table 1*).

Myoelectric activity patterns of normal aorta and abdominal aortic aneurysm

Recorded myoelectric activity of the muscles of normal abdominal aorta was characterized by changes in the membrane potential which can be divided into three types called waves. The first type waves appeared on average every 128±14 min. Mean wave amplitude was 0.150±0.03 mV and mean duration was 0.43±0.05 s. They were closely correlated with the changes in blood pressure recorded by the direct method. Average heart rate calculated on the basis of pressure changes in the studied animals was 132±5 beats/min. *Fig. 4A* shows an exemplary record of the myoelectric activity of the aortic muscles, made using the DSI implants with simultaneous registration of the aortic blood pressure changes (*Fig. 4A*). The catheter measuring the blood pressure was located in the aortic lumen at the level of the electrodes recording the myoelectric activity changes. Time synchronization was visible between the appearance of the type I waves in EMG and changes in blood pressure as measured by the direct method. This clearly confirms the origin of the type I wave. Second type of waves appeared on average every 15.9±4.4 min. Their mean duration was 2.69±1.5 second. Average discharge amplitude of the type II waves was 0.205±0.157 mV. *Fig. 4B* presents a sample recording of myoelectric activity of aortic smooth muscle cells depicting the type II waves (*Fig. 4B*). There is a visible line of rising electrical potential caused by breathing (observation of the animal during the experiment). The recording was performed while the animal was standing relatively calmly. Type III waves appeared on average every 4.03±1.07 min. Their mean duration was 11.81±5.3 second. Average discharge amplitude of type III waves was 0.345±0.232 mV.

The recorded myoelectric activity of the AAA was the same as for the normal aorta section. All types of waves observed for the normal aorta were present as well, and their frequency and amplitude did not differ significantly from those recorded in the normal aorta. Moreover, no statistically significant differences were observed in aortic smooth muscle myoelectric activity in the different experimental groups.

Anatomopathological changes in the dilated aorta

Group I - small single foci of calcium deposits on the border of tunica media and adventitia were found. Small, mainly lymphocytic inflammatory infiltrations were observed near the calcium deposits (*Fig. 5A*). Damage to the endothelial cells and their vacuolisation accompanied by local disruption of the internal elastic membrane were noticed (*Fig. 5B*). The adventitia examination showed small inflammatory infiltrations of lymphocytic cells located mainly around the vessel and swelling combined with the damage and loosening of the fibers of the outer adventitia.

Table 1. Mean diameter of aneurysmal abdominal aorta in the study groups determined by ultrasound measurements. The last raw shows the results of posthumous measurements made after filling the aorta with compressed air (P≤0.01).

	Diameter of the abdominal aorta (mean ± SD [mm]) in group		
USG measurement	**I**	**II**	**III**
week 0	6.6±1.0	6.4±0.9	6.6±0.7
week 1	8.8±0,8	10.4±1.6	12.9±1.7
week 2	8.9±1.0	11.0±1.0	12.7±1.2
week 3	9.2±1.6	11.1±1.1	13.0±1.0
week 4	9.4±1.1	11.7±1.3	13.2±0.8
Relative widening (mean±S.D. [%])	37.4±8.6%	76.6±9.3%	104.2±11.3%
Posthumously	12.5±2.1	17±2.5	17.2±2.4

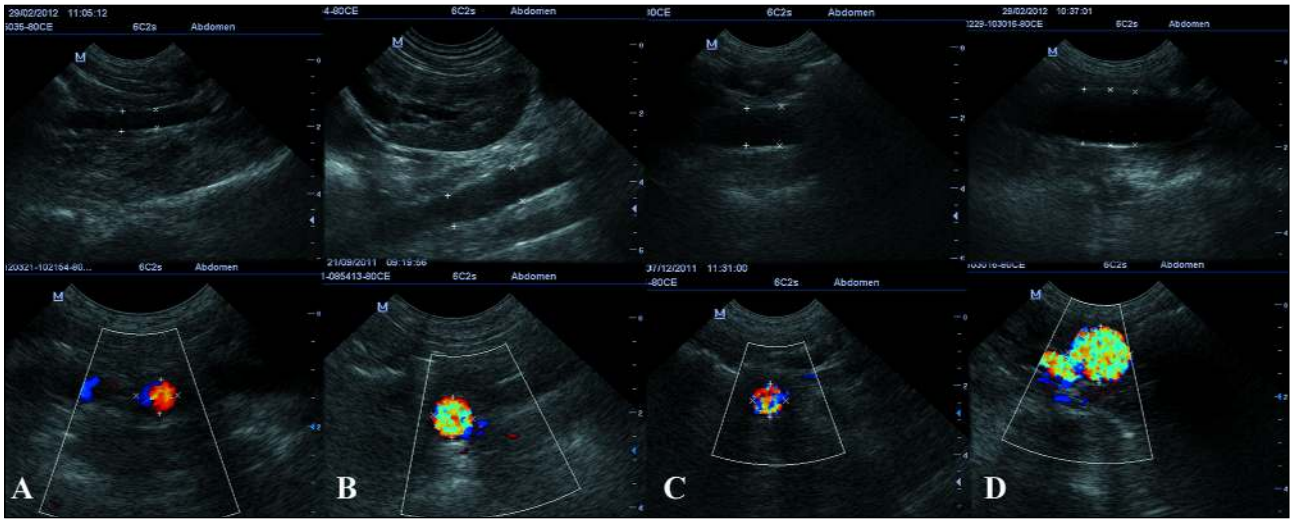

Fig. 3. Longitudinal (top) and transverse (bottom) views of the aorta obtained with ultrasound. (*A*) Normal dimensions of the abdominal aorta in pig. (*B*) A follow-up ultrasound in the 4th week after treatment of the aorta with elastase. (*C*) AAA induced with collagenase and elastase, imaging in the 2nd week after surgery. (*D*) AAA in swine after treatment with collagenase/elastase and $CaCl_2$, follow-up examination in the 2nd week.

Group II - in comparison to group I the calcium deposits on the border of tunica media and adventitia were more numerous and they were also found in deeper layers of the tunica media. Calcium deposits were accompanied by small inflammatory infiltrations composed mainly of lymphocytic cells, and those areas featured various stages of elastic fibers and smooth muscle

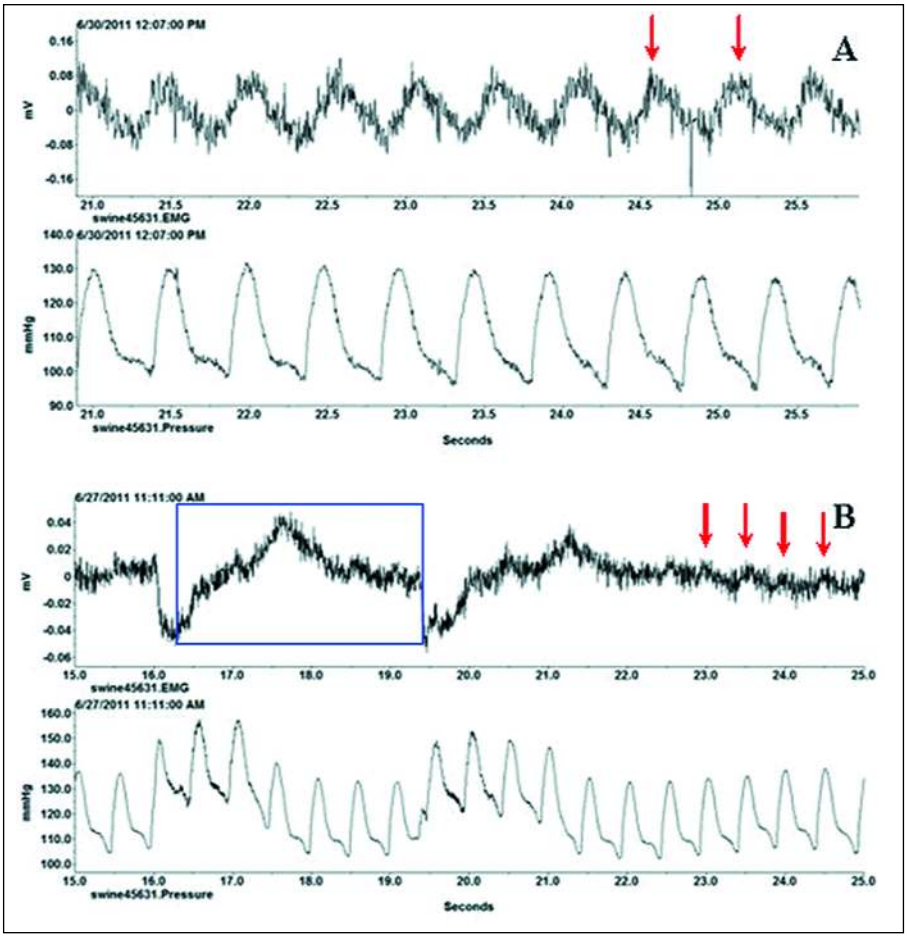

Fig. 4. Electrical activity of the abdominal aorta smooth muscle in swine. (*A*) The upper graph shows type I waves (red arrows) that are closely correlated with the pressure changes presented below. (*B*) The upper graph shows type II waves that are correlated with the breathing-induced pressure increase within the abdominal aorta (in blue border). Red arrows indicate type I waves. Bottom graph presents the blood pressure changes as measured by the direct method. The recording was performed using DSI apparatus.

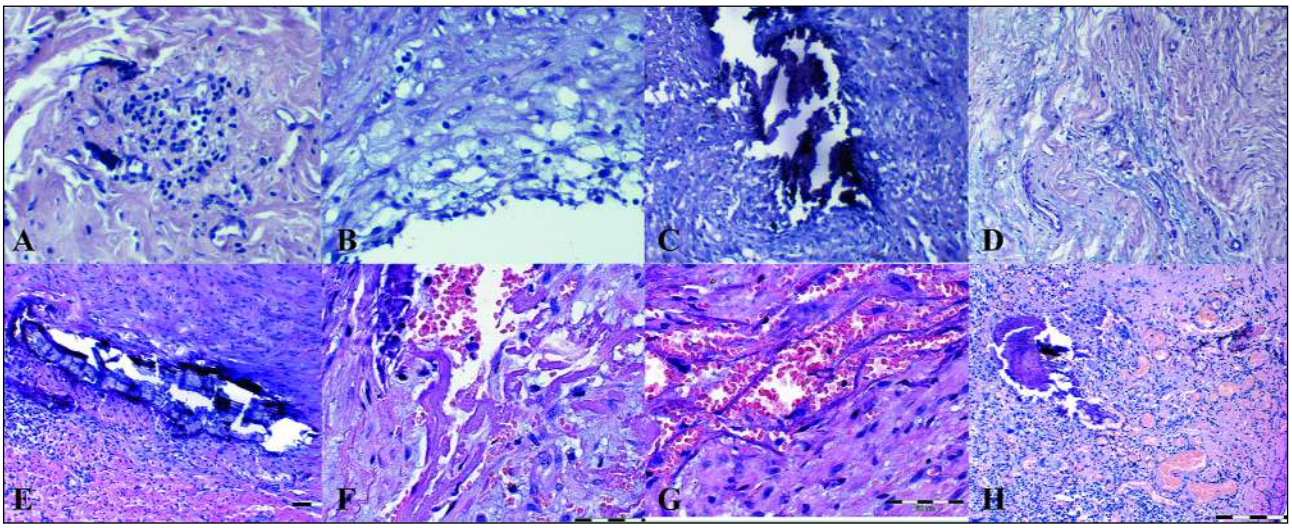

Fig. 5. Histopathological images of lessions in abdominal aorta from pigs, sections stained with hematoxylin-eosin. (*A-B*) Sections of the aorta treated with elastase, lymphocytic inflammatory infiltrations and endothelial cell loss are visible. (*C-D*) Calcium deposit accompanied by small inflammatory infiltrations and an area featured by elastic fibers and smooth muscle cells degradation in the wall of aneurysm induced with elastase/collagenase. (*E-H*) Aortic lesions in the third experimental group. (*E*) Calcium deposit on border between tunica media and internal membrane surrounded by massive lymphocytic infiltration. (*F*) Degradation and loss of the elastic fibers and smooth muscle cells in the central part of tunica media. (*G*) Massive penetration of red blood cells within the damaged tunica media. (*H*) Calcification and inflammatory infiltrates in the adventitia. Lymphocytes, neutrophils and giant cells appear. Additionally, proliferation of small blood vessels is visible.

cells degradation (*Fig. 5C, 5D*). The adventitia examination showed small inflammatory infiltrations of lymphocytic cells located mainly around the vessel. In many places the atrophy of the endothelial cells was noticed.

Group III - massive accumulation of calcium deposits mainly in the outer layers of the tunica media were detected, but there were also spots where they reached even the internal membrane. In the central part of the tunica media, and usually near the calcium lesions, significant degradation and sometimes even loss of elastic fibers and smooth muscle cells was seen. There were numerous inflammatory infiltrations around and away from calcium deposits, composed mainly of lymphocyte cells. Moreover, massive penetration of erythrocytes was observed within the damaged structures of the tunica media (*Figs. 5E-5G*). Furthermore, numerous inflammatory infiltrations within the adventitia were noticed, containing, apart from dominating lymphocyte cells, also single plasma cells and neutrophils. The inflamed adventitia also featured calcium deposits around which there were single, multinucleated giant cells, and frequent areas of massive fibrosis with proliferation of fibroblastic cells and small blood vessels (*Fig. 5H*) There were frequent necrotic spots within the superficial layer of adventitia, under which haemorrhages were visible. Pronounced loss of endothelial cells was detected.

DISCUSSION

Experimental methods of aneurysm induction can be classified into three broad categories. They comprise aneurysms in genetically predisposed animals, and aneurysms induced by physical or chemical methods (4). Animals predisposed to the aneurysm formation are turkeys (5, 6) and mice (7-9). Physical methods of aneurysm creation are a heterogeneous group of procedures including: destruction of the tunica media and adventitia (10), damage of the vessel wall with laser, dilating the lumen with incompatible angioplasty balloon, replacement of the aortic wall section with a tubular prosthesis made of a synthetic material (dacron, polyurethane), implantation of an anterior patch of artificial (dacron) or natural origin obtained from the recipient or heterologous (fragment of the cow internal jugular vein fixed in glutaraldehyde), (11-17). Chemical methods involve the application of various chemicals into the aortic lumen or on the vessel wall, causing progressive dilatation of this section of the abdominal aorta. They are widely used in smaller laboratory animals such as mice, rats and rabbits (18, 19). Elastase is one of the substances commonly used in this method of inducing abdominal aortic aneurysms. The enzyme is administered into the lumen of temporarily isolated aortic section or applied externally (20, 21). There are some attempts to improve this method by combining elastase with other substances. Collagenase is a proteolytic enzyme used together with elastase in aneurysm induction. Another solution is a combination of elastase and calcium chloride used in rats (22). Initially, the large animal model for abdominal aortic aneurysm involved dogs (10). However, for ethical reasons and due to the differences in the coagulation system between dogs and humans, the currently preferred model is a swine (Sus scrofta). There are many methods of inducing abdominal aortic aneurysms in pigs, mainly physical ones that are used for testing new surgical procedures of AAA treatment. More recent studies on aneurysm induction in swine are based on the combination of physical and chemical methods, which allows for obtaining changes more similar to naturally occurring human aneurysms and their quick induction.

The aim of this study was to demonstrate the effectiveness of selected methods of experimental induction of abdominal aortic aneurysm in swine and to adapt the EMG examination (electromyography) to record the vessel wall changes.

In the first study group in which the aneurysmal lesion was induced using a physical method (stretching the vessel wall with a catheter) and elastase application into the lumen, the result was negative. The available literature states that this method was successful in inducing aneurysmal lesion in swine carotid artery using elastase and in dilatation of the abdominal aorta sections *ex vivo*. Unfortunately, it was also found that attempts at creating abdominal aortic aneurysm in living pigs using elastase and mechanical stretching failed, despite destruction of the elastic fibers within the section treated with the enzyme (23-25). By means of mechanical stretching of the aortic wall and administering under pressure 5 ml solution containing 500 IU of elastase with a syringe and 20 min incubation of the elastase in the aortic lumen we achieved significant intraoperative dilatation of the aortic lumen. However, the lesion regressed over 1–2 weeks after the surgery. This study confirms earlier observations published by other scientists (23-25). Damage to the elastin fibers is not sufficient for the development of the aneurysmal change, despite mechanical damage to the wall and inducing transient inflammation of the vessel. Repair mechanisms are so efficient that the intraoperative dilatation is compensated and we only observed hypertrophy of the connective tissue in the place of the surgical intervention. Histopathological examination revealed small foci of calcium deposits and lymphocytic inflammatory infiltrations and endothelial cell damage accompanied by elastic membrane tear. Such changes to the aortic internal structure are not enough to sustain the aneurysmal lesion and the regenerative mechanisms act to reduce the induced aneurysm until its complete disappearance.

Modification of the method by the introduction of an additional proteolytic enzyme collagenase radically improved the results of the experiment. In the second group of animals we obtained a significant dilatation of the abdominal aortic lumen that did not regress.

The outcomes of our experiment confirm the results obtained by Hynecek *at al*. (26). By dilating the abdominal aorta with an angioplasty balloon and administering 50 ml of a solution containing 8000 IU of collagenase and 30 IU of elastase for 20 minutes the authors achieved the aorta dilatation by 62±35%, and six weeks later the diameter of the lesion imaged with MRI was 194±37% of a normal vessel. Researchers argue that the changes in concentration and organization of elastin and collagen fibers are extremely important factor in the pathogenesis of the aneurysm formation (26-29). However, the mere damage to the elastic fibers by elastase application is not sufficient to induce aneurysmal changes. Another necessary step is the addition of collagenase, which affects the structure of collagen fibers and consequently leads to the formation of the aneurysmal change. Introduction of a balloon into the vessel lumen accelerates the natural dilatation process in a blood vessel with weakened structure of elastin and collagen fibers (26). In humans this process takes many years and aneurysms occur in the second half of human life. In the case of experimental abdominal aortic aneurysm the natural process of lumen dilatation is accelerated by mechanical stretching of the vessel.

In the study conducted by our team, aneurysms, however, did not have a clear tendency to increase over time. The relative enlargement of the aorta remained at the same level throughout the control period. Such observation may be associated with the animal model. Young animals used in the experiment have a large potential for tissue regeneration. Moreover, animals did not have comorbidities such as hypertension and atherosclerosis, which are considered to be predisposing factors for the aneurysm development in human. The differences in results obtained by our

research team and by other authors may be associated with distinct experimental protocols and other imaging methods used (26).

The addition of calcium chloride allowed to obtain the largest AAA in pigs, especially in the early monitoring period. We assume that $CaCl_2$ reduced tissue repair processes after acute aortic lumen enlargement by exacerbating inflammation and producing localized necrotic lesions as compared to the second group. Histopathological changes characterized by more intense inflammatory cell infiltration, more numerous calcium deposits and more pronounced loss of elastic fiber, smooth muscle cells and endothelium compared to the second group support the conclusion. Described findings are also more similar to histological changes in human aneurysms. Lack of growth over time is not fully understood. Similar factors like in the case of second group may play a role in this process. However, the use of calcium chloride caused even the decrease in relative aneurysm dimension over the follow-up period. The reason for this phenomenon may be stiffening of the aortic wall caused by areas of massive fibrosis in adventitia observed in histological sections.

Hynecek *et al*. described time-dependent changes in the histological images of experimental abdominal aortic aneurysms in pigs. Initially they observed the atrophy of endothelial and smooth muscle cells and the degradation of elastic and collagen fibers. In the sixth week endothelial regeneration was observed, accompanied by partial renewal of the muscle layer, whereas the changes in the connective tissue fibers continued and there appeared calcification foci surrounded by lymphocytes (26). The authors did not find as severe inflammatory changes as in the animals from our third study group, which clearly confirms the usefulness of calcium chloride in the experimental model of abdominal aortic aneurysm in swine. Corresponding histopathological changes were observed in abdominal aortic aneurysms in humans (30).

Very interesting results were obtained while studying the myoelectric activity of the aortic smooth muscle. We distinguished three types of registered waves related to the changes in the aortic lumen pressure. Type I waves were associated with changes in blood pressure related to the heart contractions (systolic and diastolic pressure). Type II waves reflected the changes within the chest caused by breathing movements and type III waves were caused by tonus variations of the autonomic nervous system innervating and controlling the vessel lumen (31). Mechanisms of formation of the recorded waves were explained in detail in the previous studies and the results and comprehensive discussion on their etiology were published in the paper by Czerski *et al*. (31, 32). The fact that there were no differences in VSMC myoelectric activity between AAA and normal aorta is very important.

This shows that the muscle tissue was not significantly damaged during aneurysm induction or that the electrical potential changes are transmitted along the vessel from adjoining cells. Given the results of histopathological examination which showed significant changes in the aortic smooth muscle it is supposed that the contractility of the aneurysmal aorta is impaired. However, this assumption requires further studies. Animals in which we induced AAA were young and healthy. Their organisms were equipped with high performance regenerative mechanisms. In the natural process of AAA formation in humans there are a number of factors damaging the vessel over a long period of time. Moreover, the repair mechanisms are less efficient due to the advanced age of the patients.

The presented techniques for inducing experimental abdominal aortic aneurysm can be used in numerous scientific studies on the aneurysm etiology and formation and for the development of AAA treatment methods. In the future we plan to improve the experimental model of abdominal aortic aneurysm using additional inflammatory factors, which will make the model more similar to the natural aneurysmal lesions developing in humans.

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Conflict of interests: None declared.

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