INTRODUCTION

The aim of the study is to develop differences in myoelectric activity of the aorta on the aorta-straight prosthesis interface in compared to healthy and aneurysmal sections. Experimental animal models imitating pathological processes in humans are used in studies on etiopathogenesis and disease treatment (1). One of these models is an experimentally induced abdominal aortic aneurysm (AAA) in pigs, developed in previous studies (2). The pig is an animal considered to be most similar to humans in terms of physiology and anatomy (3). The swine AAA model closely resembles the morphology of human aneurysm (4). Therefore, the induced AAA can be used for testing treatment methods and research on processes that are observed in humans with aneurysm (1, 5-7). The designed experimental model is repeatable and low-invasive to animals. There are many techniques for the imaging of abdominal aortic aneurysm, enabling the monitoring of aneurysm development in time and classification of risk of rupture. The most popular techniques include ultrasonography, contrast enhanced radiography and magnetic resonance imaging. In this study, we used an innovative method for the monitoring of changes in the muscular layer of the abdominal aorta (VSMC) based on the records of electromyographic (EMG) signals (2, 8). This method enables the observation of electrical parameters in the VSMC.

Changes in the electric potential within the VSMC are closely associated with aortic contractility. The study’s objective was to carry out a long-term observation which would allow for the development of patterns describing the contractility of the abdominal aorta and demonstrate changes in aortic contractility associated with aneurysm.

MATERIAL AND METHODS

Animals

Experiments were carried out on 10 piglets of 20 to 30 kg body weight. Five animals were assigned to each of the 2 groups. An aneurysm of the abdominal aorta was created experimentally in animals from the first and second study group. After 4 weeks, animals from the second group were subject to aneurysm repair using an aortic prosthesis. During the experiment, we measured the myoelectric activity of the muscular layer of the abdominal aorta and aneurysmal lesion with the ultrasonographic technique. Measurements of the aorta and aneurysmal lesion and histopathological analyses were carried out post-mortem. We found a statistically significant decrease in the myoelectric activity of the aorta on the aorta-straight prosthesis interface and a significant decrease in the thickness of the muscular layer of the aorta on the aorta-prosthesis interface. No similar changes were found for experimentally induced aneurysms of the abdominal aorta. A straight prosthesis graft may not be the perfect option in the treatment of abdominal aortic aneurysm, as it contributes to the remodelling of the tissue on the prosthesis-aorta interface. This may result in the relapse of an aneurysm and post-operative complications.

Key words: abdominal aortic aneurysm, aneurysm creation, aortic electromyography, swine, myoelectric activity, tissue remodeling, prosthesis
suturing an aortic prosthesis instead of the aneurysmal lesion, and electrodes for the measurement of EMG signals were implanted. The electrodes were implanted on the prosthesis-normal tissue interface, proximally and distally from the aortic prosthesis.

The myoelectric activity, in both the first and second groups, was monitored non-stop for 24 hours once a week, always on the same day of the week. We also took aortic measurements with the ultrasound technique, once a week, throughout the entire experimental period. Tests in the first group of animals were carried out for 4 weeks, and in the second group, for 8 weeks in total (4 weeks after the creation of the aneurysm and 4 weeks after grafting the prosthesis).

**Aneurysm induction procedure during operation**

An aneurysm was induced by the mechanical stretching of the abdominal aortic wall with a Foley’s catheter size 6, after previous occlusion of the blood flow inside the aorta with vascular clamps. The aortic wall was stretched for 2 minutes. 500 IU of elastase and 6000 IU collagenase, total volume 4 ml, were then administered via the catheter into the aortic lumen and incubated for 20 minutes. During that time, gauze soaked in 0.5 M calcium chloride was applied onto the aortic wall. The procedure for inducing an aneurysm was developed in previous studies (2).

**Surgical approach and anaesthesia of animals for the procedure**

Animals were operated on following the lateral surgical approach. For that purpose, the animals were immobilised and premedicated (0.1 mg/kg bw medetomidine, 10 mg/kg bw ketamine i.m.) Basal anaesthesia was carried out using a 4 mg/kg bw i.v. dose of propofol. After intubation the anaesthesia was maintained with a mixture of ketamine and relanium (i.v.) administered according to their effectiveness and enhanced using propofol.

The surgical area covered the lateral left abdominal area, dorsally in the region marked by the line of the lumbar spine, cranially by the costal arch line, caudally by the knee fold and ventrally by the teat line. An incision in the skin was made in one third of the lower part of the lateral abdominal region, 3–4 cm behind the costal arch line up to the knee fold. After reaching the muscules, an incision was made between the rectus abdominis muscle and the obliquus externus abdominis muscle. After reaching the peritoneum, the muscles were separated from the peritoneum by hand and oriented dorsally towards the lumbar spine in the retroperitoneal region up to the abdominal aorta located retroperitoneally. Direct access to the aorta was achieved by drawing the tissues aside with retractors. Before the clamping of the abdominal aorta, both in the procedure for inducing an aneurysm and prosthesis grafting, 2000 IU heparin and 4 mg dexamethasone were injected intravenously. The lesion was closed layer by layer, with the first suture layer put on the muscles of the abdominal integument (Vicryl®-2 single interrupted knot suture) and the second layer put on the subcutaneous tissue (Vicryl®-2 running suture). The third layer was a cutaneous horizontal blanket suture (Nylon® 1-0).

After procedure pain was controlled by the administration of meloxicam (0.4 ml/12 kg bw i.m.) and a preventive antibiotic (amoxicillin) was administered (1 ml/10 kg bw). For a week after the procedure, each animal received 50 mg of Clexane Forte (from Sanofi Aventis). A detailed description of the procedure is provided in another publication (9).

In the second group after 4 weeks, the animals were reoperated on in order to repair the induced aneurysmal lesion. The procedures to achieve anaesthesia and to access the abdominal aorta were identical to those used for inducing the abdominal aortic aneurysm. After reaching the aneurysmal section of the abdominal aorta, vascular clamps were placed above and below the aneurysmal lesion in order to block the flow of blood to the operation site. Before this, an intravenous 2000 IU dose of heparin was administered. The aneurysmal section of the aorta was removed and then replaced by an InterVascular OCHSNER 500 12 mm prosthesis graft. The prosthesis was attached with knot sutures to the vascular wall according to surgical practice. Blood flow in the aorta was restored. Electrodes for the measurement of the myoelectric activity of the aorta were implanted on the prosthesis-aorta interface, above and below the prosthesis. The position of the implanted prosthesis is presented in Fig. 1.

![Fig. 1. Repair of aneurysmal lesion using a straight prosthesis. The prosthesis grafted to replace the aneurysmal aortic section is seen. Electrodes for recording electrical potentials are implanted on the prosthesis-healthy tissue interface, about 5 mm cranially and caudally from the prosthesis.](image-url)
After the completed experiments, the animals were euthanized, and aneurysmal lesion was dissected during reoperation for analysis. Visualisation of the size of the aneurysmal lesion, and measurements of a healthy aorta and aneurysmal aorta. We measured the aorta diameter and the length of the induced aneurysmal lesion using the straight prosthesis. The mean diameter in millimeters of the aneurysmal section when compared to the section of the healthy aorta showed a mean dilation of the aortic lumen of 96 ± 13% (post-mortem measurements). However, the USG scan demonstrated a mean diameter of the abdominal aorta of 6.6 ± 0.7 mm. Within 4 weeks of animal life, a slight increase in the diameter (by 1.2 ± 0.3 mm) was demonstrated in the USG scan, which confirms this significant difference between the results of the USG scan and post-mortem measurement caused by animal growth.

In the experimentally induced AAA, in accordance with definition, the aorta diameter increased greatly above 50%. Post-mortem measurements of the aorta filled with compressed air showed a 160.6 ± 5.5% (P<0.001) increase in the diameter of the aneurysmal section when compared to the section of the healthy aorta. The mean diameter in millimeters of the aneurysmal section was 17.2 ± 2.4 mm (post-mortem measurement in the first experimental group). Measurements taken using ultrasound showed a mean dilation of the aortic lumen of 96 ± 13% (P<0.001) during the four-week measurement period when compared to the diameter of the healthy aorta. The mean diameter of the induced AAA, determined using ultrasound, was 12.9 ± 3 mm. During the four-week observation, we found an increase in the AAA diameter, but it was not statistically significant and resulted from animal growth. No differences between the animal groups were found for the size of the aneurysmal lesion; and therefore, the results for both groups were combined. The measurement results are presented in Table 1, and they are consistent with previous findings (2).
Myoelectric activity patterns of healthy abdominal (first group) aorta, abdominal aortic aneurysm (second group) and abdominal aorta on the aorta-aortic prosthesis interface

The recorded myoelectric activity of the muscles of healthy abdominal aorta was characterised by changes in the membrane potential, which can be divided into three types, called waves. The origin of waves recorded in the myoelectric activity of VSMC was investigated in detail in previous experiments and described in other publications (2, 8). The recorded myoelectric activity of the abdominal aortic aneurysm was identical with that of the healthy section of the aorta. All three types of waves observed in the healthy section of the aorta were recorded. Wave frequencies and amplitudes did not differ significantly from those recorded for the healthy section of the aorta (first experimental group). The recorded myoelectric activity of the healthy section of the aorta on the aorta-aortic prosthesis interface (about 5 mm distance from the aortic prosthesis) showed statistically significant differences when compared to the activity recorded for the healthy aorta and abdominal aortic aneurysm. All types of waves found in the records for the section of the healthy aorta were detected; they had much lower amplitude (statistically significant change) but the same frequency (the second group).

The results are shown in the Table 2.

Anatomopathological changes in the experimentally induced abdominal aortic aneurysm

We found massive calcium deposits, mainly in the external layers of the tunica media and on the tunica media-adventititia interface as well as within the tunica media. A pronounced degradation, with local disruption of elastic fibres and smooth muscle cells, was found in the central part of the tunica media, usually near the foci of calcium deposits. Numerous inflammatory infiltrations, mainly containing lymphocytes, were found around and outside the calcium deposits. Massive

Table 1. Mean diameters of the abdominal aortic aneurysm measured using ultrasound (both experimental groups). Post-mortem measurements of the aorta filled with compressed air (first experimental group) are presented in the last row. The table presents statistically significant results as compared to the healthy aorta (P £ 0.01).

<table>
<thead>
<tr>
<th>USG time</th>
<th>Diameter of the abdominal aorta mean ± S.D. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>week 0</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>week 1</td>
<td>12.9 ± 1.7</td>
</tr>
<tr>
<td>week 2</td>
<td>12.7 ± 1.2</td>
</tr>
<tr>
<td>week 3</td>
<td>13.0 ± 1.0</td>
</tr>
<tr>
<td>week 4</td>
<td>13.2 ± 0.8</td>
</tr>
<tr>
<td>post-mortem</td>
<td>17.2 ± 2.4</td>
</tr>
</tbody>
</table>

Table 2. Patterns of myoelectric activity in the experimental groups. The table presents statistically significant results as compared to the control group. * P £ 0.005.

<table>
<thead>
<tr>
<th>The control group</th>
<th>First-order waves</th>
<th>The second-order waves</th>
<th>The third-order waves</th>
</tr>
</thead>
<tbody>
<tr>
<td>frequencies (min)</td>
<td>128 ± 14</td>
<td>15.9 ± 4.4</td>
<td>4.03 ± 1.07</td>
</tr>
<tr>
<td>amplitude (mV)</td>
<td>0.150 ± 0.03</td>
<td>0.205 ± 0.157</td>
<td>0.345 ± 0.232</td>
</tr>
<tr>
<td>duration (s)</td>
<td>0.43 ± 0.05</td>
<td>2.69 ± 1.5</td>
<td>11.81 ± 5.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1st group</th>
<th>First-order waves</th>
<th>The second-order waves</th>
<th>The third-order waves</th>
</tr>
</thead>
<tbody>
<tr>
<td>frequencies (min)</td>
<td>126 ± 12</td>
<td>14.9 ± 4.9</td>
<td>4.12 ± 1.04</td>
</tr>
<tr>
<td>amplitude (mV)</td>
<td>0.138 ± 0.05</td>
<td>0.209 ± 0.147</td>
<td>0.365 ± 0.286</td>
</tr>
<tr>
<td>duration (s)</td>
<td>0.39 ± 0.04</td>
<td>2.75 ± 1.3</td>
<td>10.91 ± 5.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II group</th>
<th>First-order waves</th>
<th>The second-order waves</th>
<th>The third-order waves</th>
</tr>
</thead>
<tbody>
<tr>
<td>frequencies (min)</td>
<td>111 ± 18</td>
<td>13.2 ± 3.8</td>
<td>3.93 ± 1.12</td>
</tr>
<tr>
<td>amplitude (mV)</td>
<td>0.98 ± 0.06*</td>
<td>0.135 ± 0.067*</td>
<td>0.145 ± 0.064*</td>
</tr>
<tr>
<td>duration (s)</td>
<td>0.38 ± 0.06</td>
<td>3.45 ± 0.9</td>
<td>10.83 ± 6.2</td>
</tr>
</tbody>
</table>

Fig. 2. Hematoxylin-eosin staining (A and C) and van Gieson (B) staining. (A) thickness of the muscular layer of the healthy aorta, mean 690 µm. (B) thickness of the muscular layer of the abdominal aortic aneurysm, mean 630 µm. (C) thickness of the muscular layer of the aorta near the aortic prosthesis, about 5 mm distance, mean 530 µm.

Myoelectric activity patterns of healthy abdominal (first group) aorta, abdominal aortic aneurysm (second group) and abdominal aorta on the aorta-aortic prosthesis interface

Anatomopathological changes in the experimentally induced abdominal aortic aneurysm
penetration of erythrocytes within the damaged structures of the tunica media was also found. Furthermore, numerous inflammatory infiltrations within the adventitia were observed, containing mainly lymphocytic cells, but also single plasma cells and neutrophils. The inflamed adventitia also showed calcium deposits, around which there were single, multinuclear giant cells and frequent areas of massive fibrosis with a proliferation of fibroblastic cells and small blood vessels. There were frequent necrotic spots within the superficial layer of adventitia, under which haemorrhages were visible. A pronounced loss of endothelial cells was detected. The observed changes were consistent with findings from previous studies (2).

**Histopathological changes on the abdominal aorta-simple prosthesis interface and changes in the thickness of the muscle layer in dissected specimens**

Histopathological changes observed in the dissected specimens at about 5 mm from the aortic prosthesis did not differ significantly from those observed in the experimentally induced abdominal aortic aneurysm. The only difference found was an increased per cent share of connective tissue caused by massive fibrosis and proliferation of fibroblasts and small blood vessels. We found a significant reduction in the thickness of the muscular layer of the aorta due to degradation. The following differences in the thickness of the muscular layer were observed: the mean thickness of the muscular layer in the healthy aorta was 690 µm (Fig. 2A), 630 µm in the experimentally induced aneurysm (Fig. 2B) and 530 µm near the prosthesis (Fig. 2C). The differences of in aortic thickness are caused by elastic fibers and smooth muscle cells degradation, presence of calcium deposits, inflammatory infiltrations containing lymphocytic cells and local necrotic lesions. The histopathological changes observed in abdominal aorta aneurysm were similar with those described in previous paper (2).

**DISCUSSION**

An experimentally induced abdominal aortic aneurysm seems to be a good model for research into the etiopathogenesis of an abdominal aortic aneurysm in humans and for methods for the repair of an aneurysmal lesion using a prosthesis or stent graft (2, 10). One of these models is an experimentally induced aneurysm of the abdominal aorta in pigs, developed and tested in previous studies (2). Mechanically and enzymatically induced AAA will never be consistent with aortic aneurysm created over the years under natural conditions, but at the moment, it is the best option to conduct the invasive study on the pathology. The model is repeatable and stable. In the experiments, we achieved a dilation of abdominal aortic lumen greater than 50% of the healthy aorta diameter. This parameter classifies the induced change as an aortic aneurysm (2, 8, 11). We demonstrated an increase in the diameter of AAA by 71 ± 3.5% (P≤0.001) (post-mortem measurement in the first experimental group) and by 82 ± 15% (P≤0.001) (ultrasound measurement, mean for both experimental groups) in comparison to the diameter of the healthy aorta. The induced aneurysmal lesion showed no symptoms of regression. The results of the experiment confirm the findings from previous studies (2) and experiments by other researchers (12-15).

Changes in the wall of the abdominal aorta were monitored by recording myoelectric activity. Previous studies were focused on the myoelectric activity of the healthy abdominal aorta (8) and abdominal aortic aneurysm (2). The recorded myoelectric activity is characterised by the appearance of three types of waves: first-, second- and third-order. The origin of waves was explained in other papers (2, 8). First-order waves are closely associated with the heart rate because of their strong correlation with changes in blood pressure, measured using an invasive method and ECG records (2, 8). Blood injected into the arteries during systole results in increased blood pressure and dilation of the vessel lumen. Stretching of muscle cells is followed by their contraction determined by reflex. The contraction is recorded as changes in the membrane potential of VSMC in the form of first-order waves. Another characteristic feature of VSMC is its contraction in response to electrical, but also mechanical, chemical and hormonal, stimuli. This was confirmed in many studies carried out on the smooth muscular layer of the gastrointestinal, reproductive and urogenital systems, and on the smooth muscular layer of the abdominal aorta and a cow’s udder (16). Second-order waves are associated with breathing. They are produced as a result of changing pressure in the chest caused by working respiratory muscles. This process also influences changes in systemic blood pressure (2, 8). VSMC also responds to these changes by changing the membrane potential, recorded as second-order waves. Shallow breathing in subjects during rest or induced narcotic sleep results in reduced amplitude and frequency of second-order waves or their absence in records (8).

Third-order waves are produced as a result of changes in the potential of the sympathetic nerves of the autonomic system regulating the lumen of arteries and thus influencing changes in blood pressure (2, 8, 17-20).

In the experimentally induced AAA, the recorded myoelectric activity of the abdominal aorta did not differ significantly (P≤0.005) when compared to patterns recorded during control experiments on the healthy aorta. This suggests that the muscular tissue was not seriously damaged in terms of functional parameters during the induction and development of an aneurysm or that changing electrical potentials are transmitted along the blood vessel from intact surrounding cells. The histopathological examination revealed changes in the VSMC of the aorta in the experimentally induced abdominal aortic aneurysm. The mean thickness of the muscular layer in the healthy aorta was 690 µm and 630 µm in the experimentally induced aneurysm. The difference in the mean thickness of the muscular layer was 60 µm. Such a small, statistically insignificant change in the thickness by 8.7% did not result in changes of the recorded patterns of myoelectric activity.

In an aneurysm repaired using a straight prosthesis, we observed lower values of myoelectric activity recorded for VSMC. All types of waves found in records for the section of healthy aorta were detected; they had much lower amplitude but the same frequency. The mean amplitude of discharges (0.98 ± 0.06 mV) recorded as first-order waves was 45% lower in comparison to the amplitude for the healthy section of the aorta (P≤0.005). The mean amplitude of discharges for second-order waves was 34.2% lower in comparison to the waves recorded for the healthy aorta (P≤0.005). The mean amplitude of discharges for third-order waves was 57.9% lower in comparison to the healthy aorta (P≤0.005).

Reduction in the amplitude of discharges in the recorded myoelectric activity of the abdominal aorta on the prosthesis interface was caused by the reduced amount of muscular tissue and dysfunction caused by the increased amount of fibrous connective tissue. The observed changes inhibited vascular contractility (21). This was confirmed by the results from measurement of the muscular layer of VSMC on the prosthesis-aorta interface. The thickness of VSMC reduced by 23.18% in comparison to the mean thickness measured for the healthy aorta (a decrease of 160 µm, P≤0.0005). In addition, a reduction in the amplitude of third-order waves was caused by damage to sympathetic innervation of the abdominal aorta during the surgical repair of the aneurysmal lesion using an aortic
prosthesis. During aneurysm repair, the section of the abdominal aorta to be removed has to be separated from the adjacent tissues. As a result, nerves in this part of the vessel become damaged during dissection. This results in the reduced potential of impulses from the nervous system and disorders in the regulation of aortic lumen. The muscular layer of the aorta is insufficiently stimulated and thus has a lower potential. This was confirmed by the observations of the reduced amplitude of records third-order waves in comparison to the corresponding section of the healthy aorta.

The use of electromyographic records may allow for better understanding of the mechanism responsible for regulating the lumen in both the healthy and aneurysm aorta. In our study, we demonstrated statistically significant differences in the myoelectric activity of the abdominal aorta on the aorta-straight prosthesis interface in comparison to the healthy aorta and aneurysmal aorta. This proves the motoric dysfunction of the interface, because the myoelectric activity is closely correlated with contractility. However, it should be remembered, that the fibrosis and sympathetic denervation may be a consequence of implantation of the tube in the aortic wall and after certain period changes might recover. Nevertheless, differences in the properties of contractile sections may be one of the reasons for relapsing aneurysms. As we conclude from the study, the implantation of the straight prosthesis may not be a perfect option in the treatment of an abdominal aortic aneurysm because of the contribute to remodelling of the tissue on the prosthesis-aorta interface. Alternatively, the studies of using extracellular matrix as a vascular prosthesis seem to be promising. Biologic scaffold materials are commonly used in regenerative medicine and characterized by the acceleration of neovascularization and low immune response to the graft.

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