Effects of removal of the adventitia of the descending aorta and structural alterations in the tunica media in pigs

Efeitos da remoção da túnica adventícia da aorta descendente em suínos

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ABSTRACT

Objective: To investigate the effects of removal of the adventitia on the tunica media in a pig model. **Methods:** The experiment was performed in eight pigs. The adventitia of the descending aorta was removed. The aortic segment was removed following euthanasia at two, four, six and eight weeks,. Next, slices of the aorta were stained with hematoxylin and eosin (HE) and Weigert - Van Gieson**. Results**: After two weeks there was a slight cellular breakdown in the outer third of the media. After four weeks structural breakdown of elastic fibers was observed in the outer two thirds of the same layer. In six weeks, several areas of necrosis and almost complete disruption of elastic fibers were identified. Finally, after eight weeks, there was fibrosis of the entire wall, with disruption of the internal elastic lamina. **Conclusion:** The removal of the adventitia leads to degeneration of the media, determining loss of the normal structure of the aortic wall that is variable in its location, intensity and shape, depending on the length and duration of the ischemic insult.

Key words: Aorta. Vasa vasorum. Microcirculation. Ischemia. Dissection.

INTRODUCTION

Aortic dissection is the most devastating catastrophe among cardiovascular diseases, but the mechanism by which the it occurs is not fully understood. There are several factors to be considered, such as hemodynamic disorders and factors related to mechanical and geometrical properties of the aorta[1]. The Vasa Vasorum (VV) have an important role in various diseases, as insufficient blood flow through the aortic wall is involved in the pathophysiology of atherosclerosis and may contribute to necrosis of the aortic tunica media.

The VV are small arteries that penetrate the arterial wall both from the luminal surface (VV internae) and by the adventitia (VV externae). The VV from the adventitia branch into smaller vessels that supply the outer layers of the tunica media[2, 3].

This study aims to demonstrate the histological alterations that occur in the aortic wall over time after the interruption of blood flow from the VV in the descending aorta in a porcine model.

METHODS

The experiment was performed with eight young female Landrace-Large White pigs, with a mean age of eight weeks, weighing on average 25 kg (21 - 27 kg). One animal was excluded from the analysis due to death secondary to rupture of the thoracic aorta and hypovolemic shock during removal of the adventitia of the descending aorta. The seven remaining animals were divided into four groups accordingly with the timing of euthanasia: two weeks after lesion (three animals), four weeks (two animals), six weeks (one animal) and eight weeks (one animal). The experiment was approved by the ethics committee of the Federal University of Rio Grande do Sul (UFRGS) and the Porto Alegre Clinics Hospital (HCPA) -–Protocol: 06548.

Surgical technique

The pigs were pre medicated with acepran (0.1mg/Kg), midazolam (0.3 mg / kg) and morphine (0.5 mg / kg). Venous access was obtained in the marginal vein of the ear for administration of medication and fluids. The

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animals were pre-oxygenated for five minutes before induction of anesthesia with propofol (1 mg/kg). They were then ventilated with oxygen via endotracheal intubation. General anesthesia was maintained by the infusion of propofol (0.8 mg/kg/min) and monitored by means of electrocardiogram and oximetry. Through a left lateral thoracic incision into the left 6th intercostal space, the pleural cavity was entered and the mid portion of the descending aorta was dissected. A five-cm adventitia flap was removed and the intercostal arteries ligated. After removal of the adventitia, the aorta was wrapped with bovine pericardium (Figure 1). A drain with continuous aspiration was put into place before closure of the chest wall.

Histological analysis

Euthanasia was performed accordingly to the same anesthetic protocol previously described. Aortic segments wrapped in bovine pericardium were removed together with about five cm of the proximal and distal aorta. The samples were then immersed in 10% buffered formalin and sent to the pathology laboratory. Next, a slice of each segment (proximal, middle and distal) of the aortas was stained with hematoxylin and eosin (HE) and Weigert - van Gieson (to highlight collagen and elastic fibers).

The stained slices were analyzed using a PL-A662 Pixelink camera coupled to Axiothotz Zeiss lens with 25x, 50x and 100x magnification. Assessment of the histological parameters and morphometric measurement of the histological sections were performed by a pathologist with expertise in arterial pathology and blinded to the selected groups.

RESULTS

During the removal of the aorta an intense fibrotic sheath around the injured segment was observed, but thanks to the use of the bovine pericardium there was little adherence to adjacent tissues.

After two weeks, subendothelial fibrosis was observed but the internal elastic lamina was preserved, as were the inner two thirds of the media. There was a slight cellular breakdown in the outer third of the media, with some lymphocytes and neutrophils. In addition, foci of angiogenesis were identified in the outer third of the media.

In animals sacrificed after four weeks, the subendothelial fibrosis with internal elastic lamina remained unchanged and the inner third of the tunica media was preserved. There was structural breakdown of elastic and collagen fibers in the outer two thirds of the tunica media. Lymphocyte and neutrophil infiltration and foci of angiogenesis were observed in the outer two thirds of the media (Figure 2 and 3).

After six weeks, the following findings were identified: diffuse foci of necrosis of the aortic wall with subendothelial fibrosis. Regarding inflammation, there was lymphocytic, histiocytic and neutrophilic infiltration, with necrosis throughout the entire tunica media. Foci of angiogenesis were observed throughout the tunica media and in the subendothelial portion.

Finally, after eight weeks, there was fibrosis of the entire wall of the aorta, with disruption of the internal elastic lamina. We observed an area of moderate inflammation near the endothelium. As for angiogenesis, foci were observed throughout the tunica media and in the subendothelial portion (Figure 4 and 5).

In summary, there was a progressive increase in the degree of injury, which started in the outer portion of the media and progressed toward the inner layers with time.

DISCUSSION

Vascular inflammation is a complex process that involves migration, extravasation and local activation of mononuclear leukocytes in the vessel wall[4]. The cellular infiltrate of macrophages and lymphocytes represents the major cell types in human aneurysms and are located mainly in the tunica adventitia[5]. Besides promoting leukocyte infiltration and cytokine production, the adventitial layer is highly reactive and may play a significant role in the formation of aneurysms. It is believed that the adventitial layer has a key role in the genesis and maintenance of vascular inflammation seen in aneurysms and atherosclerotic vascular disease[6, 7].

The three-dimensional study of VV was made possible by micro-computed tomography studies[8,9], as well as a better understanding of the anatomy and distribution by micro-arteriography. The adventitial VV connect to each

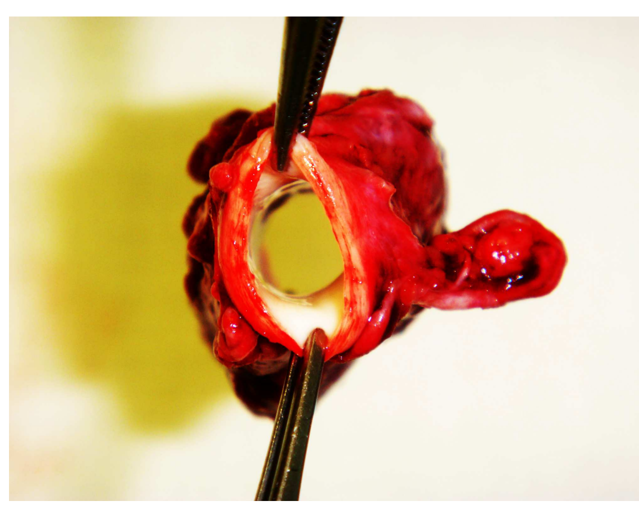

Figure 1 - *Descending thoracic aorta after removal of the adventitia, being covered by bovine pericardium.*

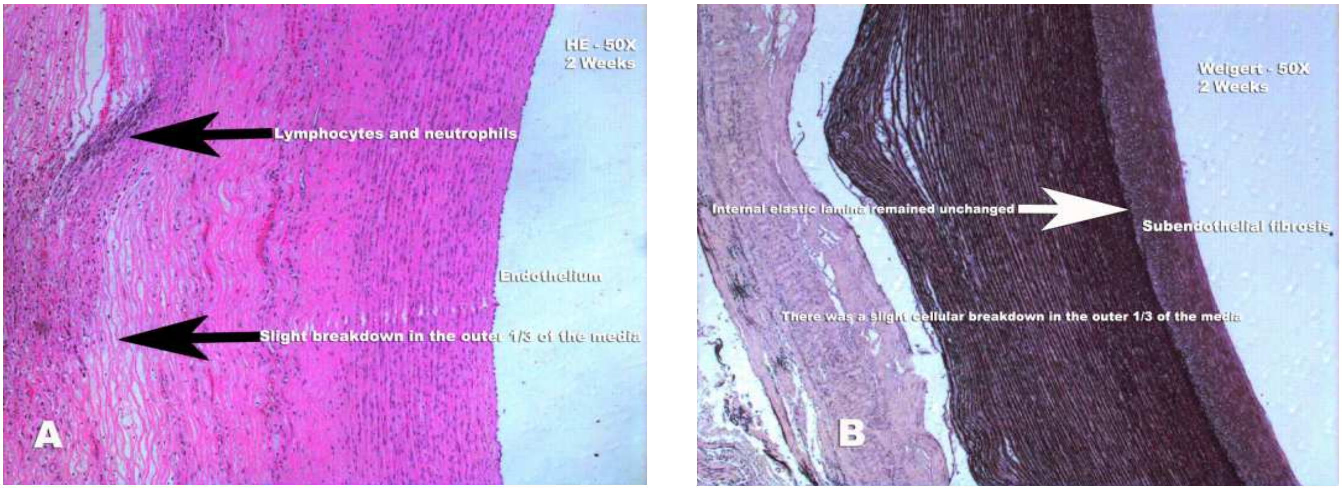

Figure 2 - *After two weeks: **A** - slight cellular breakdown in the outer third of the media, with some lymphocytes and neutrophils **B** - subendothelial fibrosis observed, but preserved internal elastic lamina, as the inner two thirds of media.*

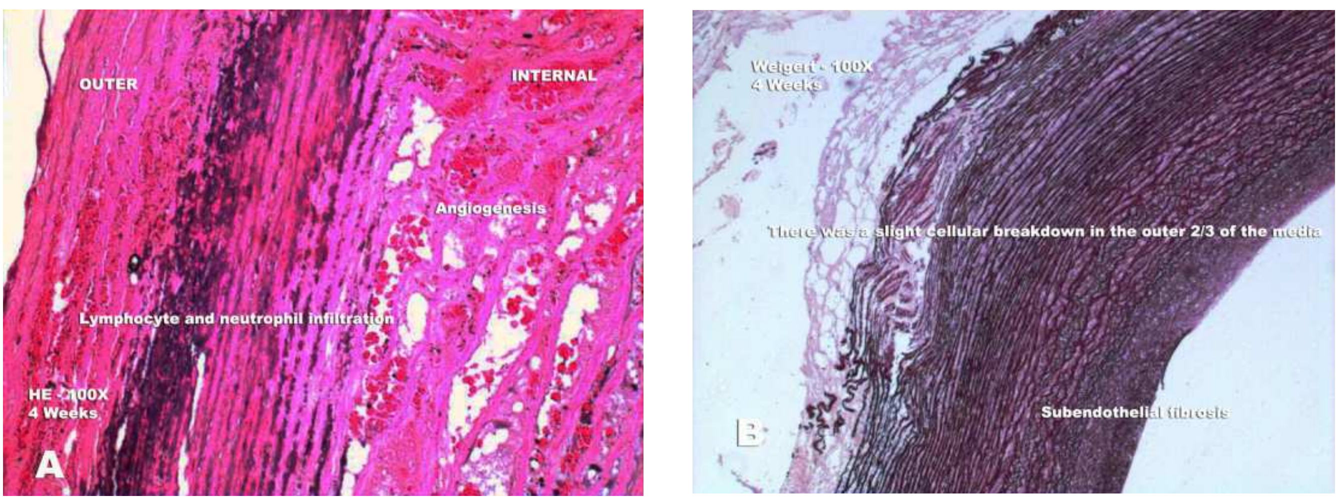

Figure 3 – *Findings after four weeks: **A** - lymphocyte and neutrophil infiltration and foci of angiogenesis observed in the outer two thirds of media **B** - subendothelial fibrosis with preservation of the internal elastic lamina and of the inner third of the tunica media with structural breakdown of elastic and collagen fibers in the outer two thirds of the same layer.*

other by means of a plexus, but behave like terminal arteries. Embolization of VV reduces the density of these vessels at that point and increases the number of branches in areas previously less irrigated[10]. This feature may impact the spatial distribution of perfusion and drainage of the arterial wall.

The mechanism by which the dissection occurs is not yet fully understood. There are several factors to be considered, such as hemodynamic disorders and factors related to the mechanical and geometrical properties of the aorta (anisotropy and the structural organization of the aortic wall)[1].

There are two main mechanical factors that have influence in aortic dissection: anisotropy (the variation in pressure at different points of the aortic wall) and the structural organization of normal aorta wall, which is composed of different layers, i.e., the wall is not homogeneous.

Anisotropy with subsequent disruption of layers in the aortic wall is the main mechanism of aortic dissection. The immediate consequence of this anisotropy is modification of local stress in the aortic wall, more intense in the media. The entry injury commonly occurs at the interface of layers, especially if there is loss of structural integrity at that point. The elastic properties of the aortic wall are determined mainly by the components of the aortic media. The elastin-collagen ratio, smooth muscle cells, the composition of the extracellular matrix, as well as wall thickening, all play an important role in the elastic properties of the aortic wall. Based on these mechanical principles of the aortic wall, Stefanadis *et al.*[11] developed an experimental model of ischemia of the arterial wall by the interruption of the VV, removing the periaortic fat from the ascending aorta of dogs, and assessed the changes in elasticity of the aortic wall. This study, which evaluated the changes with two weeks of ischemia, showed a decrease in the elasticity

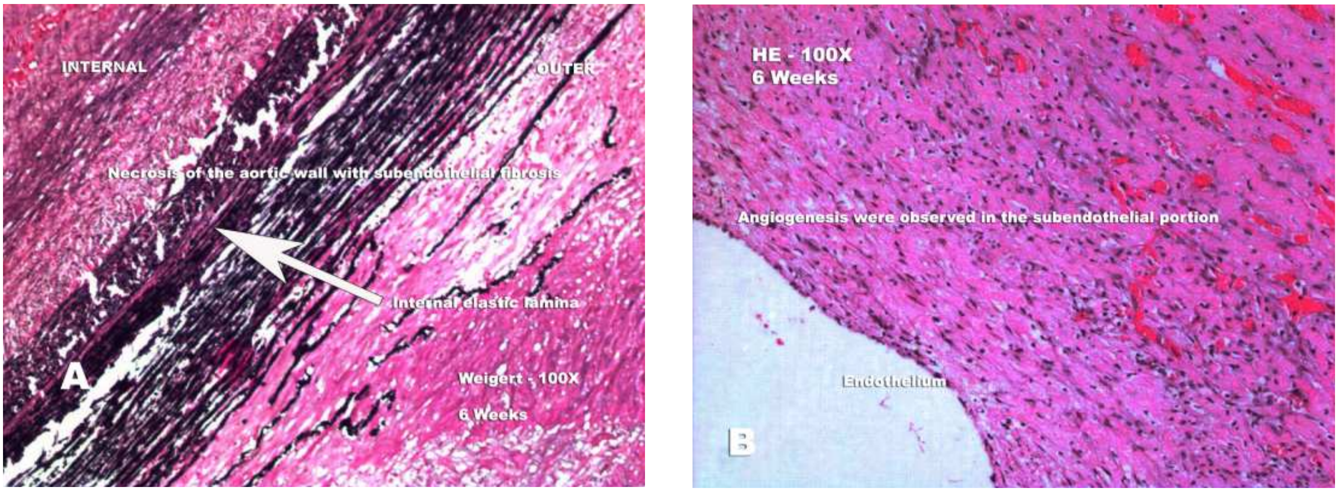

Figure 4 – *Findings after six weeks:* ***A*** *- diffuse foci of necrosis of the aortic wall, with subendothelial fibrosis* ***B*** *- foci of angiogenesis observed throughout the tunica media and in the subendothelial portion.*

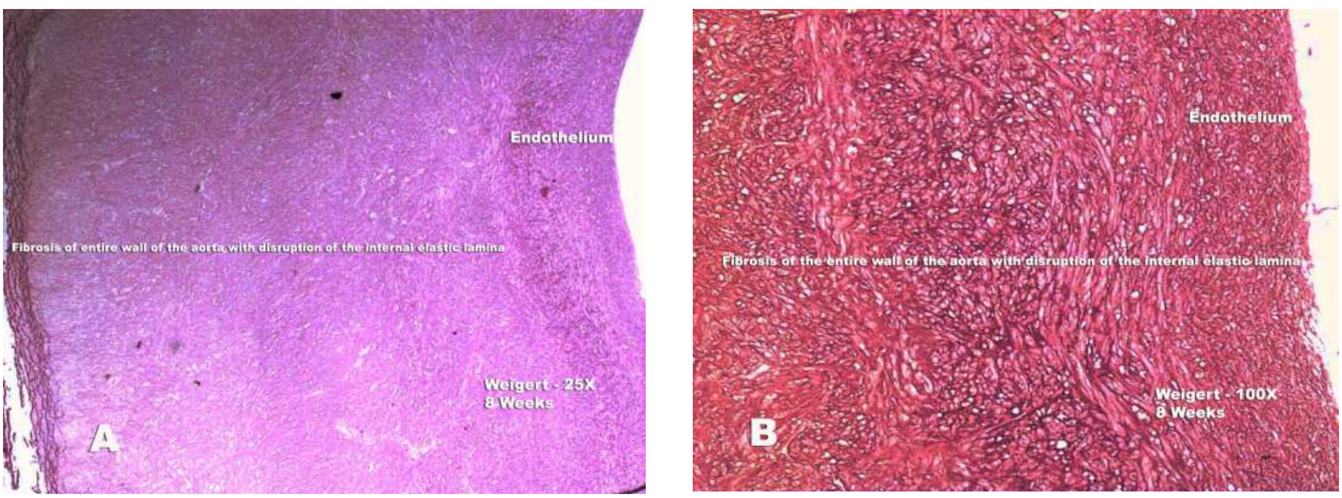

Figure 5 – *Findings after eight weeks:* ***A*** *- ischemic insult – fibrosis of the entire wall of the aorta with disruption of the internal elastic lamina* ***B*** *- ischemic insult – fibrosis of the entire wall of the aorta, with disruption of the internal elastic lamina.*

of the aortic wall, necrosis of the tunica media accompanied by disarrangement and fragmentation of elastic fibers and of the ratio of collagen to elastin. These results confirm findings from previous studies[12, 13]. Other investigations also suggest that the decreased blood flow in the aortic wall during a hypertensive crisis may contribute to necrosis of the tunica media of the aorta[14]. Stefanadis *et al.*[11] also demonstrated, besides the breakdown of collagen and elastin fibers, an increase in the stiffness of the ascending aorta in dogs two weeks after the removal of the VV. These results were confirmed latter by the work of Angouras *et al.*[15], who studied the morphological alterations secondary to interruption of VV by removing the adventitia of the descending thoracic aorta in pigs. After two weeks, ischemia of the aortic wall led to morphological alterations in the content of collagen and elastin in the outer third of the media, resulting in stiffening in this region. In the latter study, a non-porous and inert material (polyvinyl chloride) was used to cover the injured segment of the aorta in order to avoid adherence to adjacent tissues. In this study, we chose to wrap the aorta with bovine pericardium because it is a more biocompatible, still non-porous, graft.

In this study we used the Weigert-van Gieson method to better analyze elastic and collagen fibers. Elastic fibers are stained in black by Weigert's stain (basic fuchsine / resorcinol), and collagen fibers in red by van Gieson's stain (acid fuchsine). The background tissue, regardless of the type, is stained yellow by the picric acid. Nuclei are stained in red by Weigert's iron hematoxylin.

Unlike the findings reported in the study by Angouras *et al.*[15] and Stefanadis *et al.*[11], our study demonstrated the presence of subendothelial fibrosis with a mild inflammatory infiltrate of lymphocytes and neutrophils at the outermost part of the media, as well as sparse foci of angiogenesis in the outer layer, since two weeks after injury.

In this study, the histological alterations that occur in the aortic wall in a longer term were demonstrated. To our knowledge no other study has evaluated the ischemic alterations in the aortic wall beyond two weeks of ischemia. It could be seen that the interruption of blood flow from the VV in the aortic wall led to structural disarrangement, with loss of elastic fibers in the tunica media that began in the outer portions (two weeks) and that, over time (four, six and eight weeks), the ischemic insult led to a progressive disarrangement of the inner layers of the arterial tunica media, until there was complete laceration of the internal elastic lamina and fibrosis of the entire wall of the aorta, with almost complete destruction of elastic fibers. Probably the role of the VV internae (from the luminal surface) in irrigating the inner third of the media is less important than it was expected.

CONCLUSION

The removal of aorta adventitia leads to degeneration of the media, determining loss of the normal structure of the aortic wall that is variable in its location, intensity and shape, depending on the length and duration of the ischemic insult.

R E S U M O

Objetivo: *Investigar os efeitos da remoção da adventícia da aorta em suínos.* ***Métodos:*** *O experimento foi realizado com oito suínos. Removeu-se a camada adventícia da aorta descendente. Após a eutanásia com duas, quatro, seis e oito semanas, o segmento aórtico era removido. Após, eram feitos cortes histológicos com a coloração de hematoxilina e eosina (HE) e pelo método de* Weigert – Van Gieson. ***Resultados****: Após duas semanas identificou-se um leve desarranjo do terço externo da túnica média. Nos animais sacrificados após quatro semanas observou-se um desarranjo estrutural dos terços externos da túnica média. Após seis semanas observou-se necrose da parede aórtica. Finalmente, após oito semanas além da fibrose da parede aórtica identificou-se a destruição da lâmina elástica interna.* ***Conclusão:*** *A remoção da adventícia da aorta em suínos levou à alterações degenerativas da média, determinando perda da estrutura da parede aórtica que é variável em sua localização, intensidade e forma, dependendo do tempo a partir do qual se estabeleceu a lesão isquêmica.*

Descritores*: Aorta. Vasa vasorum. Microcirculação. Isquemia. Dissecação.*

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