Stable experimental model of carotid artery saccular aneurysm in swine using the internal jugular vein

Modelo experimental estável de aneurisma sacular em artéria carótida de suínos utilizando veia jugular interna

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A B S T R A C T

Objective: To develop an experimental model of stable saccular aneurysm in carotid of pigs using the internal jugular vein. **Methods**: In 12 healthy pigs, weighing between 25 and 50kg, five males and seven females, we made a right common carotid artery aneurysm. After elliptical arteriotomy, we carried out a terminolateral anastomosis with the distal stump of the internal jugular vein. Aneurysm volume was calculated so that the value did not exceed 27 times the area of the arteriotomy. After six days angiography and microscopic examination were performed to assess patency of the aneurysm and the presence of total or partial thrombosis. **Results**: There was a significant weight gain of pigs in the time interval between the manufacture of the aneurysm and angiography (p = 0.04). Aneurysmal patency was observed in ten pigs (83%). Operative wound infections occurred in two animals (16.6%), both with early onset, three days after the making of the aneurysm. Histological analysis showed aneurysm thrombus partially occluding the light in nine pigs (75%). In these animals, it was observed that on average 9% of the aneurysmal diameter was filled with thrombi. **Conclusion**: It was possible to develop a stable experimental model of saccular aneurysms in pig carotid artery by use of the internal jugular vein.

Key words: Aneurysm. Jugular veins. Carotid arteries. Animal experimentation. Swine.

INTRODUCTION

The two most important factors for the emergence of aneurysms in cerebral vessels are the vulnerability of the arterial wall and increased hemodynamic stress. Histopathologic studies using hematoxylin and eosin show that lack of the internal elastic lamina, associated with normal intima, media and adventitia, as well as the presence of polymorphonuclear cells, plasma cells and lymphocytes, are present in these aneurysms. Despite advances in the knowledge of this disease, the pathophysiologic processes involved in its formation currently remain unknown and controversial [1,2]. The saccular aneurysm type is the most frequent, being present in about 80 to 90% of cases [2,3].

It is estimated that 0.5% to 6% of the population is affected by a brain aneurysm, and of these, about 15-31% have multiple lesions [4,5]. In families with two or more affected individuals, the prevalence reaches 10% [3]. Rupture of saccular aneurysm is the major cause of non-traumatic subarachnoid hemorrhage, accounting for approximately 85% of cases [6,7]. It has a mortality rate between 32 and 67% and leads to long-term physical sequelae in 10 to 20% of survivors[4]. A cerebral hemorrhage is fatal in 10-15% of patients before receiving any medical attention in the hospital, and those who survive often are left with some neurological or cognitive impairment [8]. If untreated, these aneurysms have averaged 3.5% of rebleeding rate each year in the first decade, with mortality due to late rebleeding of 67% [9,10].

The ruptured brain aneurysm most often affects patients between 55 and 60 years of age, being rare in childhood, when it occurs in this age group, it is usually present in individuals over ten-years-old [11].

The most common symptoms of brain aneurysms are the result of the secondary cerebral subarachnoid hemorrhage. The onset is sudden, with strong headache, nausea, vomiting, neck stiffness and decreased level of consciousness [8].

There is great difficulty in studying this disease in terms of etiology, pathophysiology and treatment, due in part to being extremely rare in animals, which would potentially serve as natural experimental models [12]. Thus, greater knowledge about its behavior and treatment depends on the availability of experimental research that resembles its morphology and physiology [13,14]. Currently, the long-term results of endovascular embolization with coils have varying degrees of recanalization after treatment. Thus, the development of new coils, with greater thrombotic potential, also depends on the existence of experimental models [15].

The aim of this study was to produce a stable experimental model of saccular aneurysm in the common carotid artery of pigs using the internal jugular vein, with features that allow its use in the development of endovascular devices and training of new surgeons.

METHODS

This research was a descriptive study for the development of an experimental model in animals approved by the Ethics Committee of the University of Health Sciences of Alagoas (UNCISAL), Maceió, Alagoas State – AL, Brazil, under protocol 62-A, and the Ethics Committee on Human Studies and Animal Studies, Federal University of Vale do São Francisco (UNIVASF), under protocol 03.031.103.

The survey was conducted with 12 Landrace pigs, aged between eight and ten weeks, and was divided into two phases. The first consisted of making an aneurysm in the right common carotid artery, and was held initially at the Center for Experimental Surgery and vivarium at UNCISAL, being completed in the Laboratory of Experimental Surgery at UNIVASF. The second phase occurred after six days at the Centre of Experimental Surgery and vivarium at UNCISAL for animals initially operated at this institution, and at the Veterinarian Hospital at UNIVASF, for the remaining animals. This second phase consisted of angiography and aneurysm resection for histological study.

In both phases, the animals underwent general anesthesia before surgical procedures. We employed the pre-anesthetic agents atropine 0.04 mg/kg subcutaneously, and after ten minutes, 15mg/kg ketamine and midazolam, 0.2 mg/kg intramuscularly. Then the marginal ear vein was punctured with a 20 gauge catheter for the infusion of saline

(0.9%) to be initiated 20ml/kg/hour for induction. Anesthesia was induced with thiopental sodium at a dose of 12.5 mg/kg until loss of tracheal reflex. After induction, the pig was intubated with an endotracheal tube and ventilated with a tidal volume of 15ml/kg, often 12-15 breaths per minute. Inhalation anesthesia was maintained with isoflurane and ventilation with 100% O2. During the anesthetic procedure, the following parameters were evaluated by noninvasive monitoring: heart rate, respiratory rate, and systolic and diastolic blood pressures.

In the first phase, the animal was placed in dorsal recumbency and subjected to injection of 1 g of cephalothin intravenously. Then we proceeded to the midline incision in the anterior neck, extending 5 cm below the mandible to the beginning of the sternum. After dissecting the muscle planes, we addressed the common carotid artery and the right jugular vein. Unfractionated heparin 100U/kg was administered intravenously. We proceeded to the longitudinal elliptical incision in the common carotid artery with a longitudinal diameter of 7mm and a transverse diameter of 2mm. The opening area of the blood was calculated based on the formula of an ellipse: $A = \pi . 3.5 . 1$ (A = area; π = 3.14159265; 3.5 = half of the longitudinal diameter; 1 = half of the transverse diameter), where the value was recorded in mm^2 (Figure 1).

Regarding the internal jugular vein, it was cross-sectioned, with ligation of the proximal stump using a 3-0 cotton thread. The following step was a terminolateral anastomosis between the distal jugular stump and the common carotid artery, with 6-0 polypropylene sutures, thus fashioning an arteriovenous fistula. We then ligated the fistula at such a distance from the anastomosis that the volume of the formed aneurysm was 27 times the value of the area of elliptical arteriotomy. The aneurysm volume, in mm3, was calculated based on the volume of a cylinder according to the formula $V = \pi . r^2 . H$ (V = volume; π = 3.14159265, r = radius H = height) (Figure
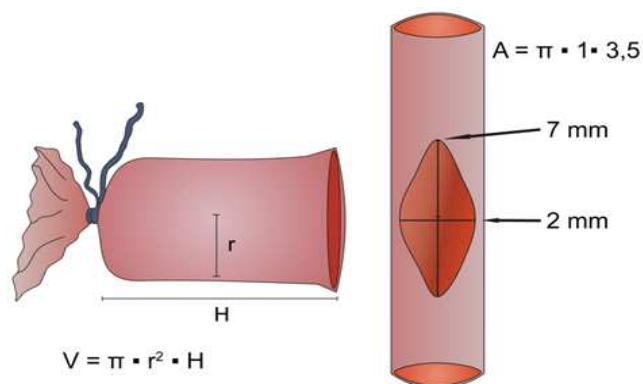

Figure 1 - *Calculation of the arteriotomy area and volume of the venous pouch. A = area; π = 3.14159265; 3.5 = half the value of the longitudinal length; 1 = half the value of the cross-sectional diameter; V = volume; r = radius; H = height.*

1). The filling of the aneurysm with blood was observed and the neck incision was closed in layers. Inhalation anesthesia was discontinued and the pig was awakened from anesthesia.

The primary variable defined was the aneurysm patency frequency. Secondary variables analyzed were: diameter of the common carotid artery, internal jugular vein diameter, time of common carotid artery clamping, length of venous purse, aneurysm sac volume, frequency of bleeding, hematoma, rupture, pseudoaneurysm and infection, time of onset of infection, frequency of dehiscence of skin suture, neurological disorder, thrombosis at histopathology and proportion of the aneurysm lumen filled with thrombi.

To evaluate the frequency of aneurysm patency, pigs underwent control angiography six days after the making of the carotid aneurysm. Midline incision was made in the anterior neck extending 5cm below the jaw to the top of the sternum. After dissection of the muscle planes, we assessed the common carotid artery proximally, inserted a number 6 Foley catheter in the arterial lumen, injected 20ml of iodinated contrast and angiography was done using simple radiography apparatus. After, we performed an *en bloq* resection containing the common carotid artery aneurysm to evaluate the frequency of thrombosis through histological analysis and the percentage of the lumen filled with aneurysmal thrombus. Next, the pig was euthanized with toxic dose of thiopental sodium (4mg/kg) and the infusion of a long-lasting muscle relaxant (pancuronium).

Aneurysms were referred to immersion in paraffin, microtome and lamination; cuts were made transversely, obtaining three slides of each aneurysm: one from the proximal portion, one from the middle portion and lastly one from the distal portion. Staining was carried out with hematoxylin-eosin. The analysis of each specimen was made in optical microscopy by the same pathologist, who verified the existence of thrombus and its histological characteristics. Next, the percentage of aneurysmal lumen occupied the thrombus was calculated. Each of the three slides of each aneurysm was photographed under a microscope (Figure 2). Through photography, using the ImageJ software 1.44, we measured the total aneurysmal lumen area and the one occupied by the thrombus, thus being able to estimate the percentage of the area of the aneurysmal lumen filled with thrombi in each slide. Then, the percentages of occupied aneurysmal lumen of the three slides were summed to obtained the estimated percentage of the overall aneurysmal lumen filled with thrombi. The aneurysmal lumen in each of the three blades was considered to be weighted 33.33% of the total lumen of the aneurysm.

We used a confidence interval of 95%. For numeric variables, we used the Student t test. To study the correlation between categorical variables, we used the Fisher exact test. The p value was considered statistically significant when <0.05.
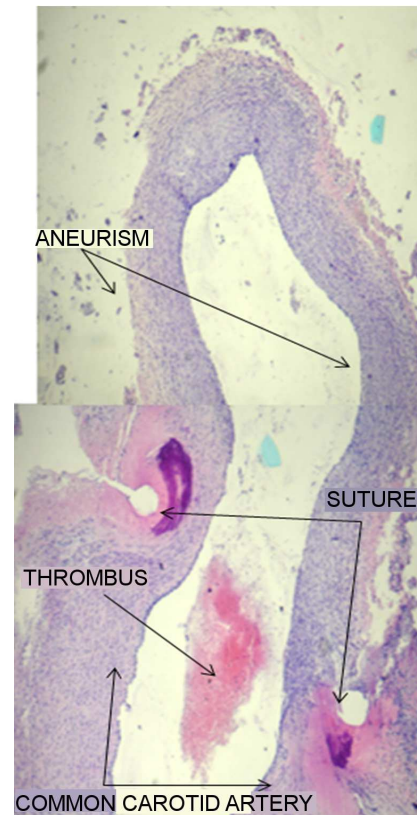

Figure 2 - *Characteristics of the histological slide of the aneurysm (HE 50X).*

RESULTS

The 12 animals submitted in the making of the aneurysm survived the experiment. We used seven females (58.3%) and five males (41.6%).

The average weight of the animals during the preparation of the aneurysm was 38.8 ± 5.4 kg, whilst during angiography, 40 ± 6.3 kg. There was a significant weight gain in the time interval between the manufacture of the aneurysm and angiography (p = 0.04).

There were no major complications, such as bleeding, hematoma, pseudoaneurysm or neurological disorder. Infections were observed in two animals' wounds (16.6%), both with early onset on the third day following the operation.

The average time of the making of the aneurysm was 130.7 ± seven minutes, and the average clamping was 33.8 ± 1.3 minutes (Table 1). We analyzed the diameter of the common carotid artery and jugular vein, as well as the length and volume of the vein pouch (Table 1).

Regarding the primary variable, we observed that ten (83%) aneurysms were patent at angiography (Figure 3). Aneurysmal thrombosis was observed at angiography in two animals (16.6%), one of which showed a partial thrombus filling the lumen, and the other, total thrombosis. These two animals were females.

Upon histological analysis of the aneurysms, there were partially occluding thrombi in nine animals (75%). It was observed that on average 9% of full aneurysmal lumens were filled with thrombi; 55.5% of the cases of thrombosis in this analysis occurred in females and 44.4% in males.

In the two animals with diagnosis of thrombosis at angiography (100%), histological analysis confirmed it. On the other hand, of the nine animals with thrombosis diagnosed by histopathology, two (22.2%) had it displayed on angiography; they did not have the highest percentages of aneurysmal lumen

The nine animals with aneurysmal thrombosis after histological analysis showed thrombi characteristics of recent formation, with clusters of Red Blood Cells being permeated by mononuclear cells. We did not observe areas of organization or fibrin deposits (Figure 4).

DISCUSSION

The experimental animal models have been used for decades, both in the study of the natural history of arterial aneurysms and in the evaluation of treatment outcome [16].

These models attempt to reproduce the histological, geometrical, hemodynamic and pathophysiological characteristics of cerebral aneurysms in humans [17]. These models should be suitable for hemodynamic and pathological studies and to evaluate the efficacy of therapeutic procedures [18,19]. Five types of animals have been used to surgically construct these models of aneurysm: rabbit, mouse, sheep, dog and pig [10,12,20-23].

Small animals have disadvantages, such as the need often microscope for handling. Furthermore, the arterial access site is often sacrificed at the end of each angiogram, and the narrow lumen of the vessel hinders the insertion of endovascular devices that are represented by simultaneous use of multiple microcatheters [24,25].

In canines, the fibrinolytic system of the blood is very active when compared to humans. Experiments have shown that clots occluding arteries of dogs t unclogged in about 12 hours, which may be the explanation for the high incidence of aneurysms patency in these experimental models. However, this characteristic becomes a natural disadvantage to study the efficacy of endovascular devices and makes it difficult to extrapolate the results of studies in a canine model for treatment of human aneurysms. This is particularly important in procedures that require blood thrombosis around microballoons and coils for occlusion of the aneurysm sac.

Table 1 -   Average number of secondary variables.

Variables	Mean	95% Confidence Interval	
		Lower Limit	Upper Limit
Cooking Time aneurysm (min)	130.75	115.33	146.16
Clamping time (min)	33.83	30.77	36.89
Diameter of the common carotid artery (mm)	5.33	4.83	5.82
Internal jugular vein diameter (mm)	6.00	5.61	6.38
Length of the venous pouch (mm)	12.10	10.88	13.31
Volume of the venous pouch (mm ^{3})	337.5	306.04	368.95

Table 2 -   Distribution of thrombosis.

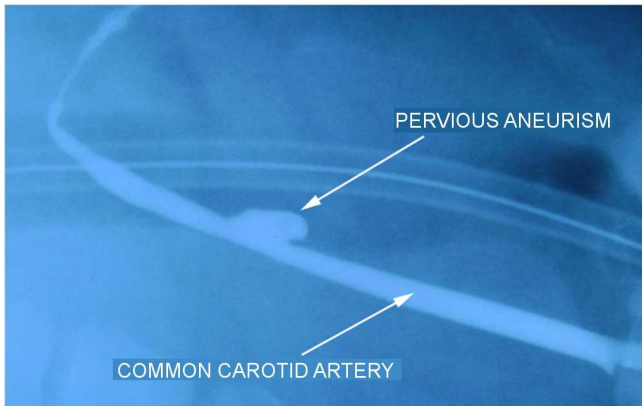| Pig	Thrombosis at angiography	Aneurysmal lumen with thrombosis in histological analysis (%)
1		
2	Total	13.7
3		
4		19.66
5		
6		6.23
7	Partial	15.16
8		3.33
9		7.93
10		20.52
11		20.89
12		0.67

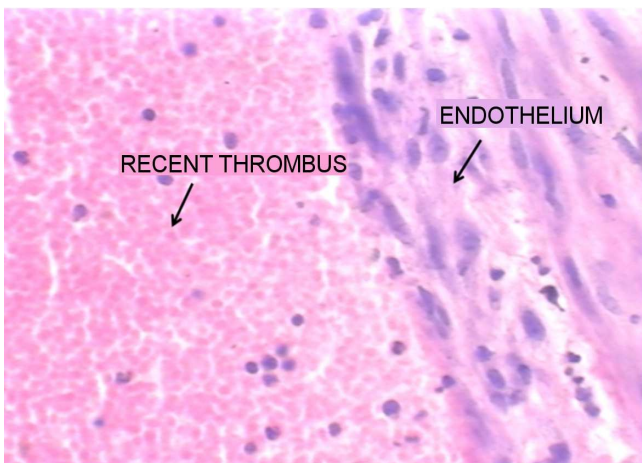***Figure 3 -*** *Angiography showing aneurysm patency.*

Figure 4 - *Histological appearance of the thrombus.*

We chose pigs because they possess a fibrinolytic system and coagulation similar to human's [12]. Moreover, they are easy to handle in the laboratory, the neck vessels are larger, making surgical management and testing endovascular devices easy; they are also ethically acceptable [2,13,21].

Comparative studies have demonstrated that the coagulation and fibrinolytic systems in non-human primates have more similarity with the human one when compared to dogs and pigs. However, the use experimental primates has become difficult due to ethical barriers [16].

In this work, there were no superiority or important differences related to gender, in agreement with the literature, which did not report such differences either [13].

We observed significant weight gain (p = 0.04) of pigs in the interval between the making of the aneurysm and control angiography, which can be limiting to technical studies that require long term follow-up. Genetically-modified animals, with delayed growth, could allow this follow-up. Low cost and easy handling are important in the choice of the experimental model. Since we used animals with a mean weight of 38.8 kg and short follow-up, there were no major difficulties in this regard.

In this study, we observed no major complication after making of the aneurysm, unlike some authors, which show rates of up to 14.2% of bleeding [13,25]. The rate of surgical wound infection (16.6%) was similar to those found in studies using dogs (11.1%), and was considered a good result, considering that pigs appear to have less hygiene dogs [26].

The average diameter of the common carotid artery was 5.3 mm, and is therefore compatible with devices for diagnostics and embolization used in humans.

Patency of the aneurysms was 83.4%. Prolonged patency is an important feature in the choice of the experimental model. The longer the experimental aneurysm remains pervious, possibly the greater its macro and microscopic similarities with the chronic aneurysms present in humans. Furthermore, there is the possibility to study their growth rate and time to rupture. Another important aspect is the possibility to fill this time interval with programmed embolization aimed at testing new coils and the training of new surgeons.

The techniques used to create the experimental aneurysms are varied [27]. Most work with pigs, using anastomosis techniques similar to the one of this study, using the external jugular [13,28,29]. In some, there was spontaneous thrombosis of the aneurysm immediately after its making, which led to immediate change of technique [28]. In others, there were rates of up to 50% of thrombosis after 14 days of follow-up [13]. In some of these works, the aneurysm was made and promptly embolized, with no study in the short or medium term [29]. Byrne created fistulae between the external jugular vein and common carotid artery of pigs in 2004 and, after 14 days, they was occluded by endovascular approach, forming a venous pouch. In this experiment, he observed patency of the aneurysm in one pig studied after 14 days [21].

Some studies used anastomosis techniques similar to this study's, with the external jugular vein in dogs, associated with postoperative aspirin daily administration, not observing thrombosis after 14 days of follow-up [26]. There are reports using external carotid artery and jugular vein in combination with postoperative aspirin, with only 8% of thrombosis after four weeks [25].

Part of the published literature shows no standardization in relation to surgical technique, size of aneurysms and follow-up time of these aneurysms, complicating the analysis [23,30].

In our study, histological analysis did not show the total and near-total thromboses viewed by angiography. In these two pigs, thrombi were small after histological evaluation. As these thrombi had histological features of recent formation, characterized by cluster of red blood cells permeated by mononuclear cells, they may have been broken easily during histological sections. Regarding the recent aspect of the thrombus, there was agreement with the literature, which shows the organization of thrombus only after about ten to 14 days after formed [15].

Histological evaluation detected more thrombi than angiograms, showing greater sensitivity of the former method when compared to latter.

The experimental model in pigs used in this study demonstrated anatomical similarity with the human cerebral circulation aneurysms. There was a low incidence of spontaneous thrombosis within six days of follow-up, allowing its use for testing new endovascular therapeutic devices, as well as for the training of surgeons in the meantime. However, work is needed with longer follow-up time, thus allowing a more accurate assessment of the long-term patency of these aneurysms.

Although most studies used external jugular vein, our model showed indices of thrombosis and complications similar to, or even lower than, other series. We believe that, as the access is the same for the common carotid artery and internal jugular vein, surgical trauma is less, reducing the rate of complications.

In conclusion, a stable experimental model of saccular aneurysms of the swine carotid artery could be developed using the internal jugular vein.

R E S U M O

Objetivo: Desenvolver um modelo experimental estável de aneurisma sacular em carótida de suínos utilizando veia jugular interna. **Métodos:** Em 12 suínos sadios, com peso variando entre 25 e 50kg, cinco machos e sete fêmeas, foi confeccionado aneurisma na artéria carótida comum direita. Após arteriotomia elíptica, foi realizada anastomose terminolateral com coto distal de veia jugular interna. O volume do aneurisma era calculado de maneira que o valor não excedesse em 27 vezes o valor da área da arteriotomia. Após seis dias, era realizada angiografia e análise microscópica do aneurisma para avaliar perviedade e trombose parcial ou total. **Resultados:** Houve ganho de peso significativo dos suínos no intervalo de tempo entre a confecção do aneurisma e a angiografia (p = 0,04). Foi observada perviedade aneurismática em dez suínos (83%). Ocorreram infecções de feridas operatórias em dois animais (16,6%), ambas com início de aparecimento em três dias após a confecção do aneurisma. Análise histológica dos aneurismas mostrou trombos ocluindo parcialmente a luz em nove suínos (75%). Nesses animais, observou-se que, em média, 9% da luz aneurismática estava preenchida por trombos. **Conclusão:** Pôde ser desenvolvido um modelo experimental estável de aneurisma sacular em carótida de suínos utilizando veia jugular interna.

Descritores: Aneurisma. Veias jugulares. Artérias carótidas. Experimentação animal. Suínos.

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