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Experimental study of femoral vein reconstruction with sugarcane biopolymer tubular graft.

Estudo experimental de reconstrução da veia femoral com enxerto tubular do bipolímero de cana-de-açúcar.

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A B S T R A C T

Objective: to evaluate, through Doppler flowmetry, venography, histology and clinical evolution, the use of sugarcane biopolymer (BP) tubular grafts in the reconstruction of femoral veins in dogs. **Methods:** we submitted eight adult dogs to femoral vein reconstruction, on the left with BP tubular graft and on the right with autologous vein. In the postoperative period, the animals underwent clinical evaluation and femoral vein Doppler flowmetry. After 360 days, we reoperated the dogs and submitted them to femoral vein phlebography with iodinated contrast. We removed the segments of the femoral veins containing the grafts and sent them for histopathological evaluation. **Results:** *t*he dogs did not present hemorrhage, hematoma, surgical wound infection or operated limb edema. One animal had superficial venous dilatation in the left inguinal region. Phlebography performed 360 days after the first surgery showed that three (37.5%) BP grafts and seven (87.5%) grafts from the control group (C) were patent. In the histopathological evaluation, we found an inflammatory reaction, with neutrophils and lymphocytes on the external surface of both groups. In the intimal layer of the grafts and in the outer layer in the two groups, we observed fibrosis. **Conclusion:** based on the results obtained with the experimental model used, BP presents potential to be used as a tubular graft for venous revascularization. However, new research must be performed to confirm its efficacy in the revascularization of medium and large diameter veins, which could allow its use in clinical practice.

Keywords: Vascular Grafting. Femoral Vein. Saccharum. Bioprosthesis. Vascular Surgical Procedures. Dogs.

INTRODUCTION

Venous reconstruction surgery, especially when involving the use of vascular substitutes, poses a major challenge to the vascular surgeon, since the veins can easily lose their cylindrical shape. Currently, the most applied venous reconstructive surgical techniques use vascular substitutes in tubular form or as patches[1-3].

The vascular substitutes used in venous reconstruction may be autologous, biological or synthetic. Autologous substitutes are the most cost-effective, but have limited use by the availability of autologous veins in each patient. Synthetic substitutes, such as those of Dacron and e-PTFE are expensive, less resistant to infection, and are used in the reconstruction of large diameter veins[4-10].

A special class of material produced from substances synthesized by different types of microorganisms has been recently studied. These microorganisms exhibit organic tissue characteristics, such as flexibility and resistance to rupture. An example of this type of material is an extracellular polysaccharide, produced through bacterial synthesis from sugarcane molasses, developed at the Biopolymers Laboratory of the Sugarcane Experiment Station of the Federal Rural University of Pernambuco. The Sugarcane Biopolymer (BP) presents flexibility, resistance to rupture, besides being biocompatible and presenting low cytotoxicity[11-17].

Recently the BP membrane has been successfully used as a patch in arterioplasties and venoplasties in the femoral vessels of dogs, with no reported cases of thrombosis, rupture or rejection[16,17].

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The development in the production and technological innovation of BP materials resulted in the creation of tubes, which can be applied, at low cost, in the area of vascular surgery as tubular venous grafts.

The purpose of this study was to evaluate, through Doppler flowmetry, venography, histology and clinical evolution, the use of BP tubular grafts in the reconstruction of femoral veins in dogs.

METHODS

We used eight adult dogs, six males, with a mean weight of 16.8kg in this study. We kept the animals at the Experimental Surgery Nucleus of the Center of Health Sciences of the Federal University of Pernambuco (NCE-CCS-UFPE). This research was approved by the Animal Experimentation Ethics Committee of the Center of Biological Sciences of the Federal University of Pernambuco (CEEA-UFPE), under the procedure 23076.000105/2012-22. It is in accordance with the norms in force in Brazil, especially Law n# 11,749, of 2008, regulated by Decree n# 6899, of July 15, 2009, which deals with animal use for scientific purposes.

Prior to undergoing any surgical procedure, all animals received: 1) Duramune Max 10® for immunization against the distemper virus, coronavirus type 2, adenovirus and parvovirus CPV2b, parainfluenza virus, and against Leptospira (*L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa* and *L. Pomona*); 2) antirabic vaccine. As vermicide, the dogs were given subcutaneous Ancilex® at a dosage of 1ml/5kg, for hookworm, spirocercosis and toxocariasis. They remained in the vivarium under surveillance for a period of 21 days before being submitted to the experimental procedure. Preoperatively and throughout the experiment period, they were fed daily with water and 6kg of chow specific for adult dogs. The study design used was the randomized controlled trial.

We divided the experiment into two groups: one control group consisting of eight right femoral veins with autologous femoral vein graft, and the experimental group, consisting of eight left femoral veins with the BP tubular graft. In this research, each animal was its own control. During the study period, the animals were conducted by trained technicians and veterinarians following international standards for animal research.

Before surgery, the animals fasted for 12 hours. Anesthesia was performed with thiopental sodium (12.5mg/kg), ketamine hydrochloride (2.5mg/kg) and pancuronium bromide (0.2mg/kg). Each animal was then placed in dorsal decubitus and submitted to orotracheal intubation. Next, trichotomy and antisepsis of the inguinal regions were performed, and the femoral veins were accessed through a 5cm long longitudinal inguinal incision. After exposure of the femoral veins, we resected a 10mm-long segment from the left femoral vein, which we then reconstructed with the BP tubular graft through continuous, terminal-terminal, proximal and distal sutures with cardiovascular 7.0 polypropylene suture. We performed the same procedure in the right femoral vein, but we made its reconstruction with a 10mm venous segment taken from the left femoral vein. The dogs were followed for 360 days after surgery through clinical evaluation and doppler flowmetry.

In the first week, the animals received daily clinical evaluation, and from the eighth day on, the evaluation was weekly until the 30th postoperative day. Thereafter, we evaluated the animals monthly until the end of the evaluation period. After 360 days monitoring, we reoperated the dogs following the same conditions of anesthesia and access of the femoral veins of the first surgery. After exposure of the femoral veins and puncture of the internal saphenous veins, right and left, we performed a phlebography. After the phlebography, we sacrificed

the animals with a toxic dose of the anesthetic and removed the left and right femoral vein segments containing the BP and control grafts.

For the histological evaluation, the removed venous segments were fixed in 10% neutral formalin. The material was then stained with hematoxylin and eosin and Masson's trichrome for microscopic study. The specimens were processed in the Laboratory of Histopathology Research of the Post-Graduation in Pathological Anatomy of the Federal University of Pernambuco.

For data analysis, we computed absolute and percentage distributions of the statistical measures, mean, median, standard deviation, variation coefficient and minimum and maximum values (descriptive statistics techniques), frequency percentages and the respective variants distributions. We applied the chi-square test for homogeneity to assess whether the distribution of variables differed between groups. In cases where the assumptions of the chi-square test were not satisfied, we used the Fisher's exact test. We considered the significance level of 5% in all the conclusions. We entered the data in an Excel spreadsheet and analyzed it using the Statistical Package for Social Sciences (SPSS) software, version 18.

RESULTS

All eight animals survived the established 360-day period for clinical observation and second surgery. During the observation period, the dogs had no clinical signs of hemorrhage or hematoma in the operative wound. There were also no signs of wound infection or edema in the operated limbs in both groups. One dog presented dilation of the superficial veins in the limb and left inguinal region (BP) (Table 1). The postoperative Doppler flow measurement showed the presence of phasic flow with the respiration in the femoral veins in all dogs evaluated on the first postoperative (POD). The flow pattern changed, losing the characteristic of being phasic with breathing and becoming continuous in the BP group as follows: in the eighth POD, one dog presented continuous flow, in the 30th POD, two dogs, and 360 days after the surgery, five dogs. In the control group, we observed that in the eighth and thirtieth day the dogs maintained the phasic flow with respiration, but 360 days after surgery one dog presented continuous pattern flow (Table 1).

Table 1. *Distribution of Doppler flow, edema and superficial venous dilatation at different moments of the evaluation.*

	1st day		8th day		30th day		360th day	
	BP	Control	BP	Control	BP	Control	BP	Control
Phasic Flow								
Yes	8 (100%)	8 (100%)	7 (87.5%)	8 (100%)	6 (75%)	8 (100%)	3 (37.5%)	7 (87.5%)
No	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)	2 (25%)	0 (0%)	5 (62.5%)	1 (12.5%)
*p-value**	-**		1,000		0467		0119	
Edema								
Yes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)
*p-value**	-**		-**		-**		-**	
Superficial Venous Dilatation								
Yes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)	0 (0%)
No	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	7 (87.5%)	8 (100%)
*p-value**	-**		-**		-**		1.000	

** p-value of Fisher's exact test (if p-value <0.05, the distribution of the factor under study is homogeneous between the moments of analysis); ** Could not be calculated, therefore, the absence/presence was constant at the moments evaluated.*

Phlebography performed 360 days after the first surgery showed that three (37.5%) BP and seven (87.5%) grafts of the control group were patent (Figure 1).

Figure 1. *Phlebography after 360 days: radiopaque needles highlight the grafts.*

At the macroscopic evaluation, the femoral veins in both groups were externally lined by a thick layer of loose connective tissue and showed scarring adherence to neighboring tissues. We observed no change in staining or consistency of the BP graft (Figure 2). Histological evaluation of the venous segments showed that the inner layer of the grafts was continuous with the intima layer of the recipient femoral vein and formed by a thick layer of fibrous tissue firmly adhered to the internal surface of the grafts (Figure 3). The external surface of the grafts in the BP group was surrounded by a layer of connective tissue rich in lymphocytes, neutrophils and rare macrophages firmly adhered to the grafts. This layer with inflammatory cells was coated externally by a thick layer of fibrous tissue. The implants of the control group were also externally coated by a layer of inflammatory cells, but these occurred less than in the experimental group, demonstrating a mild inflammatory reaction. In the control group, the inflammatory cell layer was also coated externally by a thick layer of fibrous tissue. We found no foci of degenerative process or areas of calcification.

Figure 2. *Femoral vein appearance in the BP group after 360 days.*

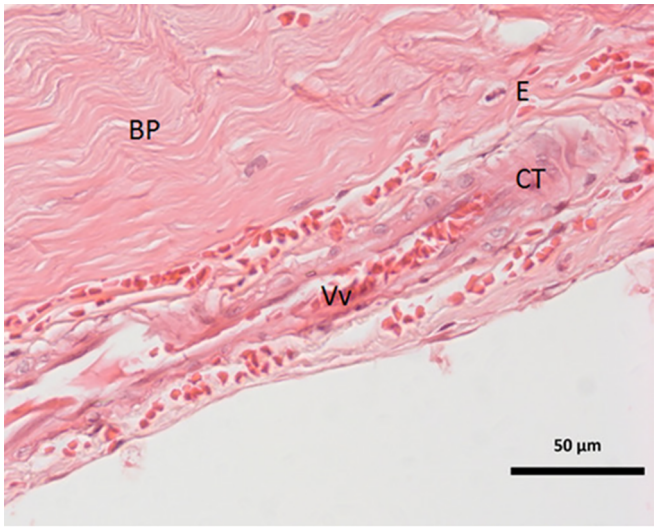

Figure 3. *Internal surface of the BP graft. Vv- vasa-vasorum; E- endothelium; CT- connective tissue.*

DISCUSSION

Dogs have been used as experimental models in several scientific studies. Some authors describe that the behavior of vascular prostheses in dogs is what most resembles humans. This explains their preference as an animal model commonly used for evaluation of vascular grafts[16-21].

Due to both the simplicity of the access and diameter, we chose the femoral veins for the accomplishment of these experiments. In femoral vein exposure there is no trauma to the abdominal musculature, which is often related to pain and limitation of movements in the postoperative period, and there is no manipulation of the intra-abdominal organs, as occurs in surgery on the inferior vena cava. There is a lower risk of severe hemodynamic changes and, in addition, the extensive network of collateral veins in this region ensures, in most cases, limb drainage when femoral vein thrombosis occurs[16,17,20].

Although the BP graft presented some properties different from those of an autologous vein, such as minor elasticity, there was no difficulty in performing the suture. This feature has already been demonstrated in other studies that used the BP membrane as a patch in angioplasties of the femoral arteries and veins of dogs[16,17].

Another aspect of the present study is that the control group comprised the femoral veins contralateral to that of the BP graft implants. This allowed a smaller number of animals to be used in the study, and consequently sacrificed, which is in accordance with current bioethical principles. In addition, the fact that each animal is its own control made the groups more homogeneous, as well as in other studies that used the same principle, as in the cases of biopolymer patches in veins and femoral arteries of dogs, and in the use of latex as prosthesis in the femoral artery also of dogs[16,17].

The observation time of the groups was 360 days. This period is in agreement with the observed in several experimental works that evaluate the use of vascular substitutes in animal models. This occurs because the healing process of the vascular grafts, which involves its incorporation by the adjacent organic tissues, occurs between 180 and 360 days after the implant[3,20,21].

The complementary exams used in this study for the evaluation of the venous grafts were Doppler flowmetry and ascending phlebography. Doppler flowmetry is a noninvasive test that uses ultrasound principles to assess venous flow, but does not provide information on vein morphology. Several experimental studies have shown that Doppler flowmetry can be used in postoperative evaluations in reconstructive vascular surgery. Phlebography is an invasive examination that uses iodinated contrast and allows studying both venous physiology and morphology, so it is considered the gold standard for the evaluation of the venous system and has been widely used in experimental models that use animals to test vascular substitutes[16,17]. The association between clinical evaluation, Doppler flowmetry and phlebography allowed a high sensitivity in the diagnosis of thrombosis and venous graft patency.

The use of these exams in association has been described in other experimental studies evaluating venous grafts[16,17].

The main complications resulting from the use of tubular venous grafts are thrombosis, rupture and infection of the surgical wound. Several authors have reported the occurrence of these complications. The rate of thrombosis found in the literature with reconstructions using synthetic vena cava substitutes varies from 20 to 30% after one year of surgery. In the reconstruction of the iliac veins in humans, which present a smaller caliber than the vena cava using synthetic e-PTFE prosthesis, the one-year thrombosis rate is about 50%, while in endovascular reconstructions, also of the iliac veins, the rate of thrombosis after one year ranges from 70 to 80%[7-10]. Greca's study, which used a patch with a biological graft in the vena cava of dogs, revealed that after 40 days, only one of the 16 studied dogs had partial thrombosis, and anticoagulation was performed in the transoperative period[3]. In the present study, we used no anticoagulation, which may have contributed to the higher rate of thrombosis in the BP group.

We identified no cases of infection or hemorrhage in the operative wound in both groups, findings that have been reported in other experimental studies[16,17]. The development of chronic inflammatory reaction on the external surface and the presence of fibrosis on the internal surface were observed in all cases of the control group and in the experimental group, similar to that found in the literature[16,17].

The healing process of the BP grafts occurred in a similar way to that of the autologous vein grafts with regard to the presence of inflammatory cells on their external surface and fibrosis on both surfaces, but in the experimental group there was a more important inflammatory reaction than in the control one. We found no phagocytic cells in the inflammatory reaction of the external surface of the grafts of either group. The absence of these inflammatory cells confirms the low antigenic content of both grafts, since the presence of phagocytic cells has the objective of defending the organism against an aggressive agent. We observed no foci of calcification in the biopolymer prosthesis, as observed in heterologous grafts[20-23].

The inflammatory reaction observed in this study was concentrated in the periphery of the implants, both in the autologous graft and in the BP prosthesis. We also observed that the intima and adventitia layers were firmly adhered, respectively, to the inner and outer faces of the biopolymer prosthesis. This aspect has already been described in previous studies that used BP membrane patches in arterioplasties and femoral venoplasties in dogs[10,11].

Advances in the surgical treatment of chronic acute venous lesions have been slowly evolving. The results obtained in this study represent a perspective for venous reconstruction surgery in humans. Due to its low cost, the use of BP grafts may bring new possibilities for the treatment of several venous diseases. New research must be carried out to confirm and improve the results found.

R E S U M O

Objetivo: *avaliar, através de dopplerfluxometria, de venografia, de histologia e de evolução clínica, o uso de enxertos tubulares de biopolímero de cana-de-açúcar (BP) na reconstrução de veias femorais em cães.* **Métodos:** *oito cães adultos foram submetidos à reconstrução de veia femoral, à esquerda com enxerto tubular de BP e à direita com veia autóloga. No período pós-operatório, os animais foram submetidos à avaliação clínica e dopplerfluxometria das veias femorais. Após 360 dias, os cães foram reoperados e submetidos à flebografia das veias femorais com contraste iodado. Os segmentos das veias femorais contendo os enxertos foram retirados e enviados para avaliação histopatológica.* **Resultados:** *os cães não apresentaram hemorragia, hematoma, infecção da ferida operatória ou edema dos membros operados. Um animal apresentou dilatação venosa superficial na região inguinal esquerda. A flebografia realizada 360 dias após a primeira cirurgia demonstrou que três (37,5%) enxertos de BP e sete (87,5%) do grupo controle (C) estavam pérvios. Na avaliação histopatológica foi encontrada uma reação inflamatória com neutrófilos e linfócitos na superfície externa de ambos os grupos. Na camada íntima de revestimento dos enxertos e na camada externa nos dois grupos, foi encontrada fibrose.* **Conclusão:** *com base nos resultados obtidos com o modelo experimental utilizado, conclui-se que a BP apresenta potencial para ser utilizado como enxerto tubular para revascularização venosa, porém novas pesquisas precisam ser realizadas para confirmar a sua eficácia na revascularização de veias de médio e grande calibre, o que poderia permitir o seu uso na prática clínica.*

Descritores: *Enxerto Vascular. Veia Femoral. Saccharum. Bioprótese. Procedimentos Cirúrgicos Vasculares. Cães.*

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