ORIGINAL ARTICLE

A new vascular substitute: femoral artery angioplasty in dogs using sugarcane biopolymer membrane patch – hemodynamic and histopathologic evaluation

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RESUMO

Contexto: A obtenção de um substituto arterial ideal para o emprego nas reconstruções das artérias de pequeno e médio calibre é ainda o principal objetivo da maioria das pesquisas cientificas desenvolvidas no campo dos substitutos vasculares. A membrana de biopolímero de cana-de-açúcar pode ser de grande utilidade para a realização das reconstruções arteriais em vasos de calibre inferior a 4 mm e assim permitir o tratamento de doenças que afetam milhões de pessoas no Brasil e em todo o mundo.

Objetivo: Avaliar do ponto de vista hemodinâmico e histopatológico o comportamento da membrana do biopolímero de cana-de-açúcar quando utilizada com remendo em arterioplastias femorais em cães.

Método: Oito cães adultos mestiços sob anestesia geral foram submetidos no Núcleo de Cirurgia Experimental do Centro de Ciências da Saúde-UFPE a velocimetria Doppler percutânea das artérias femorais direita e esquerda para controle pré-operatório. Sob condições de assepsia e anti-sepsia, os cães foram submetidos a arterioplastia femoral bilateral com remendos da membrana de biopolímero de cana-de-açúcar no lado esquerdo e de PTFE expandido (e-PTFE) no lado direito. Na primeira semana pós-operatória, os cães foram submetidos a avaliação clínica diária e semanal a partir do oitavo dia. A avaliação clínica consistiu no exame dos pulsos femorais, na avaliação da marcha e na observação da presença de tumor pulsátil, hematoma ou hemorragia e infecção da ferida operatória. Após 180 dias das arterioplastias, sob anestesia geral, procedeu-se nova fluxometria Doppler percutânea das artérias femorais. Os animais foram submetidos à dissecção das artérias femorais, medido o diâmetro arterial e realizada velocimetria Doppler trans-operatória em pontos proximal e distal à arterioplastia. A seguir foi realizada laparotomia e exposição da aorta abdominal para acesso arteriográfico. Os segmentos das artérias femorais com os remendos foram retirados para estudo histopatológico e os animais foram mortos com dose tóxica de anestésico.

Resultados: No período de avaliação de 180 dias, nos dois grupos, não foram observados casos de infecção da ferida operatória, dilatação, ruptura, falso-aneurisma ou trombose. Nos dois grupos foi encontrada, na superfície externa dos remendos, uma resposta inflamatória crônica com neutrófilos e linfócitos além de fibrose. Na superfície interna dos remendos, em ambos os grupos, foi encontrada fibrose. No grupo controle ocorreu invasão dos remendos de e-PTFE por fibroblastos.

Conclusões: Com base nos resultados obtidos com o modelo experimental utilizado, durante o período de observação de 180 dias, conclui-se que a membrana do biopolímero de cana-de-açúcar constitui-se em um substituto arterial adequado quando utilizado sob forma de remendos em artérias femorais de cães.

Palavras-chave: Prótese vascular, implante de prótese vascular, bioprótese e cana-de-açúcar.

ABSTRACT

Background: Achievement of an ideal arterial substitute to be used in the reconstruction of small- and medium-caliber arteries is still the main objective of most scientific research studies developed in the area of vascular substitutes. Sugarcane biopolymer membrane could be extremely useful to perform vascular reconstruction in arteries with diameter smaller than 4 mm, allowing treatment of diseases that affect millions of people in Brazil and worldwide.

Objective: To evaluate the hemodynamic and histopathologic behavior of sugarcane biopolymer membrane when used as a patch in femoral artery angioplasty in dogs.

Method: Eight adult mongrel dogs, under general anesthesia, underwent percutaneous Doppler velocimetry of the left and right femoral arteries for preoperative control at the Health Sciences Center Experimental Research Laboratory of Universidade Federal de Pernambuco. After being disinfected, the dogs underwent femoral artery patch angioplasty using a sugarcane membrane biopolymer patch on the left side and an expanded PTFE (e-PTFE) patch on the right side. The dogs underwent daily clinical evaluation for the first week and weekly thereafter. Clinical evaluation consisted of an examination of femoral artery pulses, gait assessment and verification as to whether there was any pulsatile tumor, bruising, hemorrhage or surgical wound infection. One hundred and eighty days after the angioplasties, percutaneous Doppler velocimetry of the femoral arteries was performed under general anesthesia. Next, the dogs underwent dissection of the femoral arteries, measurement of the arterial diameter and perioperative Doppler velocimetry proximally and distally to the artery angioplasty. Laparotomy was then performed to expose the abdominal aorta for angiographic access. The femoral artery segments with patches were harvested for histopathologic analysis, and the animals were sacrificed under a toxic dosage of anesthetic.

Results: At 180 days, no cases of surgical wound infection, aneurysm, rupture, pseudoaneurysm or thrombosis were observed in either group. A chronic inflammatory reaction with lymphocytes and neutrophils was seen in both groups on the outer surface of patches. Fibrosis was seen on the inner and outer surfaces of the patches. Invasion by fibroblasts of all e-PTFE patches occurred in the control group.

Conclusions: Based on the results obtained with the experimental model employed, for a 180-day period, it may be concluded that the sugarcane biopolymer membrane is an adequate arterial substitute when used as a patch in femoral artery angioplasty in dogs.

Keywords: Vascular prosthesis, vascular prosthesis implantation, bioprosthesis, sugarcane.

Introduction

Closure of arteriotomies, using varied techniques, may cause reduction in vascular lumen, especially in small-diameter arteries. That reduction is a limiting factor for arterial reconstruction that can, however, be avoided using a patch.[1-5]

The concept of patch angioplasty was first established by Carrel & Guthrie in 1906. Despite having their foundations well established, this surgical technique only started being widely used after the description performed by Dos Santos, in 1946, of the endarterectomy technique and of the studies by Senning and Crawford et al., in 1959, who independently reported experimental and clinical use of patch grafts, confirming the hypothesis that they widened vascular lumen and avoided stenosis after an arteriotomy.[2-4,6]

Several types of materials have been used as patches in angioplasties. Nowadays, the most widely used are autogenous veins, biological grafts of bovine pericardium and synthetic Dacron and expanded polytetrafluoroethylene (e-PTFE) patches. Autogenous veins are more indicated for small- and medium-diameter angioplasties, whereas the bovine pericardium and synthetic patches are more used in large-diameter arteries.[7-11]

An extracellular polysaccharide produced by bacterial synthesis based on sugar cane molasses has been recently developed at Estação Experimental de Cana-de-Açúcar de Carpina of Universidade Federal Rural de Pernambuco.[12]

The membranes formed based on that polymer were processed for application as surgical graft at the Experimental Surgery Sector of the Center of Health Sciences at Universidade Federal de Pernambuco. They were evaluated by laboratory tests and presented flexibility, low toxicity, biocompatibility and resistance against rupture.[13]

Such membrane has been used in many areas of experimental surgery: healing of skin wounds; in ureteral reconstruction; as a substitute of tympanic membrane; among others. However, there is no report of studies on its behavior as an arterial substitute, except in a master's thesis developed by our group.[14-18]

Following the line of research using the sugarcane biopolymer membrane in several experiments of reconstructive surgery of organs and tissues, this study aims at evaluating, from the hemodynamic and histopathologic perspective, the behavior of that membrane as an arterial wall substitute as patches in the femoral artery of dogs.

Method

Between January 2005 and June 2006, eight dogs (*Canis familiaris*) were used, one female and seven males, mean body weight of 15.4 kg.

The animals were kept in an experimental laboratory for 21 days before starting the study. They were given chow diet, rabies vaccine and vermicide.

Fasting for solid foods occurred over the 24 hours preceding the surgery for all animals.

The dogs were anesthetized using endovenous sodium thiopental (12.5 mg/kg), ketamine chloride

(2.5 mg/kg) and pancuronium bromide (0.2 mg/kg). After inguinal trichotomy, percutaneous Doppler velocimetry was performed in the femoral arteries and, next, disinfection using Povidine®, and then the surgical sites were disinfected.

Longitudinal 5-cm skin incisions were then performed in inguinal regions to expose the left and right femoral arteries. After exposure, arterial diameters were measured using a manual pachymeter.

Next, longitudinal arteriotomies were performed by removing an elliptic fragment of the arterial wall measuring 1.5 cm in diameter and a diameter equal to 1/3 of the arterial diameter. To correct arterial wall defects, femoral angioplasties using e-PTFE patches on the right side and sugarcane biopolymer membrane patches on the left side were performed using a cardiovascular polypropylene continuous 7-0 suture (Figure 1).

Figure 1 - Femoral angioplasty using sugarcane membrane patch (note that the femoral vein submitted to a sugarcane biopolymer membrane patch was analyzed in another study)

After anesthetic recovery, the animals were sent to independent cages in the experimental laboratory. Solid foods were released 8 hours after the surgery.

Over the first postoperative week, the dogs were submitted to daily and weekly clinical assessment starting on the eighth day. Clinical assessment consisted of the examination of femoral pulses, walking and presence of pulsatile tumor, hematoma or hemorrhage and surgical wound infection. Six months after the surgery, the dogs were submitted to a new surgery. Preoperative cares and anesthetic technique were repeated. A new percutaneous Doppler velocimetry of the femoral arteries was performed, as well as inguinal incisions to expose femoral arteries. Next, perioperative Doppler velocimetry of the femoral arteries in distal and proximal sites to the angioplasty were performed and the measurements of arterial diameter repeated using a manual pachymeter.

Next, the dogs were submitted to laparotomy for exposure and puncture of the abdominal aorta using an 18-gauge Jelco®, followed by iodinated-contrast arteriographies (Conray®). After arteriography and macroscopic evaluation, all arterial segments having patches were removed for histopathologic tests. The material was stained with hematoxylin-eosin and picrosirius (Figure 2).

Figure 2 - Arteriography showing patent femoral arteries (arrows showing the site of patches)

The experiments performed were distributed into two randomly established groups: Group I, eight arterial patches using the biopolymer (experimental group); Group II, eight arterial patches using e-PTFE (control group). In this study, each animal was its own control.

The research protocol was sent for appraisal of the Bioethics Committee at Universidade Federal de Pernambuco, which approved it without restrictions.

Significance level used for all statistical tests was 5%. The data were included in an Excel spreadsheet and analyzed using the software *Statistical Package for the Social Sciences* (SPSS) version 11.

Sample size of each group was determined considering the comparative objective of significant difference between groups as to mean variation in diameter (before and after arterioplasty). Significance level was set in 5.0% (test error). Test power had an 80% level. Minimal acceptable difference between both groups was 0.005 cm (or 0.5 mm) in relation to variation in diameter before and after arterioplasty. That is the difference based on which significant difference should be obtained. Ratio between groups was 1.00, aiming at having equal sample sizes in both groups.

Results

All animals survived the established period of 180 days for clinical observation and second surgery. There were no cases of infection, dilatation or aneurysm, rupture or pseudoaneurysm and thrombosis in any group (Figure 3).

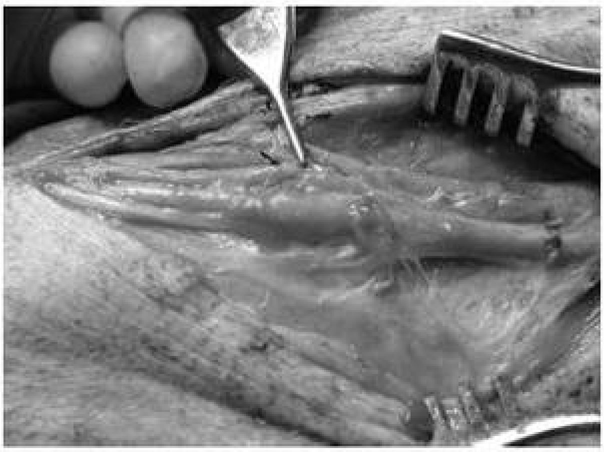

Figure 3 - Femoral artery containing sugarcane membrane patch after 180 days

In assessments before and after the arterioplasty, Doppler sign in the left and right femoral arteries was present and had three-phase wave flow.

Arteriography showed that the femoral arteries in the control and experimental group had no thrombosis. The main arterial flow occurred through those vessels, and there was no development of irregular pattern in collateral circulation. There were no dilatations or pseudoaneurysms in both groups.

Measurement of arterial diameters before and 180 days after angioplasties did not show statistically significant differences ($p > 0.05$, using the signed-rank Wilcoxon test) (Table 1).

Table 1 - Statistics of femoral artery diameters before and after angioplasty, according to material used (group)

	Material used		
Variables	Experimental (n = 8)	PTFE (n = 8)	p
Femoral artery diameter before angioplasty			
Mean*	0.375	0.394	$p^{\dagger} = 0.180$
Median*	0.375	0.375	
Standard deviation*	0.046	0.073	
VC (%)	12.344	18.511	
Minimum*	0.300	0.300	
Maximum*	0.450	0.500	
Femoral artery after angioplasty			
Mean*	0.381	0.394	$p^{\dagger} = 0.157$
Median*	0.400	0.400	
Standard deviation*	0.037	0.050	
VC (%)	9.758	12.585	
Minimum*	0.300	0.300	
Maximum*	0.400	0.450	
Variation (before – after)	0.006	0.000	$p^{\dagger} = 0.317$
Mean*			
Median*	0.000	0.000	
Standard deviation*	0.032	0.038	
VC (%)			
Minimum*	-0.050	-0.050	
Maximum*	0.050	0.050	
p	$p^{\dagger} = 0.564$	$p^{\dagger} = 1,000$	

VC = variation coefficient.
* Measured in cm.
† Using the signed-rank Wilcoxon test.

Histological evaluation showed presence of a chronic inflammatory reaction in all patches of both groups mediated by lymphocytes, neutrophils with no statistically significant differences between groups (Fisher's exact test), as well as fibrosis in its external surface. There was formation of a new intimal layer continuing the receptive artery in the internal surface, consisted of fibrosis. The new adventitia and intimal layers were strongly attached to wall patches. In both groups it was not possible to confirm presence of endothelial cells in the internal surface, since the immunohistological test was not used (Figure 4).

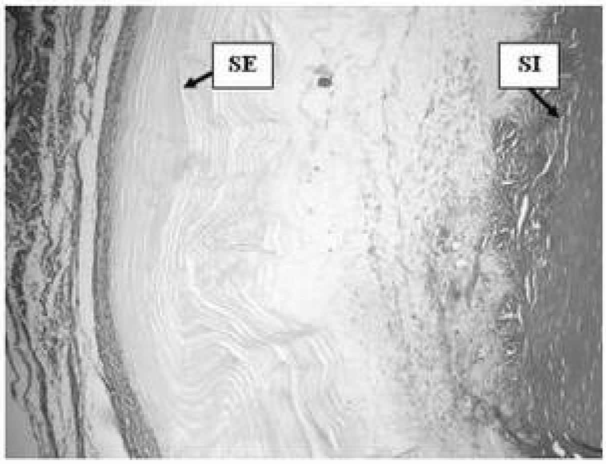

Figure 4 - Sugarcane biopolymer membrane patch (magnification 3.5 x in an optical microscope, stained with picrosirius) with chronic inflammation in the internal surface (SE) and fibrosis in the internal surface (SI)

In both groups there were no focuses of degenerative process or calcification areas. In the control group using e-PTFE patches, invasion by fibroblasts occurred in all cases (Figure 5).

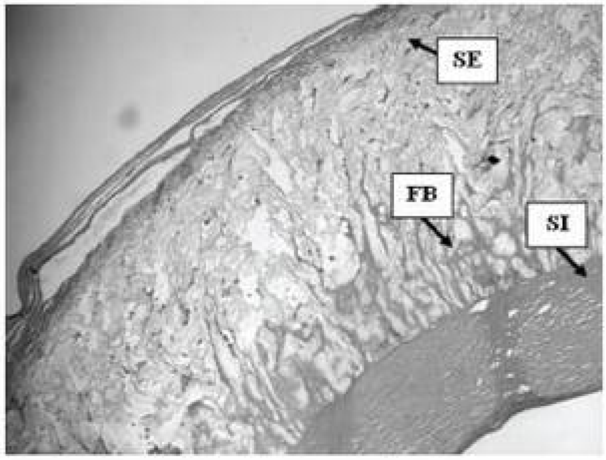

Figure 5 - Invasion of e-PTFE patch (magnification 3.5 x in an optical microscope, stained with hematoxylin-eosin) by fibroblasts (FB) and chronic inflammatory reaction in its external surface (SE). Fibrosis in the internal surface (SI)

Discussion

All current substitutes have major limitations. Autogenous veins are scarce, biological grafts have high rates of aneurysmal dilatation and calcification, and synthetic grafts are expensive and have low resistance to infection. Thus, search for an ideal substitute remains, especially for application in small- and medium-diameter arteries. This material must have proper technical qualities to be used in human beings, in association with a low production cost, so that it can be widely used.[7-11]

Nowadays, many research studies have evaluated use of new materials for substitution of blood vessels. Some of them have been directed to the study of materials produced based on substances synthesized by microorganisms, such as bacterial cellulose, which was used in the production of tubular grafts by Klemm et al., in 2001, for substitution of the carotid artery in rats.[19]

Patch angioplasty is a reconstruction technique that can be easily performed, which is the reason why it has been used in experimental studies to evaluate new arterial patches.[20-29]

The main complications resulting from the use of arterial patches are the same seen in tubular grafts: thrombosis, rupture and pseudoaneurysm, dilatation and surgical wound infection.[20-29]

Such complications did not occur in the present study. The results were confirmed by clinical assessment and by Doppler velocimetry, as well as by arteriography. Absence of dilatation in the femoral arteries after implantation of the patches was also confirmed by measurement of femoral artery diameters at the patch implantation site. Association between Doppler flowmetry and arteriography allowed proper assessment of collateral circulation, since it allowed analysis both of morphology and characteristics of blood flow in femoral arteries.

Complications resulting from use of arterial patches can be caused by failure of material used as a patch, as observed by Pena & Husni, who reported 20% of thrombosis in femoral angioplasties with Dacron patches in dogs and by McCready et al., when using porcine intestinal submucosa as a patch in carotid angioplasties and obtaining 10% of pseudoaneurysms.[24,29]

Similarly, Menon et al. found 35% of thrombosis in femoral and carotid angioplasties using Dacron patches compared with 25% of thrombosis using autogenous vein patches, showing superiority of the latter.[22]

The healing process of sugarcane membrane patches was characterized by presence of a chronic inflammatory reaction in the external surface mediated by lymphocytes and neutrophils, as well as fibrosis. In the internal surface of these patches, there was development of an intimal layer formed by fibrosis. That process is similar to that observed in healing of synthetic materials, such as e-PTFE.[9-11,28]

Also similarly to e-PTFE patches, focuses of degenerative process with loss of integrity were not observed in sugarcane membrane patches, as in autogenous artery or vein patches. In both groups, rare presence of macrophages confirms low rate of antigens in those materials. There were no focuses of calcification, as observed in biological graft healing.[20,22,24,28]

Differently of e-PTFE patches, sugarcane membrane patches did not allow invasion by fibroblasts. Penetration of cells in e-PTFE patches occurs due to presence of micropores in their structure. That process has been described in the literature and is important for a complete healing of patches. The sugarcane biopolymer membrane has no pores and, therefore, did not allow invasion by fibroblasts, but the new intimal and adventitia layers were strongly attached, respectively, to the internal and external patch walls.[9-11,30,31]

Conclusion

Based on the results obtained with the experimental model used, over a 180-day observation period, it can be concluded that the sugarcane biopolymer membrane is a proper arterial substitute when used as patches in the femoral artery of dogs.

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