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ORIGINAL

Induction of vascular lesion in an experimental rabbit model: technical information and incidents

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Abstract
Objective: To describe the process for inducing atherogenic lesions in rabbits, to show the damage to vessels caused by two different caliber balloon catheters, and to show the usefulness of ultrasonography in the quantification of vascular damage.
Material and methods: We used 36 rabbits. We studied the levels of triglycerides and cholesterol and examined the aorta and iliac arteries by ultrasonography. We performed arteriography and induced a vascular lesion by denuding the left iliac artery with a balloon catheter: group A 2.5 mm diameter balloon catheter, group B 3 mm diameter balloon catheter. After 8 weeks on a hyperlipidic diet, biochemical and ultrasonographic measurements were repeated.
Results: Cholesterol before the diet: 37.96 ± 19.3 mg/dL and after the diet: 1761 ± 296.91 mg/dL. The baseline ultrasonographic measurements of vessel diameter were: aorta 4.1 ± 0.7 mm, right iliac artery 3 ± 0.3 mm, left iliac artery 3 ± 0.4 mm. After vascular damage and 8 weeks hyperlipidic diet, in group A the ultrasonographic measurements of vessel diameter were: aortic lumen 2.78 ± 1.21 mm, right iliac artery 2.18 ± 0.81 mm, and the left iliac artery 1.16 ± 0.63 mm; in group B, the aortic lumen measured 3.07 ± 1.06 mm, the right iliac artery 2.53 ± 0.9 mm, and the left iliac artery 1.39 ± 1.1 mm.
Four rabbits died; in the 32 remaining rabbits, morbidity was higher with a 3 mm balloon catheter.
Conclusion: After denudation with a balloon catheter and a hyperlipidic diet, the rabbits developed arterial stenosis. The damage with a 2.5 mm diameter catheter reduces complications.

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PALABRAS CLAVE
Arterioesclerosis;
Angioplastia;
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Técnicas endovasculares;
Radiología intervencionista

El conejo como modelo de experimentación. Inducción de lesión vascular: técnica e incidencias

Resumen
Objetivo: Describir el proceso para conseguir la lesión aterogénica en el conejo, mostrar el daño vascular producido por 2 catéteres de balón de diferente calibre y el valor de la ecografía para su cuantificación.
Material y métodos: Se emplearon 36 conejos. Se estudiaron los valores de triglicéridos y colesterol, y se realizó una ecografía de aorta e ilíacas. Se realizó una arteriografía y se indujo la lesión vascular denudando la arteria ilíaca izquierda con catéter de balón: grupo A, catéter-balón 2,5 mm, y grupo B catéter-balón 3 mm de diámetro. Tras 8 semanas con dieta hiperlipídica se realizaron nuevas mediciones bioquímicas y ecográficas.
Resultados: Colesterol antes de la dieta 37,96 ± 19,3 mg/dl, y tras la dieta 1,761 ± 296,91 mg/dl. Los hallazgos ecográficos mostraron un diámetro de la aorta de 4,1 ± 0,7 mm, de la arteria ilíaca derecha de 3 ± 0,3 mm, de la arteria ilíaca izquierda de 3 ± 0,4 mm. Tras el daño vascular y 8 semanas de dieta, en el grupo A la luz de la aorta era de 2,78 ± 1,21 mm, la arteria ilíaca derecha medía 2,18 ± 0,81 mm y la arteria ilíaca izquierda 1,16 ± 0,63 mm; en el grupo B la luz de la aorta fue de 3,07 ± 1,06 mm, la arteria ilíaca derecha 2,53 ± 0,9 mm y la arteria ilíaca izquierda 1,39 ± 1,1 mm.
Murieron 4 conejos y de los 32 restantes hubo más morbimortalidad con el catéter de balón de 3 mm.
Conclusión: Tras la denudación con catéter de balón y dieta los conejos desarrollan estenosis de la arteria. El daño con catéter de 2,5 mm de diámetro disminuye las complicaciones.

Introduction

Atherosclerosis and its cardiovascular complications are the leading cause of mortality in developed countries. Studies in humans and animals indicate that it is initiated by changes in the endothelial function of vessels.[1]

Rabbits have been used as an experimental model for studying cardiovascular diseases; they quickly develop atheromatous lesions if they are fed a high cholesterol diet.[2,3] These lesions have a higher lipid and macrophage content than those seen in humans. In addition, induced vascular damage makes them more stable and uniform in size and distribution.[4]

The aim of this study is to describe the process used to achieve atherogenic injury in the rabbit, including the diet and the balloon catheter technique to get a reproducible vascular lesion in the iliac artery,, to show the vascular damage produced by 2 balloon catheters of different size, and to analyze the value of ultrasound in the quantification of vascular damage.

Materials and methods

This study involved 36 New Zealand white rabbits (18 males and 18 females), 3 months of age and weighing approximately 6.6 pounds. Rabbits were obtained from the experimental farm at the Department of Animal Science of the Polytechnic University of Valencia and were maintained according to Spanish legislation for the maintenance of animals used in experimentation and other scientific purposes, reflected in paragraph 3 of Article 11 of the Spanish Real Decreto 223/1988 on March 14th, and the European Union regulations regarding Experimentation and Animal Protection (86/609/CCE).

Physical examination of the rabbits

Upon receipt, the animals underwent a general physical examination to demonstrate their health and eligibility for the study. They were assessed for general alertness, heart and lung auscultation, weight, blood tests and ultrasound examination to verify the integrity of both the aorta and common iliac.

Sedation

Sedation was performed by injecting the dorsal muscles of the back with xylazine (Xylagesic 2%Calier®) at 3 mg/kg and ketamine (Imalgene 1000, Merial®) at 20 mg/kg. Morphine was added (morphine 2%, Braun®) as an analgesic at 0.2 mg/kg. Once the animal was sedated and motionless, the outside of the rabbit's ear was shaved and an intravenous catheter was placed in the marginal ear vein. Blood was extracted and hematological and biochemical parameters were measured. Parameters considered of greatest importance for our study were cholesterol and triglyceride levels.

Ultrasound scan

We used a high-resolution Acuson 128 XP/10® ultrasound using 5 and 7.5 MHz transducers. The left caudal paravertebral abdominal area was shaved to ensure good contact between the transducer and the skin. The animal was positioned in the right lateral position so that a suitable acoustic window for ultrasound recording of the aorta and left common iliac artery was available. The last section of the abdominal aorta and the start of the left common iliac artery were identified in two-dimensional ultrasound to obtain the diameter of the aorta and iliac artery in a longitudinal section. Color Doppler was used to verify the correct flow of the aorta and iliac and the absence of alterations.

Arterial catheterization technique

The aim of the catheterization was to use an angioplasty balloon to achieve arterial damage (denudation technique) in the left common iliac artery. This artery was selected because its diameter is similar to the coronary and the infrapopliteal arteries' diameters in humans. These vessels are the most affected by arteriosclerotic diseases and would be most applicable to humans. The procedure consisted of 3 phases: anesthesia and positioning of the rabbit, surgical externalization of the carotid and catheterization using the Seldinger technique.

1. *Anesthesia and positioning of the rabbit.* The induction of anesthesia was performed with propofol (Propofol® Lipuro Braun) at a dose of 3 mg/kg. Maintenance of the anesthesia was done with a continuous perfusion of propofol (perfusor® fm, Braun) at a dose of 15 ml/h.
   The rabbit was positioned in the supine position, the forelimbs were caudally extended and the animal was immobilized using sandbags (fig. 1). The neck was extended and was taped in that position; each animal was identified with lead letters. The area of interest was cleaned and disinfected with povidone-iodine solution and isolated with a sterile cloth.
2. *Surgical externalization of the carotid artery.* The approach was performed through a skin incision in the middle of the rabbit's neck with a No. 10 scalpel. The superficial muscle of the neck was incised with scissors and just below appear the sternocephalicus and sternohyoid muscles, which were cut longitudinally along the intramuscular line and lateralized, revealing the trachea. Once the trachea was moved laterally, the carotid sheath was exposed. The carotid sheath is composed of the carotid artery, the vagosympathetic trunk and the internal jugular vein. The carotid was carefully isolated with a hemostat forceps (mosquito), allowing it to remained exteriorized and be prepared for catheterization.
3. *Catheterization by Seldinger's technique.* The carotid artery was punctured using an 18 g needle abbocath and a hydrophilic guide was introduced (0.035 J 180 cm Terumo®) a few centimeters. A fluoroscopy (Bennet® X-ray Technologies) guidance was used as the tip of the guide was moved into the descending thoracic aorta because the guide tends to go into the heart spontaneously. The fluoroscopic anatomic level used as a reference for the rotation of the tip of the guide was the first rib. Once the guide was in the abdominal aorta, an introducer was inserted (Radiofocus Introducer® II 4 fr, Terumo) until it was fully placed into the abdominal aorta. Sodium heparine was injected through the introducer at a dose of 25 IU/ml to prevent thrombosis.
   To ensure the normality of the arteries, an arteriogram of the abdominal aorta with all its visceral branches as well as the pelvic area was performed through the introducer using 10 ml of iohexol (350® Omnigraf Schering-Plow) diluted to 50% in heparinized saline (fig. 2).

Figure 1 Positioning of the rabbit.

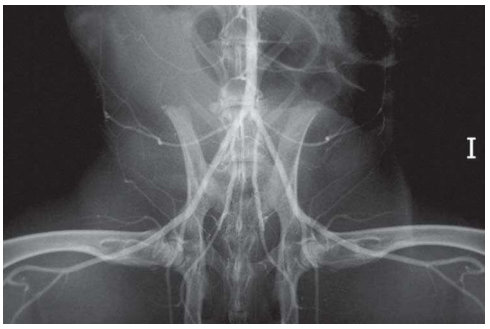

Figure 2 Rabbit normal arteriography.

Artery denudation using a balloon catheter technique

Angioplasty catheters (Symmetry® balloon dilatation catheter, Boston Scientific®) were introduced directly through the introducer. All catheters were 20 mm in length. A guide was not used because the catheters navigate well and reache the left common iliac artery with minimum manipulation.

The rabbits were split up in two different groups as follows: group A consisted of 20 animals that underwent vascular denudation using a balloon of 2.5 mm maximum expansion, and group B was made up of 12 animals in which vascular denudation was performed by inflating the balloon to 3 mm thickness. The technique of endothelial denudation, as described by other authors,[5,6] lied in inflating the balloon in the area to be injured and, once inflated, perform short and firm movements backwards and forwards (4 times for 2 sec), causing the desired endothelial damage. The balloon catheter was then deflated and removed, and the introducer was used to perform a new angiography to assess the effect on the vessels and rule out complications (fig. 3).

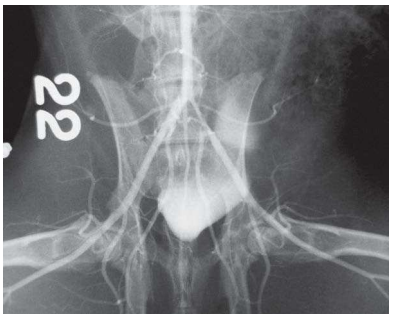

Figure 3 Immediate effect of denudation of the iliac artery. The decrease in size is due to spasms that occur after arterial damage.

Table 1 Composition of hyperlipidemia diet

Ingredients	%
Barley	12.0
Wheat bran	30.0
Sunflower cake	10.0
Sunflower seed	1.5
Alfalfa hay	28.0
Beet pulp	4.0
Cereal straw	4.0
Sugarcane molasses	1.0
Butter	0.5
Palm oil*	6.0
Cholesterol	1.0
Sodium Bicarbonate	0.6
Calcium carbonate	0.6
Sodium chloride	0.3
Corrector of vitamins and trace elements	0.5

Italicized ingredients were added to the diet.
*GRACOM, Silla, Valencia, Spain.

The carotid artery was closed, with monofilament absorbable suture (Safilo® 5 / 0, Braun®) in order to maintain the lumen patency. The muscles were sutured in layers, and the skin with intradermal sutures, both with absorbable multifilament material (Safilo® 3 / 0, Braun®).

Postoperative therapy was 1 mg/kg meloxicam (Metacam®, Schering-Plow®), 1 mg/kg enrofloxacin (Baytril®, Bayer®) and 0.05 mg/kg buprenorphine (Buprex®, Schering-Plow®).

Hyperlipidemic diet

After surgery, animals were fed a hyperlipidemic diet *ad libitum* for 8 weeks. The composition of the diet is shown in the table 1. Note the addition of 1%pure cholesterol crystals and 6%palm oil to increase the atherogenic effect.

After the eight weeks, a new Doppler ultrasound was performed in the aorta and the right and left primitive iliac arteries, evaluating the response of the arteries to both the diet and intra-arterial injury in the left iliac artery. A new blood sample was performed and laboratory analytic parameters were assessed again.

Statistical analysis

We used descriptive statistics and the χ^2 test for comparison of variables. Differences were considered statistically significant when a $p < 0.05$ was obtained.

Results

Thirty-two (78.13%) out of thirty-six animals beginning the study reached the catheterization and angioplasty process, but only 25 finished it, representing an overall survival rate of 69.44%.

To simplify the results, we have analyzed the data by dividing it into two different phases: anesthetic management and surgical management.

Anesthetic management. The sedation protocol used was successful, with no casualties and with satisfactory results. Anesthetic induction with Propofol produced 4 casualties in a total of 36 animals, or 11.11%.

Surgical management. The results were divided into two working groups:

— In group A (consisting of 20 animals in which we used an angioplasty balloon 2.5 mm in diameter), 17 animals (85%) survived to the postoperative stage and 3 did not (2 because of carotid bleeding during catheterization and 1 because of iliac artery rupture).
— In group B (consisting of 12 animals in which we used an angioplasty balloon 3 mm in diameter), 8 animals (66.67%) survived to the postoperative stage and 4 did not (3 because of rupture of the left iliac artery after handling with the ball and 1 because of aortic rupture) (fig. 4).

The analytical results of the biochemical tests at baseline and after 8 weeks on the atherogenic diet are shown in table 2, highlighting the significant increase in blood cholesterol levels, without triglyceride level differences. There were also no differences in the cholesterol and triglyceride levels between males and females.

The results of the ultrasound examination by measuring the light vessel, both basal and after arterial injury and diet-induced hyperlipidemia are shown in table 3. There was no significant difference between both groups in the diameters of the aorta and both iliac arteries. Differences between male and female rabbits were not significant either.

Figure 4 Rupture of the left iliac artery with extravasations of contrast.

Table 2 Analytical results

	Pre atherogenic diet	Post atherogenic diet
Plasma cholesterol (mg/dl)	37.96 ± 19.3	1,791 ± 296.91*
Plasma triglycerides (mg/dl)	30.04 ± 11.01	36.16 ± 10.20

*Statistically significant, $p < 0.01$.

Table 3 Ultrasound results

	Light aorta (mm)	Light right aorta iliac (mm)	Light left aorta iliac (mm)
Baseline examination	4.1 ± 0.7	3 ± 0.3	3 ± 0.4
Group A After vascular damage + 8 weeks diet	2.78 ± 1.21	2.18 ± 0.81[a]	1.16 ± 0.63[a]
Group B. After vascular damage + 8 weeks diet	3.07 ± 1.06	2.53 ± 0.9[a]	1.39 ± 1.1[a,b]

There was no significant differences between group A and group B, $p > 0.05$.
[a]Statistically significant differences ($p < 0.05$) between left and right iliac arteries, demonstrating the effectiveness of vascular damage.
[b]Two arteries are the goal of complete obstruction.

However, the left iliac artery luminal diameter, where angioplasty was used for denudation, was significant decreased compared to the right iliac artery.

Discussion

The rabbit is used as a laboratory animal because of its ease of handling, its accessibility and in its physiological similarity to humans.[5,7,8] In recent studies, ultrasound has proved to be very useful in assessing the changes caused by atherosclerotic plaques in main arteries because it is a noninvasive tool that does not endanger the animal's life.[9]

The anesthetic management used in the sedation protocol was very effective, as no deaths were seen in our group of animals. In contrast, induction of anesthesia with propofol caused 4 deaths, most likely because rabbits are very sensitive to stress, which leads to physiological changes such as severe tachycardia, which makes anesthetic accidents very common.[10]

Two deaths (the second and eighth rabbits) during surgical manipulation were due to carotid hemorrhage at the time of puncture, and they were attributed to the learning curve, as no deaths occurred after rabbit #8. As shown previously, atheromatous lesions develop rapidly in rabbits if they are fed a hypercholesterolemic diet,[2,3] as shown by the increase in basal cholesterol levels after the special diet. Injuries that occurs in the vessel accelerate the formation of atherosclerotic plaques, making them more stable and uniform in size and distribution.[4] In the model of arterial denudation used in this study, angioplasty causes a narrowing of the artery and subsequent damage by compression of the media, intramural hemorrhage and dissection of the intima and/or medium layer, leading to an inflammatory response. Restenosis is a combination of mural thrombus filling the dissection plates and forming a cell growth in addition to collagen deposits and elastin in the intima, media and adventitia of the artery.[11] This is demonstrated in this study by ultrasound as the lumen diameter of the injured iliac artery was smaller than the contralateral.

The choice of angioplasty balloon size used to produce vascular damage is important because the 2.5 mm balloon produced fewer complications than the 3 mm diameter balloon catheter. Because the different sizes caused atheromatous lesions of similar magnitude, we suggest using a balloon of smaller diameter than the healthy artery to minimize complications. It should be noted that the angiographic image of stenosis immediately after arterial denudation is due to arterial spasm as a vessel response to injury and more reliable values are obtained with ultrasound 8 weeks later (fig. 3).

In conclusion, the rabbit is a good animal model for atherogenesis. In addition, vascular damage with balloon angioplasty is best done with a ball of smaller diameter rather than the vessel itself. Ultrasound is considered the diagnostic method of choice for assessing the arterial response after vascular damage.

Conflict of interest

The authors declare no conflicts of interest.

Authoring

M.D. Ferrer conceived and designed the study and participated in data collection and interpretation.

E. Esteban and F. Liste contributed to the conception and design of the study and critically review drafts.

J M. Carrillo and J.J. Ramos helped in obtaining data for the study.

M.T. Balastegui contributed to data analysis and helped in drafting.

O. Cosin critically reviewed the content and relevance of the article.

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