Original Article

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Development of a Rabbit Iliac Arterial Stenosis Model Using a Controlled Cholesterol Diet and Pullover Balloon Injury

콜레스테롤 식이 및 내막 손상을 통한 토끼 장골동맥 협착 전임상 모델 개발

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Purpose This study aimed to develop a rabbit iliac stenosis model and evaluate the effects of different mechanical injury techniques on the degree of arterial stenosis.

Materials and Methods Eighteen rabbits were divided into three groups: cholesterol-fed with pullover balloon injury (group A; $n = 6$), cholesterol-fed with localized balloon dilatation (group B; $n = 6$), and chow-diet with pullover balloon injury (group C; $n = 6$). After baseline angiography, the left iliac arteries of all rabbits were injured with a 3 × 10 mm noncompliant balloon using either a wide pullover technique (groups A and C) or a localized balloon dilatation technique (group B). A nine-week follow-up angiography was performed, and the angiographic late lumen loss and percentage of stenosis were compared.

Results Group A exhibited the most severe late lumen loss (A vs. B, 0.67 ± 0.13 vs. 0.04 ± 0.13 mm, $p < 0.0001$; A vs. C, 0.67 ± 0.13 vs. 0.26 ± 0.29 mm, $p < 0.05$; stenosis percentage 32.02% ± 6.54%). In contrast, group B showed a minimal percentage of stenosis (1.75% ± 6.55%).

Conclusion Pullover-balloon injury can lead to significant iliac artery stenosis in rabbits with controlled hypercholesterolemia. This model may be useful for elucidating the pathogenesis of atherosclerosis and for evaluating the efficacy of novel therapeutic interventions.

Index terms Hypercholesterolemia; Angiography; Peripheral Arterial Disease

INTRODUCTION

Peripheral arterial disease (PAD) is a significant health problem affecting more than 200 million people worldwide (1-3). PAD is the third leading cause of atherosclerotic cardiovascular morbidity after coronary artery disease and stroke (1). Therefore, animal models of atherosclerosis are crucial for evaluating the efficacy of new endovascular devices and outcomes of interventional procedures. Rabbits are commonly used for this because they are relatively easy to acquire and handle, inexpensive to purchase and maintain, and have well-understood genetic characteristics (4). New Zealand White rabbits have been extensively used in preclinical interventional studies because of their straight external iliac artery, relatively uniform proximal-to-distal luminal diameter, and absence of branching vessels (5). The external iliac artery is particularly useful for placing experimental devices, such as drug-eluting stents and drug-coated balloons. However, the use of cholesterol-fed rabbit models in studies of PAD has several limitations. Cholesterol-fed rabbits primarily develop plaques in the aortic arch and descending thoracic aorta (6-8). Plaque formation in the iliac arteries and abdominal aorta, which is typical in humans, is not readily observed in cholesterol-fed rabbits (9).

High serum cholesterol levels over a long period are required for long-term plaque development. An intensive high-cholesterol diet can lead to hepatic failure or failure to thrive in rabbit models (10-12). Therefore, further research is necessary to develop preclinical animal models that accurately simulate human atherosclerosis for testing and evaluating intravascular therapies.

Therefore, we aimed to develop a rabbit model of iliac artery stenosis using controlled hypercholesterolemia and assess the degree of iliac artery stenosis according to different mechanical endothelial injury techniques.

MATERIALS AND METHODS

ANIMAL MODEL

This study was approved by the Institutional Animal Care and Use Committee of Seoul National University Bundang Hospital (No. 2020-01334). Eighteen male New Zealand White rabbits weighing 3000–3500 g were used in this study. All rabbits were housed in cages with a 12-hour light/dark cycle and ad libitum access to water. The rabbits were randomly divided into three groups: cholesterol-fed with pullover balloon injury (group A; n = 6), cholesterol-fed with localized balloon dilatation injury (group B; n = 6), and standard chow-diet with pullover balloon injury (group C; n = 6) (Fig. 1). The cholesterol-fed groups (A and B) were fed an atherogenic diet comprising 0.5% cholesterol and 6% corn oil for the first five weeks, followed by a diet with lower cholesterol (0.025%) for another five weeks to prevent liver failure (10-12).

BALLOON INJURY TECHNIQUES

Balloon injury was induced in all groups one week after initiation of the cholesterol diet. A 3-mm balloon catheter was used as the average diameter of the rabbit iliac artery according to angiography was < 3 mm. The left iliac artery of each rabbit was injured with a 3 × 10 mm balloon catheter (Genoss Co., Ltd., Suwon, South Korea) with nominal pressure (8 ATM) using either the pullover technique (groups A and C) or localized balloon dilatation technique

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(group B). Pullover balloon injury was induced by inflating the balloon at the common femoral artery and rapidly (< 1 s) pushing it up to the common iliac artery to maximize the shear stress on the vascular endothelium of the external iliac artery. In contrast, localized balloon dilatation injury was induced by stepwise balloon inflation–deflation over the entire external iliac artery segment to precisely generate barotrauma at the targeted location (Fig. 2).

After arterial injury, the animals in groups A and B continued the atherogenic diet for another four weeks, followed by a low-cholesterol (0.025%) diet for the remainder of the study period.

ANGIOGRAPHY

All rabbits underwent angiography of the bilateral aortoiliac and femorotibial arteries using a portable C-arm (BV Pulsera; Philips, Amsterdam, Netherlands) at baseline (after the first week) and after nine weeks. Angiographic lumen diameter and late lumen loss were evaluated by an independent observer using ImageJ version 1.44 (National Institutes of Health, Bethesda, MD, USA). Quantitative analysis of the angiographic parameters was performed by two radiologists (L.J.H. and H.D.M.). Radiopaque vascular tape (Stent Guide; LeMaitre, Burlington, MA, USA) was used as the reference standard for vessel length and diameter. The rabbits were anesthetized with an intramuscular injection of 0.2 mL/kg of

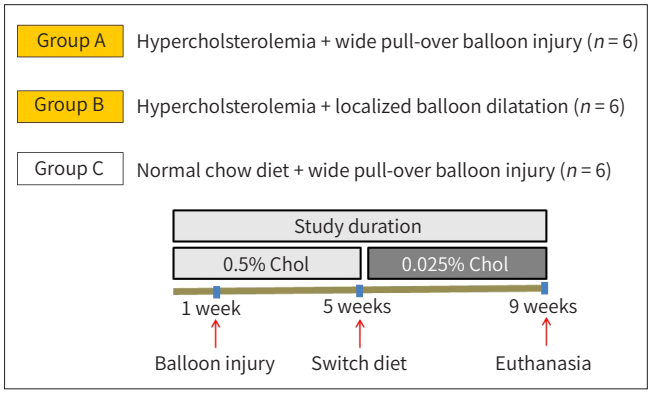
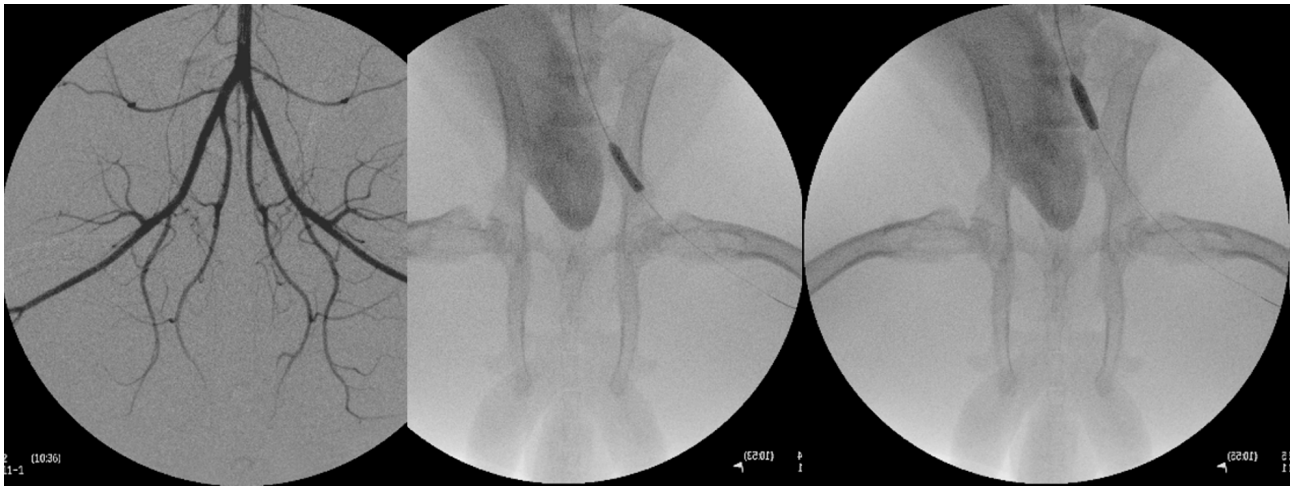


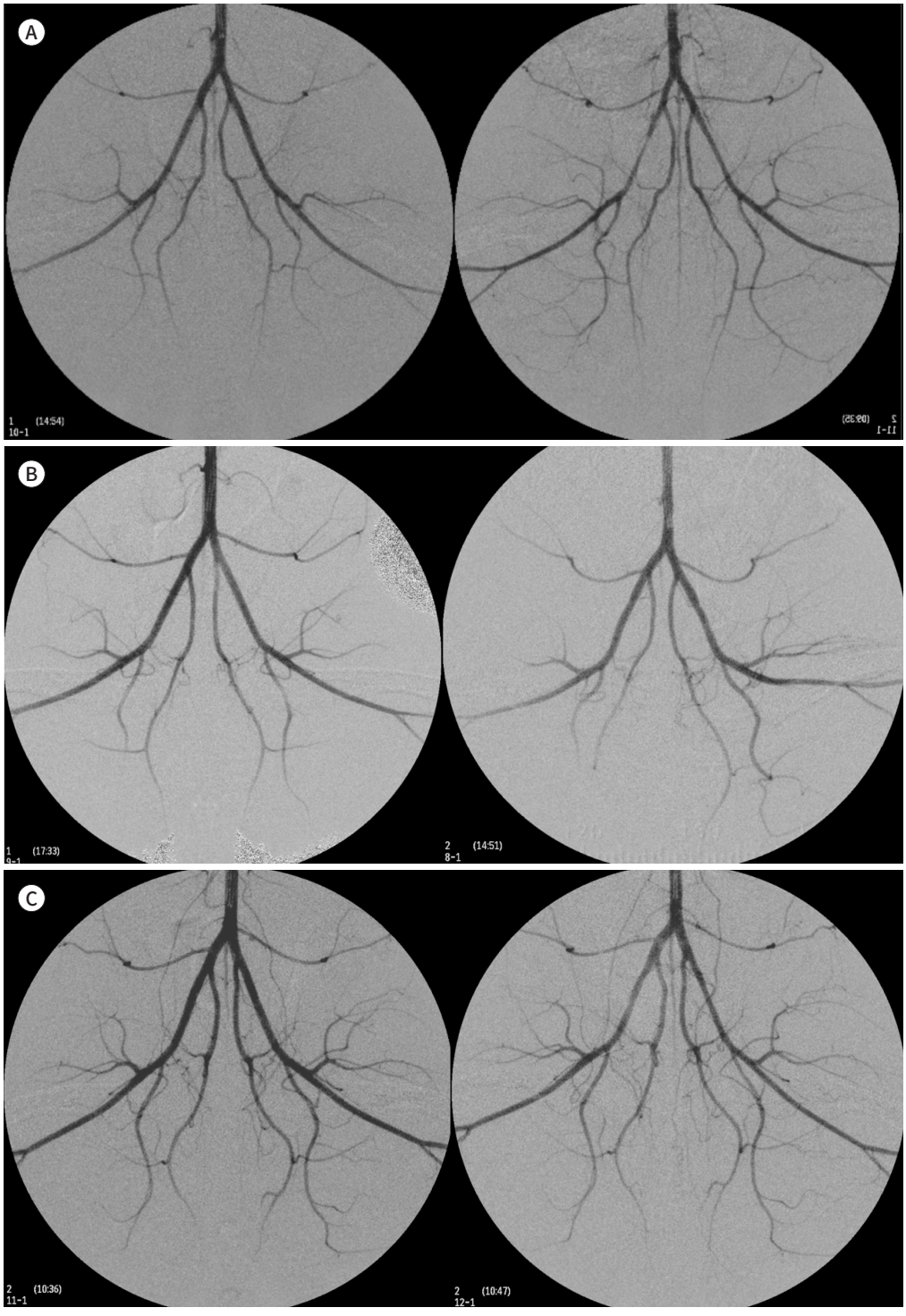
Fig. 1. Schematic timetable of diets and balloon injury for each group.

Fig. 2. A serial angiographic image demonstrating the pullover balloon injury technique performed on the left common iliac artery of the rabbits.



tiletamine (Zoletil; Virbac, Carros, France) plus 0.1 mL/kg of xylazine (Rompun; Bayer, Leverkusen, Germany), and placed in the supine position. Left lateral neck access was used to expose the common carotid artery. A 4-Fr vascular sheath (Radiofocus; Terumo Corp., To-

Fig. 3. Representative angiographic images of each group at baseline (left column) and 9 weeks (right column) (A: group A, B: group B, C: group C).



kyo, Japan) was used to puncture and engage the arteries, and a heparin solution (100 IU/kg) was injected to prevent thrombus formation after sheath insertion. After angiographic evaluation, all animals were euthanized, and the injured vessels were harvested. One injured artery each from groups A and C was randomly selected and prepared for hematoxylin & eosin and RAM-11 staining to evaluate cellular proliferation and atheroma development.

STATISTICAL ANALYSIS

All data are presented as mean ± standard deviation. The angiographic lumen diameter and angiographic parameters were analyzed using the Kruskal–Wallis and Mann–Whitney U tests with Bonferroni correction. Data processing and analyses were performed using the SPSS (version 21.0; SPSS Inc., IBM Corp., Chicago, IL, USA). Statistical significance was set at a two-sided $p < 0.05$.

RESULTS

Iliac artery injury was induced in all rabbits. Twelve rabbits' arteries (groups A and C) were injured using the pullover technique, whereas six (group B) underwent a localized balloon dilatation technique. All of the animals survived until the end of the experiment.

ANGIOGRAPHIC LATE LOSS AND PERCENTAGE STENOSIS

Angiographic stenosis was observed in all groups (Fig. 3, Table 1). At the 9-week follow-up,

Table 1. Luminal Diameter and Angiographic Stenosis Rate of Each Group

Parameters	Group A	Group B	Group C
Baseline luminal diameter (mm)	2.08 ± 0.05	2.09 ± 0.18	2.32 ± 0.11
Follow-up luminal diameter (mm)	1.41 ± 0.15	2.02 ± 0.22	2.07 ± 0.37
Angiographic late loss (mm)	0.67 ± 0.13	0.04 ± 0.13	0.26 ± 0.29
Angiographic stenosis (%)	32.02 ± 6.54	1.75 ± 6.55	11.25 ± 12.97

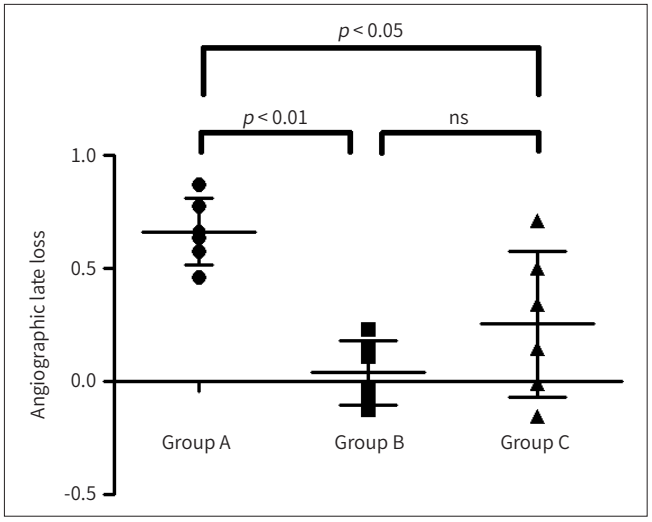
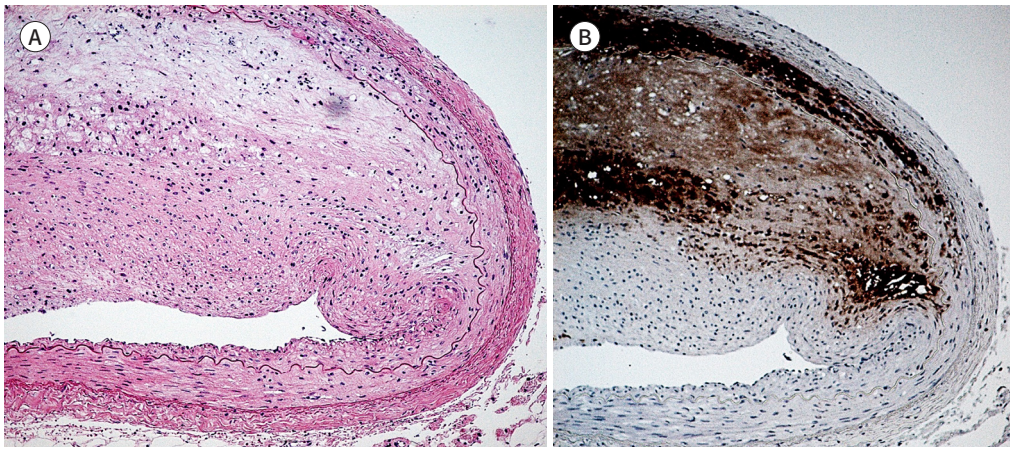


Fig. 4. Angiographic late loss in groups A, B, and C.
ns = not significant

Fig. 5. Representative photomicrograph (hematoxylin & eosin [A] and anti-rabbit macrophage staining [B], × 40) of the transverse section of group A shows intimal hyperplasia with macrophage infiltration.



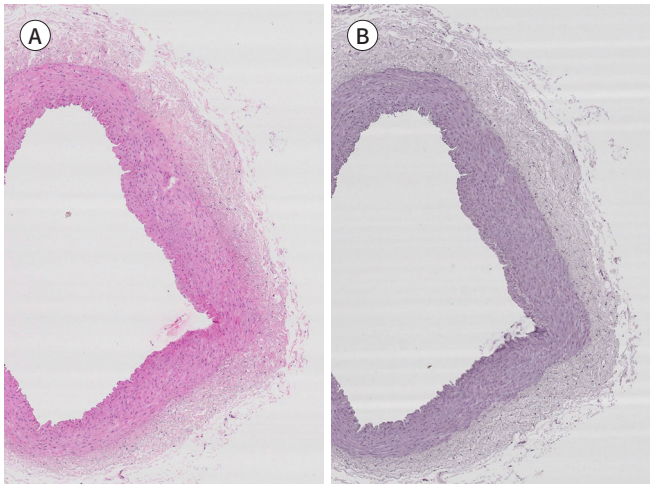


Fig. 6. Representative photomicrograph (hematoxylin & eosin [A] and anti-rabbit macrophage staining [B], × 40) of the transverse section of group C shows smooth muscle thickening without intimal hyperplasia or macrophage infiltration.

group A showed significant late lumen loss compared to groups B and C (A vs. B, 0.67 ± 0.13 vs. 0.04 ± 0.13 mm, $p = 0.008$; A vs. C, 0.67 ± 0.13 vs. 0.26 ± 0.29 mm, $p = 0.04$). There was no significant difference in late loss between groups B and C ($p = 0.166$) (Fig. 4). Group A had the greatest angiographic stenosis rate (32.02% ± 6.54%), which was significantly higher than group B ($p = 0.008$). There was no significant difference in the percentage of stenosis between groups A and B ($p = 0.073$) or B and C ($p = 0.336$). The histological sections from the middle of the injured left iliac artery in group A showed marked intimal hyperplasia with macrophage infiltration, whereas those from group C showed smooth muscle proliferation without neointima formation or macrophage infiltration (Figs. 5, 6).

DISCUSSION

In this study, we developed a novel experimental protocol to effectively induce atherosclerotic iliac artery stenosis in rabbits by controlling hypercholesterolemia and balloon injury using the pullover technique.

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The rabbit iliac artery model has been widely used as a preclinical testbed for evaluating newly developed stents or balloons for treating atherosclerotic coronary or peripheral artery diseases (13-15). However, most previous studies used only normal iliac arteries or rabbit models of atherosclerosis induced by high-cholesterol diets. However, these methods have not yet been standardized. Moreover, most studies did not measure the degree of stenosis of the iliac artery during the experiments (16, 17).

In this study, endothelial denudation using the pullover technique (groups A and C) effectively induced iliac artery stenosis. Furthermore, pullover injury combined with controlled hypercholesterolemia (group A) showed the highest percentage of stenosis, with relatively low interindividual variability.

A controlled cholesterol diet has been used to develop an atherosclerotic animal model that avoids fatal liver failure and loss (10-12). However, a hypercholesterolemic diet alone may not be sufficient to induce significant stenosis in rabbits. In our study, the percentage of stenosis in group B was only 1.75% $\pm$ 6.55%. Therefore, appropriate endothelial injury may be required to form atherosclerotic lesions in the index artery, as observed by comparing groups A and B.

Injury to the arterial endothelial layer triggers an inflammatory response leading to the recruitment of immune cells, such as monocytes and macrophages, to the injury site (18). Immune cells accumulate cholesterol and other lipids to form foam cells, which are hallmarks of early atherosclerotic lesions (18). Over time, the accumulation of foam cells, smooth muscle cells, and extracellular matrix components leads to advanced atherosclerotic plaques (19). Similar to previous research, the section from the injured artery in group A showed significant intimal hyperplasia with macrophage infiltration, indicating advanced atheroma formation. In contrast, sections from group C displayed smooth muscle proliferation without neointima formation or macrophage infiltration (Figs. 5, 6).

This study showed that the pullover technique resulted in subsequent injury that may have initiated atherosclerosis; however, balloon dilatation was insufficient. Injury is a crucial component of atherosclerosis because it causes inflammation during the early stages of the disease (20, 21). Without this mechanism, atherosclerotic plaque formation may require a significantly higher hypercholesterolemia threshold.

The experimental protocol used in this study, which combines the pullover technique with controlled hypercholesterolemia, offers a practical standardized approach for inducing atherosclerotic iliac artery stenosis in rabbits; it can be used to elucidate the pathogenesis and treatment response of atherosclerosis. This study may contribute to the development of novel therapeutic strategies for atherosclerosis.

This study has several limitations. First, there are differences in lipid metabolism between rabbits and humans; therefore, the composition of atherosclerotic plaques may vary (22). However, our model, which induces a more pathological state than the normal vessel model, can be used to evaluate the efficacy of drug-eluting stents and drug-coated balloons. Secondly, the sample size was relatively small. Nevertheless, we observed statistically significant differences in angiographic stenosis and the lowest interindividual variability in group A.

In conclusion, the pullover balloon injury technique induced significant iliac arterial stenosis in rabbits with controlled hypercholesterolemia. This model may be useful for eluci-

dating the pathogenesis of atherosclerosis and for evaluating the efficacy of novel therapeutic interventions.

Author Contributions

Conceptualization, L.C., L.J.H., Y.C.J.; data curation, L.J.H.; formal analysis, M.H.D., L.C., L.J.H., K.M.; funding acquisition, L.J.H.; investigation, L.C., L.J.H.; methodology, M.H.D., L.C., L.J.H.; project administration, L.J.H.; software, L.C., K.M.; supervision, L.J.H., K.K.Y., Y.C.J.; visualization, Y.C.J., K.M.; writing—original draft, M.H.D., L.C., L.J.H.; and writing—review & editing, all authors.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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콜레스테롤 식이 및 내막 손상을 통한 토끼 장골동맥 협착 전임상 모델 개발

민 훈[1] · 이종호[1] · 이재환[1,2,3]* · 김건영[1] · 윤창진[1,2,3] · 김민욱[4]

목적 콜레스테롤 식이 및 기계적 혈관 내막 손상 유발을 통한 토끼 장골동맥 협착 모델을 개발하고 서로 다른 내막 손상 방법에 따른 협착 유발 정도를 평가하고자 한다.

대상과 방법 18마리의 토끼를 콜레스테롤 식이 후 풍선으로 당김 손상(pullover injury)을 가한 군(group A, 6마리), 콜레스테롤 식이 후 국소 확장 손상(localized balloon dilatation) 군(group B, 6마리), 일반 사료 식이 후 당김 손상을 가한 군(group C, 6마리)로 나누었다. 모든 군에서 혈관조영술을 시행하고 좌측 장골동맥에 직경 3 mm, 길이 10 mm의 비순응성(non-compliant) 풍선 카테터를 이용하여 당김 손상(group A, C) 또는 국소 확장 손상(group B)를 가하였다. 실험 시작 후 9주째 추적 혈관조영술을 시행하여 혈관조영술상 장골동맥의 협착 정도(후기 내강 손실, 협착 비율)을 정량적으로 비교 평가하였다.

결과 A군이 9주째 추적 혈관조영술에서 가장 심한 후기 내강 손실을 보였고 32.02% ± 6.54%의 협착이 확인되었다(A군 vs. B군: 0.67 ± 0.13 mm vs. 0.04 ± 0.13 mm, $p < 0.0001$; A군 vs. C군: 0.67 ± 0.13 mm vs. 0.26 ± 0.29 mm, $p < 0.05$). B군에서는 혈관조영술상 협착이 1.75% ± 6.55%로 매우 낮게 나타났다.

결론 콜레스테롤 식이 및 당김 손상 기법을 이용한 기계적 내막 손상이 토끼 장골 동맥에서 유의한 협착을 유발함을 확인하였다. 이 전임상 모델은 전임상 말초동맥 질환의 질병 모델로 사용될 수 있을 것으로 예상된다.

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