Experimental Model of Atherosclerosis with Lesions in Aorto-Iliac Axis in Apoe -/- Mice

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Acknowledgements and Credits:

This work has been carried out thanks to the Research Institute of the La Paz University Hospital (IdiPaz) (Madrid, Spain), whose staff has closely followed the study and thanks to the Research Foundation of the La Paz Hospital and the Autónoma University (Madrid, Spain).

Funding Information:

To carry out this research we have had the support of the Research Institute of the Hospital Universitario La Paz (IdiPaz) (Madrid, Spain), which has the infrastructure (animal facility), material and resources for the recruitment, treatment and monitoring of animal models, as well as institutional support for the analysis of the results obtained.

Private funding was obtained for the purchase of the animals and the La Paz Hospital Research Foundation IdiPaz and the Autónoma University (Madrid, Spain) have made their facilities available to us (Experimental Surgery Service, animal facility, medication, etc.) free of charge for they are researchers hired at these institutions. The financing needs translate into the purchase of the experimental animals and the diet necessary to complete the murine model of arteriosclerosis.

Conflicts of Interest:

The authors have no conflict of interest.

1. ABSTRACT

***Introduction**: Atherosclerosis is an inflammatory chronic disease which has an elevated prevalence. The mouse model is the most commonly used.*

***Materials and methods**: An experimental and descriptive study of a group of 20 male mice C57BL/6J KO apoE -/- fed high-fat diet during 14 weeks was carried out. Analysis of the aorto-iliac axis and the presence of advanced atherosclerotic lesions affecting the subjects, as well as levels of triglycerids and total cholesterol. Weight of each subject was measured every week.*

***Results**: There is a global tendency in all the subjects to gain weight and have higher levels of triglycerids and total cholesterol at the end of the study. Most subjects developed advanced atherosclerotic lesions in histological samples.*

***Discussion:** Most studies have analyzed lesions in aortic root, but these don´t allow to know the severity of the disease. In the same line as other studies, we have successfully obtained atherosclerotic lesions.*

Conclusions: This experimental model with genetically modified mice provides the development of advanced atherosclerotic lesions in the aorto-iliac axis after high-fat diet during 14 weeks and a correlation with alteration of the lipoprotein profile in plasma.

***Keywords:** Experimental Model, Atherosclerosis, Aorto-Iliac Axis*

2. MAIN TEXT

1. Introduction

Atherosclerosis is a systemic disease which causes coronary heart disease, cerebrovascular disease, and peripheral arterial disease. This set of cardiovascular diseases is the leading cause of death in the world (1). It has its origin in a chronic inflammatory process that causes the deposit of lipids and cells from the immune system in the arterial wall (2). To carry out research on different drugs that take action at this level, in the initial phases, we use animal models to assess their effects. Although studies have been carried out based on rabbit, pig and primate atherosclerotic models (3), the most developed model for both ease of handling and lower cost has been the mouse model, which, in addition, allows to be genetically modified (4,5). Beverly Paigen studied the vulnerability of ten mouse breeds to atherosclerosis on a high-fat diet, being the most vulnerable breed C57BL/6J, which developed type I, type II and even type IV lesions of the American Heart Association Classification (3,6). In 1992, the first genetically modified Knock Out (KO) mouse model with genetic deletion of apolipoprotein E (apoE -/-) and KO Low-Density Lipoprotein Receptor (LDL-R) mouse were developed. The former are more hyperlipemic models that develop more severe lesions which even appear with a normal diet throughout the arterial tree. An increase in the thickness of the lipid nucleus occurs in mice fed a high-fat diet (in general, they develop more advanced lesions). Cholesterol in KO apoE -/- mice is mobilized in the form of Very Low-Density Lipoproteins (VLDL), mainly (7). Our main aim is to present a descriptive experimental model for obtaining arteriosclerotic lesions in C57BL/6J KO apoE -/- mice, analyzing the aorto-iliac axis, and the existence of advanced atherosclerotic lesions in the subjects, after having been subjected for a long period of time on a high-fat diet. This research helps us to obtain atherosclerotic mice with lesions in the aortic bifurcation in a reliable and economical way.

2. Materials y methods

We have developed an experimental and descriptive study based on a murine model of a single group of genetically modified mice.

2.1. Animals and Experimental Model

Twenty male C57BL/6J KO apoE -/- mice of 6 weeks of age were obtained from the Janvier Labs Center. They were kept in a controlled facility, in 4 cages with 5 mice in each cage, daily cycles of 12 hours light / 12 hours darkness, at a temperature of 22º C +/- 2º C and free access to water and food. The animals are identified by ear tags under slight inhalation anaesthesia with sevofluorane. To carry out the procedures, an optical microscope equipment, microsurgical material and anaesthetic equipment were needed (Figure 1). When mice reached 10 weeks of age, Paigen diet was iniciated, which consists of a modified high-fat diet based on 16% fat; 1.25% cholesterol; 0.5% sodium cholate for 14 weeks. All animals were weighed weekly with the average weight of the subjects calculated. The procedures were adjusted to the current regulations on the use of animals in research and teaching Royal Decree 53/2013 and European Directive EU 63/201. They were approved by the center's Animal Welfare Committee and authorized by the Madrid Province under Proex CEI 85-1599-A257.

2.2. Lipid and Cholesterol Levels

Blood samples were collected before starting the high-fat diet, after 10 weeks of diet and at the end point of the study. The plasma levels of triglycerides and total cholesterol were determined. The extraction of the samples was carried out under sedation. Puncture of the maxillary region with a 25G needle is performed to obtain a blood sample. Subsequently, they were centrifuged at 3000 revolutions per minute for 10 minutes.

The corresponding identification of each tube was made in relation to each mouse sample. It was kept in a refrigerator at a temperature of -80ºC. The average of the determinations for weeks 0, 10 and 14 was calculated.

2.3. Histological Samples

After 14 weeks, the animals are sacrificed. With the animal in the supine position, a transperitoneal approach to the abdominal aorta and aortic bifurcation was performed, using a median laparotomy under microscope vision. After careful dissection, the infrarenal abdominal aorta and both iliac arteries were sectioned, removed, and stored in containers with 4% liquid formaldehyde solution. Subsequently, the fixation and inclusion in paraffin was carried out for the preparation of the samples. They were sectioned and adhered to a slide that was stained posteriorly with hematoxylin-eosin (HE) and Masson's trichrome stain.

An analysis of the areas of the total wall of the vessel and the atheroma plaque of each sample was carried out with HE and Masson's trichrome staining obtained using Fiji ImageJ software, taking only those images photographed with a magnification of 200x and represented in Pixels2 .

A score was carried out indicating the existence or not of the following elements: atheroma plaque (yes / no), cholesterol crystals (yes / no), foam cells (yes / no), inflammatory cells (yes / no) and internal elastic lamina breakage (yes / no).

3. Results

During the study, 4 mice were lost to follow-up due to death of unknown cause (1 at week 11, 2 at week 12, and 1 at week 13). This yields a non-negligible mortality in the 20% model.

As previously mentioned, the weight of the subjects is measured weekly. A clear trend towards weight gain in subjects with a high-fat diet during the study (Graph 1).

Taking blood samples was a difficult task, since the animal must be sedated and immobilized to perform the puncture in the maxillary region. In most cases, the samples were insufficient and required adequate dilutions; and, in other cases, they coagulated. The average values for triglyceride and cholesterol levels were obtained from the samples for week 0, week 10 and week 14 of the study. The results obtained showed incresing levels of triglycerides and total cholesterol of the analyzed samples during the study, as shown in the graphs (Graphs 2 and 3).

The histological samples obtained from the aorto-iliac axis showed the formation of atheroma plaques in the arterial wall in 43.8% of the subjects, with cholesterol crystal deposits in 18.8%. The mean arterial wall area obtained was 843,712.13 Pixels2 and the mean area of the atheroma plaque was 365,100.33 Pixels2. In 43.8% of the subjects, the presence of foam cells and infiltration with inflammatory cells were observed, as well as the rupture of the internal elastic lamina both in HE and Masson's trichrome stains. The subjects that developed lesions were mostly atheromatous lesions in advanced stages (Figures 2 and 3).

4. Discussion

The development of atherosclerotic lesions in genetically modified mice model, especially KO apoE -/-, has been extensively studied (8–11). Most of the studies have analyzed the histological lesions that appear in the aortic root in this type of mice on a high-fat diet, since it is the location where they appear earlier and which allows shorter studies to be carried out. Over time, these lesions spread along the arterial tree, appearing more advanced and more severe lesions, similar to those that appear in humans. Tangilara et al. described a model for the macroscopic quantification of atheroma plaques using Sudan IV staining and HE staining for the microscopic quantification of lesions in the aortic root (12). In other studies, the analysis of atherosclerotic lesions is carried out only at the level of the aortic root, which does not allow us to know the severity of the disease, therefore, the analysis of the complete arterial tree would be necessary (5,9). There is no standardized model for evaluating atherosclerotic lesions in murine models. Our objective with this study is to achieve a reliable and economical model of arteriosclerosis in the abdominal aorta for vascular studies.

In our model we have focused on analyzing the aorto-iliac axis from the immediately infrarenal aorta, which is one of the anatomical regions of the central arterial axis most affected in humans. We have observed advanced atherosclerotic lesions in most of the subjects which developed lesions in our study, after having been subjected to a high-fat diet for a long period of time. This matches the results in published studies (5). Likewise, the analytical results obtained over time show us an increase of the triglycerides and total cholesterol parameters, previously shown in the graphs. In published murine models, total cholesterol levels in apoE -/- mice fed the normal diet reach 600 mg / dL, while apoE -/- mice fed the Western diet (containing 21% fat and 0, 15% cholesterol) reach 1,300-2,000 mg / dL of total cholesterol (13). As mentioned previously, the results obtained in our study should be interpreted with caution, since there were several samples that could not be analyzed under the appropriate conditions.

The main limitations of this study lie in the arterial morphology of the mouse, which presents some differences with the human being, this is the absence of vasa vasorum and a thinner middle layer (3). Even so, it represents an easy model to handle given its small size and the speed to quickly develop lesions in order to study the effect of different drugs which take action at this level. Another limitation of this study was the disparity in obtaining plasma samples that could lead to interferences in the results. Finally, although samples of the aorto-iliac axis were obtained from the infrarenal aorta, given the difficult dissection under microscope vision, there was no homogeneous reference point to perform the section of the aorta, and therefore no point where to start the histological sections.

5. Conclusion

This experimental murine model using genetically modified mice provides advanced arteriosclerotic lesions in the aortic bifurcation after 14 weeks of a high-fat diet, at the same time, there is a correlation with the alteration of the lipoprotein profile in plasma.

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Figure 1. Mice cage with free access to water and food, microsurgical equipment with microscope and anesthetic equipment.

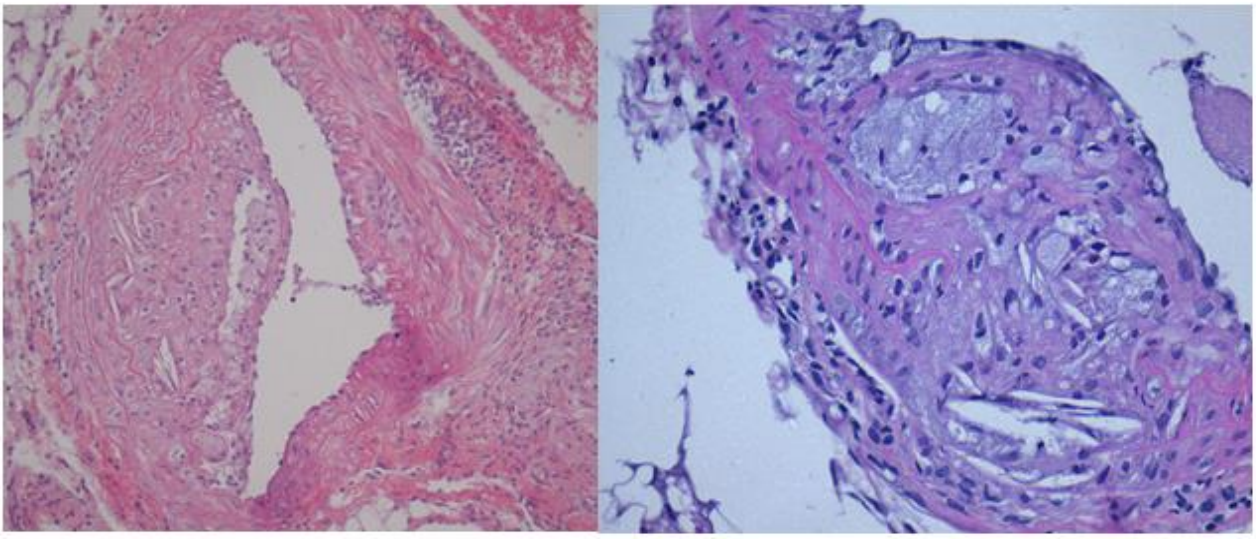


Figure 2. Arterial cross section with hematoxylin-eosin staining. Atheroma plaque with abundant inflammatory cells and cholesterol deposits. The image on the left at 20x and the image on the right at 40x.

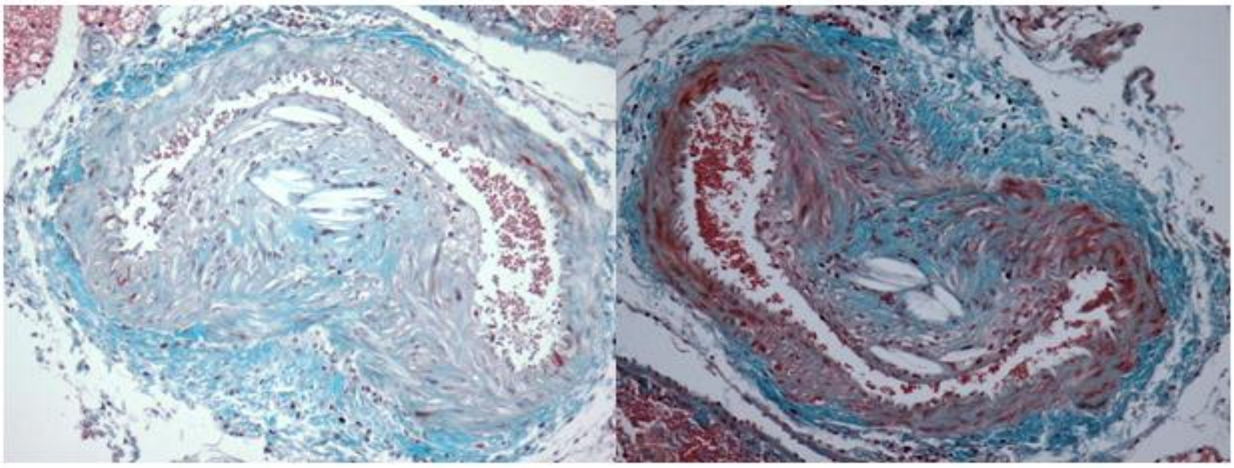
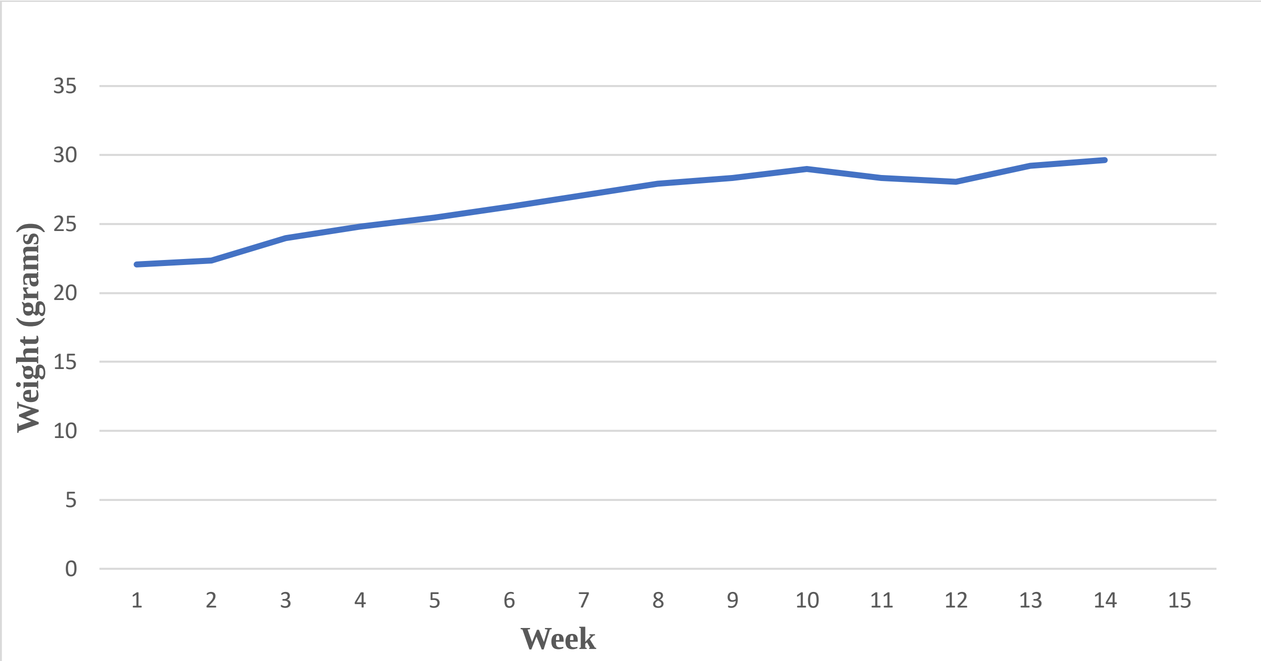
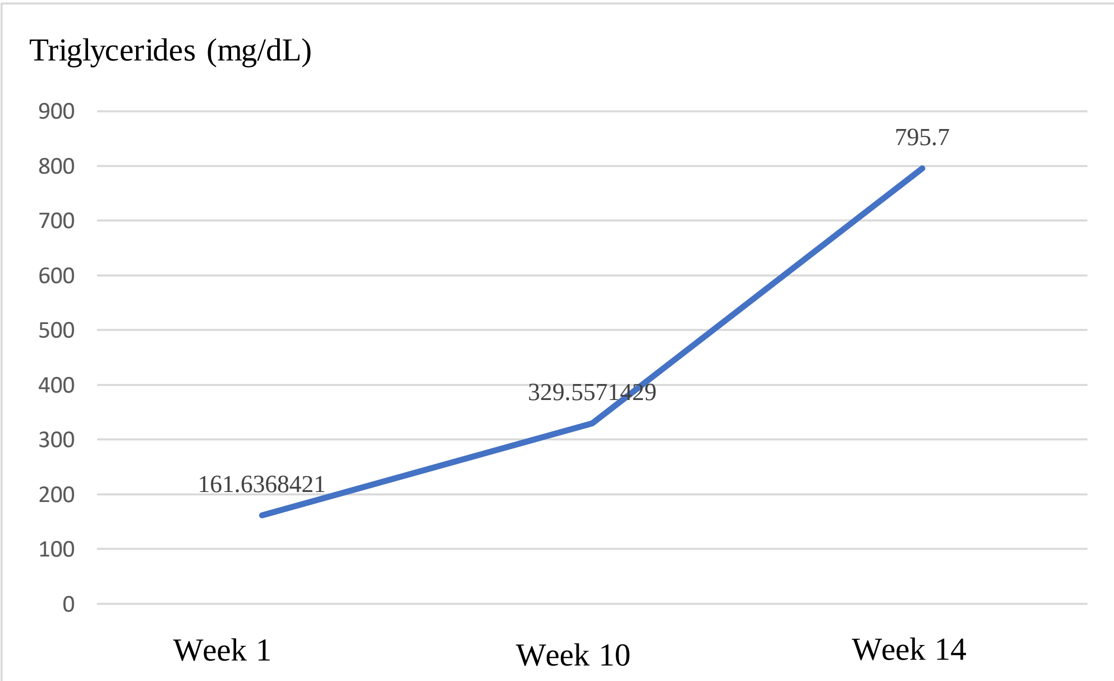


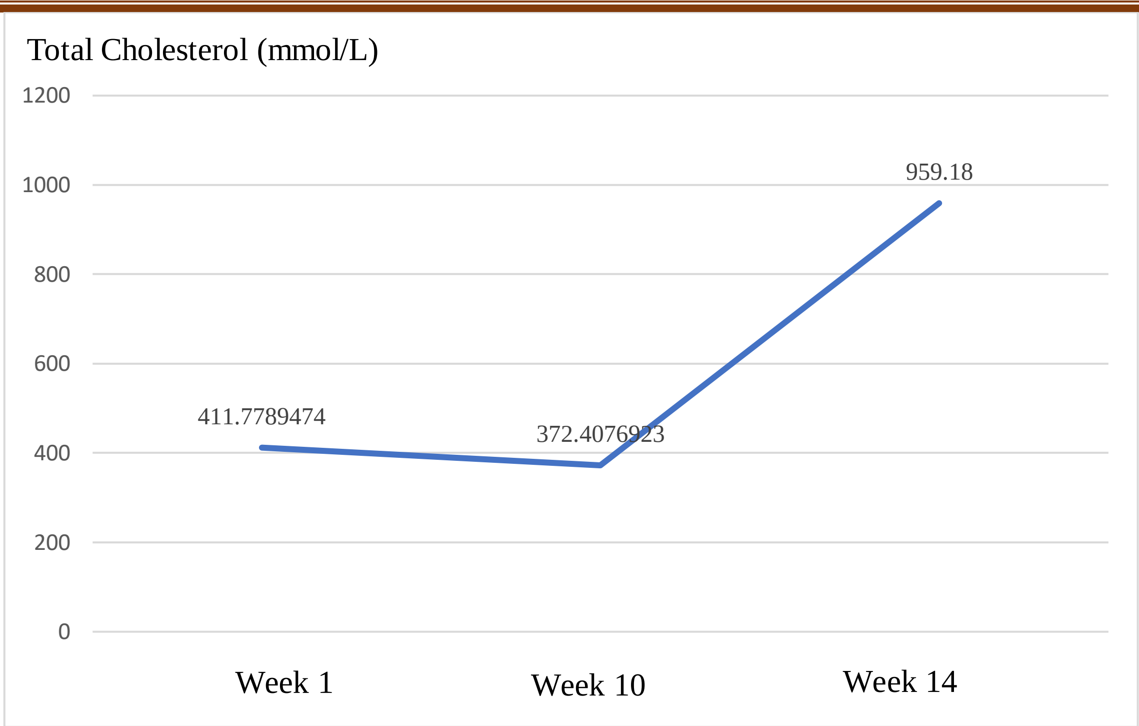
Figure 3. Arterial cross section with Masson's trichrome staining. Atheroma plaque with a necrotic nucleus with cholesterol deposits, abundant inflammatory cells and a fibrous layer. Both images in 20x.



Graph 1. Average weekly weight of the subjects. X = Week. Y = Weight (grams).



Graph 2. Triglyceride levels.



Graph 3. Total colesterol levels.