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BRIEF REPORT

Exploring the effects of the atherosclerosis progression and the choice of affected arteries in the design of experiments with Apolipoprotein E-deficient mice

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KEYWORDS
Apolipoprotein E;
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Animal models

Abstract The objective of this study is to explore the longitudinal progression of atherosclerosis and the correlation between methods to measure the lesion in apolipoprotein E-deficient mice. Atherosclerosis progression was assessed by measurements of foam cell-rich depositions in their proximal aortas, and/or in surgically excised arteries, to assess the histological luminal narrowing. A longitudinal study was performed by comparing the values for carotid, aorta, and femoral and iliac arteries using common histological techniques. There were no significant differences in progression between different arteries, but correlation with the classical measurement of atherosclerosis in the aortic root was poor. Each laboratory requires specific standardization. Carotid arteries were sensitive to atherosclerosis in these mice, and progression was exponential. In conclusion, morphometric data show the importance of the choice of the duration of treatment, the appropriate controls, and the age at which to begin the experiments.

PALABRAS CLAVE
Apolipoproteína E;
Aterosclerosis;
Enfermedad arterial periférica;
Modelos animales

La exploración de los efectos de la progresión de la aterosclerosis y la elección de las arterias afectadas en el diseño de experimentos con ratones deficientes en apolipoproteína E

Resumen El objetivo de este estudio es explorar la progresión longitudinal de la aterosclerosis y la correlación entre los métodos para medir la lesión en los ratones deficientes en

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apolipoproteína E. La progresión de la aterosclerosis se evaluó mediante mediciones de depósiciones ricas en células espumosas en las aortas proximales y/o en las arterias extirpadas quirúrgicamente para evaluar histológicamente el estrechamiento luminal. Se realizó un estudio longitudinal, y los valores para la carótida, la aorta, las arterias femorales e ilíacas se compararon mediante técnicas histológicas comunes. No hubo diferencias significativas en la progresión entre las diferentes arterias, pero la correlación con la medición clásica de la aterosclerosis en la raíz aórtica era pobre. Cada laboratorio requiere su normalización específica. Las arterias carótidas fueron sensibles a la aterosclerosis en estos ratones y la progresión fue exponencial. En conclusión, los datos morfométricos muestran la importancia en la elección de la duración del tratamiento, los controles apropiados y la edad a la cual comenzar los experimentos.

Introduction

Atherosclerosis is the single most important contributor to cardiovascular disease. Mechanistic studies are difficult in humans for obvious reasons and the number of unanswered questions is remarkable. However, emerging evidence, mainly obtained in the study of carotid atherosclerosis, suggests that regression and/or stabilization of atherosclerosis in humans are achievable goals.[1] Promising procedures such as to increase the efflux of lipids from plaques or to facilitate the emigration of foam cells out of the arterial wall only can be currently envisioned in animal models but further progress will probably require better models and/or more conclusive interpretations.[2,3] Current atherosclerosis-susceptible animal models have provided valuable insights in preclinical studies, but in this stage of knowledge, further achievements will require a complete understanding of the limiting factors.

Atherosclerosis in humans is a lifelong, insidious disease, in which it is difficult to explore the course and the effects of continuing management. To prove, discard or improve certain hypotheses, rodent models of atherosclerosis, and in particular knockout (KO) models, are extremely useful but they are highly susceptible to liver injury, and results are not interchangeable, poor reproducibility is common and limitations are difficult to appreciate under some circumstances.[4,5] After the generation of Apolipoprotein E (Apo E) deficient mice, the generated prospects were high and have certainly provided useful data.[6–8] The main advantage of these mice is likely the seemingly spontaneous development of atherosclerosis. Furthermore, as compared with other models, there is no need for dietary or surgical manipulations but a consensus view is difficult to achieve due to different opinions in the proper design of experiments.[3,9,10] Extrapolations are also difficult. For example, the deficiency of Apo E is responsible of lipoprotein alterations not described in humans and it is unlikely that these modified mice could replicate the age-related distribution of lesions in humans.[11]

In this study, we explore a particular concern on how to reach decisions related to the duration of pharmacological treatments and the age at which to begin the experiments, which are troublesome factors in the design of preclinical studies. The longitudinal analysis of atherosclerosis progression in different sites and the correlation between methods to measure the lesion development may clarify important issues to improve validity of the conclusions.

Materials and methods

All procedures were carried out in accordance with institutional guidelines (CEIA, 2014-237). We measured the atherosclerotic lesions in collected samples from experimental Apo E-deficient mice sacrificed at 10 and 24 weeks of age (8 males and 8 females at each time-point). This is the duration study commonly used in experiments according to available literature and we also used a common method to score lesions just beyond the aortic sinus.[12,13] Values were compared to those obtained histologically in carotid arteries. Samples were embedded in paraffin and stained with haematoxylin and eosin. The area of the lumen was quantified by using AnaliSYS™ (Soft Imaging System, Münster, Germany). To avoid the influence of high-fat diets, mice in a C57BL/6J background obtained by inbreeding of mice purchased to Jackson Laboratory were housed under standard conditions and given a commercial, low-fat, mouse diet (14% protein rodent maintenance diet, Harlan, Barcelona, Spain). Results prompted us to design a longitudinal study with male mice allocated in 6 groups ($n = 5$, each) and sacrificed at 16, 24, 36, 44, 50 and 60 weeks to remove aorta and main peripheral arteries to histologically analyze atherosclerotic lesions.

Results and discussion

We confirm that atherosclerosis progression in female mice is higher than observed in male mice in measurements beyond the aortic sinus. In contrast, this difference was not appreciable with serial histological sectioning of carotid arteries. Therefore, gender should be considered a confounding factor and, as such, it should be clearly stated in the description of results. When comparing the measurement of atherosclerosis progression in the aortic sinus and in carotid arteries, values of the Pearson's correlation coefficient were not significantly different from zero at the