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Pathogenetic role of hypercholesterolemia in a novel preclinical model of vascular injury in pigs☆

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ABSTRACT

Objective: Most strategies against intimal hyperplasia developed in several preclinical models failed in terms of clinical application, often due to a discrepancy between animal and human disease. The aim of this study was to setup for the first time a porcine vascular injury model with mild hypercholesterolemia able to significantly increase the degree of stenosis resembling human settings and investigate the pathogenetic role of hypercholesterolemia on protective genes and inflammatory response affecting matrix deposition and cell proliferation.
Methods: Pigs were fed with standard (SD, $n=7$) or high-cholesterol diet (HCD, $n=7$) for 120 days. A balloon angioplasty injury was induced in carotid arteries.
Results: Hypercholesterolemia induced a mild significant increase of total and LDL cholesterolemia. HCD significantly increased the degree of stenosis ($48\pm3\%$ vs. $13\pm4\%$, $p=0.001$), with induction of cell proliferation, matrix deposition, TGF-β1/TGFβRII and MMP2 expression and reduction of collagen. The reduced expression of the protective gene heme oxygenase-1 and inducible-nitric oxide synthase in HCD was associated to a systemic inflammation with a significant increase in circulating leukocytes, serum IFN-γ and TNF-α and a local inflammatory response with an increase of CD3-positive cell infiltrates. There was a significant correlation between CD3 infiltrates and the degree of stenosis.
Conclusion: We developed for the first time a porcine vascular injury model with mild hypercholesterolemia able to significantly increase the degree of stenosis and showed the pathogenetic role of hypercholesterolemia on intimal hyperplasia. New therapeutical strategies to prevent restenosis can be tested in this preclinical hypercholesterolemic model resembling human disease.

1. Introduction

Hypercholesterolemia is one of the most important risk factors in vascular diseases. Nearly 50% of adults have total cholesterol concentrations at the level that the National Cholesterol Education Program (NCEP) expert panel considers "borderline-high risk" [1].

Restenosis limits the success of many vascular interventions, including bypass grafting, endarterectomy and stenting [2]. Several preclinical models of restenosis have been developed for the study of the pathophysiology and research for innovative treatments [3]. The possibility to translate the results in humans depends on how preclinical models can mimic human diseases in terms of pathogenesis.

Intimal hyperplasia (IH) is characterized by vascular injury resulting from cell proliferation, matrix deposition, systemic and local inflammation. The proliferation and migration of vascular smooth muscle cells (VSMCs) in response to various inflammatory stimuli is one of the features of restenosis [4]. When VSMCs acquire the capability to migrate they switch their phenotype from a contractile to a synthetic state, being able to synthesize more extracellular matrix (ECM). Transforming growth factor-beta 1 (TGF-β1) contributes to restenosis stimulating neointimal cell proliferation and ECM synthesis and remodeling, with an enhancement of matrix metalloproteinase 2 (MMP-2) expression [5].

Current evidences support a central role of inflammation in all phases of the vascular disease. The inflammatory response following vascular injury has been studied at cellular and molecular level

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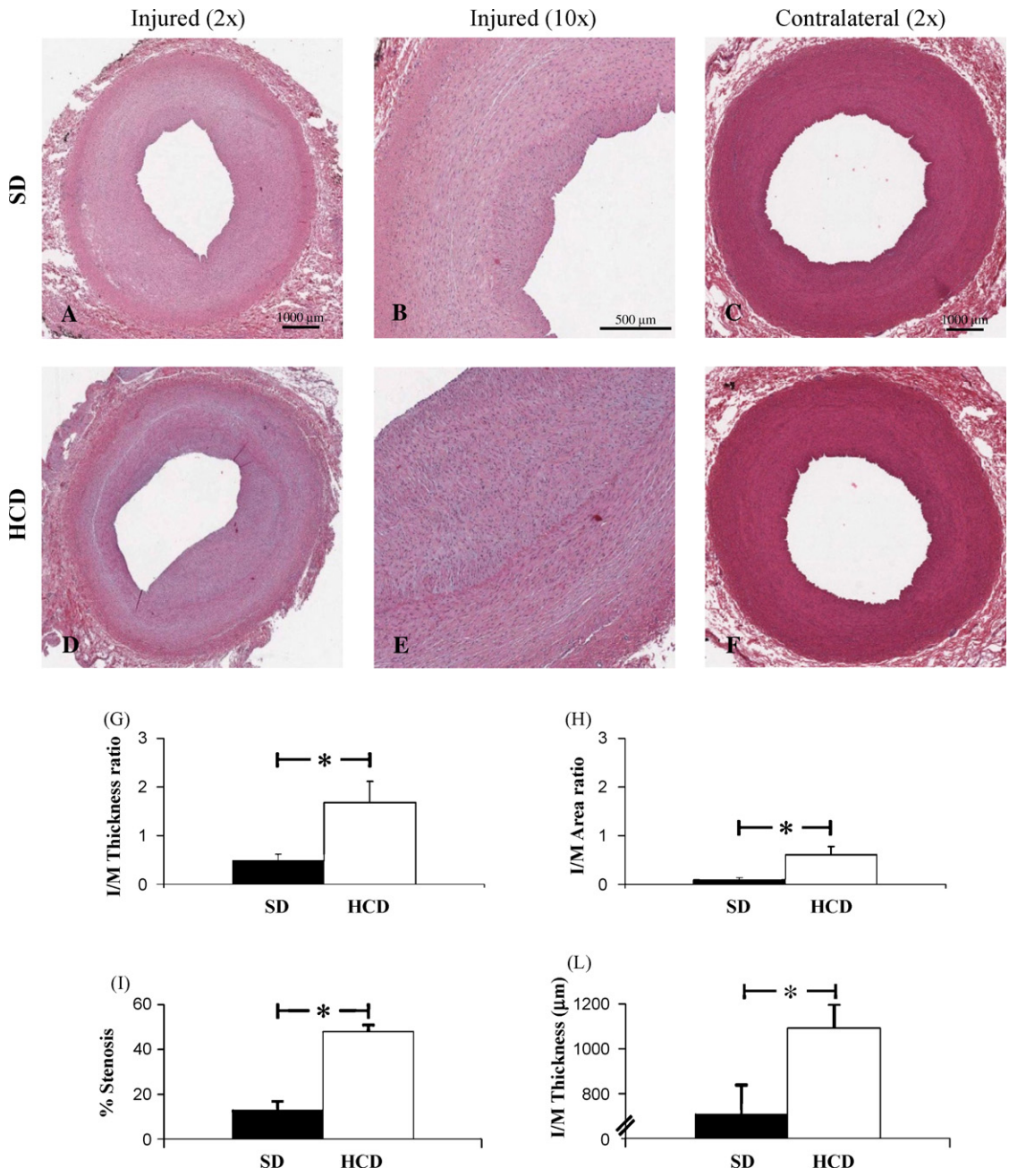

Fig. 1. Hypercholesterolemia exacerbated intimal hyperplasia after vascular injury in pigs. Balloon angioplasty induced a vascular injury in carotid of both SD and HCD pigs, without affecting contralateral vessels (A and F). HCD significantly increased I/M thickness ratio, I/M area ratio, the degree of stenosis and IM thickness in carotid arteries harvested 60 days after BI respect to SD (G–L). Magnification 2× (A, C, D and F) and 10× (B and E). $^{*}p < 0.05$.

[6], demonstrating the pathogenetic role of circulating acute-phase reactants, such as TNF-α [7].

Nitric oxide (NO) and heme oxygenase-1 (HO-1) are protective molecules on vasculature with antioxidant and anti-inflammatory activity [8]. The assessment of HO-1 and inducible-nitric oxide synthase (iNOS) expression in a preclinical model of vascular injury will shed light on the role of these protective genes [8] in the pathogenesis of restenosis, similarly to transplant-associated vasculopathy, allowing to identify new therapeutical targets [8].

There is a huge amount of studies on the pathogenesis of vascular injury aiming at the development of new therapeutical strategies, but unfortunately most of the results later proved ineffective in patients. One of the reasons is that current animal models do not accurately reflect the pathogenesis in humans [9]. An additional reason for false-positive preclinical results may arise from the evaluation of histopathologic parameters, such as the intima-media thickness ratio, without also considering clinically validated parameters, such as the degree of stenosis and intima-media thickness, widely used in clinical trials [10].

The impact of hypercholesterolemia and inflammation has received considerable attention in the last decade, but the mechanism by which borderline hypercholesterolemia exacerbates vascular injury in a clinically relevant preclinical model has never been assessed.

The aim of this study was to setup for the first time a porcine vascular injury model with mild hypercholesterolemia resembling human settings able to significantly increase the degree of steno-