EXPLORATION OF MECHANISMS LEADING TO PLAQUE INSTABILITY IN A RABBIT ATHEROSCLEROTIC MODEL. PRELIMINARY DATA

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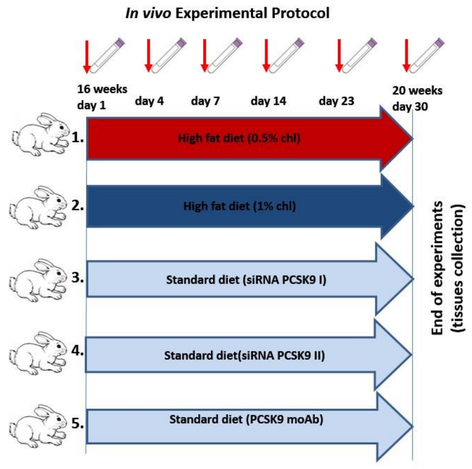
INTRODUCTION

Background. Atherosclerosis is a lipoprotein-driven disease that leads to plaque formation at specific sites of the arterial tree through intimal inflammation, fibrosis, necrosis, and calcification. Sometimes such plaques may suddenly cause life-threatening coronary thrombosis clinically described as acute coronary syndrome.

Hypothesis. An atherosclerotic rabbit model, with a lipoprotein metabolism similar to the human patient, have been proposed to explore the mechanisms leading to plaque instability. We expect to identify an unambiguous alarmin expression pattern, associated to specific atherogenic signalling pathways that may explain the residual risk of cardiovascular-related events and associated mortality.

Aim. The project will explore the plaque instability and the early signs of risks and/or protective factors in coronary disease by using an experimental design comprising both the classical pharmacology (statin administration) and the newest generation of molecular biology approaches (RNA silencing or biological active agents such as PCSK9 antibodies). In preliminary studies the hypercholesterolemic food concentration and treatment to be used was evaluated.

METHODS

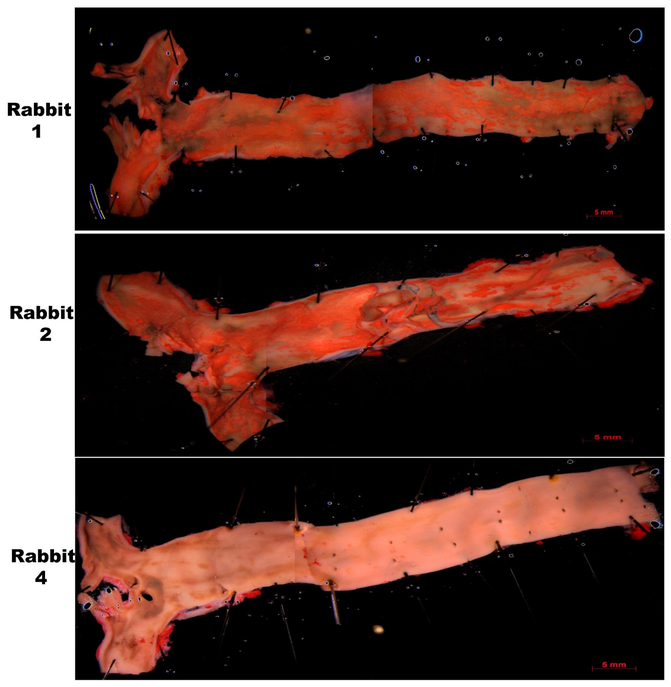


A schematic of experimental design

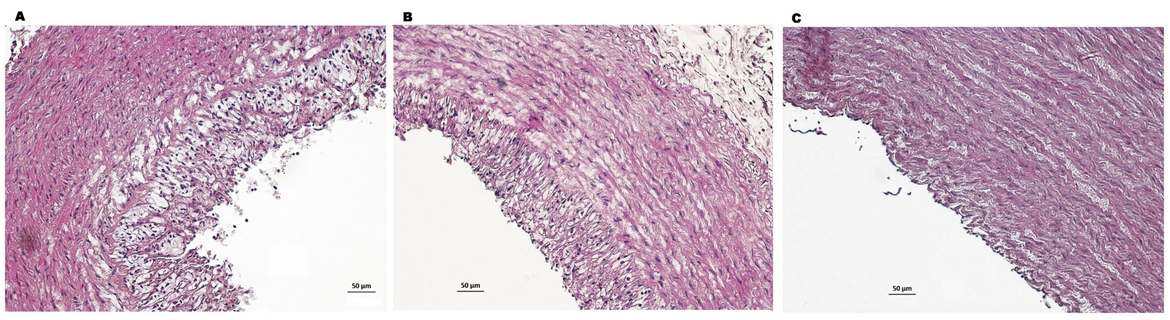
Adult male New Zeeland White rabbits (16 weeks-old) were randomly split into 5 groups: two animals were fed a diet containing either 0.5% or 1% high cholesterol diet and three animals were fed a standard diet together with inhibition of PCSK9 for 4 weeks. The inhibition of PCSK9 were obtained either with siRNA (molecule I or II) or using monoclonal antibodies against PCSK9 (Evolocumab, Repatha, AMGEN).

RESULTS

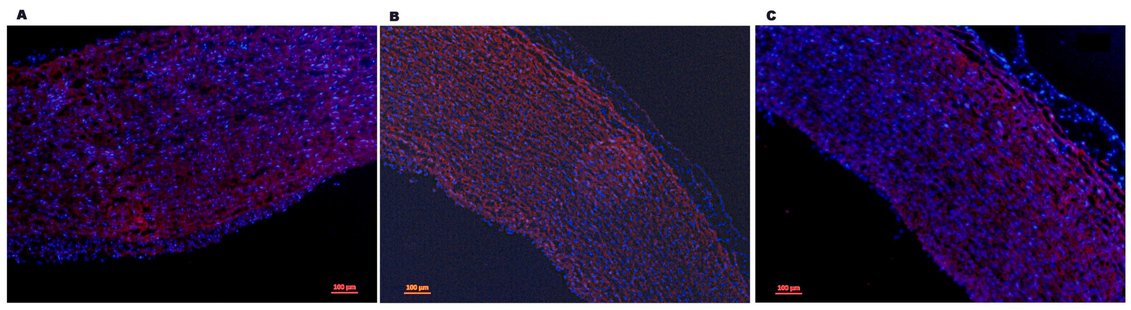
Hyperlipidemic diet induces atherosclerotic plaque progression



The presence of atherosclerotic lesions were assessed by Oil Red O staining. Representative images of aortic atherosclerosis in cholesterol-fed rabbits (rabbit 1 and rabbit 2), comparative with rabbit 4 that were fed a standard diet.

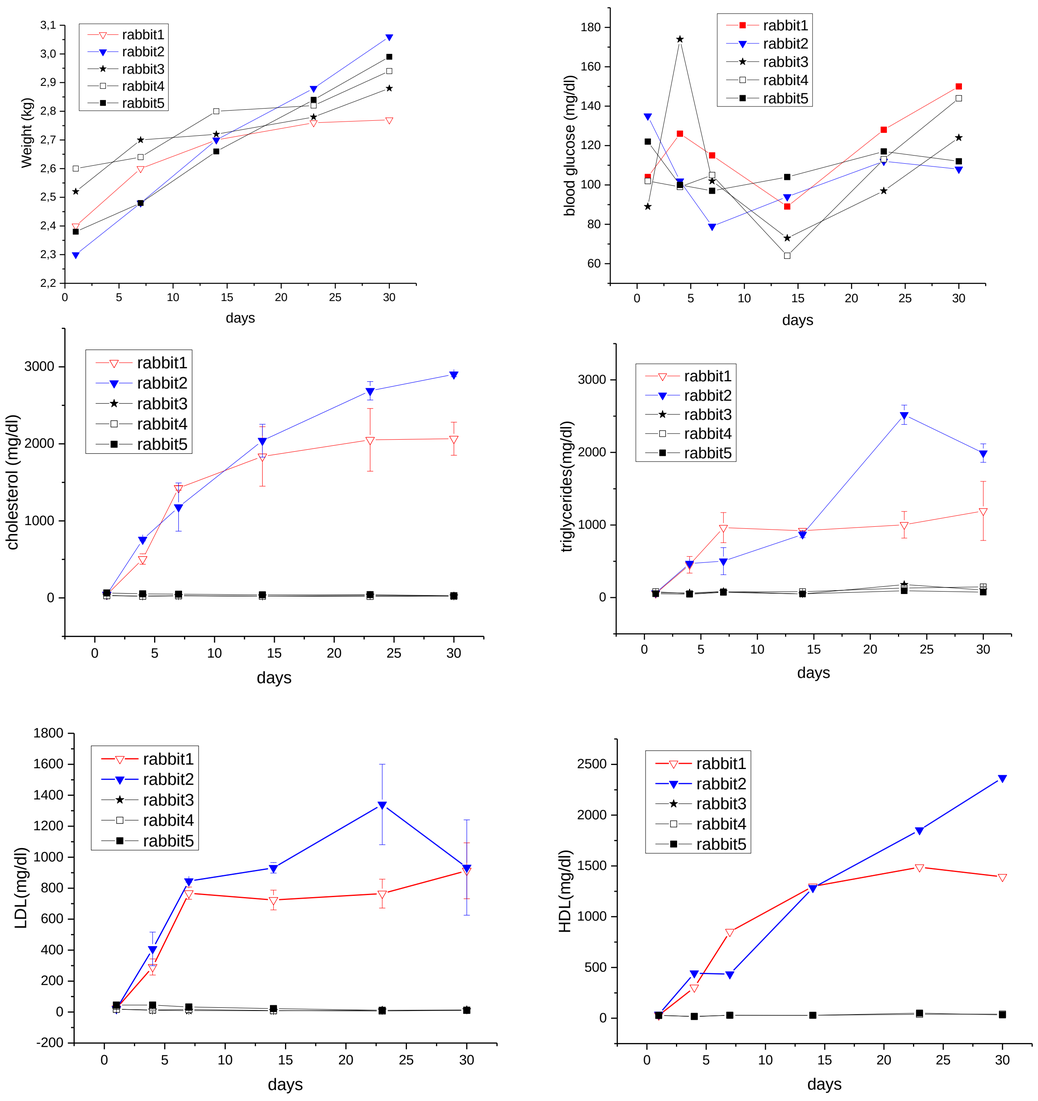


Microscopically atherosclerotic lesions. The presence of atherosclerotic lesions were assessed by hematoxylin and eosin staining, that is essential to evaluate the quality of the lesions (A, B) such as cellular and extracellular matrix component features and plaque vulnerability.

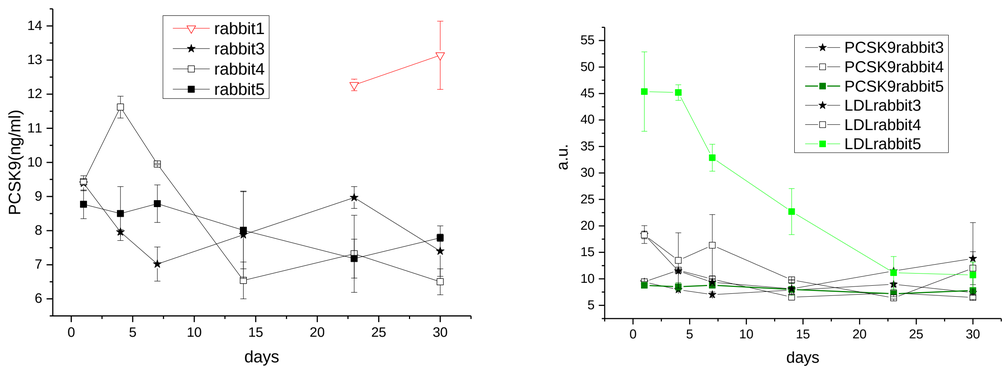


Immunohistochemical investigations of the lesions. In the present study, we found that irrespective of the cholesterol concentration diet the atherosclerotic plaques (A, B) higher expression of α-smooth muscle actin were detected than in the animals fed a standard diet (C).

Hyperlipidemic diet induces a significant increase in lipid parameters in the plasma



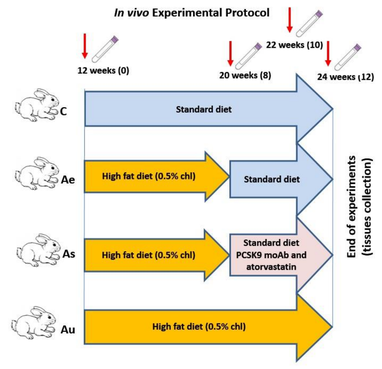
Body weight, blood glucose and plasma lipid profiles. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and triglyceride levels were measured by using Dialab kits.



Levels of PCSK9 protein in plasma samples were detected by ELISA kit. The administration of anti PCSK9 antibody showed a decrease of the plasma PCSK9 protein and LDL in the treated animals.

CONCLUSIONS

The preliminary results suggest that hypercholesterolemic diet induces atherosclerotic plaque progression in rabbit model and for more advanced lesions, the 0.5% cholesterol diet will be extended to 12 weeks. The inhibition with anti PCSK9 demonstrated to be functioning in the decreasing of LDL plasma and could be successfully used in the proposed study.



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