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LDL apheresis activates the complement system and the cytokine network, whereas PCSK9 inhibition with evolocumab induces no inflammatory response

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Inflammation;
Evolocumab

BACKGROUND: Low-density lipoprotein (LDL) apheresis is an extracorporeal treatment modality used in high-risk coronary patients. It may, however, induce complement activation and downstream inflammation due to bio-incompatibility.

OBJECTIVE: We explored changes in soluble inflammatory markers when changing from LDL apheresis to the novel PCSK9 inhibitor evolocumab.

METHODS: Three patients with familial hypercholesterolemia participated. Blood samples (EDTA plasma) for complement activation and markers of inflammation were obtained before (baseline) and after LDL apheresis week at 0 and before biweekly administration of evolocumab at weeks 1, 3, 5, and 7. Complement activation was measured by ELISA and cytokines by multiplex technology.

RESULTS: Complement activation products C3a and Bb were both significantly higher after LDL apheresis compared to baseline ($P = .01$), returned to baseline levels before administration of evolocumab and remained low through week 7. C4d was unchanged during LDL apheresis, whereas TCC was slightly higher after apheresis compared to baseline and week 7 without statistical difference. MCP-1 was higher after LDL apheresis compared to baseline ($P = .04$), returned to baseline levels before administration of evolocumab and remained low through week 7. There were minor changes for other cytokines including TNF, IFN-γ, MIP-1α, MIP-1β, with some higher and some lower after apheresis; however, none of these changes were statistically significant. Fibrinogen and CRP were lower after LDL apheresis and had returned to levels comparable to baseline at week 7, statistically significant however only for fibrinogen.

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CONCLUSIONS: LDL apheresis activated the alternative complement system significantly as reflected by an increase in C3a and Bb. PCSK9 inhibition did not affect complement or cytokines during 7 weeks follow-up.

Introduction

Low-density lipoprotein (LDL) apheresis is an established treatment in familial hypercholesterolemia when drugs cannot adequately control cholesterol levels or when side effects or contraindications preclude the use of oral medication.[1] It has been shown to efficiently reduce LDL as well as clinical endpoints.[2] However, the apheresis filters may also activate various parts of the innate immune system,[3] including the complement system, which in turn may prove to be disadvantageous in the process of atherosclerosis.[4] Complement activation can occur through either the classical, the lectin, or the alternative pathway,[5] and we have previously shown that the latter is responsible for most of the activation in LDL apheresis.[3] Either activation pathway leads to the cleavage of C3 to C3a and C3b in the common pathway. This in turn cleaves C5 into C5a and C5b and induces the formation of the terminal complement complex (TCC) in the terminal pathway.

Recently, various proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have been approved for the treatment of hypercholesterolemia, and in larger trials, they have been shown to reduce LDL cholesterol by 50%–60% and Lp(a) by 20%.[6,7] They also appear to reduce cardiovascular events,[8] although results from clinical trials especially designed to investigate hard endpoints can first be expected in late 2016 and 2017. We have recently shown that when patients with familial hypercholesterolemia (FH) established in long-term, weekly LDL apheresis were switched to PCSK9 inhibition by biweekly subcutaneous administration of the monoclonal antibody evolocumab, LDL levels were equally well controlled.[9] Moreover, evolocumab did not lower high-density lipoprotein (HDL), in contrast to what was seen after apheresis. In the present article, we report on the effects of such a transition on soluble markers of the innate immune system.

Material and methods

The study design and results regarding lipid parameters have recently been published.[9] In brief, it was an observational study with three FH patients established in long-term LDL apheresis. Treatment was converted to a PCSK9 inhibitor (evolocumab), and the patients were examined immediately before and after their last apheresis treatment (week 0), after 1 week (immediately before the first evolocumab injection [week 1]), then biweekly before administration of evolocumab (weeks 3, 5, and 7).

Patients and ethics

The patients had genetically confirmed heterozygous FH (C210 G mutation in the LDL receptor gene, a common FH mutation affecting the binding domain of the LDL receptor). There were two women and one man (53 ± 3 years) who all had angiographically verified coronary artery disease. They were intolerant to statins due to myalgia, and they did not use any type of lipid-lowering medication. The patients had been treated weekly with LDL apheresis for 135 ± 13 months. Baseline characteristics of the patients are shown in Table 1. All three patients signed informed consent and the local ethics committee approved the study.

LDL apheresis

The last LDL apheresis (week 0 of the study) was performed with LDL column Cascadeflo-EC-50W (Asahi Kasei Medical Europe) after previous plasma separation with Plasmaflo OP-50 (Asahi Kasei Medical Europe), using the Infomed HF440 apheresis machine (Infomed SA, Geneva, Switzerland). Anticoagulation was obtained by heparin.

Evolocumab treatment

Evolocumab was administered according to the manufacturer's instructions in week 1, 3, 5, and 7 with the recommended dose of 140 mg subcutaneously (autoinjector). The injections were performed by the patients in the hospital, supervised by experienced nurses, after demonstrations with dummy autoinjectors.

Blood samples and analyses

Fasting blood samples were obtained from the arteriovenous fistula (week 0) or by standard venipuncture

Table 1 Baseline characteristics of the three study subjects

Age, y (SD)	53 (3)
Gender (males/females), n	1/2
Coronary heart disease, n	3
C210 G mutation, n	3
Months in apheresis (SD)	135 (13)
Historically high LDL, mmol/L (SD)	10.3 (0.8)
LDL in apheresis, pretreatment, mmol/L (SD)	5.5 (0.9)
HDL in apheresis, pretreatment, mmol/L (SD)	1.0 (0.2)
Lp(a) in apheresis, pretreatment, mg/L (SD)	484 (76)

Data as mean (standard deviation) or numbers (n).