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Complement profile and activation mechanisms by different LDL apheresis systems

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ABSTRACT

Extracorporeal removal of low-density lipoprotein (LDL) cholesterol by means of selective LDL apheresis is indicated in otherwise uncontrolled familial hypercholesterolemia. During blood–biomaterial interaction other constituents than the LDL particles are affected, including the complement system. We set up an ex vivo model in which human whole blood was passed through an LDL apheresis system with one of three different apheresis columns: whole blood adsorption, plasma adsorption and plasma filtration. The concentrations of complement activation products revealed distinctly different patterns of activation and adsorption by the different systems. Evaluated as the final common terminal complement complex (TCC) the whole blood system was inert, in contrast to the plasma systems, which generated substantial and equal amounts of TCC. Initial classical pathway activation was revealed equally for both plasma systems as increases in the C1rs–C1inh complex and C4d. Alternative pathway activation (Bb) was most pronounced for the plasma adsorption system. Although the anaphylatoxins (C3a and C5a) were equally generated by the two plasma separation systems, they were efficiently adsorbed to the plasma adsorption column before the "outlet", whereas they were left free in the plasma in the filtration system. Consequently, during blood–biomaterial interaction in LDL apheresis the complement system is modulated in different manners depending on the device composition.

1. Introduction

Heterozygous familial hypercholesterolemia is common and, due to high levels of low-density lipoprotein (LDL) cholesterol, carries a high risk of premature atherosclerosis if not treated [1]. In most cases the disease is controlled by lipid lowering medication, but in some instances extracorporeal treatment by means of LDL apheresis is necessary [2]. This treatment is highly effective in reducing LDL cholesterol and clinical end-points [2,3]. The artificial surfaces may, however, affect other constituents of the blood in an adverse manner. Studies on blood–biomaterial interaction during extracorporeal treatment have demonstrated that complement activation may be triggered by biomaterial surfaces [4], and studies in hemodialysis have shown that hemodialysis membranes trigger the complement system [5]. The biocompatibility of dialysis membranes is also linked to clinical end-points [6]. Studies indicate that the alternative pathway (AP) of complement activation is important when foreign surfaces interact with blood [7,8]. The alternative pathway can be activated directly by the surface or amplified after initial activation by classical or lectin pathway activation [4,9], in both cases playing a pivotal role in the degree of activation beyond C3. Notably, even if the biomaterial surfaces induce complement activation, the membranes may also adsorb complement factors such as C3a and C5a [10]. Consequently, it is the net result after extracorporeal treatment that is of clinical importance. This is in accordance with the definition of biocompatibility as being "the ability of a material to perform with an appropriate host response in a specific application" [11], recently revised to "Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy" [12].

Complement activation may be of particular clinical importance for patients undergoing long-term, potentially lifelong, LDL apheresis treatment as the complement system plays a role in the development of atherosclerosis [13]. Whereas activation of the initial

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phase (C1–C3) might contribute to the clearance of plaque debris, the later phase (C5–C9) may enhance inflammation and lead to plaque destabilization. Our previous work has indicated that three LDL apheresis columns affect biomarkers, including complement activation products, differently [14,15]. This prompted us to set up an ex vivo study investigating in detail the complement compatibility of these LDL apheresis columns by repeatedly circulating whole blood from healthy donors through the systems while sampling at sites prior to and after plasma separation and column passage. Total complement activation was evaluated by measuring the fluid phase terminal C5b–9 complex (TCC). The initial pathway activation mechanisms were revealed by determining the levels of C1rs–C1 inhibitor complexes (C1rs–C1inh) for the classical pathway, C4d for the classical and the lectin pathways, and Bb for the alternative pathway. The "inflammatory load" from the common activation of all pathways was evaluated as the amounts of the potent anaphylatoxins C3a and C5a.

2. Materials and methods

2.1. Ethics

The local ethics committee approved the study and all blood donors signed an informed consent.

2.2. Donors

Blood was drawn from six healthy donors (three males and three females). Each individual donated 450 ml of blood three times at approximately one month intervals.

2.3. LDL apheresis

The following devices were compared: Liposorber1 D DL-75 (DL-75) (Kaneka Corp.n, Osaka, Japan); Liposorber1 LA-15 (LA-15) (Kaneka Corp.); Cascadeflo EC-50W (EC-50W) (Asahi Kasei Medical Europe). Treatment with the DL-75 and LA-15 columns was conducted using a Kaneka MA-03 (Kaneka Corp.) machine. The OctoNova (MeSys Gmbh, Hannover, Germany) machine was used in treatment with the EC-50W column.

DL-75 is a whole blood adsorption column and part of the Liposorber D system. This column utilizes dextran sulfate cellulose beads for adsorption of LDL cholesterol. It is modified with regard to the particle size and allows for perfusion and adsorption of LDL cholesterol directly from whole blood. The flow rate was 30 ml min^{-1}. LA-15 is a dextran sulfate cellulose adsorption column which removes LDL cholesterol from plasma. It is based on electrostatic binding between positively charged apolipoprotein B (ApoB) and the negative charges of dextran. The flow rate was 100 ml min^{-1}, the plasma flow rate 20 ml min^{-1}. Cascadeflo EC-50W is a lipid filtration system eliminating LDL cholesterol on the basis of molecular weight and three-dimensional structure. The flow rate was 100 ml min^{-1}, the plasma flow rate 20 ml min^{-1}.

The plasma separation column used in both LA-15 and EC-50W is a PlasmaFlo OP-05 W column (Asahi Kasei Medical Europe) and hence the column is identical for both the plasma separation based systems.

Anticoagulation is mandatory during clinical apheresis treatment. In whole blood apheresis (DL-75) acid citrate dextrose-A is used, while in the two plasma systems (LA-15 and EC-50W) heparin is commonly used. In the current ex vivo model lepirudin was used, as it has been demonstrated that lepirudin does not affect the complement system [16].

A total of 18 ex vivo LDL apheresis treatments were performed, for six donors on the three different LDL apheresis columns (DL-75, LA-15 and EC-50W). Treatment time was 240 min for each column.

2.4. Ex vivo model

25 mg lepirudin (Refludan, Celgene, Marburg, Germany) in 50 ml of 0.9% NaCl was added to a 600 ml Blood-Pack Unit without anticoagulant (Fenwal, Lake Zürich, IL). The freshly donated whole blood (450 ml) was immediately transported to the research laboratory (transportation time <5 min). 50 ml was transferred to a control bag (sample control (SC)) similar to that mentioned above (Fig. 1). The main bag served as a reservoir for the closed circuit. The blood reservoir and the control bag were then placed in a temperature controlled heater (Binder, Binder GmBH, Tuttlingen, Germany) set at 37 °C, with constant movement by means of a modified test tube rotator (Rock 'n Roller, Labinco BV, Breda, The Netherlands). The reservoir was attached to the LDL apheresis system (DL-75, Fig. 1A, LA-15 and EC-50W, Fig. 1B).

Blood samples were obtained from the control bag (SC) before LDL apheresis (T_0), and then during apheresis at 15, 30, 60, 120, 180 and 240 min at the positions shown in Fig. 1. All blood samples from the different positions were drawn simultaneously (within a time frame of 1 min) for each sample time.

2.5. Blood samples and analyses

Samples were drawn into tubes containing EDTA (to block any further complement activation) to a final concentration of 10 mM

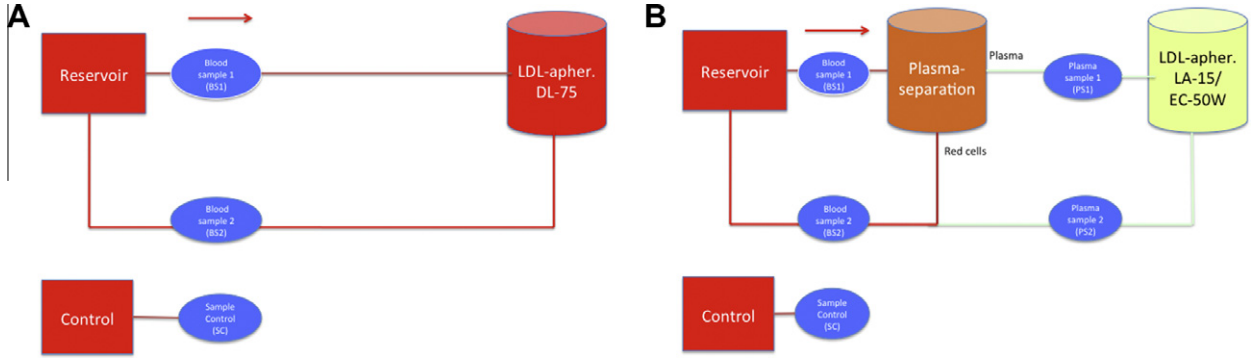

Fig. 1. Schematic drawing of the ex vivo model with blood sample postitions. The reservoir denotes the whole blood bag to which leipuridin was added. (A) Blood samples were obtained from a position after the reservoir (BS1) and then after the LDL apheresis column DL-75 (BS2). A red arrow shows the direction of flow in the system. The control bag was kept on the test tube rotator next to the reservoir and samples were drawn directly from this bag (SC). (B) The columns LA-15 and EC-50W, which required plasma separation before LDL apheresis. The sample sites were before plasma separation (BS1), after plasma separation (PS1), after LDL apheresis (PS2), and after red cells (from plasma separation) and plasma (after LDL apheresis) were combined (BS2), indicating the position where the treated blood would be returned to the patient in a clinical setting. Again, the direction of flow is denoted with a red arrow. As for the whole blood system, samples from the control bag are indicated by SC.