Control of IBMIR Induced by Fresh and Cryopreserved Hepatocytes by Low Molecular Weight Dextran Sulfate Versus Heparin

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Rapid destruction of hepatocytes after hepatocyte transplantation has hampered the application of this procedure clinically. The instant blood-mediated inflammatory reaction (IBMIR) is a plausible underlying cause for this cell loss. The present study was designed to evaluate the capacity of low molecular weight dextran sulfate (LMW-DS) to control these initial reactions from the innate immune system. Fresh and cryopreserved hepatocytes were tested in an in vitro whole-blood model using ABO-compatible blood. The ability to elicit IBMIR and the capacity of LMW-DS (100 µg/ml) to attenuate the degree of activation of the cascade systems were monitored. The effect was also compared to conventional anticoagulant therapy using unfractionated heparin (1 IU/ml). Both fresh and freeze–thawed hepatocytes elicited IBMIR to the same extent. LMW-DS reduced the platelet loss and maintained the cell counts at the same degree as unfractionated heparin, but controlled the coagulation and complement systems significantly more efficiently than heparin. LMW-DS also attenuated the IBMIR elicited by freeze–thawed cells. Therefore, LMW-DS inhibits the cascade systems and maintains the cell counts in blood triggered by both fresh and cryopreserved hepatocytes in direct contact with ABO-matched blood. LMW-DS at a previously used and clinically applicable concentration (100 µg/ml) inhibits IBMIR in vitro and is therefore a potential IBMIR inhibitor in hepatocyte transplantation.

Key words: Innate immunity; IBMIR; Thromboinflammation; Hepatocyte transplantation; Low molecular weight dextran sulfate (LMW-DS)

INTRODUCTION

Minimal invasive methods to treat a variety of medical conditions are continuously under development; in the future, orthotopic liver transplantation (OLT) in some current approaches may be replaced by less invasive cell transplantation procedures. In particular, hepatocyte transplantation (Hctx) shares many of the advantages of other minimally invasive procedures, being less traumatic for the patient and more cost effective for healthcare systems. Hctx would also address the problem of finding a donor at the appropriate time if cells from cryopreserved cell banks could be used. However, despite decades of research, Hctx has thus far had only limited clinical application.

The first hundred reported cases of clinical Hctx were recently reviewed, and although the cell transplant had an ameliorative effect in several cases, no patient was found to be fully cured by this method. The main reason for this failure is the poor engraftment of the transplanted hepatocytes (Hcs); in fact, up to 70% of the transplanted cells are initially cleared in an event that is not prevented by regular heparin and immunosuppressive treatment and might be caused by the innate immune system.

During the transplantation procedure, Hcs are infused directly into the recipient's circulation through the portal vein and are distributed distally into the sinusoids, where they become entrapped. Until the Hcs are integrated into the liver plate, a process that extends over approximately 16–20 h, the cells are in direct contact with the recipient's blood and may trigger innate immune reactions.

We have previously characterized a thromboinflammatory reaction termed the instant blood-mediated inflammatory reaction (IBMIR), an innate immune effect that is elicited by Hcs that are in contact with ABO-matched blood. In vitro perfusion experiments have established that Hcs in contact with blood regularly activate the complement and coagulation systems and induce clot...
formation in conjunction with the recruitment of neutrophils. Within an hour, the Hcs are entrapped in a clot infiltrated by neutrophils. Tissue factor (TF) expressed on the Hcs' surface initiates the coagulation cascade through the TF pathway\(^\text{7–9}\). A patient who was treated by Hctx and monitored for the same parameters during the infusion of Hcs showed a corresponding response\(^1\).

The underlying nature of the thromboinflammation, which results in cell destruction and low engraftment, is complex and involves both cellular components and several cascade systems that share mutual connections and amplification loops. The cascade systems are composed similarly with inactive zymogens that are proteolytically cleaved to yield downstream-acting serine proteases and are simultaneously locally activated in response to danger signals\(^9\). Complement can also contribute to a local enhancement of coagulation through the action of complement factor (C) 5a, which can induce TF expression on leukocytes\(^1\) and upregulation of plasminogen-activator inhibitor (PAI-1) in mast cells\(^10\). Mannan-binding lectin-associated serine protease-2 (MASP-2) cleaves prothrombin to thrombin, activated factor XII (FXIIa) can cleave complement factor C1, and thrombin can cleave C3 and C5\(^1\). Furthermore, both MASP-1 and MASP-2, which are key molecules in the activation of the lectin pathway of the complement system, are triggered by fibrin\(^1\).

Thus, all these data suggest that it would be attractive to be able to control the upstream triggering of these inflammatory reactions in order to increase the survival of transplanted cells.

Our preliminary experiments with low molecular weight dextran sulfate (LMW-DS) have indicated that this substance might have potential for interfering with the cascade systems that result in IBMRI\(^7\). The objectives of the present study were to further explore this issue. LMW-DS is a synthetic polymer made from 17%–20% \(\alpha\)-glucose monomers, with an average molecular weight of 5 kDa. LMW-DS exerts an anticoagulatory effect through inactivation of FXIa and FXIIa by enhancing the effect of C1 inhibitor (C1-INH)\(^1\). C1-INH also suppresses complement activation in vitro and in vivo\(^15,16\). Other effects of LMW-DS on the innate immunity include an inhibition of toll-like receptor (TLR) 2 and 4, which diminishes NK-cell activation and the maturation of monocyte-derived dendritic cells\(^17,18\). In an unknown way, LMW-DS also interferes with the adhesion of platelets\(^19\). LMW-DS has already demonstrated positive effects in improving islet transplantation\(^20–22\).

**MATERIALS AND METHODS**

**Ethics**

All experimental procedures were conducted in compliance with Swedish law and regulations and approved by the regional Ethics Committee (Dno 2004:M-223). Informed consent was obtained from each donor of human tissue.

**Human Hcs**

Liver tissue was obtained from patients undergoing partial liver resection for primary or secondary tumors. Human Hcs were isolated and handled as previously described\(^23\), based on a three-step perfusion technique according to Strom et al.\(^24\). The isolated liver cells were stored overnight at 4°C in University of Wisconsin (UW) preservation solution (Viaspan; Bristol-Myers Squibb, New York, NY, USA) or cryopreserved.

**Cryopreservation and Thawing of Hcs**

Hcs (1.5 \(\times\) 10\(^7\)) were centrifuged at 50 \(\times\) g for 5 min at 4°C. The supernatant was removed, and ice-cold UW solution was added to the pellet to produce a volume of 4.5 ml; the preparations were then moved to ice-cold cryovials. Dimethyl sulfoxide (DMSO; WAK-Chemie Medical GmbH, Steinbach, Germany) was added dropwise to each vial to a total volume of 5 ml. After 5 min on ice, the cell suspensions were cryopreserved by means of a computer-controlled rate freezer according to a freezing protocol previously described\(^25\). The cells were kept overnight in liquid nitrogen. Before the experiments, the cells were rapidly thawed in a 37°C water bath with gentle agitaton and then transferred to an ice-cold tube. Dilution of the cell suspension and the cryoprotectant with William’s medium E (WME) (Sigma-Aldrich, St. Louis, MO, USA) was performed dropwise on ice, following the times recommended by Steinberg et al.\(^26\).

**Preparation of the Hcs Prior to the Tubing Loop Experiment**

After thawing and dilution, the cryopreserved cells were washed twice and dissolved in WME and kept at 4°C. The Hcs stored in UW solution were also washed twice and then handled in the same way as the cryopreserved cells. Hcs were only used if the reevaluated viability of the cells was \(\geq 70\%\). When necessary, the cells were enriched through a Percoll (GE Healthcare Life Sciences, Uppsala, Sweden) density gradient\(^27\).  

**Human Blood and Platelet-Poor Plasma**

All human blood was obtained from healthy volunteers who had received no medication for at least 2 weeks prior to the experiments. Blood was collected in an open system in which all surfaces that came into contact with blood were coated with immobilized heparin (CHCTM, Corline, Uppsala, Sweden). Platelet-poor plasma (PPP) was obtained from lepirudin (final concentration, 50 \(\mu\)g/ml; Schering AG, Saksa, Germany) anticoagulated blood
that had been centrifuged twice at 3,400×g for 15 min at room temperature (RT).

**Tubing Loop Model**

A previously described Chandler loop model mimicking the portal blood flow was used\(^ {28,29} \). In brief, the loops were made from polyvinyl chloride tubing coated with immobilized heparin (CHC\(^ \text{TM} \)), with an inner diameter of 6.3 mm. The pieces of tubing were placed on a rocking device to generate blood flow of 45 ml/min, within a 37°C incubator. Each loop was loaded with 7 ml of fresh blood, ABO compatible with the Hc donor. A series of experiments were performed; in each experiment, at least one negative control loop was used, containing only blood and 100 µl of WME. Hcs (1 × 10\(^ 5 \)) in 100 µl of WME were added to each of the other loops, and to certain loops LMW-DS (pK Chemicals, Køge, Denmark) or heparin (Leo Pharma AB, Malmö, Sweden) was also added. Prior to and during the experiments, 1 ml of blood was collected from each loop at 5, 15, 30, and 60 min into ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich)-containing tubes (final concentration: 10 mM).

This model was used in different experimental setups.

**Assay to Study the Effects of LMW-DS on Freshly Isolated Hcs**. Two different doses of LMW-DS were used: 100 and 1,000 µg/ml. The LMW-DS was added at the same time that the cells were introduced into the loop. The doses of LMW-DS were extracted from previous studies\(^ {20,22} \).

**Assay to Compare the Ability of Heparin and LMW-DS to Control the IBMIR**. Experiments were performed on fresh liver cells. The effects of both 100 and 1,000 µg/ml of LMW-DS were compared to heparin (Leo) in two different concentrations, 0.5 and 1 IU/ml. The tested doses of heparin (Leo) are within clinical therapeutic applicable concentrations, but the higher dose is in the upper clinical range\(^ {20,26} \). Both substances were added at the time that the Hcs were introduced into the loops. In each series of experiments, the same cells were tested toward the same blood donor in order to compare the two substances.

**Assay for Thromboinflammation Elicited by Freeze–Thawed Hcs Compared to Fresh Hcs**. Freshly isolated Hcs were divided into two portions and stored overnight (14–18 h): one was cold stored in UW solution prior to the experiments, and the other was cryopreserved and thawed prior to use in an experiment. In each series of experiments, the same cells (freeze–thawed or cold stored in UW solution) were tested at the same time toward the same blood from the same ABO-compatible blood donor (Fig. 3A).

**Assay of the Ability of LMW-DS to Control the IBMIR Elicited in Freeze–Thawed Hcs**. The same setup was used as in the previous experimental setup with fresh and freeze–thawed Hcs, but 100 µg/ml of LMW-DS was used in some of the loops.

**Blood Cell Count**

The blood samples (collected in EDTA-containing tubes) retrieved from the loop experiments were immediately used to obtain cell counts in a Coulter AcT differential analyzer (Beckman Coulter, Miami, FL, USA). The remaining samples were centrifuged at 3,000×g for 20 min at 4°C. Plasma was collected and stored at −70°C until analyzed.

**Fibrin-Activated Serine Proteases and the Inhibitory Effect of LMW-DS**

Initially fibrin was prepared by the addition of 0.05 international units (IU) of thrombin (Hoffman-La Roche, Basel, Switzerland) to 400 µg of fibrinogen (Haemochrom Diagnostica, Mölndal, Sweden) at a ratio of 1:2,650 (mol/mol). Clots were formed in polypropylene Eppendorf tubes for 30 min at 37°C and then sonicated.

To study fibrin activation of serine proteases, PPP was further incubated for 30 min at 37°C with 20 µg/ml fibrin (positive control). To analyze the effects of LMW-DS, parallel experiments were performed in which samples were preincubated for 30 min at 37°C with 100 µg/ml LMW-DS before the addition of fibrin. PPP without fibrin and LMW-DS were used as negative controls. Activation of the samples was stopped by the addition of EDTA (10 nM). The samples were then centrifuged at 3,400×g for 15 min at 4°C, and the collected PPP was used for the detection assays.

**Enzyme Immunoassays**

Generation of blood activation markers was measured by enzyme-linked immunosorbent assay (ELISA). The rationale behind this approach is that the serine proteases can only form stable complexes with the serine protease inhibitors (serpins) after they have been activated and thus are capable of cleaving the bait region of the serpins [antithrombin (AT) and C1-INH]. Thrombin–AT (TAT) complexes were measured with commercial ELISA kits (Behringwerke, Marburg, Germany), and FXIa/AT, FXIIa/AT, and FXIIIa/C1-INH were all analyzed according to Sanchez et al.\(^ {31} \), and kallikrein/AT and kallikrein/C1-INH according to Bäck et al.\(^ {32} \). MASP-1/AT, MASP-2/AT, MASP-1/C1-INH, and MASP-2/C1-INH were analyzed as described by Kozarcanin et al.\(^ {11} \). To measure complement activation, C3a and sc5b-9 generation in plasma supernatants was measured by ELISA according to the method of Nilsson Ek Dahl et al.\(^ {33} \).
Effect of LMW-DS on Hc Metabolic Function

Hcs were cultured as previously described\(^{22}\). In half of the cultures, 100 µg/ml LMW-DS was added. Samples were collected from the incubation medium and analyzed for albumin secretion, ureogenesis, and midazolam 1-hydroxylation according to Donato et al.\(^{34}\).

Statistical Analysis

All data, except in Figure 2, are presented as means± standard error of the mean (SEM). For all statistical calculations, Prism, version 5.0 for Macintosh, was used (GraphPad Software Inc., La Jolla, CA, USA). Data from the loop experiments, when normally distributed, were evaluated with Student’s two-tailed matched-paired \(t\)-test. Data in Figure 2 were evaluated using the Wilcoxon nonparametric two-tailed matched-paired test and presented as median and interquartile range (IQR). Values of \(p<0.05\) were considered significant.

RESULTS

Inhibition of Hc-Triggered IBMIR by LMW-DS

LMW-DS was tested at two different doses, with fresh Hcs being perifused for 60 min in ABO-matched blood in the tubing loop model. At both 100 and 1,000 µg/ml, LMW-DS influenced all the measured parameters. Because of the overall effectiveness of the lower dose, only results with this dose are presented (Fig. 1). The level of TAT, reflecting thrombin generation, was only 2% of the level in the positive control tubing with perifused Hcs without any additives (Fig. 1A). Activation of FXII was also significantly suppressed (\(p<0.05\)) by the addition of LMW-DS (Fig. 1C). There was a tendency toward decreased activation of FXI and kallikrein, but the effect did not reach significance (Fig. 1B and D). More than 70% of the platelet count was maintained during the whole experiment when 100 µg/ml of LMW-DS was added to the loops, and the initial level of polymorphonucleocytes (PMNs) was kept constant throughout the observation time (Fig. 1E and F). Complement activation, reflected by C3a and sC5b-9 formation, was significantly decreased by treatment with LMW-DS, but the values for MASP-1/AT were not significantly influenced (Fig. 1G–I). In loops without LMW-DS, all the hallmarks of the IBMIR were observed (Fig. 1A–I).

Inhibition of Hc-Triggered Thromboinflammation by Heparin Compared to LMW-DS

Loop experiments were performed in order to determine whether LMW-DS was equal to or more efficient than conventional anticoagulant therapy (e.g., heparin) in controlling the cascade systems causing cell destruction. Heparin (Leo) had almost no effect at the lower concentration of 0.05 IU/ml, so results are shown for 1 IU/ml and compared to 100 µg/ml LMW-DS. The heparin (Leo)-treated sample maintained 62% of the original platelet count throughout the perifusion, a level that was significantly (\(p<0.01\)) higher than that in the control tubing loop without any additions (only 25% of platelets remaining); there was no significant difference between the heparin (Leo) and LMW-DS treatments in terms of the decrease in platelets. There was also no significant difference between heparin (Leo) and LMW-DS treatment in terms of changes in other cell counts during the experiment, and overall only small changes were observed (Fig. 2A). With regard to the capacity to control the cascade system, LMW-DS was found to be significantly more efficient than heparin (Leo). TAT, FXIIa/AT, C3a, and sC5b-9 formation were all significantly lower in loops containing LMW-DS when compared to loops with heparin (Leo). Heparin (Leo) had no significant effect in terms of reduction of C3a and sC5b-9 when compared to loops with Hcs alone.

Thromboinflammation Triggered by Freeze–Thawed Hcs and Inhibition With LMW-DS

Given the complexity of innate immunity and the fact that different types of surfaces elicit different responses, it is not clear that results from fresh cells are transferable to freeze–thawed cells. Therefore, we designed experiments in which all parameters were kept constant except for the freeze–thawing step to investigate whether the two sets of results would be comparable (Fig. 3A). The triggering of thromboinflammation from freeze–thawed Hcs and cold-stored Hcs were compared after 60 min of perifusion in the blood loop model. Both groups had the same degree of consumption of platelets along with pronounced activation of the coagulation system reflected by the accumulation of TAT (Fig. 3B). Equivalent complement activation, increasing C3a levels, was registered in the two groups. We did not find any significant differences between cold-stored and freeze–thawed Hcs in terms of the tendency to induce thromboinflammatory reactions. Inhibition studies in which LMW-DS was added to the loops at the concentration 100 µg/ml showed that it controlled the cascade systems and cell consumption equally well in freeze–thawed Hcs as in cold-stored Hcs (Fig. 3B).

Fibrin-Activated Serine Proteases and the Inhibitory Effect of LMW-DS

PPP incubated with 20 µg/ml of fibrin revealed triggering of the contact activation system, reflected by the formation of FXIIa-serpin and kallikrein-serpin complexes. Preincubation of the PPP with 100 µg/ml of LMW-DS before the addition of fibrin clearly interfered
Figure 1. Inhibition of fresh hepatocyte (Hc)-triggered IBMIR by LMW-DS in the tubing model after 60 min of perifusion. Human Hcs incubated in the tubing model with ABO-compatible blood. Results are given for preexperimental values and values after 60 min for loops containing Hcs and loops containing Hcs with the addition of 100 µg/ml low molecular weight dextran sulfate (LMW-DS). (A–D) Activation of coagulation and inhibition of LMW-DS. Thrombin-antithrombin (TAT) and activated factor XII (FXIIa)/AT were significantly restored by LMW-DS ($p < 0.05$). (E, F) Changes in cell count after inhibition with LMW-DS. Both platelet and polymorphonucleocyte (PMN) counts were significantly controlled by LMW-DS ($p < 0.05$). (G–I) Inhibition of Hc-triggered complement parameters by LMW-DS. C3a and sC5b-9 were significantly controlled ($p < 0.05$). Changes in MASP-1/AT levels did not reach significance. Data are means ± standard error of the mean (SEM). Lower number ($n$) of observations: the amount of retrieved plasma did not allow analysis of all parameters in all experiments. Inclusion of parameters not previously reported from preliminary experiments with LMW-DS gave a higher $n$ for some parameters.
with this activation and therefore generated lower levels of serine proteases/serpin complexes (Fig. 4A and B). Serpin complexes formed with the lectin complement pathway serine proteases, MASP-1 and MASP-2, were also triggered by fibrin activation of PPP. LMW-DS preincubation caused an equally pronounced decrease in MASP/serpin complexes as it did for the contact activation system (Fig. 4C and D).

**Effect of LMW-DS on Hc Metabolic Function**

Fresh Hcs in culture were evaluated for changes in metabolic function when 100 µg/ml LMW-DS was added to the medium. There was no difference in metabolic function in cells cultured with the presence of LMW-DS compared to cells in medium without additions (Fig. 5).

**DISCUSSION**

The hepatic microcirculation is normally regarded as tolerogenic, given that tolerance can be induced to antigens introduced via the portal circulation but not if the same antigens are introduced elsewhere. However, when Hcs are infused into the portal vein, they do not induce tolerance, but instead are rather rapidly cleared by the innate immunity, an activity that is not prevented by ordinary immunosuppressive treatment. These inflammatory reactions are attributed to the IBMIR that is elicited when transplanted cells activate the blood-borne cascade systems. The IBMIR is mainly triggered by TF expressed on the Hcs surfaces and is further complicated by the fact that TF molecules on Hcs are already largely preloaded with endogenous FVII/FVIIa. There is also an abundant cross-talk between the coagulation and complement systems, and therefore the optimal strategy for prohibiting the instant triggering of thromboinflammation is to target both the complement and coagulation systems during the transplantation procedure.
CONTROL OF IBMIR INDUCED BY LMW-DS

system that is triggered by Hcs in contact with blood. LMW-DS was also able to effectively control the complement activation, an effect that was not achieved with heparin (Leo). In this study, LMW-DS was overall significantly superior in controlling the activation of the cascade systems triggered by Hcs compared to heparin (Leo). However, the cellular levels were restored to levels above those of the negative control tubing by use of either substance.

Fresh Hcs are generally considered to be preferred for transplantation, with a better metabolic profile and superior viability. Nevertheless, cryopreserved Hcs are regularly used in Hctx, since they offer the advantage of being readily available and better characterized before transplantation. In addition to the initial enzymatic digestion, cryopreserved Hcs have undergone two stressful critical procedures, freezing and thawing, and it is not clear what impact this handling has on the tendency to elicit IBMIR. Therefore, we designed experiments where we used isolated Hcs from the same donor that were either cold stored in UW solution or freeze thawed. All other parameters were kept constant since the cells were matched to the same blood donor, and the subsequent experiments were performed at the same time. Our results indicated that the cryopreserved Hcs induced the IBMIR in contact with ABO-compatible blood to the same extent as did fresh Hcs, and LMW-DS was equally effective in preventing the IBMIR in both types of Hcs.

The current study also showed that LMW-DS has the potential to interact with and reduce fibrin-induced activation of the serine proteases FXIIa and kallikrein in the
Figure 4. Fibrin-activated serine proteases and the inhibitory effect of LMW-DS. Fibrin activation of serine proteases in platelet poor plasma (PPP); formed serpin complexes are shown with black bars. White bars indicate start values. Gray bars show the amount of serine protease/serpin complexes formed after preincubation of PPP with 100 µg/ml LMW-DS ($n=3$).

Figure 5. Hcs in culture were analyzed according to metabolic function±LMW-DS. (A) Albumin secretion into the culture medium during 24 h±the addition of 100 µg/ml LMW-DS in WME. (B) Production of urea during 1 h as a product of metabolism of 10 mM NCL±the addition of 100 µg/ml LMW-DS in WME. Boxes indicate medians and IQR. Whiskers indicate the 5–95 percentiles. (C) Cytochrome P450 function (3A4); 1-hydroxylation of midazolam determined by high-performance liquid chromatography (HPLC). Values are means±SEM. There was no significant difference between the two groups with and without the addition of LMW-DS; $n=4$ (each experiment in triplicate).
contact activation system, as well as the MASP-1 and MASP-2 of the complement system. It is likely that the capacity of LMW-DS to interfere with the contact activation system and control the complement system that was not found for heparin (Leo) is reflected in the registered superiority compared to heparin (Leo) in controlling the coagulation system triggered by Hcs.

Taken together, our data indicate that LMW-DS had equally good capacity to control IBMIR from both fresh and freeze–thawed Hcs and was superior to heparin (Leo) in controlling the cascade systems and especially the complement system in thromboinflammatory reactions.

The LMW-DS concentration tested here is in the same range as the levels previously used in a phase I study in which LMW-DS was shown to be safe when infused intravenously, with no adverse effects; furthermore, the tested dose kept the activated partial thromboplastin time (APTT) under 150 s\(^{20–22}\). Avoiding intense anticoagulation is particularly important in patients with liver failure, who often have impaired coagulation and untoward bleeding.

The current study is a limited in vitro study, but the results are positive, and the upstream inhibition of the cascade systems achieved with LMW-DS makes it potentially attractive for clinical applications. LMW-DS inhibits coagulation at the level of FXIa, and since one molecule of FXIa generates up to 1,000 molecules of thrombin\(^{42}\), a broad downstream effect is generated. The complement system also has a large inborn amplification potential, and therefore upstream inhibition is desirable.

With respect to the complexity and redundancy of the immune system, there are probably numerous factors to consider before the detrimental effects of innate immunity can be completely controlled. The environment into which the Hcs are infused is a minefield: the sinusoids are heavily loaded with immune cells, particularly innate immune cells that maintain the balance between tolerance and activation\(^{43}\). Furthermore, the liver produces numerous acute-phase proteins, complement factors, and soluble pathogen-recognition receptors and is therefore predominantly an innate immune organ\(^{44}\). Given these conditions, it is essential to evoke as little reaction as possible from the innate immunity in the initial phase, in order to improve the initial percentage of engrafted cells and also to diminish the linkage to adaptive immunity.

The results of the present study demonstrate that LMW-DS has the potential to control various innate thromboinflammatory responses evoked by Hcs in contact with blood and was overall more efficient than heparin (Leo) in controlling the cascade systems. LMW-DS is therefore a promising substance to replace heparin in order to achieve a better control of IBMIR, with less risk of bleeding, in the context of Hctx. This issue would be interesting to investigate further in in vivo studies.

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