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Heparin coating reduces cell activation and mediator release in an in vitro venovenous bypass model for liver transplantation

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Abstract We used an in vitro model for venovenous bypass in a prospective, randomized study to analyze the effect on leukocytes cell activation after coating the total blood contact surface with covalently bound heparin. In ten experiments heparin-coated circuits were used, and in ten other experiments noncoated circuits were used. Monocyte cytokine production and neutrophil myeloperoxidase release were analyzed. Monocytes were isolated using anti-CD14 paramagnetic beads, and oligo(dT)$_{25}$ beads were used to isolate mRNA before subsequent reverse transcription and semiquantitative amplification of various cytokines in order to determine time-related changes in expression during bypass. After 2 h, mRNAs for IL-1β and IL-6 were highly upregulated in noncoated compared to heparin-coated circuits. Little or no change was seen in the expression of other cytokines. IL-1β and IL-6 were measured in plasma after 12 h and reflected the upregulated mRNAs in noncoated circuits. A significantly reduced release of myeloperoxidase was observed in coated versus noncoated circuits. This indicates that heparin-coated surfaces reduce cellular activation and the release of inflammatory mediators.

Key words Liver transplantation, venovenous bypass, heparin coating · Venovenous bypass, heparin coating, liver transplantation · Heparin coating, venovenous bypass, liver transplantation · Leukocyte activation, cytokines, liver transplantation

Introduction

Venovenous bypass (VVBP) is used routinely during human orthotopic liver transplantation (OLT) to prevent venous stasis in the lower part of the body and splanchnic venous pooling during the anhepatic phase [7, 12, 15]. Both noncoated and heparin-coated VVBP are in use worldwide, but no comparative studies have been performed in order to evaluate these systems. In contrast, several studies have been performed, on heparin coating in cardiopulmonary bypass (CPB) [9, 13, 20]. Unlike VVBP, in CPB an oxygenator and cardiotomy suction are used. As a result, data obtained from CPB studies cannot be directly transferred to VVBP.

In CPB, activation of the body's defense systems has been observed upon exposure of blood to artificial surfaces of the bypass components. Such activation involves both the humoral cascades, such as the kallikrein-kinin, coagulation, fibrinolysis, and complement systems, and cellular activation [4]. Activation of circulating cells plays an important role in the inflammatory response. Release of myeloperoxidase (MPO), a cationic iron-containing enzyme mainly found in the primary granules of neutrophil granulocytes, takes place during CPB [19] and is used as a marker for neutrophil activation [10, 18]. Mononuclear cell activation leads to the release of several cytokines. In response to injury, trauma, or infection, cytokines play an important role in the

acute phase. The proinflammatory cytokines IL-1, IL-6, and TNFα participate in a synergetic manner to mediate inflammation in this acute phase response [3].

The membrane protein mCD14 acts mainly as a receptor for the complex of endotoxin/lipopolysaccharide (LPS) bound to LPS-binding protein (LBP) and is primarily found on monocytes and macrophages [17]. The activated mCD14 receptor subsequently activates an intracellular signal cascade, which results in stimulation of the mRNA synthesis of a number of mediators, including proinflammatory cytokines, nitrogen monoxide, and platelet-activating factor. In addition, a soluble form of the receptor (sCD14), found in plasma and other body fluids, mainly activates endothelial and epithelial cells [17].

In the present study, we evaluated whether coating the inner surface of the VVBP bypass circuits with covalently bound heparin affects biocompatibility. Activation of CD14-positive (+) cells (mainly monocytes/macrophages) was measured by their mRNA expression and protein release of cytokines. In addition, we analyzed activation of neutrophile granulocytes by measuring the release of MPO in plasma. Our data clearly indicate that the heparin-coated surface is biologically active, reducing cellular activation and the release of important inflammatory mediators.

Materials and methods

In vitro venovenous bypass model

We recently described a modification of conventional VVBP in OLT [12]. In the present study, we used this VVBP system in an in vitro model. The bypass circuit consisted of a custom pack liver set (model CB 4649, 3/8″ silicone tubings; Medtronic, Minn., USA) with a centrifugal pump (Biomedicus BP-80; Medtronic) maintaining a constant flow of 2 l/min throughout the experiments. A heat exchanger (D720 Helios-A CP; Dideco, Mirandola, Italy) was introduced into the bypass circuit to keep a steady blood temperature of 37.5 °C. In ten experiments, all components of the circuits were covalently coated with a water-insoluble heparin complex using the Carmeda method [5], whereas in ten other experiments noncoated, but otherwise identical, sets were used.

Briefly, one unit (500 ml) of fresh (within 2 h) venous CPD-treated, anticoagulated, whole donor blood (The Blood Center, The National Hospital, Oslo, Norway) was diluted with 500 ml Ringer acetate (Braun, B. Braun Melsungen, Melsungen, Germany). A portion of the diluted blood was incubated in a corresponding set of tubings at 37.5 °C, without circuit flow but with slow rotation to keep the blood homogeneous, and used as a control for spontaneous cell activation. Blood samples were collected at time intervals for cell isolation (0, 0.5, 1, 2, 3, 12, 24, 48 h) and for plasma sampling (0, 3, 12, 24, 48 h). The zero samples were collected both from whole blood before dilution and from blood diluted with Ringer acetate.

Isolation of CD14-positive cells

Dynabeads M-450 CD14, designed to isolate human myelomonocytes (monocytes, macrophages, and one granulocyte subset; Dynal, Oslo, Norway), were used to isolate pure CD14-positive (CD14 +) cells from the blood samples collected both from the circuits and from the control blood. Fifty microliters of Dynabeads (4×10^8 beads/ml) was prewashed in 200 µl ice-cold PBS (0.07 M sodium phosphate, 1.4 M sodium chloride, 6% sodium citrate, pH 7.3) and resuspended in 100 µl PBS. Five hundred microliters of full blood (time 0, undiluted) or 1 ml blood diluted in Ringer acetate (time 0 diluted, 0.5, 1, 2, 3, 12, 24, and 48 h samples) was added to the beads. The test tubes were incubated with gentle rotation for 10 min at 4 °C and were subsequently placed on a magnet (Dynal MPC) for 3 min. The supernatant was removed and the beads/cells were washed twice in 400 µl ice-cold PBS and transferred to a new tube in the last wash. The supernatant was removed by placing the tubes on the magnet and the cells were lysed by adding 500 µl lysis/binding buffer [100 mM TRIS-HCl, pH 8.0, 500 mM LiCl, 10 mM EDTA, pH 8.0, 1% LiDS (SDS), 5 mM dithiothreitol]. The lysates/beads were used directly for mRNA isolation or frozen at –20 °C.

Messenger RNA isolation

Dynabeads mRNA DIRECT kit (Dynal) was used for isolation of mRNA. Unless otherwise indicated, all steps were performed on ice. Fifty microliters of oligo$(dT)_{25}$ Dynabeads (5 mg/ml) was prewashed in lysis/binding buffer before the buffer was removed. Simultaneously, the CD14 Dynabeads/cell lysates were placed on the magnet and the supernatant from the CD14 tubes was transferred to the oligo$(dT)_{25}$ tubes. The test tubes were gently rotated for 3–5 min at room temperature before a short spin (1–2 s). The tubes were placed on the magnet for 2 min before removal of the supernatant. The beads/mRNA were washed twice in washing buffer 1 (10 mM TRIS-HCl, pH 8.0, 0.15 mM LiCl, 1 mM EDTA, 0.1% LiDS) and subsequently twice in washing buffer 2 (10 mM TRIS-HCl, pH 8.0, 0.15 mM LiCl, 1 mM EDTA). Beads/mRNA were transferred into new tubes in the last wash, the supernatants were removed, and the beads were washed once in diethylpyrocarbonate (DEPC)-treated dH_2O. Finally, the beads/mRNA were resuspended in 20 µl DEPC dH_2O and used directly in RT-PCR or frozen in 10 µl aliquots at –20 °C.

Reverse transcription and polymerase chain reaction

Semiquantitative analyses of cytokine mRNA expression were performed using reverse transcription (RT) and, subsequently, polymerase chain reaction (PCR) in a PCR Cycler (GeneAmp 9600; Perkin Elmer/Cetus Corp., Norwalk, Conn., USA). Synthesis of cDNA was performed by RT directly on the mRNA attached to the oligo$(dT)_{25}$ beads using a GeneAmp RNA PCR Kit (Perkin Elmer). Two microliters of the mRNA/beads was added to a 20-µl solution containing 5 mM $MgCl_2$, 50 mM TRIS-HCl, pH 8.3, 10 mM KCl, 1 mM dGTP, 1 mM dATP, 1 mM dTTP, 1 mM dCTP, 1 U/µl RNAse inhibitor, and 2.5 U/µl MuLV reverse transcriptase. RT was carried out for 1 h at 37 °C, followed by denaturation at 99 °C for 5 min.

Subsequently, PCR was performed on 10 µl RT/cDNA mix and amplified using 25 pmol RT-PCR Amplimer Sets for IL-1β, IL-2, IL-6, IL-8, and IL-10 (Clontech Laboratories, Palo Alto, Calif., USA). TNFα and control β-actin primers were manufactured by

Pharmacia Biotech (Uppsala, Sweden). The TNFα primers had the sequences: 5'primer, 5'GAGTGACAAGCCTTAGCCCATG-TTGTAGCA-3' and 3'primer; 5'GCAATGATCCCAAAGTAG-ACCTGCCCAGACT-3' [8]; the β-actin primers had the sequences: 5'primer, 5'TGACGGGGTCACCCACACTGTGCCCATC-TA-3' and 3'primer, 5'CTAGAAGCATTTGCGGTGGACGAT-GGAGGG-3') [6]. As controls for the individual interleukin primer sets as well as for TNFα, a positive control delivered by the manufacturer (Clontech) was used, whereas human genomic DNA was used as a control for β-actin. PCR was carried out in a 50-μl solution containing 1.25 U AmpliTaq Gold polymerase (Perkin Elmer). The conditions used for all reactions were polymerase activation at 94 °C for 12 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min 30 s, and synthesis at 72 °C for 1 min 30 s, followed by 10-min elongation at 72 °C after the last cycle.

PCR products were identified by gel electrophoresis in 1 % agarose gel containing 0.5 μg/ml ethidium bromide and visualized on a UV screen. Correct product lengths of the different PCR products [IL-1β, 802 base pairs (bp); IL-2, 305 bp; IL-6, 628 bp; IL-8, 289 bp; IL-10, 328 bp; TNFα, 443 bp; and β-actin, 660 bp] were verified using a 100-bp DNA Ladder (Life Technologies, Gaithersburg, Md., USA).

Enzyme immunoassays for IL-1β and IL-6

Ethylenediaminetetraacetic acid-anticoagulated blood samples were collected at 0, 3, 12, 24, and 48 h before cooling, centrifugation, and plasma storage at –70 °C. The plasma was analyzed for IL-1β and IL-6 using enzyme immunoassay kits (R&D Systems, Minneapolis, Minn., USA) according to the manufacturer's recommendations. Data are given as median and 95 % confidence intervals (CI) of pg/ml cytokine in eight experiments for IL-1β (four noncoated and four coated) and in 12 experiments for IL-6 (6 noncoated and 6 coated).

Neutrophile granulocyte activation

Activation of neutrophile granulocytes was measured by the release of myeloperoxidase (MPO) in plasma. The MPO assays were performed in all 20 experiments according to a sensitive and specific double antibody enzyme immunoassay for MPO from human granulocytes, described in detail elsewhere [19]. Data were corrected for the dilution of blood in Ringer acetate and are indicated as median and 95 % CI in μg/l MPO.

Endotoxin measurements

Endotoxin was measured in plasma samples from all 20 experiments with the Limulus Amoebocyte Lysate (LAL) test (COA-TEST Endotoxin; Chromogenix, Mölndal, Sweden). The procedure for the kinetic method followed the guidelines of the manufacturer. Endotoxin values below 10 ng/l were considered negative.

Statistical analysis

Descriptive statistics are given as median and 95 % CI. The MPO data were analyzed using a repeated measures analysis of variance (Anova); the Friedman test was used for multiple comparisons within the groups and the Kruskal-Wallis test was used for multiple comparisons between the groups. A P value less than 0.05 was considered significant.

Results

CD14 + cell activation and cytokine mRNA expression

We used an in vitro VVBP model to compare noncoated and heparin-coated systems in the evaluation of cellular activation. Figure 1 shows the cytokine mRNA expression in noncoated systems (*panel A*) compared to the corresponding expression in heparin-coated systems (*panel B*). The mRNAs for IL-1β and IL-6 showed the greatest differences between noncoated and coated circuits, and between activation in the circuit and spontaneous activation.

A generally very low basal expression of IL-1β mRNA was detected in CD14 + cells. During flow in noncoated circuits, high induction of IL-1β mRNA was found after 2 h with persistent expression to 48 h (Fig. 1A, IL-1β circuit). In contrast, IL-1β mRNA in spontaneously activated cells showed a slight increase after 1 h, whereafter the mRNA signal declined (Fig. 1A, IL-1β spontaneous). In contrast, no induction of IL-1β mRNA was seen in cells isolated from heparin-coated circuits or in the corresponding spontaneous activation samples (Fig. 1B, IL-1β).

No basal expression of IL-6 mRNA was detected in CD14 + cells at time zero, either in whole blood or in blood diluted with Ringer acetate. IL-6 mRNA was strongly induced after 2 h and remained elevated for up to 48 h in noncoated circuits (Fig. 1A, IL-6 circuit). The time point for this induction varied to some extent between the different blood donors (after 2 or 3 h), although the same semiquantitative strong expression was seen in all experiments using noncoated circuits. In the spontaneous activation controls, a slight increase in IL-6 mRNA was detected after 24 h and remained for up to 48 h (Fig. 1A, IL-6 spontaneous). In comparison, negligible levels of IL-6 mRNA were detected both in the circuits and in the spontaneous activation controls when heparin-coated tubings were used (Fig. 1B, IL-6). The expression of IL-6 receptor (IL-6R) was also investigated in the same samples. We found a fairly strong basal expression but no induction of IL-6R in samples from the circuits or from the controls, with or without heparin coating (data not shown).

IL-2 mRNA was not detectable in either noncoated or heparin-coated circuits or controls (Fig. 1A, B; IL-2). In contrast, high basal expression of IL-8 was found, that did not seem to be influenced by flow, time, or the inner surface of the tubings (Fig. 1A, B; IL-8). The expression of IL-10 mRNA showed a very low basal level but was induced after 3 h in noncoated circuits (Fig. 1A, IL-10) and after 12 h in heparin-coated circuits (Fig. 1B, IL-10). This induction was higher in noncoated than in coated circuits. Interestingly, induction of IL-10 showed semiquantitatively stronger expression in the noncoated and coated controls than in their correspond-

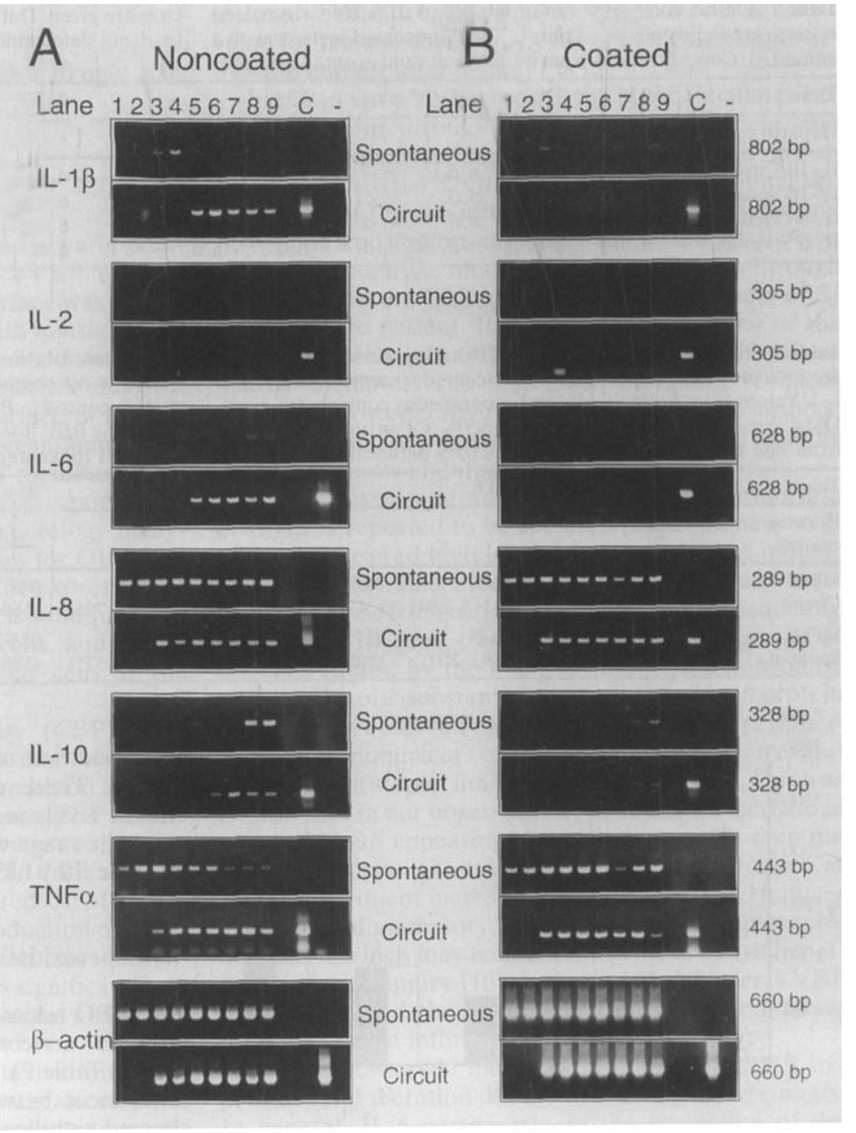

Fig. 1 A, B Agarose gel electrophoresis for semiquantitative determination of amplified cytokine mRNAs in CD14 + cells isolated from noncoated (**A**) or heparin-coated (**B**) systems. Lane numbering refers to the time points for cell isolation: lane 1, 0 h undiluted blood; lane 2, 0 h blood diluted 1:2 in Ringer acetate; lane 3, 0.5 h; lane 4, 1 h; lane 5, 2 h; lane 6, 3 h; lane 7, 12 h; lane 8, 24 h, and lane 9, 48 h. In addition, *C* denotes the control reaction using a cytokine-specific DNA template, whereas a dash (–) denotes a control where the reaction mix contains dH_2O instead of template. The amplified cytokines are, from the top, IL-1β, IL-2, IL-6, IL-8, IL-10, TNFα. The β-actin control is shown at the bottom. The spontaneous expression is shown above the expression in the circuit for each PCR product. The sizes in base pairs (*bp*) of the amplified products are shown to the right. Data are in each case representative for 3–5 separate experiments

ing circuits, appearing after 12 h (Fig. 1A, B; IL-10 spontaneous).

A medium basal expression of TNFα was seen that increased slightly with time in noncoated circuits (Fig. 1A, TNFα circuit). The expression in spontaneously activated cells (Fig. 1A, TNFα spontaneous) increased at first but subsequently decreased. In the coated circuits, a similar expression pattern was found for TNFα (Fig. 1B, TNFα).

The β-actin controls showed similar levels of expression, independent of coating, flow, or time. In all of the coated circuit samples shown (Fig. 1B), the mRNA isolated from the spontaneous activation control after 12 h (lane 7) showed some degradation, reflected by a slightly lower expression pattern of cytokines and β-actin control in this lane in the respective figures (Fig. 1B, lane 7).

IL-1β and IL-6 in plasma

To evaluate the production of IL-1β and IL-6 proteins, we performed enzyme immunoassays on plasma samples collected at 0, 3, 12, 24, and 48 h. Neither IL-1β nor IL-6 was detected in plasma from the experiments in which heparin-coated circuits were used (Table 1). In contrast, induction of protein expression was found for both cytokines using noncoated circuits. Whereas IL-1β mRNA increased after 2 h, detectable levels in plas-

Table 1 Plasma concentrations of IL-1β and IL-6. Heparin-coated systems are indicated with a plus (+) and noncoated systems with a minus (−). Concentrations from both circuits and spontaneous controls are given. Data are indicated as median and 95% CI in pg/ml. (*n. d.* not detectable)

Time	0 h		3 h		12 h		24 h		48 h	
Heparin coating	+	−	+	−	+	−	+	−	+	−
IL-1β/Circuit	n.d.	n.d.	n.d.	n.d.	n.d.	18 (14–29)	n.d.	62 (41–570)	n.d.	138 (77–1200)
IL-1β/Spontan.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IL-6/Circuit	n.d.	n.d.	n.d.	n.d.	n.d.	41 (6–166)	n.d.	737 (104–9697)	n.d.	2140 (307–21 176)
IL-6/Spontan.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2 Plasma concentrations of MPO. Heparin-coated systems are indicated with a plus (+) and noncoated systems with a minus (−). Values from both circuits and spontaneous controls are given. Data are indicated as median and 95% CI in μg/l. Activation from one time point to the next: $P < 0.05$ between 0 and 3 h for coated controls, $P < 0.001$ for all other groups; at 3 h: $P < 0.001$ for coated vs noncoated circuit, $P < 0.05$ for noncoated circuit vs noncoated controls, $P < 0.01$ for coated vs noncoated controls; at 12 h: $P < 0.01$ for noncoated circuits vs noncoated controls, $P < 0.001$ for coated vs noncoated circuits

Time	0 h		3 h		12 h		24 h		48h	
Heparin coating	+	−	+	−	+	−	+	−	+	−
MPO/ Circuit	110 (80–145)	141 (87–220)	170 (115–289)	397 (312–490)	266 (188–441)	642 (514–795)	945 (566–1536)	926 (705–1167)	2728 (2129–3798)	1734 (1421–3687)
MPO/ Spontan.	110 (80–145)	141 (87–220)	145 (93–200)	257 (198–337)	252 (166–352)	324 (216–571)	935 (548–1649)	992 (707–1191)	2530 (1691–3876)	2585 (2058–3501)

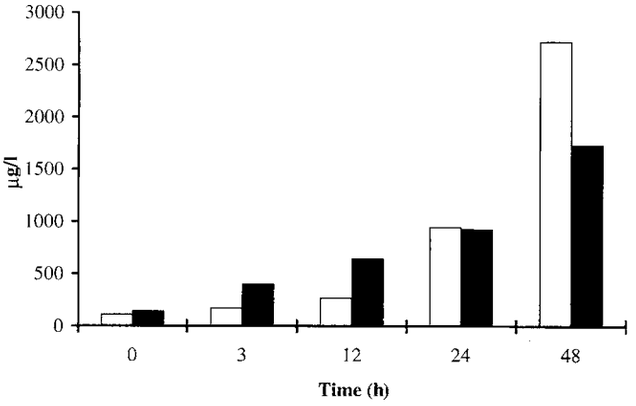

Fig. 2 MPO in plasma from coated vs noncoated circuits. Data are indicated as median in μg/l MPO. □ Heparin coated; ■ noncoated

ma were measured after 12 h [18 (14–29) pg/ml] and increased approximately threefold at 24 h [62 (41–570) pg/ml], with a maximum expression and eightfold induction at 48 h [138 (77–1200) pg/ml] (Table 1). Similarly, whereas IL-6 mRNA levels increased after 2 h (or 3 h), the corresponding IL-6 levels in plasma were detected after 12 h [41 (6–166) pg/ml] and increased approximately 18-fold at 24 h [737 (104–9697) pg/ml], with a maximum level and a 52-fold induction at 48 h [2140 (307–21 176) pg/ml] (Table 1). Strong variations in IL-1β and IL-6 plasma levels between the different blood donors were observed.

In addition, we analyzed soluble CD14 (sCD14) in plasma. These data did not show any differences in sCD14 levels in noncoated versus heparin-coated circuits; the range was 1–4 μg/ml, similar to the sCD14 levels in healthy blood donors (data not shown).

Myeloperoxidase release

The MPO release from neutrophils increased with time both in noncoated and in heparin-coated systems (Fig. 2, Table 2). Statistical analyses showed significant differences between the different groups. All groups showed significant differences from one time point to the next ($P < 0.05$ for coated controls from 0 to 3 h, $P < 0.001$ for all other comparisons).

Comparisons between the groups showed significant differences after 3 h ($P < 0.001$ between coated versus noncoated circuits; $P < 0.05$ between circuits and spontaneous controls in noncoated systems; and $P < 0.01$ between coated and noncoated spontaneous controls) and after 12 h, whereas no significant differences were observed at time zero or after 24 and 48 h (Table 2). At 12 h, we found no differences between coated circuits and corresponding controls or between coated versus noncoated spontaneous controls, whereas we did for noncoated circuits versus controls ($P < 0.01$) and for coated versus noncoated circuits ($P < 0.001$; Table 2). In short, noncoated circuits showed significant differences compared to all other groups.

Plasma endotoxin levels

No plasma endotoxin concentrations above 10 ng/l were detected in any experiment.

Discussion

In this study we compared noncoated and heparin-coated extracorporeal circuit models for VVBP used in OLT. In the coated circuits, the inner surface was coated with a polyethyleneimine polymer with partially degraded heparin fragments end point-attached and covalently bound to the polymer [5]. Our mRNA and protein data clearly show that the inner surface of the bypass circuits has profound influence on inflammatory cell activation measured as amounts of mediators produced by the cells. To our knowledge, this report is the first systematic, comparative, molecular biology analysis of cytokine profiles in a VVBP model for OLT. The methodology of measuring changes in the expression of individual cytokine mRNAs over time is semiquantitative although the relative number of cells, and subsequently the mRNA per unit number of cells, is the same throughout the study.

Studies of cardiopulmonary bypass (CBP) have clearly shown that the body's defense systems are strongly activated upon contact between blood and the artificial surface of the bypass components. CBP involving an oxygenator, which greatly contributes to the reactions observed, is not present in VVBP. In CBP, a reduction in granulocyte activation, measured as MPO release, has been observed upon the introduction of heparin to the inner surface [20]. In line with this observation, we show that the release of MPO is significantly reduced at 3 and 12 h in heparin-coated versus noncoated VVBP in our in vitro model.

MPO is released after granulocyte activation and is responsible for the production of free radicals. Heparin may activate platelets, which in turn may activate granulocytes [1]. In our study, heparin seems to have had the opposite effect since heparin-coated circuits had significantly lower MPO levels after 3 and 12 h than did the noncoated ones. In addition, in CPB there is reduced complement activation in heparin-coated compared to noncoated circuits, and this is probably the reason for the decreased MPO release [19]. Endotoxin is another potent granulocyte activator. All of the experiments were controlled and found to be negative for endotoxin. Thus, endotoxin was not responsible for the MPO release. Neutrophils survive only a few hours in vitro. Although the blood was used within 2 h after donation, the length of the experiment (48 h) was beyond the lifespan of neutrophils, which might explain the higher MPO levels at 24 and 48 h in coated versus noncoated circuits due to cell death and subsequent cell content leakage. Therefore, our in vitro VVBP model is not of value for the measurement of MPO as a granulocyte activation marker after 12 h.

In our in vitro VVBP model, the blood circulates 120 times per hour through the pump, in the tubing, and through the heat exchanger. During OLT, VVBP is normally used for 1–4 h. Our observed transcriptional upregulation of the proinflammatory cytokines IL-1β and IL-6 appeared within the time range for an in vivo VVBP, although the blood only passes 20 times through the VVBP during OLT, assuming a blood volume of 6 l in an average patient. It is likely that triggering of the immune defense systems and the production of inflammatory mediators can affect the transplant recipient and cause postoperative complications. In this respect, the present in vitro study is a supplement to an ongoing study in patients undergoing OLT.

In monocytes and macrophages, a large proportion of IL-1β is reported to be secreted [2]. Consistent with this, we measured high levels of secreted IL-1β in plasma from the noncoated circuits. This increase most probably arose from activation of cells and subsequently induced IL-1β gene transcription due to triggering of the cells caused by the inner surface of the noncoated circuits. Despite substantial transcription, IL-1β protein has not been observed in the supernatant from peripheral blood mononuclear cells [11], and this has been evaluated as a first step in limiting IL-1β activity [2]. This was not the case in our observations; we found an increase in secreted IL-1β appearing at a later time point than the mRNA increase (2 and 12 h, respectively), consistent with a subsequent increase in translation. IL-1β induces a general inflammatory response, including neutrophil activation, which may result in the progression of hepatic reperfusion injury [16]. Increased IL-1β after VVBP using noncoated circuits in liver transplantation, as we observed, might influence this reperfusion injury.

We also observed increases in both the mRNA expression and secretion of IL-6 in the noncoated circuits. In general, IL-6 participates in the regulation of the acute phase and immune responses [14]. The rapid and marked induction of IL-6 is probably crucial to its signalling role that alerts the body to the presence of tissue damage or injury of any kind. Although we observed elevated levels of IL-6 in noncoated circuits throughout the study, the corresponding IL-6 receptor (IL-6R) mRNA levels were unchanged but showed a high basal expression (data not shown). A constitutive high expression of the receptor compared to the mediator probably allows triggering of the IL-6 signalling cascade as soon as the level of the mediator increases.

A standard amount of anti-CD14 and poly(dT)$_{25}$ beads was used in all experiments, with or without heparin coating; in addition, the number of white blood cells was counted in some of the experiments and found to be similar. In this regard, our semiquantitative analyses

of IL-1β and IL-6 in noncoated versus heparin-coated circuits are reliable.

Neither IL-2, IL-8, nor TNFα showed regulated expression in the present study. In contrast, IL-10 mRNA increased at the latest time points (24 and 48 h) in both circuit types although a more pronounced expression was observed in the noncoated ones. The induction of this anti-inflammatory cytokine was probably a response to the aforementioned elevation of the proinflammatory cytokines IL-1β and IL-6. Presumably, heparin did mediate its effect by inhibiting cell adhesion to the tubings, and thereby, causing less cell activation. Since positive selection of cells using anti-CD14 paramagnetic beads was used in both the noncoated and heparin-coated experiments, this selection was not responsible for the observed cell activation.

Considering the role of cytokines in the transplantation process, it should be emphasized that several of these mediators are normally present in vivo, allowing amplification or inhibition of their expression during certain conditions. These considerations have to be taken into account when anticytokine treatment is suggested. In this respect, organ or tissue-directed targeting of the specific anticytokine, e.g., by gene therapy, has to be evaluated. Future studies should focus on unraveling the factor(s) that may influence the serious complications (sepsis, acute rejection) seen after liver transplantation. Our findings demonstrate that coating the inner surface with covalently bound heparin produces a biologically active surface that reduces activation of white blood cells. This in turn, influences the production and release of potent inflammatory mediators by these cells. Thus, the introduction of heparin-coated circuits in VVBP during OLT may help to prevent postoperative complications.

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