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ORIGINAL ARTICLE

Tissue Factor-Dependent Procoagulant Activity of Isolated Human Hepatocytes: Relevance to Liver Cell Transplantation

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Liver cell transplantation (LCT) aims to correct inborn liver function defects by infusing metabolically active cells into the diseased liver. Further improvement in LCT might depend on the prevention of early loss of transplanted cells. As tissue factor (TF)-dependent activation of coagulation was found to contribute to a low rate of beta cell engraftment in islet transplantation, we investigated the potential procoagulant activity (PCA) of hepatocyte preparations. TF expression on hepatocyte preparations was assessed by flow cytometry, reverse-transcription polymerase chain reaction and immunofluorescence. PCA depending on TF was evaluated in human plasma and in whole blood systems. Coagulation parameters were followed by routine techniques in a LCT recipient Crigler-Najjar patient. We determined that hepatocytes express soluble and membrane-bound forms of TF. We showed that hepatocytes exert a TF-dependent PCA. In parallel, delayed increase in D-dimer levels was observed following the hepatocyte infusions in the Crigler-Najjar patient. Furthermore, in vitro experiments demonstrated that TF-dependent PCA of hepatocytes is inhibited by N-acetyl-L-cysteine. In conclusion, hepatocytes exert TF-dependent PCA, which may contribute to early loss of infused cells. Addition of N-acetyl-L-cysteine to the suspensions of hepatocytes might be beneficial in LCT by inhibiting activation of coagulation. ***Liver Transpl 13:599-606, 2007.*** © **2007 AASLD.**

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In various human inborn metabolic diseases, liver cell transplantation (LCT) can provide medium-term partial metabolic control.[1-5] LCT also aims to bring immediate temporary functional support for the treatment of patients affected by fulminant hepatic failure, although demonstration of its efficacy remains difficult in this condition.[6] Increasing the quality and the replicative advantage of the transplanted cells is likely to improve the percentage of engraftment and subsequent repopulation of the recipient liver by the infused cell population.

Pancreatic islet transplantation, a therapeutic modality for type 1 diabetes, induces activation of coagulation cascade. Tissue factor (TF) synthesized by beta cells and duct cells contaminating clinical islet preparations is directly implicated in this procoagulant activity (PCA).[7,8] This may compromise the outcome of islet transplantation by early graft loss.[9,10] Therefore, the first aim of this study was to determine if isolated hepatocytes, fresh or cryopreserved, also express TF. After demonstrating the presence of TF in hepatocytes, we analyzed the PCA of hepatocyte suspension in several in vitro coagulation assays. Following in vitro demonstration of TF-dependent PCA, we further explored coagulation parameters of a 9-month-old Crigler-Najjar patient before and after cell infusions. Since we recently observed that N-acetyl-L-cysteine (NAC) derivative inhibits TF-dependent PCA of islet preparations,[11] we

Abbreviations: LCT, liver cell transplantation; TF, tissue factor; PCA, procoagulant activity; RT-PCR, reverse-transcription polymerase chain reaction; NAC, N-acetyl-L-cysteine; mAb, monoclonal antibody; mRNA, messenger ribonucleic acid; F.E.U., fibrinogen equivalent unit; as-TF: alternatively spliced variant of TF; Ig, immunoglobulin.

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also investigated the potential of NAC to inhibit in vitro PCA associated with hepatocyte suspensions.

MATERIALS AND METHODS

The protocol, including all experiments on human and animal samples, the blood investigation in the patient, and the LCT protocol were approved by the institution ethical review board.

Cell Preparations

Liver isolation and hepatocyte cryopreservation/thawing procedures were previously published in detail.[4,5] After isolation or thawing, cells were suspended in a stable solution of plasmatic proteins (85% of albumin) containing bicarbonate (1 mmol/L), glucose (11 mmol/L), and 10 U/mL heparin (infusion medium). When not specified, experiments were performed with human cryopreserved/thawed hepatocytes (Trypan blue viability around 80%).

The human adenocarcinoma cell line CAPAN-2 was used as a positive TF control in the several experiments as previously described.[8]

TF Expression of Human Hepatocyte Suspension

Immunofluorescence studies were performed to evaluate the presence of TF. For this, fresh hepatocytes were placed on cover slips and fixed by paraformaldehyde 4% (Merck, Darmstadt, Germany) for 20 minutes. Then, these cells were incubated with Triton X-100 (Sigma, Bornem, Belgium) 1% in Tris base sodium buffer (50 mmol/L Tris-HCl pH 7.4 and 150 mmol/L NaCl) (Organics [VWR], Leuven, Belgium) for 15 minutes and then with milk 3% in Tris base sodium buffer for 1 hour.[5] The primary antibodies, monoclonal mouse anti-human cytokeratin-18 antibody (Progen, Heidelberg, Germany) and polyclonal rabbit anti-TF monoclonal antibody (mAb) (immunoglobulin [Ig]G1 n4509; American Diagnostica, Andresy, France) were diluted (1/100 and 1/50, respectively) in Tris base sodium and incubated with cells for 1 hour. The secondary antibodies used were cyanine 3-conjugated anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA) and fluorescein isothiocyanate conjugated anti-rabbit IgG (Sigma). The nuclei were revealed by 4′, 6-diamidino-2-phenylindole (DAPI; Sigma) staining. Negative experimental controls were performed (absence of primary or secondary antibodies).

Presence of the classical membrane-bound (TF) and the recently new described soluble (alternatively spliced TF; as-TF) forms was analyzed by reverse-transcription polymerase chain reaction (RT-PCR). Messenger ribonucleic acid (mRNA) was extracted from 0.5×10^6 hepatocytes using the Tripure isolation reagent kit (Roche Applied Science, Brussels, Belgium) following the manufacturer's instructions. One-step RT-PCR was performed on a Thermocycler instrument (Applied Biosystems, Lennik, Belgium) with primers synthesized at Invitrogen. RT-PCR for TF or glyceraldehyde 3-phosphate dehydrogenase was realized with the following primers:

TF sense primer, 5′-TGAATGTGACCGTAGAAGATGA-3′;

TF antisense primer, 5′-GGAGTTCTCCTTCCAGCTCT-3′;[8]

Glyceraldehyde 3-phosphate dehydrogenase: sense primer, 5′-CGGACTCAACGGATTTGGTCGTAT-3′;

Glyceraldehyde 3-phosphate dehydrogenase: antisense primer, 5′-AGCCTTCTCCATGGTGGT-3′.

Products were separated by electrophoresis on 1% agarose gel and visualized with ethidium bromide under ultraviolet lamp.

We also realized a real-time RT-PCR for TF, as-TF, and β-actin on a Lightcycler instrument (Roche Applied Science) with primers synthesized at Biosource Europe, Nivelles.

Belgium as previously described.[8] mRNA was extracted from 0.5×10^6 hepatocytes with the MagNA Pure mRNA extraction kit on the MagNA Pure instrument (Roche Applied Science) following the manufacturer's instructions. Primers were as follows:

TF sense primer: 5′-GGGAATTCAGAGAAATATTCTACATCA-3′;

TF antisense primer: 5′-TAGCCAGGATGATGACAAGGA-3′;

as-TF sense primer: 5′-TCTTCAAGTTCAGGAAAGAAATATTCT-3′;

as-TF antisense primer: 5′-CCAGGATGATGACAAGGATGA-3′;

probe: 5′-TGGAGCTGTGGTATTTGTGGTCA-3′

β-actin sense primer: 5′-GGTCAGAAGGATTCCTATG-3′;

β-actin antisense primer: 5′-GGTCTCAAACATGATCTGGG-3′

The presence of membrane-bound TF was also confirmed by flow cytometry analysis. Therefore, cells were washed in phosphate-buffered saline supplemented with 1% bovine serum albumin and 10% pooled human serum and incubated for 20 minutes at +4°C with the fluorescein isothiocyanate-conjugated IgG1 mAb against TF no. 4508CJ (American Diagnostica) or the corresponding isotype-matched control mAb (Dako, Heverlee, Belgium). Cell fluorescence was analyzed using a Cyan flow cytometer (Dako).

TF-Dependent PCA of Human Hepatocyte Suspension

PCA Assay

PCA was determined in duplicate by a single-stage clotting assay on cell suspensions in phosphate-buffered saline. A total of 100 μL of each sample was incubated at +37°C for 1 minute with 100 μL of normal citrated plasma before the initiation of clotting by the addition of 100 μL of 25 mmol/L $CaCl_2$. Clotting time was recorded with a KC10 apparatus (Amelung, Lemgo, Germany) and PCA in mU/mL was determined by reference to a standard curve generated by serial dilutions of a commercial rabbit thromboplastin (Excel SA; Organon Teknica, Turnhout, Belgium). The amount of thrombo-

plastin that yielded a clotting time of 12.4 seconds was assigned a value of 1 unit. To determine the role of the TF/factor VII pathway in the PCA, additional experiments were performed using factor VII–deficient plasma (Dade Behring, Marburg, Germany). The number of cells was determined by simple counting.

Tubing loop model

A whole-blood experiment protocol was adapted from a model previously described.[8,10] Loops made of polyvinylchloride tubing (inner diameter 6.3 mm, length 390 mm) and treated with a Corline heparin surface were purchased from Corline (Uppsala, Sweden). Loops were supplemented with cell samples suspended in phosphate-buffered saline before blood addition. To ascertain the role of TF in this model, cells (0.5×10^6 hepatocytes) were preincubated at room temperature for 10 minutes with either 0.2 mg/mL mAb anti-human TF IgG1 (American Diagnostica) or 0.2 mg/mL mAb mouse IgG1 (clone 11711.11; RnD Systems, Abingdon, United Kingdom) before extensive washing in phosphate-buffered saline and tubing loop assay. Five mL of non-anti-coagulated blood from healthy volunteers was then added to each loop. To generate a blood flow of about 45 mL/minute, loop devices were placed on a platform rocker inside a +37°C incubator. Blood samples were collected into ethylene diamine tetraacetic acid (4.1 mmol/L final concentration) and citrate (12.9 mmol/L final concentration) tubes before and 30 minutes after start. Platelets were counted on a CellDyn 4000 automate (Abbott Laboratories, Abbott Park, IL) and D-dimers were evaluated by immunoturbidimetric assay (Liatest D-DI; Diagnostica Stago, Asnières sur Seine, France).

Viscometry measurement

ReoRox4 is a free oscillation rheometer, a device that enables to monitor blood viscosity over time in small blood volumes.[12] It is based on the recording of magnetically-induced oscillations; during the coagulation process, increase in viscosity (as a consequence of coagulation) results in a transient increase of oscillation damping. Human citrated blood samples were recalcified extemporaneously with a solution of 25 mmol/L Ca^{2+}. Reaction was started by adding 400 μL human citrated blood to cups containing 210 μL of Ca^{2+} solution and increasing the number of hepatocytes suspended in 40 μL of phosphate-buffered saline.

Where specified, graded doses (1-25 mmol/L) of NAC (Lysomucil; Zambon, Brussels, Belgium) were added.

Follow-Up of Coagulation Parameters in a LCT Recipient Child

A 9-month-old girl (7.5 kg) with Crigler-Najjar disease was referred to our unit for LCT. A Broviac catheter device was inserted surgically in the portal system, allowing repetitive infusions as reported.[13] The child received 2.6 billion cells (2.2 billion cryopreserved/thawed cells) in 14 successive infusions (2 infusions per day) over 2 weeks, in 3 steps of infusions (3 consecutive days for the first step, 2 consecutive days for the 2 others). Each cell-infused suspension contained 60 to a maximum of 250×10^6 cells, suspended in 25 mL of the infusion medium, given at a rate of 50 mL/minute. We followed the coagulation parameters, including fibrinogen, international normalized ratio of prothrombin (prothrombin time), activated partial thromboplastin time, D-dimers on a CA7000 automated coagulation analyzer (Sysmex, Barchon, Belgium), and platelets on a Advia 120 analyzer (Bayer, Brussels, Belgium); before infusion, after 15 minutes of infusion, and at the end of infusion in peripheral blood. We also followed those parameters in portal blood at the end of infusions.

Statistics

Results are expressed as mean ± standard error of the mean or mean ± standard deviation, and statistically significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) differences were assessed by Wilcoxon or Mann-Whitney tests.

RESULTS

Cryopreserved Hepatocytes Express Functional TF

TF expression was first documented on fresh hepatocytes by immunofluorescence. As shown in Figure 1A, we found that all cells express TF constitutively (uniform cytoplasmic staining). In parallel, the coexpression of TF and cytokeratin-18 of these cells confirmed their hepatocyte lineage. We also assessed the expression of TF at the mRNA level on hepatocytes using RT-PCR (Fig. 1B). Interestingly, both the membrane form and the alternatively-spliced variant of TF mRNA were expressed. In additional experiments, we used real-time RT-PCR to quantify TF and as-TF mRNA levels in hepatocytes. As shown in Figure 1C, the as-TF variant was predominantly expressed with an ~2-fold magnitude. Furthermore, flow cytometry analysis of hepatocytes confirmed a positive and specific staining for TF (Fig. 1D).

We then assessed PCA of hepatocytes in human plasma. As shown in Figure 2A, we observed that hepatocyte preparations exert significant PCA that was dependent on the number of cells engaged. The PCA of the hepatocyte suspensions was factor VII–dependent since it was not observed in factor VII–depleted plasma and demonstrated the involvement of TF (Fig. 2B).

PCA of Hepatocytes in Whole Blood Preclinical Model

To further investigate the PCA of hepatocytes in a model closer to the in vivo situation, we adapted the tubing loop system that was used to demonstrate the thrombotic reaction induced by islet preparations[7] and duct cells.[8] This model is based on the injection of non-anti-coagulated blood in plastic loops in which the inner surface was coated with heparin to prevent contact-

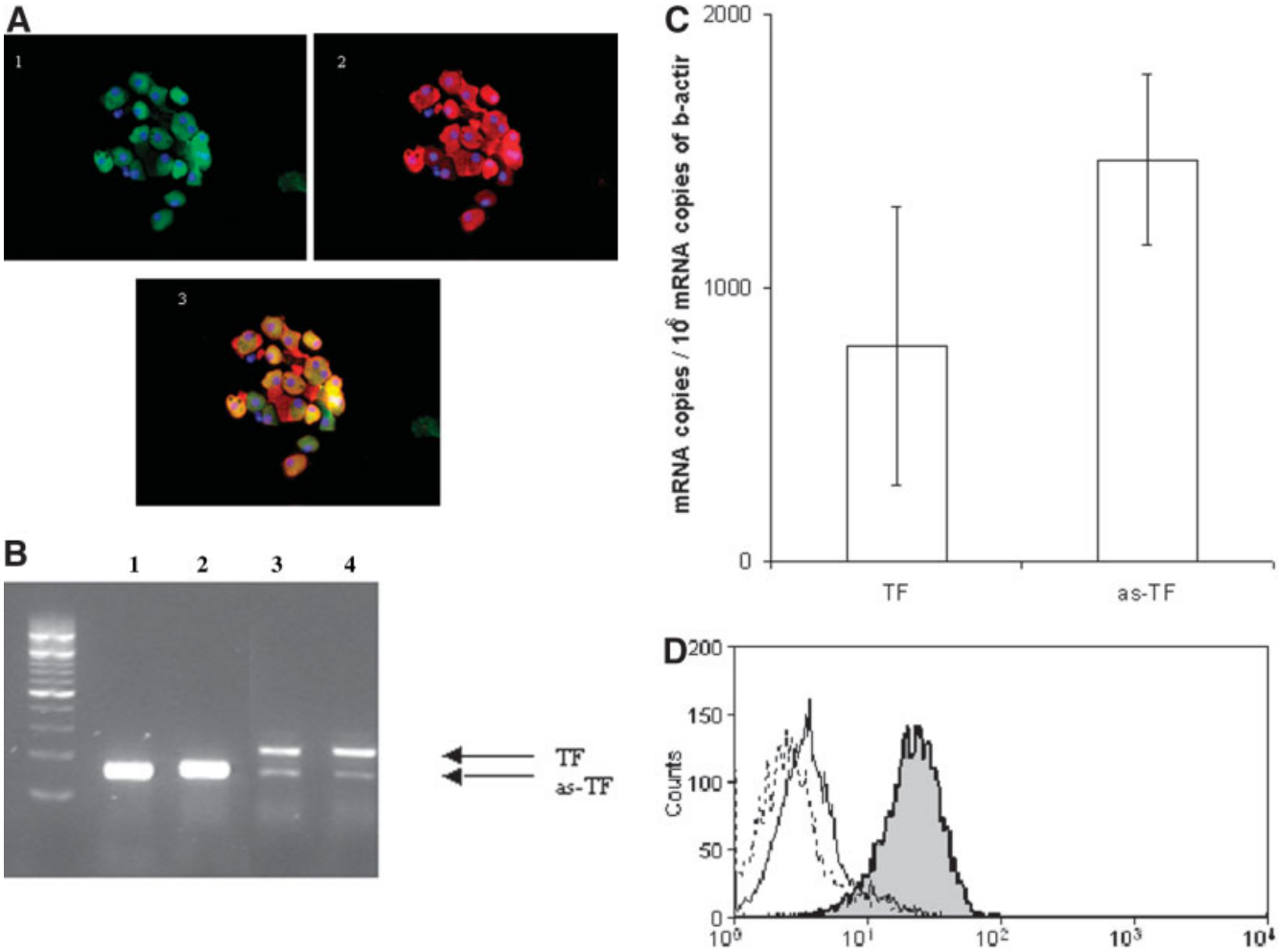

Figure 1. TF expression on hepatocytes. (A) Immunofluorescence for (1) TF (green) and (2) cytokeratin-18 (red) was performed on fresh hepatocytes placed on cover slips and fixed by paraformaldehyde. (3) A merging image of CK-18 and TF staining is also presented (magnification 20×). The nuclei were revealed by (DAPI) (blue) staining. (B) mRNA extracted from 2 cell preparations was analyzed by RT-PCR for glyceraldehyde 3-phosphate dehydrogenase (1-2) and for TF (3-4). The conditions 1 and 3, and 2 and 4 were obtained from fresh and cryopreserved/thawed cells, respectively. One representative experiment out of 3 is shown. Arrows indicate the size of either classical mRNA (407 bp) encoding the membrane form (TF) or the as-TF (247 bp) encoding the soluble form. (C) Quantification of TF and as-TF mRNA using real-time RT-PCR. Results are expressed per 10^6 mRNA copies of β-actin. Data represent mean ± standard error of the mean of 5 independent experiments. (D) Hepatocytes were analyzed by flow cytometry after staining by either anti-TF mAb (gray histogram), corresponding control isotype mAb (white histogram, thick line) or unstained cells (white histogram, dotted line). One representative experiment out of 3 is shown.

dependent blood coagulation, thus allowing the investigation of coagulation in the presence of platelets. Blood coagulation was assessed by macroscopic examination for the presence of clots and by monitoring platelet counts and D-dimer levels, which decrease or increase as a consequence of coagulation activation, respectively. We show that 5×10^5 hepatocytes added to 5 mL of blood were sufficient to induce clot formation within 30 minutes. This was associated with a dramatic drop in platelet counts (Fig. 3A) and increased D-dimer levels (Fig. 3B). To demonstrate the role of TF, pretreatment of hepatocytes with an anti-TF mAb was performed in these settings; this led to the prevention of the formation of visible clots, prevention of the drop in platelet counts, and prevention of the increase in D-dimer levels. This was not observed with an isotype-matched control mAb (Table 1).

The PCA of hepatocytes in whole blood was also assessed by free oscillating rheometry, another global coagulation measurement technique. As shown in Figure 4, we were able to confirm that the addition of increasing number of hepatocytes to citrated blood in the presence of Ca^+ resulted in the activation of coagulation. We observed that as few as 10^4 cells were sufficient to promote a statistically significant induction of human blood coagulation.

D-Dimers Increased Following LCT

To demonstrate PCA of infused hepatocytes in vivo, we also evaluated coagulation parameters in an LCT recipient patient. When the Broviac was surgically placed in the portal system and before infusions, D-dimer levels (ng/mL fibrinogen equivalent unit [F.E.U.]) were mea-

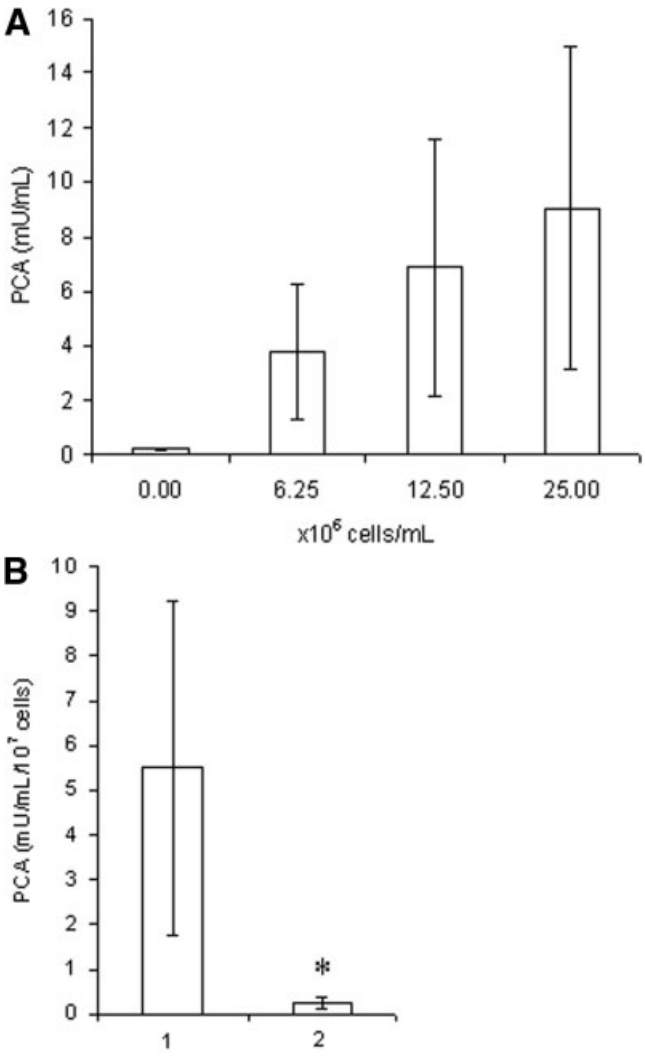

Figure 2. TF-dependent PCA of hepatocytes in plasma. (A) PCA was assessed in plasma after addition of hepatocytes (6.25, 12.50, 25.00 × 10^6/mL). Data represent mean ± standard error of the mean of 4 independent experiments. *P < 0.05 compared with normal citrated plasma. (B) PCA of hepatocytes in presence of factor VII–deficient plasma (2) was determined and compared to PCA assessed in human plasma (1). Data represent mean ± standard error of the mean of 4 independent experiments. *P < 0.05 compared with normal citrated plasma.

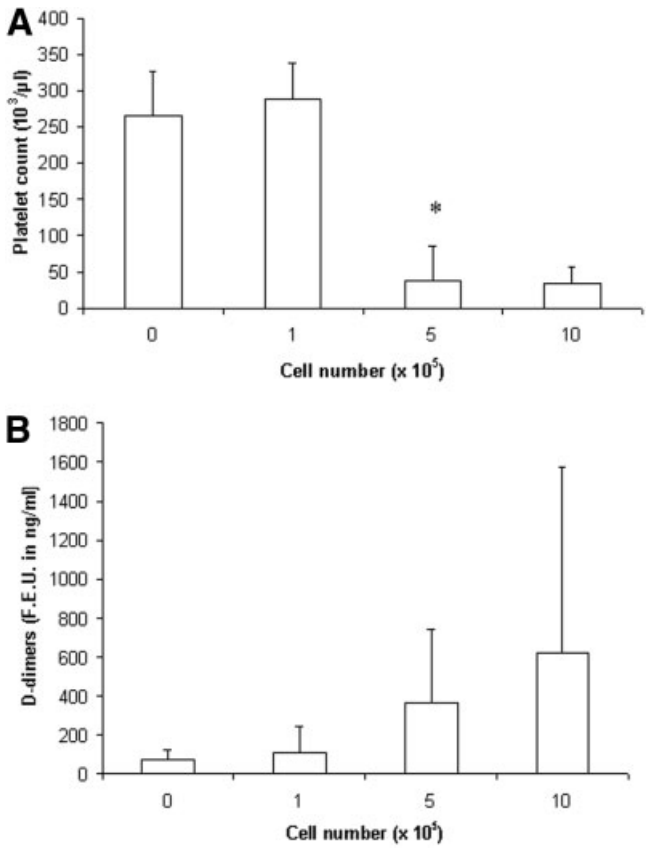

Figure 3. PCA of hepatocytes in whole blood (tubing loop model). Hepatocytes were added to 5 mL whole blood incubated in tubing loops under agitation at +37°C. Platelet count (A) and D-dimer levels (B) were monitored after 30 minutes of incubation in the absence or presence of increasing concentrations of hepatocytes (10^5, 5 × 10^5, and 10^6). Data represent mean ± standard deviation of 3 to 5 independent experiments. *P < 0.05 compared with platelets count in absence of cells.

sured in Crigler-Najjar patient peripheral blood and were considered as normal (335 ng/mL F.E.U., normal values: <500 ng/mL F.E.U.). The D-dimer values remained within normal laboratory range during the first of the 14 infusions in peripheral and portal blood (378 ng/mL F.E.U. in peripheral blood, 344 ng/mL F.E.U. in portal blood). Interestingly, we observed an increase in D-dimer levels the day following the 2 first infusions, before infusions of the second day (first step) (865 ng/mL F.E.U. in peripheral blood). No further increase in D-dimer values was observed during the infusions of the second day (873 ng/mL F.E.U. in peripheral blood, and 855 ng/mL F.E.U. in portal blood.) The increase in D-dimer levels was also confirmed on blood samples taken the day after the second step infusions, at which time D-dimer values up to 1,374 ng/mL F.E.U. were measured. One week after all the infusions, the D-dimer levels returned within normal values (397 ng/mL F.E.U.). All the other coagulation parameters remained within normal laboratory ranges during and after the infusions, in peripheral or portal blood (data not shown).

NAC Inhibits TF-Related PCA of Hepatocytes Without Inducing Toxicity

According to the modulation of the hemostatic parameters described in healthy subjects after intravenous infusion with NAC,[14] this compound was tested for its capacity to inhibit the PCA induced by hepatocytes. We first investigated the capacity of graded doses of NAC to impair the PCA in human plasma. As shown in Figure 5A, we observed a dose-dependent inhibition of hepatocyte-induced PCA with an effect already statistically significant at 10 mmol/L. We then assessed the capacity of graded doses of NAC to impair the PCA in the

TABLE 1. TF-Dependent PCA of Hepatocytes in Whole Blood (Tubing Loop Model)

Cells	Time (minutes)	mAb	Platelet count ($10^3/\mu L$)		D-dimers (ng/mL F.E.U.)	
			Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	0	No	294.5	294.5	85.0	85.0
0	30	No	252.0	252.0	105.0	105.0
5×10^5	30	No	36.3	1.6	1265.0	870.0
5×10^5	30	Anti-TF	241.2	385.0	305.0	415.0
5×10^5	30	Isotype control	0.7	2.8	965.0	980.0

NOTE: Hepatocytes (5×10^5), pretreated for 10 minutes at room temperature with anti-TF mAb or isotype-matched control mAb, were added to 5 mL of whole blood and incubated in tubing loops under agitation at +37°C. Platelet count and D-dimer levels were monitored prior to the assay and after 30 minutes incubation in the presence or absence of cells. Results of 2 experiments performed on 2 independent blood samples are shown. Data represent the mean value for platelet count and D-dimer levels.

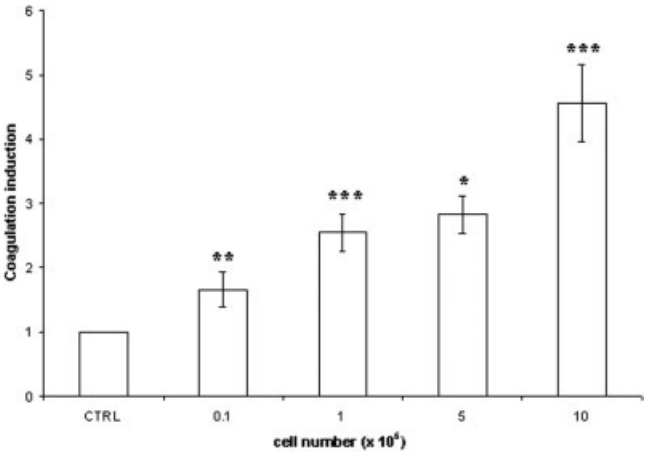

Figure 4. PCA of hepatocytes in whole blood (ReoRox model). Viscosity was assessed using free oscillating rheometry (ReoRox4) in 600 μL of recalcified human citrated peripheral blood after addition of hepatocytes (10^4, 10^5, 5×10^5, and 10^6). Data represent mean ± standard error of the mean of 4 to 8 independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control (absence of hepatocytes). Coagulation induction fold was calculated by dividing the coagulation time of the blood in absence of hepatocytes by coagulation time of the blood with the determined number of cells.

human whole blood model. NAC dose-dependently prevented the drop in platelet count induced by hepatocytes and the increase in D-dimer levels. The effect of NAC was already significant at the dose of 10 mmol/L (Fig. 5B).

These inhibitions were not due to a toxic effect of the compound as no toxicity related to several concentrations of NAC was evidenced in murine hepatocytes (n = 3).

DISCUSSION

The important finding of this study is the demonstration of hepatocyte suspension TF-dependent PCA. This in vitro observation has a practical consequence in vivo, since we observed a modification of the coagulation parameters, delayed D-dimer increase, in a LCT recipient patient. Based on its ability to inhibit TF-dependent PCA of hepatocyte suspension, we may further consider, to ameliorate LCT engraftment, addition of NAC to the hepatocyte suspension.

Indeed, low cell engraftment rate represents a major limitation of LCT, resulting in limited correction of the metabolic inborn defect.[5] Activation of the coagulation cascade was evidenced and associated with negative clinical outcome after pancreatic islet transplantation. It has been demonstrated that islet preparations, beta-cells, and contaminating duct cells exert a TF-dependent PCA. Activation of coagulation could not only lead to thrombotic events, but also elicit inflammatory reactions involving upregulation of adhesion molecule expression and chemokine production.[9,15] We therefore hypothesize that a similar mechanism may occur following LCT. In rat liver transplantation, PCA is postulated to be important in acute rejection (immune cell adherence, vascular thrombosis, and delayed-type hypersensitivity) of allograft.[16] In an animal model, fibrin deposition in the hepatic sinusoids, associated to a hypercoagulative state, is also demonstrated after liver transplantation.[17] In humans, modification of prothrombin plasma level might be considered to evaluate rejection after liver transplantation.[18] Furthermore, several LCT studies demonstrate, on animal models, that allogenic hepatocytes are highly immunogenic and stimulate strong cell-mediated immune responses.[19-21]

In the 1990s, 2 independent studies showed either weak or no TF expression by hepatocytes using immunohistochemistry on human liver frozen sections, although TF is known to be present in the HepG2 hepatoblastoma cell line.[22-24]

Therefore, we have first determined if isolated hepatocytes express TF. The majority of our experiments were performed on cryopreserved/thawed hepatocytes, which represent the main cell source in LCT. Both membrane-bound forms and soluble TF forms were found in isolated hepatocytes, but in low concentration as compared to islet preparations or the CAPAN cell line.[8] We also showed that expression of TF in hepatocytes induces coagulation in vitro. With the plasma PCA measuring technique, we determined that the PCA activity of the hepatocyte suspension was factor VII–de-

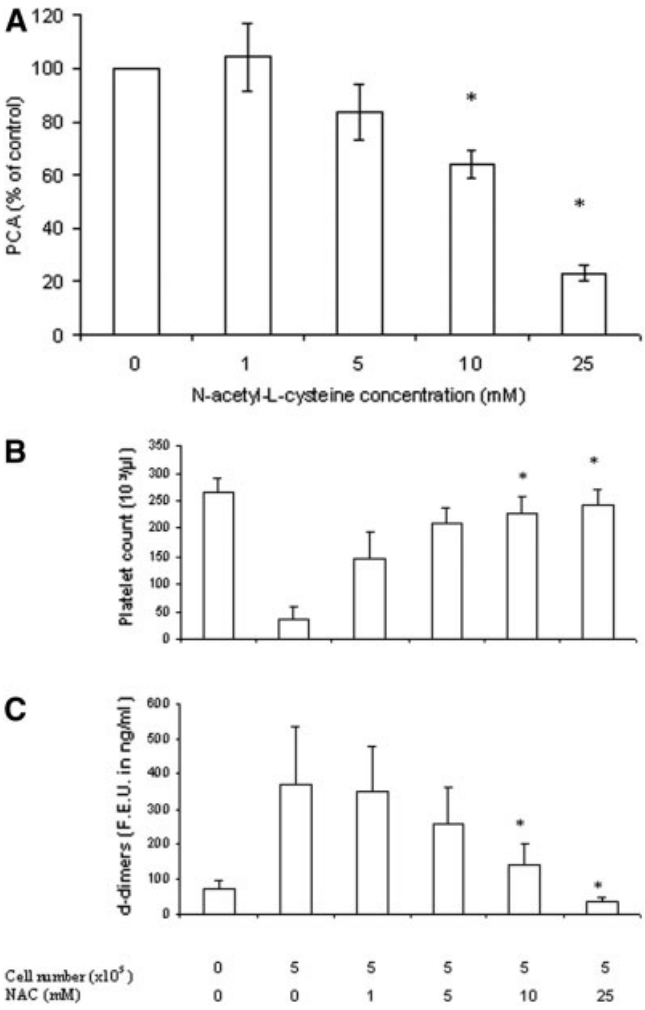

Figure 5. Modulation of TF-dependent PCA of hepatocytes by NAC. (A) PCA of hepatocyte suspension in human plasma. Graded doses of NAC were added to 90 µL of hepatocyte suspension (0.5 × 10^6/mL) before PCA was measured. Data represent mean ± standard deviation of 4 independent experiments. *$P < 0.05$ compared with absence of NAC. (B,C) 5 × 10^5 hepatocytes were added to 5 mL whole blood incubated in tubing loops under agitation at +37°C. Graded concentrations of NAC were added to the loops extemporaneously. Platelet count (B) and D-dimer (C) levels were monitored after 30 minutes of incubation. Data represent mean ± standard error of the mean of 5 independent experiments. *$P < 0.05$ compared with absence of NAC.

pendent and thus related to TF. The tubing loop technique, which mimics whole blood coagulation, allowed us to demonstrate that human hepatocytes do exert a PCA in physiologic context. Finally, we confirmed these results on whole citrated blood and in presence of Ca^+ by viscometry measurement. Taken together, these in vitro results can possibly be correlated to the portal flow modifications, the decreased number of platelets, and/or the portal presence of hepatocyte-containing thrombi described by Muraca et al.[25] after intraportal hepatocyte transplantation in the pig. We also observed a delayed D-dimer increase following hepatocyte infusions in a Crigler-Najjar patient. Interestingly, the other coagulation parameters followed were not affected by the hepatocyte infusions, suggesting that the PCA in vivo can be reduced to an infraclinical level or that cell-dependent coagulation occurs mainly in small liver sinusoids, being therefore undetectable in the high peripheral and portal flows. Similar observations, isolated increase in D-dimers, were noted during the painful coagulation crisis of venous skin malformations or after islet transplantation.[26,27]

NAC is often used in adult and pediatric treatment of paracetamol intoxication and is considered as safe in human and particularly in children.[28] Furthermore, acetaminophen cell injury in cultured mouse hepatocytes is prevented by NAC, probably through a mitochondrial permeability transition inhibition.[29,30] We also demonstrated in this study that several concentrations of NAC, lower in concentration than that used in paracetamol intoxications, were not toxic for cultured hepatic cells. Recent works also demonstrated that NAC corrects the TF-PCA on TF-expressing pancreatic islets and duct cells.[11] NAC is also proposed to ameliorate the early function of the hepatic graft, being able to limit the rate of acute rejection.[31] Clinical studies of NAC in liver transplants show better liver microcirculation without affecting early function.[32,33] Finally, NAC is known to modulate the vitamin-K–dependent hemostatic proteins and, therefore coagulation, in paracetamol overdose patients but also in healthy subjects.[14] In our study, we highlighted the anticoagulant effect of NAC, as this drug was able to prevent the TF-dependent PCA in vitro.

In conclusion, we demonstrate the TF-dependent PCA of a hepatocyte suspension. As in pancreatic islet transplantation, we may postulate that hepatocyte-related PCA may interfere with hepatocyte engraftment. This phenomenon is probably clinically expressed, after hepatocyte infusions, as we observed a delayed D-dimer increase following LCT in a patient. Further investigations are needed to evaluate the consequences of hepatocytes TF-dependent PCA on cell engraftment and rejection in vivo and to determine the potential clinical benefit of NAC.

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