Development of a Normothermic Extracorporeal Liver Perfusion System Toward Improving Viability and Function of Human Extended Criteria Donor Livers

TO THE EDITOR:

We appreciated the article by Banan et al.[1] concerning the rehabilitation of discarded livers by machine perfusion, but we think the choice of the methods defining the quality of the results of this innovative procedure deserves further discussion. Moreover, some conclusions of the authors may be premature.

The authors state that coagulation factors are produced during perfusion based on the decrease of international normalized ratio (INR), which was determined by the CoaguCheck, XS system (Roche Diagnostics, Germany) an instrument certified for entire blood and not for anticoagulated samples[2]; instead, the livers were perfused with near-expiration human blood, which contains the anticoagulant sodium citrate and what remains of its original coagulation factors. The precise meaning of INR values in these conditions is doubtful, and its decrease during perfusion may largely reflect undetermined factors, including the decline of citrate concentration, which is likely to be metabolized by the liver, the main organ responsible for citrate clearance in vivo.[3]

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About triglyceride (TG) production, the precise method adopted is not declared but routine clinical laboratory methods may be influenced by hemolysis, which occurs during machine perfusion.

To provide a basis for these considerations, we assessed coagulation factors and TG production during a procedure of extracorporeal normothermic perfusion that we conducted using a LiverAssist machine (Organ Assist, the Netherlands) on a liver procured from a 74-year-old donor, that was eventually implanted in a male patient aged 53 years. Instead of blood, we used human red blood cells suspended in the plasma substitute Gelofusine supplemented with human albumin (Alb), antibiotics, insulin, and heparin, thus not containing coagulation factors. During the perfusion (5 hours), we did not find any production of factor V (HemosIL Factor V, Werfen, Austria) nor protein C (HemosIL Protein C, Werfen, Austria). The fact that fibrinogen (FBG; antigen determination, 42.80 mg/dL) and C-reactive protein (CRP; 2.400 mg/dL), ie, 2 acute phase reactants, were released but not Alb, a negative acute phase reactant protein, suggests that FBG is released in the context of the acute phase response, which includes only some selected coagulation factors, and is expected to follow perfusion-related liver damage.

As concerns TG production, when we used the clinical laboratory assay, based on the combined reactions of the glyceroloxidase and peroxidase (SGMitalia, Rome, Italy), the results were in agreement with those of Banan et al.[1] When we repeated the analysis with the same reagent after separating the lipoproteins from the other plasma components by molecular size-exclusion chromatography (Fig. 1), TGs associated with very low density lipoprotein (VLDL) dropped from 159.50 to 3.46 mg/dL, whereas most of the reactivity to the TG reagent appeared at the molecular weight of hemoglobin (Hb), which indeed has

Time (min)	FBG (mg/dL)	CRP (mg/dL)	Alb (mg/dL)	TG (mg/dL)
60	<16.4	0.265	899	*n.d.*
120	21.40	0.864	834	2.39
180	26.10	1.360	922	2.71
240	34.70	1.970	824	3.26
300	42.80	2.400	918	3.46

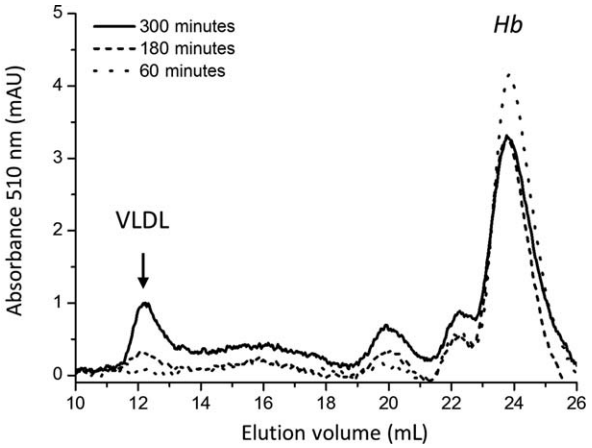

FIG. 1. The table reports the levels of FBG, CRP, VLDL-associated TG, and Alb determined during machine perfusion. FBG, CRP, and Alb concentration were determined using nephelometric assays on BNII analyzer (Siemens Healthcare, GmbH, Erlangen, Germany). Elution profile of TGs were obtained by gel filtration chromatography (Superose 6 HR 10/300 GL; GE Healthcare Europe, Little Chalfonts, UK) associated with the postcolumn injection of the reagent for TG determination. TG-specific elution profile obtained after 60 minutes (dotted line), 180 minutes (dashed line), and 300 minutes (continuous line) are shown on the right. The peak eluting at 12.2 mL corresponds to TGs associated with the VLDLs. n.d.: not detectable.

peroxidase activity and whose absorbance spectrum overlaps that of the TG assay.[4]

For these reasons, we think that adequate methods still need to be established to allow the evaluation of the performances of rehabilitated livers and to discriminate between hepatocellular function recovery and damage.

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