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Undissolved particles in UW solution cause microcirculatory disturbances after liver transplantation in the rat

Received: 1 November 1993
Received after revision: 8 June 1994
Accepted: 28 June 1994

Sir: Since Wahlberg and coworkers developed the University of Wisconsin (UW) solution for pancreas preservation [11], numerous papers have been published demonstrating that a new era of cold solid organ preservation started in 1986 [1]. Due to the ingredients in UW solution, the period of cold storage was successfully extended up to 48 h in experimental [3] and to over 20 h in human liver transplantation [5]. Orthotopic liver transplantation in rats has been performed in our lab for several years [10] and we have found UW solution to be quite superior to Euro-Collins solution with regard to the integrity of microcirculation [8, 12]. However, despite the beneficial effect of UW solution in contrast to other solutions in reducing reperfusion injury after transplantation, we recently observed transient, irregular perfusion followed by pathological leukocyte adhesion. We noted macroscopically visible small particles in the bags containing UW solution (ViaSpan, DuPont Pharma, Bad Homburg, Germany/ NBPI, the Netherlands); these were subsequently eliminated by filtering. To evaluate the relevance of these particles more systematically, we performed intravital microscopic studies after rat liver transplantation with and without filtering UW solution. In addition, we characterized the particles using mass spectrometry.

Rat liver transplantations were performed in 24 Sprague-Dawley rats according to the technique described by Kamada et al. [6]. The livers were harvested after flushing the organs with 10 ml of ice-cold UW solution via the portal vein (pressure controlled with 15–20 cm water) and subsequently stored for 20 h at 0–4 °C. In 12 of these experiments, UW solution was filtered using a bacterial filter (Sterifix 0.2 μm, Braun-Melsungen, Melsungen, Germany). Ninety minutes after completion of transplantation surgery, the rat livers were examined using intravital fluorescence microscopy, as described earlier [7, 8].

Using different lots of UW solution, irregularly perfused, spotted surfaces were observed during the first minutes after reperfusion in animals in which unfiltered UW solution had been used. In the livers that had been flushed with filtered UW solution, this irregular perfusion was macroscopically nearly completely prevented. Quantitative evaluation of the microcirculation 90 min after reperfusion indicated no significant differences with respect to the sinusoidal perfusion between the filtered group (64 % ± 1 %; mean ± SEM) and the unfiltered group (65 % ± 1 %). However, temporary sinusoidal leukocyte adhesion (adhesion time < 20 s) was significantly reduced in the filtered group (16.8 % ± 1.4 %; mean ± SEM) compared to the unfiltered group (25.8 % ± 1.9 %; $p < 0.001$). Thus, sinusoidal perfusion seems to be uneffected 90 min after reperfusion by filtering UW solution. However, the early postischemic inflammatory process, as reflected by increased leukocyte adhesion [4, 9], was attenuated by filtering UW solution.

It seems likely that small particles (20–150 μm; Fig. 1) suspended in UW solution were responsible for the disturbance of microcirculation observed during the first minutes of reperfusion. Flocky particles were macroscopically visible in all five lots examined, each containing six bags (lot number: D_3G_{21}, D_3F_{30}, D_3B_{23}, D_3CO_2, D_3LO_8, all before the guaranteed expiration date). The bags were kept at 5–6 °C in our laboratory for a period of 2 weeks to 2 months after delivery from NBPI, the Netherlands. Unstable conditions or errors in handling the bags during storage in our lab could be excluded. Professor J. H. Fischer (Experimental Medicine, University of Köln, Germany) could also observe undissolved particles in almost every commercially available lot of UW solution (personal communication).

We succeeded in isolating the particles by vacuum filtering (HA filter 0.2 μm, Millipore, Eschborn, Germany) of the sterile UW solution. Some 10–15 mg/l of a white, solid, crystalline substance (melting point about 123 °C), which was insoluble in hydrous solution, was obtained. This crystalline substance was analyzed first by mass spectrometry using a direct inlet system and electron impact ionization (70 eV). According to the mass spectrum, the substance is a mixture of palmitic and stearic acid, and high-resolution measurements gave the expected elementary composition: $C_{16}H_{32}O_2$ for palmitic and $C_{18}H_{36}O_2$ for stearic acid. For GC-MS analysis (capillary: fused silica 30 m × 0.32 mm, coated with Durabond 1, temperature program: 4 min 100 °C, then 4 min to 280 °C), the mixture was derivatived with bistrimethylsily trifluoro acetamide (BSTFA) to give the volatile fatty acid trimethylsilylesters. The chromatogram again showed a 1:1 mixture of C-16 and C-18 trimethylsilylesters as the main components, together with traces of other fatty acid esters. Mass spectra and retention were identical to those of pure references. The origin of the fatty acids remains unclear; however, we might speculate that an interaction between the numerous ingredients in UW solution and the components of the plastic bags had taken place.

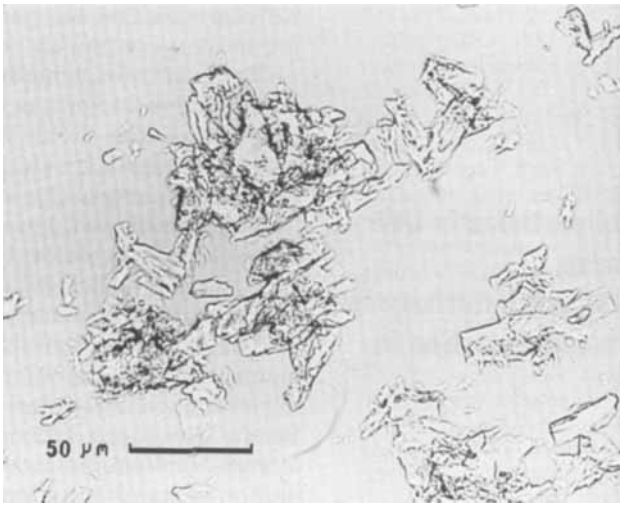

Fig. 1. Light microphotography of undissolved particles (20–150 µm) in the UW solution that cause microcirculatory disturbance in the early reperfusion period. Mass spectrometry identified the substance of palmitic and stearic acid

It is generally known that microcirculatory disturbances lead to severe organ dysfunction [2]. However, variations of the hepatic microvascular perfusion under experimental conditions using different preservation solutions have to be considered and are not necessarily related to the human situation. From our observations it can be assumed that the particles lead to an occlusion of the microvascular system, as reflected by an irregular perfusion in the first minutes of reperfusion. Even in the case of dissolution or metabolism of such fatty acids during reperfusion of the liver graft, temporarily unperfused areas of the transplanted liver graft are exposed to prolonged warm ischemia, which may lead to enhanced reperfusion injury reflected by increased leukocyte adhesion. Finally, the implications of these observations for clinical liver transplantation remain to be determined. It is, however, possible that in critical cases of poor graft function with marginal microvascular perfusion, the crystalline deposits may have clinical relevance.

Acknowledgement We thank Mr. Fuchs, from the Department of Anatomy, for the excellent microphotography.

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