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Effect of University of Wisconsin organ-preservation solution on haemorheology

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Abstract In conventional cold-storage organ preservation, the donor organ is flushed with University of Wisconsin (UW) solution at 0–4°C. The initial flush is used to wash out blood from the microcirculation to allow optimal preservation with the UW solution. The component hydroxyethyl starch (HES) of UW is known to cause relatively high viscosity and a possible interaction with blood, i.e. increased red blood cell (RBC) aggregation. The aim of this study was to investigate the influence of the HES component on the viscosity of UW and the aggregation behaviour of blood during washout. Viscosity aspects were measured with a cone-plate rheometer. HES-induced RBC aggregation was studied by means of an optical aggregation measuring device. The experiments were carried out with rat whole blood and mixtures of rat whole blood with UW-solution and UW without HES (UWmod), at 4°C. The viscosity of blood at 4°C is two-times higher than at 37°C; the UW/blood mixture at 4°C is 1.3-times more viscous than blood at 37°C; the 4°C UWmod/blood mixture equals the viscosity of blood at 37°C. The UW/blood mixture shows a ninefold increased aggregation compared with whole blood. These aggregates are larger than the diameter of the sinusoids in the rat liver. A mixture of whole blood and UWmod shows a lower aggregation than blood. Apart from an increased viscosity, HES in UW causes increased RBC aggregation. The aggregates are larger than the diameter of the sinusoids. Initial washout could be optimised by preflushing to improve the viability of the liver and to decrease delayed graft function.

Keywords RBC aggregation · Viscosity · Liver procurement · Initial washout · UW · Organ preservation · HES · Rat

Introduction

Over the past decades, liver transplantation has become a routine mode of therapy for patients suffering from end-stage liver disease. Despite major achievements in liver transplantation, more specific immunosuppressive agents and a better understanding and improved treatment of complications, preservation of the liver still remains a critical issue. To bridge the time span between donor hepatectomy and transplantation, livers are routinely preserved by static cold storage. Prior to cold storage, the liver is flushed with a hypothermic (4°C) preservation solution, in the majority of cases with the University of Wisconsin (UW) cold storage solution, before it is stored in the same solution on melting ice [1, 2].

In the past, Belzer and Southard added a colloid to a static cold storage solution, as they intended to diminish oedema formation during organ washout, and they developed one preservation solution suitable for cold storage, as well as for machine perfusion (MP) techniques [3, 4]. Until UW was introduced, static cold-storage solutions did not contain any colloid. In contrast, during continuous hypothermic perfusion, a high colloidal substance is always included to counteract extravasation of fluids due to hydrostatic pressure [5]. Based on experience with kidney preservation, a colloid, the diafiltrated hydroxyethyl starch, was included [6, 7, 8, 9]. Other reasons for the inclusion of a colloid were the better viability and outcomes of abdominal and thoracic organs preserved in colloid containing solutions [10].

Besides these beneficial effects of adding a colloid to preservation solutions, disadvantages have also been reported, especially that of the inclusion of HES in the initial washout and/or preservation solution as it tends to increase the viscosity [11, 12, 13]. A number of authors have pointed out the increased viscosity; however, at present, no studies on a true analysis of the behaviour of the UW solution in relation to blood at the time of the initial washout during organ procurement have been published.

The use of the cold-storage technique with the UW preservative allows liver preservation up to approximately 12–15 h [8, 14]. Another method for storing organs is hypothermic machine perfusion (MP), introduced by Belzer in the 1960s and shown to be beneficial in kidney preservation [1, 2]. The advantage of MP over cold storage is the continuous washout of waste products and supply of nutrients. Therefore, it might also offer better opportunities for preservation of the liver [11].

Use of the MP technique could lengthen the duration of preservation and, furthermore, it could allow the transplantation of livers retrieved from marginal or non-heart-beating donors. For an optimal result after transplantation, using machine perfusion preservation, we postulated that the initial washout and MP should use stable physiological pressures, thus minimising endothelial cell damage due to high shear stress. Therefore, in our preliminary rat experiments, portal venous washout of blood was carried out at a physiological pressure of 12 mmHg. This resulted, however, in an incomplete washout of the rat liver with UW. The repeating of the washout experiment with a modified UW without the hydroxyethyl starch (HES) component showed a better washout of blood and a complete and even distribution of the solution throughout the entire liver. An incomplete washout is avoided because it has a detrimental effect on preservation and it exposes the procured liver to increased ischaemia or reperfusion injury with a decreased functional recovery after transplantation [15, 16, 17].

Thus, our aim was to analyse the viscosity of the UW solution and the aggregation behaviour of blood due to HES. In these experiments the behaviour of the original UW solution was investigated and compared with a modified UW solution (UWmod) without HES, and both in combination with blood.

Materials and methods

Animals

Albino Oxford rats (250–350 g) were used as blood donors. In all experiments, blood (6 ml) was obtained by cardiac puncture from rats anaesthetised with halothane/O_2/N_2O. Five minutes prior to blood harvesting, 1 ml saline solution containing 500 units of heparin was administered intravenously.

Sample preparation

Blood samples were collected in heparin-coated tubes at 4°C and used immediately in the test set-ups. Rat whole blood was standardised to a haematocrit of 45% by adjustment of the amount of blood plasma; mixtures were prepared immediately before the experiments were carried out. We performed all experiments six times, using four experimental groups containing: (1) UW (ViaSpan, DuPont, Wilmington, USA), (2) UWmod (prepared in accordance with ViaSpan recipe, without addition of HES), (3) a mixture of UW and rat whole blood (1:1) and (4) a mixture of UWmod with rat whole blood (1:1). Rat whole blood at 37°C and 4°C served as first and second controls.

Viscosity

Viscosity aspects of the preservation solution were analysed by the following mechanisms:

1. Magnitude. Is the viscosity of UW influenced by HES under hypothermic conditions. What is the effect of the combination of UW and whole blood on the viscosity?
2. Non-Newtonian behaviour. Whole blood is known for its non-Newtonian behaviour, which implies that the viscosity is much higher at low shear rates than at higher shear rates [18]. Does UW itself also show this behaviour, and if so, is this effect still present and prominent when blood is combined with UW?
3. Bingham effect. Some fluids display the Bingham effect, which means that there is a stress threshold (τ_{thres}) above which the fluid will start to flow. Blood also shows the Bingham effect [18]. Does UW induce a stress threshold? Is there an increase in this stress

threshold as a consequence of the UW that could explain a poor blood washout of the liver, because (locally) the pressure is too low to overcome the stress threshold?

These viscosity measurements were done with a cone-plate rheometer (AR 1000, TA Instruments, New Castle, USA), that measured the shear stress of a 0.5 ml sample at increasing shear rates. This method allowed us to determine the viscosity (Pa s) of the solutions, defined as the quotient of shear stress (Pa) and shear rate (s^{-1}), but it also allowed us to study the behaviour of the solutions at low shear rates. By applying shear rates of up to 100 s^{-1}, we obtained shear rate–shear stress and shear rate–viscosity curves from the four solutions at 4°C and from the controls of rat whole blood at 37°C and 4°C.

Red blood cell aggregation

Corry et al. have shown that HES can increase red blood cell aggregation when it is in contact with blood [19]. Measurements to study the influence of HES chains in the UW solution on the aggregation behaviour of rat blood were carried out with a laser-assisted optical rotational cell analyser (LORCA, R&R Mechatronics, Hoorn, The Netherlands) [20, 21]. The LORCA consists of a temperature-controlled Couette system with a sample volume of 1.5 ml. It measures blood cell aggregation, using the intensity of the laser light, back-scattered by blood that is projected on the sample (Fig. 1). The amount of aggregation is displayed by the aggregation amplitude, which is defined as the difference between the intensity of back-scattered laser light at the moment of disaggregation ($t=0$) and the moment of total aggregation ($t=2$) (Fig. 1).

To represent the gradual dilution of whole blood with UW, occurring in the in situ blood washout of the organ, we chose the dilution ratios of blood with UW and UWmod to be 4:1, 3:2 and 1:1. We also measured whole blood at 4°C.

Microscopic examination

Red blood cell (RBC) aggregation in solutions of UW with whole blood (1:1) and UWmod with whole blood (1:1) at 4°C were studied by light microscopy. The solutions were processed on a glass plate as a standard smear and stained with May–Grunwald Giemsa. Microscopic examination (Leica Microsystems, Wetzlar, Germany) was carried out in combination with an image-processing program for quantification of the aggregates.

Statistical analyses

Comparison between the results was done by means of an unpaired two-tailed Student's t-test. Differences were considered to be significant for $P < 0.05$.

Results

Viscosity

The measured shear rate–viscosity and shear rate–shear stress curves for the UW and UWmod solutions, as well

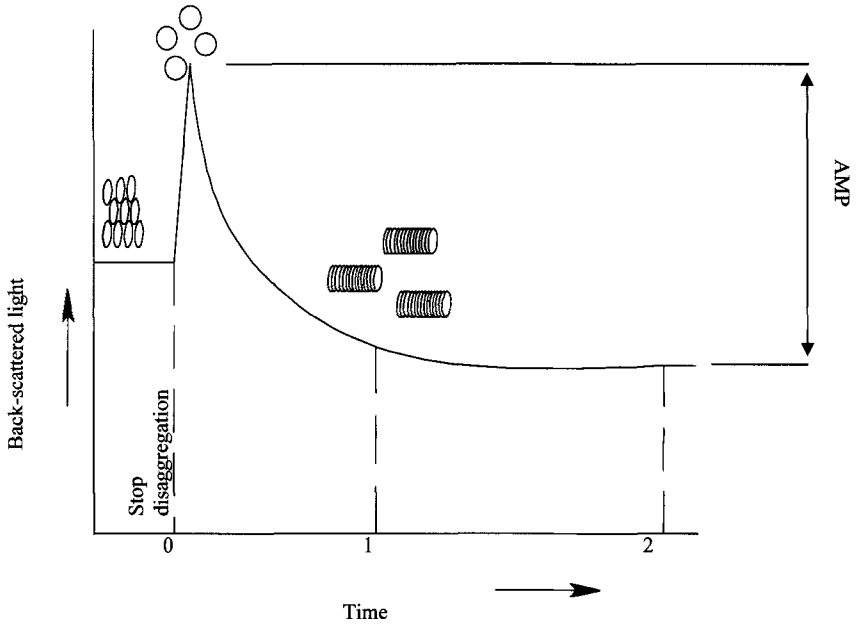

Fig. 1 Blood cell aggregation: light scatter before and after the sudden stopping of disaggregation. Erythrocyte morphology corresponding to various parts of the curve is indicated. *From left to right*: elongated flow-orientated and disaggregated blood cells, un-deformed, randomly orientated and disaggregated blood cells, rouleaux aggregation. *AMP* aggregation amplitude [10]

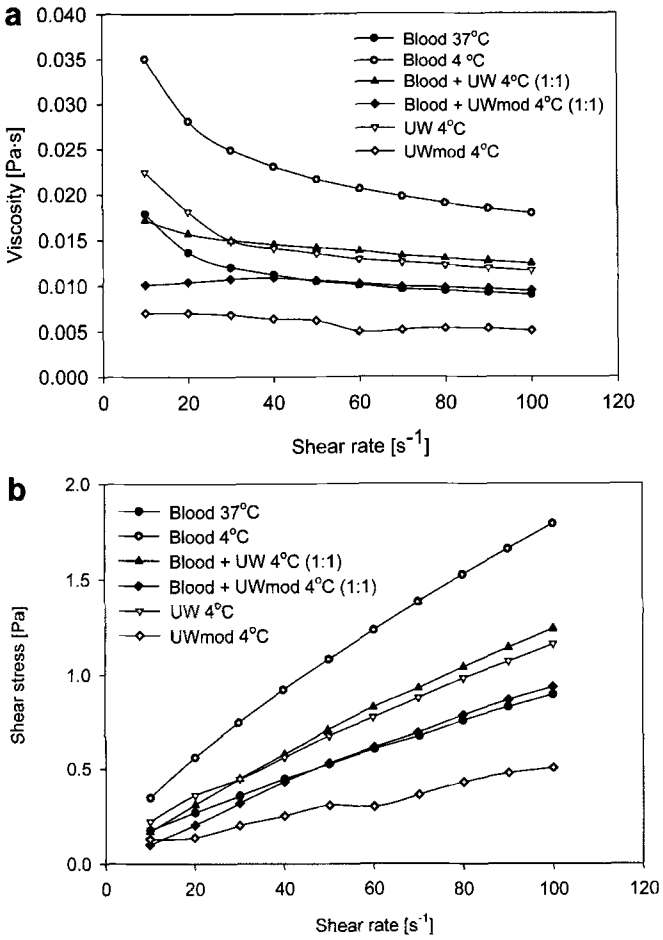

Fig. 2a, b Shear rate. Viscosity **a** and shear rate–shear stress curves **b** of UW, blood and blood–UW mixtures

as their mixture with whole blood (1:1) at 4°C, are shown in Fig. 2. For comparison, the viscous behaviour of rat whole blood at 4°C and 37°C is also shown (Fig. 2). Whole blood demonstrated its characteristic non-linear viscous behaviour, and UW showed this non-Newtonian property as well. The effect was also present in the mixture of whole blood with UW. The overall viscosity of the mixture of whole blood and UW was 1.3-times higher than that of whole blood at 37°C. UWmod showed a constant viscosity for different shear rates, and even the mixture with whole blood showed a constant viscosity, although the viscosity rate was 1.8-times higher. The low temperature caused a much higher viscosity curve for whole blood at 4°C than for that at 37°C.

The shear rate–shear stress curves demonstrated a linear relationship. The shear stresses of the solutions at zero shear rate (extrapolated from the mean) were slightly higher than zero, but no considerable increase in τ_{thres} was observed (Table 1). The viscosity of the solutions can be defined as the asymptotic value of the viscosity towards high shear rates. For a complete overview, these values are also listed in Table 1.

Table 1 Viscosity and threshold stress values of the solutions studied (*WB* = whole blood)

Parameter	Viscosity (Pa s)	τ_{thres} (Pa)
WB 37°C	$9\ 10^{-3}$ ($\pm 1.7\ 10^{-3}$)	0.03
WB 4°C	$18\ 10^{-3}$ ($\pm 3.6\ 10^{-3}$)*	0.07
UW 4°C	$11\ 10^{-3}$ ($\pm 0.4\ 10^{-3}$)*	0.001
UWmod 4°C	$5\ 10^{-3}$ ($\pm 0.4\ 10^{-3}$)*	0.009
UW + WB 4°C	$12\ 10^{-3}$ ($\pm 1.4\ 10^{-3}$)*	0.07
UWmod + WB 4°C	$9\ 10^{-3}$ ($\pm 1.2\ 10^{-3}$)	0.01

*Significant differences ($P < 0.05$) with 37°C rat whole blood

RBC aggregation

The mixtures of blood and UW of 3:2 and 1:1 showed a significant increase in aggregation when compared with rat whole blood ($P < 0.05$). The same mixtures with UWmod showed a decreasing trend in aggregation, although levels did not reach significance. The results of the aggregation measurements with the LORCA are shown in Fig. 3 as percentages of whole-blood aggregation.

Microscopic examination

A typical microscopic image of the smears of the agglutination tests of blood in a 1:1 mixture with UW or UWmod is shown in Fig. 4. The randomly distributed structure of the mixture of blood with UWmod is clearly different from the structure of the blood mixture with the original UW-solution. The aggregates that are formed in this mixture of blood and UW had a mean length of 30 µm (±8 µm) and a mean width of 18 µm (±6 µm).

Discussion

In the past, several authors used an HES-free washout solution prior to preservation with the original UW solution [22, 23, 24, 25, 26]. Pienaar et al. [27], for example, described successful machine preservation experiments, but they also used a so-called pre-flush of lactated Ringer's solution to wash out the blood from the liver. Other authors experienced difficulties in using HES in their preservation and/or initial washout solutions as well, as they have eliminated HES [11, 12, 13]. These authors suggested that these difficulties were due to the high viscosity of the UW solution, caused by HES. However, our viscosity measurements showed that

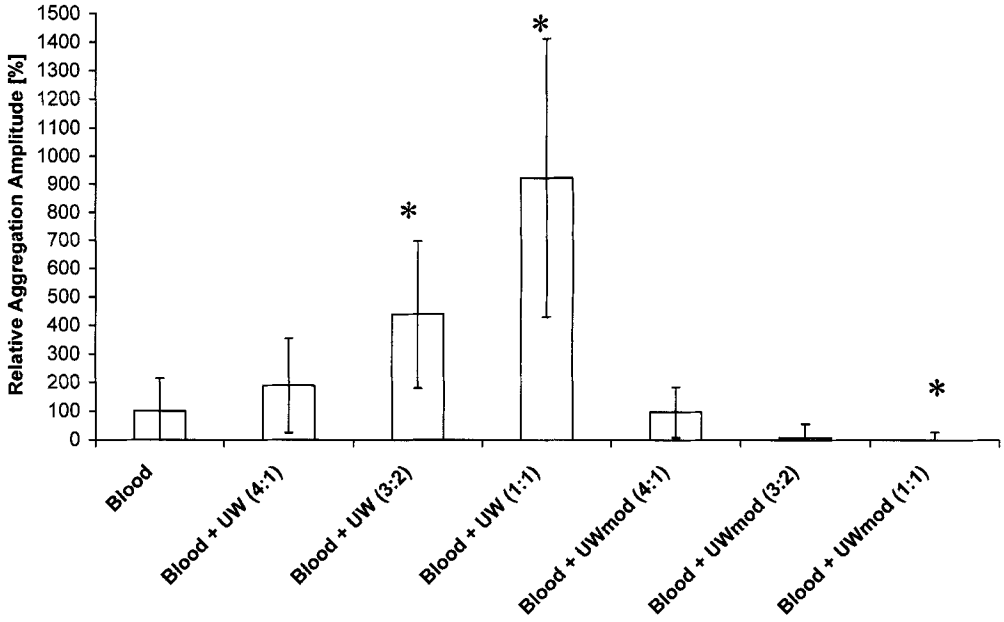

Fig. 3 Aggregation amplitudes of blood in combination with different concentrations of UW and UWmod, relative to blood. *Significant differences ($P < 0.05$) with rat whole blood

the viscosity of UW is a factor only 1.2-times higher than that of blood at 37°C. The viscosity of blood at 4°C is twice as high as that of blood at 37°C. The viscosity of the combination of UW and rat whole blood ($11 \cdot 10^{-3}$ Pa s) was found to be between the viscosity of UW at 4°C and that of rat whole blood at 4°C. The elimination of the HES from the UW significantly lowers the viscosity, as well as for UW combined with blood (1:1). Despite these results, however, high viscosity of the UW solution alone cannot be the only cause of a poor washout of blood from a rat liver. Hence, the increase in viscosity due to the use of UW at 4°C is by a factor of only 1.3 and, therefore, results in a 1.3-times lower flow at a certain washout pressure than at physiological conditions with whole blood at 37°C. It is very unlikely that this increase in viscosity is responsible for poor organ washout.

A considerable non-linear viscosity component of the UW solution is illustrated (Fig. 2). Since this phenomenon is not found in UWmod, we can conclude that this is caused by HES. In the combination of UWmod with blood, the dilution is so strong that the non-linear effect of whole blood is diminished. An increase in the threshold pressure (Bingham effect) is not present; therefore, it does not contribute to a poor organ washout (Fig. 2b).

The influence of HES chains on the behaviour of rat whole blood dominates the results of the LORCA measurements (Fig. 3). RBC aggregates are formed when UW is mixed with blood. The amplitude of aggregation increases with increasing concentration of UW. The addition of 20% UW does not have a significant effect, but the addition of 40% and 50% UW significantly increases the aggregation amplitude. The mixtures of blood and UWmod totally lack this effect. They only dilute blood, and almost no aggregation amplitude can be measured. The effect of increased RBC aggregation becomes even more apparent and clear when it is observed microscopically (Fig. 4). In these smears of the combination of whole blood and UW, large aggregates are visible. Without HES, the blood cells diffuse uniformly throughout the solution in an individual manor.

The shape and size of the aggregates induced by HES can easily block the sinusoids. The mean diameter of a sinusoid in the rat liver is approximately 10 μm; the mean length and width of the aggregates are 30 μm and 18 μm, respectively. This might cause a blockade of sinusoids that results in a poor initial washout during liver procurement, as has been recently observed in washout experiments that use non-heart-beating donor livers [15]. If it is taken into account that the average diameter of the capillaries in, for example the kidney, is approximately 10 μm, this mechanism might also play an important role in the washout and preservation of other organs.

The finding that the combination of UW and blood produces large aggregates might also play an important role in human donor organs during initial blood washout when physiological perfusion pressure is applied, since we also see this aggregation effect in human whole blood [28]. It has even been shown that the shear rates that occur in the washout procedure are not high enough to easily dissociate the formed aggregates [28]. Improvement of the initial washout procedure is, thus, of utmost importance for improving the graft quality and subsequent viability. We therefore propose to preflush the donor liver in situ with a flushing solution

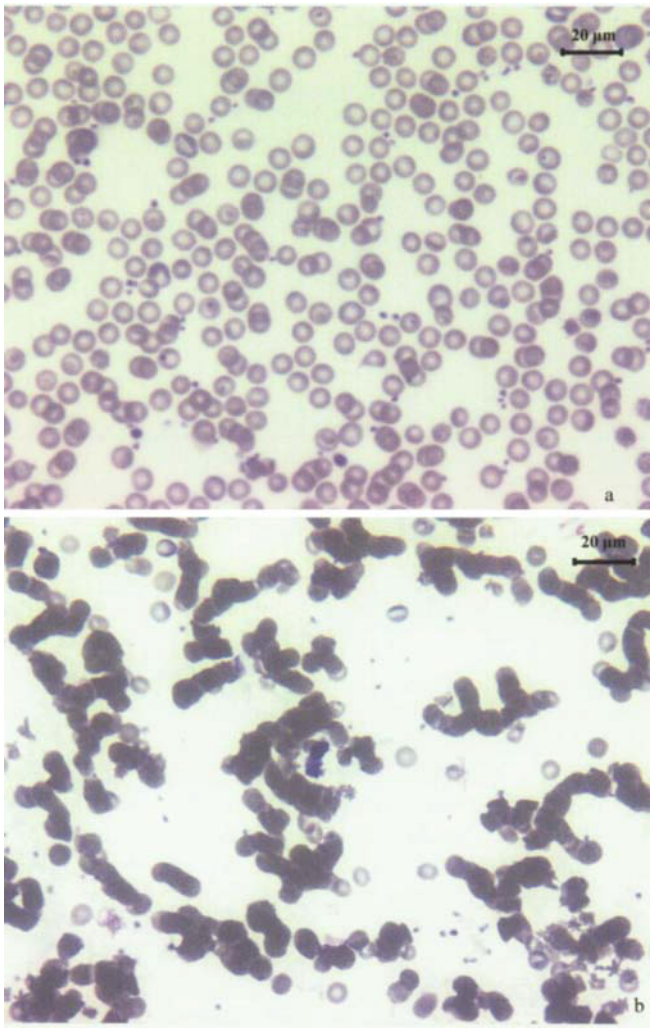

Fig. 4a, b Illustrative smears (May–Grunwald Giemsa staining) of rat blood cells in combination with **a** UWmod (1:1) and **b** UW (1:1)

without HES, or to find an alternative for HES, e.g. a non-RBC-aggregating colloid, to optimise the initial washout and subsequent organ preservation.

We conclude that poor initial washout with UW is most likely due to aggregate formation induced by HES in combination with a slightly increased viscosity of UW. Before the beneficial effects of MP can be fully utilised, initial donor liver washout has to be improved because it is strongly hampered by UW-induced RBC aggregation.

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References

1. Belzer FO, Ashby BS, Dunphy JE. 24-Hour and 72-hour preservation of canine kidneys. Lancet 1967; 2:536.
2. McAnulty JF, Vreugdenhill PK, Southard JH, Belzer FO. Use of UW cold storage solution for machine perfusion of kidneys. Transplant Proc 1990; 22:458.
3. Belzer FO, Southard JH. Principles ofsolid-organ preservation by cold storage. Transplantation 1988; 45:673.
4. Southard JH, van Gulik TM, Ametani MS, et al. Important components of the UW solution. Transplantation 1990; 49:251.
5. Biguzas M, Jablonski P, Thomas AC, et al. Evaluation of UW solution in a rat kidney preservation model. I. Effect of hydroxyethyl starch and electrolyte composition. Transplantation 1990; 49:872.
6. Ar'Rajab A, Ahren B, Sundberg R, Bengmark S. The function of a colloid in liver cold-storage preservation. Transplantation 1991; 52:34.
7. Candinas D, Largiader F, Binswanger U, Sutherland DE, Schlumpf R. A novel dextran 40-based preservation solution. Transpl Int 1996; 9:32.
8. Southard JH, Belzer FO. Organ Preservation. Annu Rev Med 1995; 46:235.
9. Sumimoto R, Jamieson NV, Wake K, Kamada N. 24-Hour rat liver preservation using UW solution and some simplified variants. Transplantation 1989; 48:1.
10. Howden BO, Jablonski P, Thomas AC, et al. Liver preservation with UW solution. I. Evidence that hydroxyethyl starch is not essential. Transplantation 1990; 49:869.
11. Dutowski P, Odermatt B, Heinrich T, et al. Hypothermic oscillating liver perfusion stimulates ATP synthesis prior to transplantation. J Surg Res 1998; 80:365.

12. Sumimoto R, Kamada N, Jamieson NV, Fukuda Y, Dohi AK. A comparison of a new solution combining histidine and lactobionate with UW solution and eurocollins for rat liver preservation. Transplantation 1991; 51:589.
13. Tojimbara T, Wicomb WN, Garcia-Kennedy R, et al. Liver transplantation from non-heart beating donors in rats: influence of viscosity and temperature of initial flushing solutions on graft function. Liver Transpl Surg 1997; 3:39.
14. Audet M, Alexandre E, Mustun A, et al. Comparative evaluation of celsior solution versus viaspan in a pig liver transplantation model. Transplantation 2001; 71:1731.
15. Minor T, Hachenberg A, Tolba R, Pauleit D, Akbar S. Fibrinolytic preflush upon liver retrieval from non-heart beating donors to enhance post-preservation viability and energetic recovery upon reperfusion. Transplantation 2001; 71:1792.
16. Ploeg RJ, Goossens D, McAnulty JF, Southard JH, Belzer FO. Successful 72-hour cold storage of dog kidneys with UW-solution. Transplantation 1988; 46:191.
17. Yamauchi JI, Richter S, Vollmar B, Menger MD, Minor T. Warm preflush with streptokinase improves microvascular procurement and tissue integrity in liver graft retrieval from non-heart-beating donors. Transplantation 2000; 69:1780.
18. Milnor WR. Hemodynamics, Williams & Wilkins, Maryland, USA, 1989.
19. Corry WD, Jackson LJ, Seaman GV. Action of hydroxyethyl starch on the flow properties of human erythrocyte suspensions. Biorheology 1983; 20:705.
20. Cicco G, van der Kleij AJ, Stingi GD, Tarallo MS, Pirrelli A. Laser-assisted optical rotational red cell analyzer (LORCA) in clinical practice. Hemorheological kinetics and tissue oxygenation. Adv Exp Med Biol 1999; 471:631.
21. Hardeman MR, Goedhart PT, Dobbe JGG, Lettinga KP. Laser-assisted optical rotational cell analyser (L.O.R.C.A.); I. A new instrument for measurement of various structural hemorheological parameters. Clin Hemorheol 1994; 14:605.
22. Iwamoto H, Matsuno N, Narumi Y, et al. Beneficial effect of machine perfusion preservation on liver transplantation from non-heart-beating donors. Transplant Proc 2000; 32:1645.
23. Kozaki K, Uchiyama M, Nemoto T, et al. Usefulness of a combination of machine perfusion and pentoxifylline for porcine liver transplantation from non-heart-beating donors with prolonged hypotension. Transplant Proc 1997; 29:3476.
24. Uchiyama M, Kozaki K, Nemoto T, et al. Liver transplantation from non-heart-beating donors: effect of machine perfusion preservation and pentoxifylline. Transplant Proc 1998; 30:3798.
25. Uchiyama M, Matsuno N, Hama K, et al. Comparison between nonpulsatile and pulsatile machine perfusion preservation in liver transplantation from non-heart-beating donors. Transplant Proc 2001; 33:936.
26. Yamamoto N, Konishi Y, Wakashiro S, et al. Seventy-two-hour preservation of porcine liver by continuous hypothermic perfusion with UW solution in comparison with simple cold storage. J Surg Res 1991; 51:288.
27. Pienaar BH, Lindell SL, Van Gulik T, Southard JH, Belzer FO. Seventy-two-hour preservation of the canine liver by machine perfusion. Transplantation. 1990; 49:258.
28. Morariu AM, van der Plaats A, van Oeveren W, et al. Hyperaggregating effect of hydroxyethyl starch components and University of Wisconsin solution on human red blood cells: a risk of impaired graft perfusion in organ procurement? Transplantation 2003; 76:37.