The Groningen hypothermic liver perfusion system for improved preservation in organ transplantation
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Chapter 4

Effect of University of Wisconsin Organ Preservation Solution on Hemorheology

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4.1 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 with improved techniques, more specific immunosuppressiva as well as a better understanding and treatment of complications, preservation of the liver still remains a critical issue. To bridge the timespan between donor hepatectomy and transplantation, livers are nowadays routinely preserved by static cold storage (CS). Prior to CS the liver is flushed with a hypothermic (4°C) preservation solution, in the majority of cases with the University of Wisconsin-Cold Storage Solution (UW), before storage in the same solution on melting ice\textsuperscript{1,2}.

In the past, Belzer and Southard added a colloid to a static CS solution as they intended to diminish edema formation during organ wash-out and develop one preservation solution suitable for both CS as well as MP techniques\textsuperscript{3,4}. Until the intro-
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duction of UW, static CS 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 in kidney preservation, a colloid, the diafiltrated hydroxyethyl starch, was included\(^6\). Other reasons to include a colloid were better viability outcomes of abdominal and thoracic organs preserved in colloid containing solutions\(^9\). Besides these beneficial effects of adding a colloid to preservation solutions, disadvantages have also been reported, especially including HES in the initial wash-out and/or preservation solution, as it tends to increase the viscosity\(^11\). Until now, a number of authors has pointed at the increased viscosity, however, no studies have ever been published that have focused on a true analysis of the behaviour of the UW solution in relation to blood at the time of the initial wash-out during organ procurement.

Using the CS technique with the UW preservative allows liver preservation up to approximately 12-15 hours\(^8,14\). Another method to store organs is hypothermic machine perfusion (MP), introduced by Belzer in the sixties and shown to be beneficial in kidney preservation\(^1,2\). The advantage of MP over CS is the continuous wash-out of waste products and supply of nutrients. It therefore might also offer better preservation opportunities for the liver\(^11\). Using the MP technique could lengthen the duration of preservation and it furthermore 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 wash-out and MP should use stable physiologic pressures thus minimising endothelial cell damage due to high shear stress. Therefore, in our preliminary rat experiments, portal venous wash-out of blood was done with a physiologic pressure of 12 mmHg. This resulted, however, in an incomplete wash-out of the rat liver with UW. Repeating the wash-out experiment with a modified UW without the hydroxyethyl starch (HES)-component showed a better wash-out of blood and a complete and even distribution of the solution throughout the entire liver. An incomplete wash-out is unwanted for since it has a detrimental effect on preservation and exposes the procured liver to increased ischemia/reperfusion injury with a decreased functional recovery after transplantation\(^15,16,17\).

Thus, the aim of this study is to analyze 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 to a modified UW solution (UWmod) without HES, and both in combination with blood.
4.2 Materials and Methods

4.2.1 Animals

Albino Oxford rats (250-350 gr) were used as blood donors. In all experiments, blood (6 ml) was obtained by cardiac puncture from anaesthetised rats using Halothane/O$_2$/N$_2$O. Five minutes prior to blood harvesting, 1 ml saline containing 500 units of heparin was administered intravenously.

4.2.2 Sample preparation

Blood samples were collected in heparin-coated tubes at 4°C and used immediately in the test set-ups. Whole rat blood was standardised to a hematocrit of 45% by adjusting the amount of blood plasma; mixtures were prepared immediately prior to the experiments. All experiments were performed in six fold, using four experimental groups containing: a) UW (ViaSpan, DuPont, Wilmington, USA), b) UWmod (prepared according to ViaSpan recipe, without addition of HES), c) a mixture of UW and whole rat blood (1:1) and d) a mixture of UWmod with whole rat blood (1:1). Whole rat blood at 37°C and 4°C served as first and second control.

4.2.3 Viscosity

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

- **Magnitude**: Is the viscosity of UW influenced by HES under hypothermic conditions and what is the effect of the combination of UW and whole blood on the viscosity?

- **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?

- **Bingham-effect**: Some fluids experience the Bingham-effect, which means that there is a threshold stress ($\tau_{thres}$) above which the fluid will start to flow. Blood also shows the Bingham-effect$^{18}$. Does UW induce a threshold stress, there an increase in this threshold stress as a consequence of the
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UW could explain a poor blood wash-out of the liver, because (locally) the pressure is too low to overcome the threshold stress ($\tau_{\text{thres}}$)?

These viscosity measurements were performed with a cone-plate rheometer (AR 1000, TA Instruments, New Castle, USA), measuring the shear stress of a 0.5 ml sample at increasing shear rates. This method allows to determine the viscosity [Pa·s] of the solutions, defined as the quotient of shear stress [Pa] and shear rate [s$^{-1}$], but also to study the behaviour of the solutions at low shear rates. By applying shear rates up to 100 s$^{-1}$, shear rate-shear stress and shear rate-viscosity curves were obtained of the four investigated solutions at 4$^\circ$C and of the controls of whole rat blood at 37$^\circ$C and 4$^\circ$C.

![Figure 4.1: Blood cell aggregation: lightscatter before and after sudden stopping disaggregation. Erythrocyte morphology corresponding to various parts of the curve is indicated. From left to right: elongated flow orientated and disaggregated blood cells, undeformed randomly orientated and disaggregated blood cells, rouleaux aggregation. (AMP is aggregation amplitude)\textsuperscript{10}.](image)

4.2.4 Red blood cell (RBC)-aggregation

Corry et al\textsuperscript{19} have shown that HES possesses the ability to increase red blood cell aggregation when it is in contact with blood. Measurements to study the influence of HES chains in the UW-solution on the aggregation behaviour of rat blood were performed using a Laser-assisted Optical Rotational Cell Analyser (LORCA, R&R...
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Mechatronics, Hoorn, The Netherlands)\textsuperscript{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 (Figure 4.1). The amount of aggregation is presented by means of 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 ($t = 2$)(Figure 4.1).

To represent the gradual dilution of whole blood with UW, occurring in the in situ blood wash-out of the organ, the dilution ratio's of blood with UW and UWmod were chosen to be 4:1, 3:2 and 1:1. Also, whole blood at 4°C was measured.

4.2.5 Microscopic examination

RBC-aggregation in solutions of UW with whole blood (1:1) and UWmod with whole blood (1:1) at 4°C were studied with 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 in combination with an image-processing program for quantification of the aggregates.

4.2.6 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 $P < 0.05$.

![Figure 4.2: Shear rate - viscosity (left) and shear rate-shear stress curves (right) of UW, blood and blood-UW mixtures.](image-url)
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4.3 Results

4.3.1 Viscosity

The measured shear rate-viscosity and shear rate-shear stress curves of the UW and UWmod solutions, as well as their mixture with whole blood (1:1) at 4°C are shown in Figure 4.2. For comparison, also the viscous behaviour of whole rat blood at 4°C and 37°C is shown in this figure. Whole blood demonstrated its characteristic non-linear viscous behaviour, and UW showed this non-Newtonian property as well. This effect was also present in the mixture of whole blood with UW. The overall viscosity of the mixture of whole blood and UW was found to be 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 1.8 times higher. The low temperature caused a much higher viscosity curve for whole blood at 4°C compared to 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 $\tau_{\text{thres}}$ was observed (Table 4.1).

Table 4.1: Viscosity and threshold stress values of the studied solutions. Significant differences ($p < 0.05$) with 37°C whole rat blood are indicated with $\ast$.

<table>
<thead>
<tr>
<th>Viscosity $\text{[Pa}\cdot\text{s]}$</th>
<th>$\tau_{\text{thres}}$ $\text{[Pa]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB 37°C</td>
<td>$9 \cdot 10^{-3} \pm 1.7 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>WB 4°C</td>
<td>$18 \cdot 10^{-3} \pm 3.6 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>UW 4°C</td>
<td>$11 \cdot 10^{-3} \pm 0.4 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>UWmod 4°C</td>
<td>$6 \cdot 10^{-3} \pm 0.4 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>UW+WB 4°C</td>
<td>$12 \cdot 10^{-3} \pm 1.4 \cdot 10^{-3}$</td>
</tr>
<tr>
<td>UWmod+WB 4°C</td>
<td>$9 \cdot 10^{-3} \pm 1.2 \cdot 10^{-3}$</td>
</tr>
</tbody>
</table>

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 4.1.

4.3.2 RBC-aggregation

The mixtures of blood and UW of 3:2 and 1:1 show a significant increase in aggregation, compared to whole rat blood ($P < 0.05$). The same mixtures with UWmod show a decreasing trend in aggregation, although levels did not reach significance. The results of the aggregation measurements with the LORCA are displayed in Figure 4.3 as percentages of whole blood aggregation.
4.3. Results

Figure 4.3: Aggregation amplitudes of blood in combination with different concentrations UW and UWmod, relative to blood. Significant differences (p < 0.05) with whole rat blood are indicated with ∗.

4.3.3 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 Figure 4.4. 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).

Figure 4.4: Illustrative smears (May-Grunwald Giemsa staining) of rat blood cells in combination with UWmod (1:1) (left) and UW (1:1) (right).
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4.4 Discussion

In the past, several authors used a HES-free wash-out solution prior to preservation with the original UW-solution\textsuperscript{22–26}. Pienaar et al\textsuperscript{27}, for example, described successful machine preservation experiments, but also reported that he used a so-called preflush of lactated Ringers solution to wash-out the blood from the liver. Others experienced difficulties using HES in their preservation and/or initial wash-out solutions as well, judged by the fact that they have eliminated HES\textsuperscript{11–13}. These authors suggested that these difficulties were due to the high viscosity of the UW solution, caused by HES. However, the viscosity measurements presented here showed that the viscosity of UW is only a factor 1.2 times higher than that of blood at 37°C. The viscosity of blood at a temperature of 4°C is doubled compared to measurements at 37°C. The viscosity of the combination of UW and whole rat blood (11 \cdot 10^{−3} Pa \cdot s) was found to be in between the viscosity of UW at 4°C and whole rat blood at 4°C. Eliminating the hydroxyethyl starch from the UW significantly lowers the viscosity, as well as for UW combined with blood (1:1). Despite these results it is, however, not justified that high viscosity of the UW-solution alone is the cause of a poor wash-out of blood from a rat liver. Hence, the increase in viscosity due to the use of UW at 4°C is only a factor of 1.3, and therefore results in a 1.3 times lower flow at a certain wash-out pressure, compared to physiologic conditions with whole blood at 37°C. It is very unlikely that this increase in viscosity is responsible for a poor organ wash-out. In Figure 4.2, a considerable non-linear viscosity component of the UW-solution is illustrated. Since this phenomenon is absent in UWmod, it can be concluded 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. According to Figure 4.2, an increase in the threshold pressure (Bingham effect) is not present, therefore not contributing to a poor organ wash-out.

The influence of HES-chains on the behaviour of whole rat blood dominates the results of the LORCA measurements (Figure 4.3). RBC aggregates are formed when UW is mixed with blood. The amplitude of aggregation is increasing with an 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 clearer when the microscopic views are observed (figure 4.4). In these smears of the combination of whole blood + UW, large aggregates are visible. Without HES, the blood cells
4.5. References

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 $\mu m$, the mean length and width of the aggregates are 30 $\mu m$ and 18 $\mu m$ respectively. This might cause a blockade of sinusoids resulting in a poor initial wash-out during liver procurement, as has been recently observed in wash-out experiments using non-heart-beating donor livers\textsuperscript{15}. Taking into account that the average diameter of the capillaries in for example, the kidney, is in the order of magnitude of 10 $\mu m$ as well, this mechanism might also play an important role in the wash-out and preservation of other organs.

The finding that the combination of UW and blood produces large aggregates may also play an important role in human donor organs during initial blood wash-out when physiologic perfusion pressures are applied, since we see this aggregation effect in whole human blood as well\textsuperscript{28}. It is even shown that the shear rates occurring in the wash-out procedure are not high enough to easily dissociate the formed aggregates\textsuperscript{28}. To improve the initial wash-out procedure it is thus of utmost importance in improving the graft quality and subsequent viability. We therefore propose to preflush the donor liver in situ with a flushing solution without HES, or to find an alternative for HES, e.g. a non-RBC- aggregating colloid, to optimise the initial wash-out and subsequent organ preservation.

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

4.5 References


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