The Groningen hypothermic liver perfusion system for improved preservation in organ transplantation
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Chapter 6

Hypothermic Machine Preservation in Liver Transplantation Revisited: Concepts and Criteria in the New Millennium


6.1 Introduction

Over the past decades liver transplantation has become a routine mode of therapy for patients suffering from end stage liver disease. To bridge the timespan between donor hepatectomy and transplantation, livers are nowadays routinely preserved by static cold storage (CS) i.e. the liver is flushed in situ with a cold preservation solution and after hepatectomy stored in a preservation solution on melting ice at 0-4°C. The decrease in temperature results in a decrease of liver metabolism. At lowered metabolic rate, the need for nutrients diminishes and production of waste products decreases substantially. Storage at 0-4°C results in 90-95% reduction of cellular metabolism, and throughout the years, 0-4°C has shown to be an adequate temperature for static CS. In the 1980’s Belzer and his co-workers optimised
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the CS technique with the development of the University of Wisconsin preservation solution (UW)\textsuperscript{5,20}. The UW-solution allows preservation of donor livers for 12 to 18 hours in the clinical setting and even storage beyond 48 hours in laboratory experiments\textsuperscript{32,33,64} compared to approximately 6 hours in EuroCollins\textsuperscript{12}. Despite the success of UW and the introduction of improved and more specific immunosuppressiva in addition to a better understanding and treatment of complications, the limits of static CS using the concept of the UW-solution appear to be reached.

To date, the majority of donor livers used for transplantation originate from brain dead donors. Livers from marginal or non-heart-beating donors are presumed to have a poorer quality and were rarely used for transplantation due to an expected decreased organ viability and associated technical complications. To overcome the present shortage of liver donors machine perfusion as a dynamic preservation method is revisited.

Already in the 1960's, Belzer\textsuperscript{3} started experiments with hypothermic machine preservation (HMP) by studying continuous perfusion of kidneys. His efforts, along with others, resulted in improved clinical results using continuous perfused kidney preservation in comparison to CS. With HMP, the use of non-heart-beating and so-called marginal kidney donors is now feasible\textsuperscript{2,15,27,46,50,63}. In the laboratory even 5-7 days successful preservation of canine kidneys can be achieved\textsuperscript{38,39}. Due to these successes and the potential to increase the donor pool and prolong storage times, continuous preservation of the liver has gained renewed interest. Based on the success of HMP of the kidney, continuous machine perfusion of the liver could contribute to better preservation of normal heart-beating donor livers, facilitate the use of marginal donor livers and nowadays also allow liver transplantation from non-heart-beating donors.

In the late 1960's Belzer\textsuperscript{4}, Slapak\textsuperscript{54} and Brettschneider\textsuperscript{10} experimented with continuous hypothermic machine perfusion of the liver in the experimental setting with results comparable or even better than livers preserved with static CS. In 1986 D'Alessandro et al\textsuperscript{15} and later Pienaar et al\textsuperscript{48} managed to transplant good quality canine livers after 72 hours preservation in a HMP dog model. Despite successful experience with continuous perfusion of kidneys and the results obtained by Pienaar with the liver, HMP has not become a standard procedure in every day practise. Moreover, until today, there is no commercially available liver perfusion machine to improve organ viability and seriously challenge the limits of liver preservation by optimising perfusion and transportation during cold storage as done with machine preservation in kidneys in dedicated centres.

This review describes the rationale for renewed efforts in liver preservation re-
6.2. Hypothermic Organ Preservation

Despite many biotechnical achievements, hypothermia is, until today, a key principle to prevent viability loss of donor livers. Thus, during preservation the metabolism is reduced. However, hypothermia also invokes cellular injury of the liver. Therefore, solutions used for hypothermic preservation have to contain protective components to prevent cold induced cellular injury. Furthermore, the solution should provide a complete initial wash-out of donor blood during the flushing period, then enable rapid cooling of the liver, maintain osmotic balance between interstitial space and vascular space and supply the liver cells with oxygen.

In the early days of liver HMP research, perfusion was established using autologous diluted blood or plasma-based solutions. Since preservation with these cellular solutions was not very successful, a shift towards acellular perfusion solutions has occurred. Slapak initially perfused livers with saline in the late 1960s, and later perfusion with cold Krebs-Henseleit bicarbonate and lactated-Ringer’s solution was reported. The UW-MP-solution, developed by Belzer and Southard, is an acellular solution used by most centres for HMP. It satisfies the physiological and biochemical requirements for a HMP solution including gluconate to prevent hypothermia induced cell swelling, phosphate to prevent intracellular acidosis, glutathione and allopurinol to inhibit oxygen free radical formation, adenosine to stimulate ATP synthesis and finally hydroxyethyl starch to keep the vasculature open and prevent edema. The UW-solution used for HMP (UW-MP) differs from the UW-solution for static cold storage (UW-CS): in UW-MP gluconate is included as an impermeant versus lactiobionate in cold storage. Using the UW-MP solution, Pienaar et al. found a 90% survival after 72 hrs HMP of dog livers. Successful experiments were also described by Boudjema et al., Kim et al. and Yamamoto et al. However, despite the success of the UW-MP solution, the same solution has negative side effects as well. The inclusion of high-molecular weight hydroxyethyl starch, for example, increases the viscosity of the UW-solution, compared to blood or solutions without a colloid (e.g. lactated-Ringer’s or Krebs-Henseleit bicarbonate). The increased viscosity of the UW-solution has been blamed for a sub-optimal wash-out of blood cells during the initial flush of the donor organ. Pirenne et al. even stated that prevention of biliary complications after transplantation...
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depends on the viscosity of the preservation solution used. Recently, we found that not so much the viscosity but instead an increased red blood cell aggregation during initial wash-out of blood due to the high-molecular weight hydroxyethyl starch appears to be the key factor\textsuperscript{70}. To eliminate the negative effects of the increased viscosity and/or the increased red blood cell aggregation, we recommend performing the initial wash-out with a low-viscous aggregation-preventing solution\textsuperscript{43,70}. This can be done by flushing with e.g. lactated-Ringer’s\textsuperscript{14,37,48,74} or EuroCollins solution\textsuperscript{30,35,67,68}, or also with UW without hydroxyethyl starch\textsuperscript{17,18}. Subsequently, organs should be perfused with the preservation solution according to the method of preservation.

Recently, Celsior, a new CS preservation solution, has been developed along the same specifications as the UW-CS solution, but lacking the colloid. Compagnon et al\textsuperscript{13} used for their continuous perfusion experiments high-molecular weight hydroxyethyl starch for osmotic balance of the Celsior-solution, hereby introducing the negative effects during initial wash-out (increased viscosity and red blood cell aggregation). Compagnon still found satisfying results, showing better viability parameters in HMP compared to cold storage.

Summarizing, an effective wash-out of blood can be achieved by pre-flushing the liver with a low-viscous aggregation-preventing solution. To restore osmotic balances, hypothermic machine preservation should be continued using the UW-MP or a UW-look-a-like solution which contains a colloid in order to prevent edema formation and maintain the protective effects for cold-induced cellular injury.

6.3 Perfusion Dynamics

6.3.1 Theoretical considerations of hypothermic machine perfusion

The liver is supplied with blood from both the portal vein and the hepatic artery. The portal vein, with a mean blood pressure of 12 mmHg, supplies the liver cells with blood coming from the splanchnic area and intestine, and contributes to 2/3 of total liver perfusion. The hepatic artery, with a pulsatile blood pressure of 120/80 mmHg supplies blood to the liver sinusoids, vessel walls, as well as to the biliary tree. It is an important factor in maintaining vessel structure and integrity of bile ducts. The blood supply of both afferent blood vessels joins in the sinusoids and leaves the liver through the hepatic veins into the inferior caval vein. When HMP of the liver intends to mimic the physiological circulation, two aspects have
to be taken into account. First, the temperature effect: using hypothermia alters vascular compliance and second, the solution effect: using different composition and properties of the preservation solution alters rheologic conditions.

The temperature effect: Lowering the temperature from 37°C to 4°C results in vasoconstriction of the hepatic vasculature and an increase in flow resistance. Hypothermia decreases the Ca\(^{++}\)-ATPase activity to virtually zero, inducing vasoconstriction of the vessels\(^{31}\). As a consequence, the decreased diameter of the vessels causes an increased resistance to flow. Furthermore, if we assume Poiseuille’s law is applicable, the resistance even increases with the fourth power of the diameter. Fortunately, it is known from hypothermic kidney perfusion that this vasoconstriction subsides in most cases in the first 30 minutes of perfusion\(^{16}\).

In addition, low temperatures also alter the physiological and biochemical characteristics of cells with loss of transmembrane ion gradients and membrane barrier functions\(^2^{23}\). As a result, endothelial cells, that play a substantial role in preservation injury, become more prone to damage\(^2^{23}\). Due to the increased sensitivity of endothelial cells to injury, detrimental factors like very high, very low and rapidly fluctuating degrees of shear stress should be avoided during organ preservation\(^9\).

The solution effect: in addition to low temperatures, liver perfusion also uses a different perfusion medium than in real life. Instead of blood, the liver is perfused with an acellular medium. The viscosity of blood at 37°C is approximately 4·10\(^{-3}\) Pa·s for high shear rates\(^{40}\). This viscosity increases for lower shear rates as the influence of blood cells becomes more apparent compared to plasma (non-Newtonian). The apparent viscosity of blood decreases in small capillaries due to a lowered wall friction, as the ratio blood cells/plasma changes in favor of plasma (Fahraeus-Lindqvist effect). In an acellular fluid the non-linear viscosity is absent, and the viscosity will approach viscosity values of pure water at 4°C, being approximately 2·10\(^{-3}\) Pa·s. In UW-solution, however, a colloid is present and it has been shown that this colloid causes a non-linear component in the viscosity\(^{70}\). It has also been demonstrated that the viscosity of UW-solution at 4°C is 11·10\(^{-3}\) Pa·s, which renders this solution three times more viscous than blood. From Poiseuille’s law,

\[
P = \frac{8\eta L}{\pi r^4} Q \quad [Pa]
\]

with \(L\) as the length of a cylindrical tube and \(r\) the radius, it can be concluded
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that pressure difference ($P$) and resulting flow ($Q$) are proportional to the dynamic viscosity ($\eta$). In other words, if the same pressure is used with another fluid with a viscosity that is three times as high, the resulting flow is three times as low. This should be taken into account when pressures or flow in hypothermic liver perfusion are defined.

6.3.2 Review of HMP techniques

In the pioneering years of liver HMP the idea was to mimic the physiological situation as good as possible. Both the hepatic artery and the portal vein were perfused by Brettschneider from Starzl's group in a continuous manner, but Slapak and Belzer used pulsatile perfusion through the hepatic artery and simultaneously non-pulsatile perfusion via the portal vein. With these methods, successful HMP has been obtained, reaching 24 hours preservation. In the following years, physiology-mimicking pumping configurations were used by other research groups. In the mid-eighties single vessel perfusion became the accepted method. Many authors limited their HMP experiments to just portal venous perfusion in a continuous manner and found good results of HMP up to 72 hours, which in fact is superior to traditional CS. The most successful liver preservation experiments were performed by Pienaar et al in Belzer and Southard’s lab in Madison, who used pulsatile perfusion of the portal vein only. They found 7/8 dogs surviving for seven or more days after transplantation of a liver preserved for 72 hr in UW-MP-solution. This is a remarkable result, taking into account that only the portal vein was perfused. Yamamoto et al also achieved successful HMP of porcine livers for 72 hours, however, they used continuous portal venous perfusion. A comparison between the two experiments is difficult, because Yamamoto did not judge the result in a transplantation model, as Pienaar did, but assessed liver viability in an isolated perfused liver (IPL) model. Over the years, the IPL model has been validated as a representative reperfusion model, despite the absence of blood cells and allorecognition in the IPL-model. Thus, it appears that in liver preservation under hypothermic conditions both pulsatile and continuous perfusion can be used through the portal vein. Recently, Compagnon et al compared different routes of perfusion in a rat liver model. A comparison of single vessel perfusion between portal venous and arterial perfusion is described and viability of the rat liver was tested in an IPL-model. They concluded that portal venous perfusion proved to be superior over static CS. Single perfusion through the hepatic artery, however, proved to be less beneficial.
6.3. Perfusion Dynamics

The perfusion characteristics of some landmark experiments throughout the years are listed in Table 6.1. The magnitude of portal perfusion pressure used varies from 2 mmHg to 25 mmHg, and flow varies from 0.14 ml/min/gr to 4.8 ml/min/gr. In all experiments, the authors, however, report superior results of HMP compared to CS. Thus, the portal vein is apparently rather insensitive to pressure and flow magnitude. Unfortunately, perfusion characteristics in these studies are often poorly defined. For better comparison of these experiments using continuous perfusion, the applied perfusion pressure and resulting flow should be described. Pulsatile perfusion is best characterised using the mean and amplitude of pressure in addition to pulse frequency. The hydraulic power, which is defined as the product of instantaneous pressure and instantaneous flow, is also a good characterisation of pulsatile perfusion. Resulting flows should also mention mean and amplitude. The majority of experiments use lower than physiologic pressures, probably on the basis of the concept that endothelial cells are more fragile under hypothermic conditions. Due to lack of data concerning pulse frequency and amplitude arterial perfusion of the liver has never been described satisfactorily. In this respect, data from kidney HMP can be helpful to assess the arterial perfusion of the liver. In the past, kidney HMP has been shown as a very successful method of pulsatile preservation using a pressure of 60/40 mmHg and pulse rate of 60 BPM, as described by several authors (Table 6.1).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Portal Vein</th>
<th>Hepatic Artery</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slapak\textsuperscript{a4}</td>
<td>Canine</td>
<td>12 mmHg (=)</td>
<td>76/23 mmHg (≈)</td>
<td>11°C</td>
</tr>
<tr>
<td>Brettschneider\textsuperscript{a10}</td>
<td>Canine</td>
<td>4.8 ml/min/gr (=)</td>
<td>1.2 ml/min/gr (≈)</td>
<td>4°C</td>
</tr>
<tr>
<td>Belzer\textsuperscript{a}</td>
<td>Porcine</td>
<td>5-8 mmHg (=)</td>
<td>60/40 mmHg (≈)</td>
<td>8-10°C</td>
</tr>
<tr>
<td>Gellert\textsuperscript{a21}</td>
<td>Porcine</td>
<td>2 mmHg (=)</td>
<td>80/40 mmHg (≈)</td>
<td>10°C</td>
</tr>
<tr>
<td>Pienaar\textsuperscript{a48}</td>
<td>Canine</td>
<td>0.5 ml/min/gr</td>
<td>0.125 ml/min/gr</td>
<td>5°C</td>
</tr>
<tr>
<td>(30 BPM=)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boudjema\textsuperscript{a7}</td>
<td>Rabbit</td>
<td>15-25 mmHg (=)</td>
<td>7°C</td>
<td></td>
</tr>
<tr>
<td>Yamamoto\textsuperscript{a7}</td>
<td>Porcine</td>
<td>0.5-0.6 ml/min/gr (=)</td>
<td>5°C</td>
<td></td>
</tr>
<tr>
<td>Rossaro\textsuperscript{a53}</td>
<td>Rat</td>
<td>0.5 ml/min/gr (=)</td>
<td>6-10°C</td>
<td></td>
</tr>
<tr>
<td>Kim\textsuperscript{a34}</td>
<td>Rat</td>
<td>11 mmHg (=)</td>
<td>4°C</td>
<td></td>
</tr>
<tr>
<td>Southard\textsuperscript{a57}</td>
<td>Rat</td>
<td>0.14 ml/min/gr (=)</td>
<td>4°C</td>
<td></td>
</tr>
<tr>
<td>Compagnon\textsuperscript{a53}</td>
<td>Rat</td>
<td>0.4 ml/min/gr (=)</td>
<td>4°C</td>
<td></td>
</tr>
</tbody>
</table>

Table 6.1: Pressure and flow values used in liver HMP. (=: continuous, ≈: pulsatile, if blank only portal perfusion was used)
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Summarizing, good results with HMP of the liver have been obtained by perfusion of the portal vein alone\textsuperscript{48,72}, while additional advantages, including better preservation of the sinusoids and biliary tree, could be gained by including perfusion of the hepatic artery\textsuperscript{49}. In a dual perfusion system of both portal vein and hepatic artery, portal perfusion could be performed using either pulsatile or non-pulsatile perfusion at a pressure ranging from 2-25 mmHg and arterial perfusion preferably in a pulsatile manner at 60/40 mmHg and a pulse rate of 60 BPM.

6.4 The Role of Oxygen in Hypothermic Preservation

One of the key factors in organ preservation is hypothermia. Hypothermia decreases the rate at which metabolism occurs. The decrease of reaction rates is represented by van't Hoff's principle and can be expressed by:

\[
Q_{10} = \left( \frac{k_1}{k_2} \right)^{\frac{10}{(t_2-t_1)}}
\]

with \(Q_{10}\) being van't Hoff's coefficient for a 10°C temperature change and \(k_1\) and \(k_2\) the reaction rates at temperatures \(t_1\) and \(t_2\) respectively. In metabolic pathways, van’t Hoff’s coefficient \(Q_{10}\) has been determined to be \(2^6,19\), resulting in a metabolism at 4°C of 10% compared to metabolism at 37°C. Fujita et al\textsuperscript{19} determined that the amount of oxygen that is consumed by a lowered metabolism is a logarithmic function of temperature. Using continuous perfusion of the portal vein with temperatures varying from 5 to 37°C, they measured a temperature (T)-dependent oxygen consumption \(V_{O_2}\) of:

\[
V_{O_2} = 0.21 \cdot 10^{0.029 \cdot T} \quad [\text{mmol/min/gr}]
\]

As the relation between oxygen consumption and temperature is logarithmic, oxygen consumption decreases with decreasing temperature, but never stops at temperatures of 0-4°C. At 4°C, metabolism still requires 0.27 mmol/min/gr liver oxygen, which implies that a certain oxygen supply during hypothermic liver perfusion is required. This finding is confirmed by a comparative study of Fujita\textsuperscript{20} of oxygen-saturated versus oxygen-deprived preservation solutions that showed that a 95% oxygen-saturated perfusate allows good viability, while a total lack of oxygen results in cellular injury and especially in endothelial cell damage. Oxygenation of
6.4. The Role of Oxygen in Hypothermic Preservation

the preservation solution, however, should be considered as a double-edged sword: oxygen is necessary for energy resynthesis but could result in an increase in reactive oxygen species as well, resulting in damage to cellular membranes. The formation of reactive oxygen species has long been considered to significantly contribute to cellular injury during only the reperfusion phase and not during the cold preservation, since during reperfusion an excessive oxygen supply to the mitochondria occurs\textsuperscript{11,72}. Recently, some reports stated that oxygen radicals are formed during preservation as well as during reperfusion\textsuperscript{41,42,47}. Therefore, the use of reactive oxygen species scavengers in a preservation solution, inhibiting formation of reactive oxygen species\textsuperscript{8,24,71} in the cold, are beneficial to organ viability after transplantation.

From the data of Fujita\textsuperscript{19} that oxygen consumption during hypothermic liver perfusion (\( V_O_2 \)) amounts to 0.27 mmol/min/gr liver, it can be determined that, using Henry’s law, the partial oxygen pressure should be:

\[
pO_2 = \frac{V_O_2 \cdot H \cdot V_{mol}}{Q} \quad [Pa]
\]

where \( Q \) is the normalised flow in ml/min/gr liver, \( H \) is Henry’s constant, denoting the solubility of oxygen in water and \( V_{mol} \) is the volume of 1 mol water (=18 ml). For most acellular preservation media, Henry’s constant for water, 2.95 \( \cdot \) 10\textsuperscript{9} Pa, can be used causing an estimated error of at most 5%.

<table>
<thead>
<tr>
<th>Reference</th>
<th>( pO_2 ) [kPa]</th>
<th>Flow [ml/min/gr]</th>
<th>( V_O_2 ) [µmol/min/gr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fujita\textsuperscript{19}</td>
<td>95</td>
<td>3-3.5</td>
<td>5.4</td>
</tr>
<tr>
<td>Compagnon\textsuperscript{13}</td>
<td>12</td>
<td>0.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Southard\textsuperscript{57}</td>
<td>13-17</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Gellert\textsuperscript{21}</td>
<td>30-40</td>
<td>0.625</td>
<td>0.041</td>
</tr>
<tr>
<td>Pienaar\textsuperscript{48}</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Slapak\textsuperscript{55}</td>
<td>21-35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rossaro\textsuperscript{53}</td>
<td>95</td>
<td>0.5</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Table 6.2: \( pO_2 \) and subsequent \( V_O_2 \) values used in liver HMP.

Some reports of successful HMP experiments have identified oxygen to be an important constituent in the preservation solution (Table 6.2). As was determined from Henry’s law, \( V_O_2 \) is directly related with \( pO_2 \) and flow. Calculating oxygen consumption, most authors do not meet the oxygen consumption value as determined by Fujita\textsuperscript{19} (Table 6.2). This indicates that results after liver HMP can be improved if a higher \( pO_2 \) during the entire perfusion period is offered.

Summarizing, even in a state of hypothermia, the liver consumes oxygen, implying
that during hypothermic preservation a certain oxygen supply is required. The pO$_2$ in the perfusate is determined by the flow rate established in relation to oxygen consumption under hypothermic conditions.

6.5 Perfusion Systems

In continuous liver HMP-research the perfusate, perfusion characteristics and oxygen supply have been studied in experimental laboratory set-ups. An overview of effective HMP-related problems including cooling, oxygenation and perfusion dynamics is shown in Figure 6.1. Choices are necessary between in-line cooling of the perfusate with a heat exchanger or cooling of the entire set-up by placing it in a refrigerator or cold room. Oxygenation is established by using an in-line oxygenator or organ surface oxygenation. Furthermore, dual pumping via both portal vein (PV) and hepatic artery (HA) or just by the portal vein alone has been performed. Arterial perfusion is generally achieved by a peristaltic pump$^{4,10,13,21,69}$. Peristaltic pumps use the principle of pushing fluid forward with compression and decompression of tubing. This is usually achieved by deforming a flexible tube, e.g. in a roller pump. A major advantage of this pumping principle is that the preservation solution has no direct contact with the pump head, thus securing sterile conditions. The consequence of the pushing forward principle is that a peristaltic pump is a flow-driven pump. Any disturbance of the flow due to an obstruction in the circuit will result in an increasing resistance and produce a high perfusion pressure. Especially in hypothermic organ perfusion this is a situation which is not desirable.

Portal venous perfusion is established by either a peristaltic pump$^{4,10,13,17,20,21,48,62}$ or by perfusion using gravity$^{36,37,69}$. Gravity perfusion is based on the principle that the height of an open reservoir above an organ is directly related with the resulting pressure at which the organ is perfused according to Bernoulli’s law. For example, if a reservoir containing UW-solution is placed 15 cm above the organ (h), a hydrostatic pressure (p) of $p = \rho \cdot g \cdot h = 11.8$ mmHg, with $\rho$ the specific gravity of UW and $g$ the gravitational coefficient, will result in a perfusion pressure ($P$) of $P = \rho \cdot g \cdot h - \frac{1}{2} \cdot \rho \cdot v^2$, with $v$ the velocity of perfusion flow. With this principle a low perfusion pressure can easily be applied, but as a consequence, this set-up is difficult to use outside the laboratory.

An easy-to-handle, portable system that incorporates these features is not yet commercially available. For HMP of kidneys now large systems exist, e.g. the Gambro kidney perfusion machine (Gambro, Stockholm, Sweden)$^{28,29,51,52,58,59}$
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and the RM3 kidney perfusion machine (Waters Medical Systems, Rochester MN, USA)\textsuperscript{61,66}, as the successor to the MOX-100\textsuperscript{1,22,44,45,75} (Figure 6.2).

The machines mentioned above basically consist of an organ chamber in which the organ is placed and a pumping system, which directs the preservation solution from a reservoir to the kidney in a pulsatile manner. In both systems, the preservation solution is delivered to the organ by means of a positive displacement (tube deforming) pump. Hypothermia is realised by cooling with ice. Oxygenation is achieved by ambient air in combination with a membrane oxygenator in the Waters machine or by a certain overpressure in the organ chamber using a small amount of medical oxygen in the Gambro machine. Although many, predominantly US, studies have reported excellent results with a lower delayed graft function after kidney transplantation than with CS, MP has not become a standard clinical method to perfuse kidneys to bridge the timespan between donor and recipient. Whether this is due to a relatively high transport weight, due to presumed operating costs or the assumption that handling of machines requires skilled personnel has remained unclear until today.

Figure 6.1: Schematic overview of experimental liver MP set-ups. (References are between brackets)
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Figure 6.2: The Gambro (left) and Waters RM3 (right) kidney perfusion machines.

6.6 Outlook on Hypothermic Machine Preservation

The challenge offered by these experimental set-ups is to design an innovative hypothermic human liver perfusion system to incorporate the mentioned features in a transportable, portable and easy-to-use system. The three key players in liver HMP are type of preservation solution, characteristics of perfusion dynamics and oxygen supply. Reviewing liver HMP experiments, the best results have been achieved with the University of Wisconsin machine preservation solution and this solution is clinically most widely used. UW-MP-solution prevents hypothermia-induced injury, but has also an increased viscosity, compared to 37°C blood, which results in a decrease in flow at physiologic pressures. The literature overview reveals that no clear conclusions can be drawn concerning the optimal perfusion characteristics, since either only perfusion pressure or perfusion flow is reported. The best estimation for perfusion of the liver is a physiological approach, i.e. on the arterial side pulsatile perfusion and on the portal side continuous perfusion is applied. Pressures could be chosen somewhat lower than physiological pressure to prevent possible endothelial cell damage under hypothermic conditions, e.g. hepatic artery 60/40 mmHg with 60 BPM and portal vein 8 mmHg. A third important observation from the literature and additional calculations suggests that oxygen is mandatory to achieve optimal preservation of the liver. The minimal amount of partial oxygen pressure [Pa] required is inversely related to the normalized flow [ml/min/gr.
To allow successful clinical application of HMP of the liver the technical considerations mentioned above have to be taken into account to improve organ viability after HMP vs CS preservation and reduce posttransplant complications. In addition, however, it is mandatory for the successful use and implementation of HMP of the liver that the technique is compatible with the standard operating procedures surgeons are using during organ procurement. The simple static cold storage technique, as the golden standard, should be the basis for the design of a HMP system, and incorporate equivalent handling, procedures and materials, with the addition of a simple dual pumping system. Furthermore, such a system should be portable to allow easy transportation and preferably be disposable, at least in part. For this purpose, according to NIOSH lifting regulations a weight of 23 kg or less should be the target\textsuperscript{73}. A last important requirement for a liver HMP system is a stand-alone working period of at least 24 hours, implying a cooling capacity to maintain hypothermic conditions within the range of 0 to 4\textdegree{}C for 24 hours. These additional criteria for a liver HMP system make it possible to comply with the existing international concept of organ sharing.

Incorporating these design criteria in a transportable system based on existing standard surgical and organ sharing procedures will successfully implement this technique into every day clinical practise and substantially contribute to improvement of donor liver quality and viability and thus shorten the waiting lists for transplantation and hopefully improve outcome after liver transplantation.

6.7 References


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