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Does machine perfusion decrease ischemia reperfusion injury?

Les machines à perfusion protègent-elles des lésions d'ischémie-reperfusion ?

D. Bon[a], P.-O. Delpech[a,b], N. Chatauret[b], T. Hauet[a,*], L. Badet[a,c], B. Barrou[a,d]

[a]*Inserm U1082, Poitiers F-86021, France ; université de Poitiers, faculté de Médecine et de Pharmacie, F-86034 Poitiers, France*
[b]*Service d'Urologie, CHU La Miletrie, 86000 Poitiers, France*
[c]*Service d'Urologie, CHU Edouard Herriot, 69000 Lyon, France*
[d]*Service d'Urologie, Service de Transplantation rénale et pancréatique, CHU la Pitié Salpêtrière, AP-HP, 75013 Paris, France*

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Ischemia-reperfusion;
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Summary
In 1990's, use of machine perfusion for organ preservation has been abandoned because of improvement of preservation solutions, efficient without perfusion, easy to use and cheaper. Since the last 15 years, a renewed interest for machine perfusion emerged based on studies performed on preclinical model and seems to make consensus in case of expanded criteria donors or deceased after cardiac death donations. We present relevant studies highlighted the efficiency of preservation with hypothermic machine perfusion compared to static cold storage. Machines for organ preservation being in constant evolution, we also summarized recent developments included direct oxygenation of the perfusat. Machine perfusion technology also enables organ reconditioning during the last hours of preservation through a short period of perfusion on hypothermia, subnormothermia or normothermia. We present significant or low advantages for machine perfusion against ischemia reperfusion injuries regarding at least one primary parameter: risk of DFG, organ function or graft survival.

*Corresponding author.
E-mail adress: t.hauet@chu-poitiers.fr (T. Hauet).

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Résumé
Dans les années 90, l'utilisation des machines de perfusion pour conservation d'organes a été abandonnée en raison de l'amélioration des solutions de conservation, efficaces sans perfusion, facile à utiliser et peu chères. Depuis les 15 dernières années, un regain d'intérêt pour les machines de perfusion est apparu, basé sur des études réalisées sur le modèle préclinique et semblant faire consensus notamment en cas de greffons issus de donneurs à critères élargis ou décédés par arrêt cardiaques (DDAC). Dans cet article, nous présentons des études pertinentes mettant en évidence l'efficacité de la préservation par perfusion hypothermique par rapport à la conservation statique hypothermique. Les machines de conservation d'organes étant en constante évolution, nous avons également résumé les derniers développements comme l'oxygénation directe du perfusat. La perfusion machine permet également un « reconditionnement » des greffons durant les dernières heures de la conservation par de courtes périodes de perfusion hypothermique, sub-normothermique ou normothermique. Nous présentons les avantages des machines à perfusion dans la lutte contre les lésions d'ischémie-reperfusion en ce qui concerne les paramètres principaux que représentent le risque de reprise retardée de fonction du greffon ou la survie du greffon.

Introduction

Preserving the function of kidney grafts is of crucial importance for effective transplantation Three major types of donors are found: living donors [1], donation after brain death (DBD) or donation after cardiac death (DCD). The other donor source is expanded criteria donor (ECD) (which includes DCD donors); these donors have co-morbidity factors, such as arterial hypertension or old age [2]. The last ten years, we observed demographic changes of donor population: decrease of DBD, increase of DCD and very slow emergence of living donation [3]. This led to development of the preservation period adapted to donor type. In this review, we mainly focused on ECD and DCD where the machine perfusion (MP) could be useful as organ underwent severe damages before preservation. We first report briefly the major steps of transplantation leading to ischemia reperfusion injuries. We then remind the evolution of the use of MP. The main section summarizes recent studies for kidney preservation with hypothermic machine perfusion (HMP) performed on swine or on human. We finally reported studies on oxygenated machine and perfusion reconditioning. Thus, a beginning of answer to the question "Does machine perfusion decrease ischemia reperfusion injury?", will be proposed.

Back to the future?

At the end of 60's, the use of machine perfusion for graft preservation emerged with the objective to increase organ preservation time [4]. In 1973, Stephenson et al. published one of the first report on the use of a Gambro perfusion machine with continuous perfusion with 4.5% albumin solution for preserving kidneys from cadaveric donor [5]. Their results were enthusiastic since satisfactory renal function has been obtained with 17 kidneys over 20 transplanted from 26 perfused up to 36 hours. Over 70's, machine preservation has been widely used especially for human cadaveric kidney [6] with clinical evidences for benefit on kidney when using continuous perfusion. But the development of new preservation solutions, easy to use, combined to potent immunosuppressive treatments, led clinical teams to slowly abandon huge pulsatile machine perfusion in favor of hypothermic static preservation even for long time period [7-9]. The interest remained for machine perfusion to increase the donor pool with organ from DCD ad ECD. It led clinical and research teams to investigate anew pulsatile machine perfusion, taking in count recent and various preservation solutions. In 1990, we observed development of new machines not only for kidney but also for liver on large animal model especially the swine [10]. Since this period, many preclinical and clinical studies, some of which presented in this paper, have been performed and testified that machine perfusion is a powerful tool for but still needs to be optimized and standardized.

Ischemia reperfusion injury in transplantation (Part I)

Ischemia – reperfusion is a complex pathophysiological process involving hypoxia and/or reoxygenation, ionic imbalance-induced edema and acidosis, oxidative stress, mitochondrial uncoupling, coagulation and endothelium activation associated with a proinflammatory immune response [11]. The main consequences of renal ischemia – reperfusion are kidney graft primary non-function (PNF) and delayed graft function (DGF) or chronic graft dysfunction, involving return of the patient to dialysis. Ischemia is caused by cessation of blood flow at the time of organ collection. This is the first step leading to kidney injury during transplantation. In all cases, ischemia is followed by reperfusion, which occurs when the graft is connected with the recipient vascular system. Reperfusion is well-known to exacerbate the cellular injuries that are initiated by ischemia. The severity of ischemic injuries found in kidneys from each donor type has a crucial effect on

graft recovery and outcome. The cause of ischemia differs according to the donor source: ischemia in kidneys from living donors is induced by a limited period of cold preservation, DBD kidneys by a longer period of hypothermic preservation and DCD kidneys by a combination of warm ischemia induced by cardiac arrest and cold preservation. As these dysfunctions are most severe in non-optimal grafts (obtained from ECD or DCD donors) new approaches are needed to improve recovery of grafts and machine perfusion preservation might be the key to perform it.

Hypothermic machine perfusion

Two commercial machine perfusions (MP) are mainly used for clinical application and preclinical experiment on large animals: the Lifeport kidney transporter from Organ Recovery System (Lifeline Scientific Inc. Itasca, IL, USA) and RM3 Renal Preservation Monitor from Waters Medical Systems (Rochester, MN, USA). Recently, Waves machine from Waters Medical obtained worldwide approval to bring a transportable solution to the RM3 technology. The solutions recommended with those machines are respectively KPS-1 and MPS, two solutions with similar composition to UW. Hypothermic machine perfusion (HMP) preservation is increasingly being used as an alternative method to static cold storage (CS) for the preservation of grafts obtained from non-optimal donors (ECD or DCD). HMP allow a flow of cold preservation solution through the vasculature of the organ in a continuous or pulsatile manner. Thus, there still is a flush of circulation through the organ and intrarenal vasoconstriction is decreased. Cortex is also irrigated and it remains a medullar microcirculation. HMP also allows reduction of cellular edema. At least, intracellular pH is maintained, waste products are removed and perfusion solution components allows low but existed metabolic rate. In the next section, we will try to present studies performed on Swine and on Human.

Preclinical studies

In 2004, Nicholson et al. proposed a renal autotransplantation model mimicking DBD or DCD conditions which was performed with addition of a warm ischemia time of 0 or 30 minutes, respectively, before hypothermic preservation in CS or in MP during 24 h. When the warm ischemia time was 30 minutes, only one animal over five survived in each group. The authors did not found any difference between those 2 survival animals. Without any warm ischemia, results clearly attested the benefit of MP for recipient. Negative impact of HMP on renal function was smaller compared to CS. The mean serum creatinine peak reached 780 µM at day 2,6 for MP and 1526 µM at day 4,8 for CS [12]. Few years later, potential benefits for kidney were evaluated on a DCD model where the renal function was measured on isolated reperfusion system of kidney during 3 hours. Intrarenal resistance measured at the reperfusion was reduced after MP preservation compared to CS with HOC (hyperosmolar citrate; Baxter Healthcare, Thetford, UK) or HTK (histidine tryptophan ketoglutarate, Custodiol®, Alsbach, Germany). The creatinine clearance was increased compared to CS with UW (Viaspan™, Merck, Wilmington, DE, USA solution). Histology data after 18 hours of perfusion were indicative for a diminution of tubular cell inflammation. After reperfusion, glomerular and tubular cell functions were improved. This preclinical study showed the actual positive effect of MP in case of DCD model [13]. HMP efficacy on ECD was confirmed on large white pig model undergoing renal autotransplantation consecutive to 30 minutes of warm ischemia. In this study, an histological evaluation showed more injury in the CS groups than in the MP groups and MP seemed to maintain brush border integrity [14].

Clinical studies

In addition to preclicinal models, clinical evidences demonstrative for HMP leverage to decrease ischemia reperfusion injuries were published. In 2005, Schold et al. tried to describe the trends in the use of HMP over 10 years'experience in the United States. The utilization rates of HMP were higher for ECD. HMP was associated to lower discard and DGF rates suggesting an important utility of HMP in kidney transplantation even if link between HMP and long-term graft survival was relatively mild [15]. In 2007, a prospective study compared HMP (227 kidneys) and CS (188 kidneys) on long term function of renal allograft. Despite longer cold ischemia time and poorer donor hemodynamics in MP group, 5-year Kaplan-Meier graft survival was better than in CS group. Moreover, MP reduced the number of patients who returned to dialysis [16]. Moers at al. confirmed those results with a one-year follow-up study. Among 654 potential donors, 336 patients matched to the study parameters and one kidney from one donor was preserved in HMP and the other kidney was preserved in CS. With HMP, risk and duration of DGF was reduced. HMP improved one year graft survival and the rate of decrease of serum creatinine level. It also led to lower serum creatinine level the two weeks following the transplantation. Regarding the other secondary end points (PNF, acute rejection or length of hospital stay), they found no difference between the two population of recipients [17]. Those studies including all types of donor, research groups also focused specifically on non-optimal donors. For the preservation of kidney donated after cardiac death, the incidence of DGF was reduced with HMP (69,5% vs 53,7%, p=0,05). The duration of DGF, even if not significant, was reduced of 4 days when using HMP and creatinine clairance at one month was increased. Finally, there was no difference regarding graft and patient survivals [18]. For kidney from ECD after brain death, a study performed on 91 donors indicates that the risk of DGF was reduced and incidence of non-function was decreased four times with HMP compared to CS. To end, graft survival was 92,3% with HMP and only 80,2% with CS [19]. One specific population represented a potential source of donor for which great efforts need to be made all old donors. In the Eurotransplant Senior programme, kidneys from donors aged over 65 years were allocated to patients on dialysis aged over 65 years. 85 donors and their 170 recipients were included into the study. For this population, DGF, one-year patient and graft survival rates were similar. One-year graft survival rate was improved only in case of patient who developed DGF where HMP was used. To finish PNF was significantly reduced in HMP group [20].

Summary

To resume, several studies have reported a reduction in DGF after HMP preservation compared with CS [21, 22]. These clinical data were supported by findings from large animal models of DCD donors in which different preservation approaches were compared; MP preservation improved kidney function and vascular reactivity more than CS. In clinical studies, MP benefits for preserving DCD kidneys are still conflicting but the tendency remains in favor to the use of MP [23, 24]. Indeed, clinical trials have shown a small but significant positive impact of MP when compared with CS in terms of both 1-year and 5-year graft survival from all types of donor. Machine perfusion preservation is particularly efficient in decreasing both PNF and DGF in ECD kidneys as well as decreasing DGF in DCD kidneys. Nevertheless, even if machine perfusion preservation might be beneficial in limiting chronic rejection, interstitial fibrosis and tubular atrophy, this approach can still be improved.

Hypothermic oxygenated perfusion

The lack of oxygen during preservation period, in static conditions or in case of perfusion, followed by a brutal recovery of oxygen is at the center of transplantation injury processes. In one hand, the lack of oxygen combined with hypothermia leads to the reduction of aerobic metabolism, situation considered as protective for the organ (no reactive oxygen species production during preservation). In the other hand, addition of oxygen during perfusion machine could allow the graft to maintain a low metabolic aerobic respiration with maintenance of ATP levels which permitted a delay of injury process.

As most commercial perfusion systems are not actively oxygenated, several options are under development to supply oxygen to the organ. The first one is to use a continuous oxygenation flow (PO_2 > 500 mmHg) *via* the perfusion system. In 2005, Minor et al. proposed to determine the potential benefits of low flow arerobic machine preservation for a porcine heterotopic transplantation model mimicking DCD conditions [25]. Renal perfusion oxygenation was evaluated at the end of the perfusion with ATP level tissue measures. Energy statuses were significantly higher in the MP group compared to the CS group. There were no difference on vascular endothelium and tubular epithelium, results obtained with electron microscopy. All kidneys preserved with machine perfusion showed spontaneous urine production while DGF was showed in all CS kidneys during the two first days after transplantation. Moreover, they did not observe a solution perfusion effect (HTK vs. UW). In 2009, Doorschodt et al. demonstrated the biological safety of a hypothermic oxygenated pulsatile perfusion called the Airdrive on an porcine autotransplantation model [26]. Microcirculation was better preserved and less morphological injuries were observed after 20h of AD compared with CS. The second option is to use artificial oxygen carriers as perfluorocarbons [27]. Hemarina-M101, an O_2-carrier with high oxygen affinity and the capacity to function at low temperatures is a interesting molecule in development as supplement to the perfusion solution [28]. Moreover, perfusion with Lifor™ *(Lifeblood Medical Inc. NJ, USA)*, a solution originally developed for liver that contains cellular nutrients and a non-protein oxygen carrier at temperatures higher than 4 °C, protected grafts in experiments using animal models of kidney transplantation conducted at room temperature [29].

Reconditioning with perfusion machine

Reconditioning consist in use of MP during the few hours before reperfusion of kidney in the recipient. Hypothermic, normothermic and subnormothermic reconditionings with MP had already been tested on swine.

Hypothermic reconditioning

In an experiment performed on pig, prior to autotransplantation, hypothermic reconditioning (HR) of kidney was carried out with a CS during 19h followed by 2h of preservation with MP at 4 °C. This group was compared to kidneys kept in CS or MP during all the preservation time of 21h [30]. The follow-up after transplantation was one week. The duration of hypothermic MP had an impact on tissue release of lactate deshydrogenase and lipid peroxidation. Those two parameters are higher in the group with higher MP time. On renal function, lower serum creatinine levels where measured when the kidney underwent a cold perfusion, without any difference between the two times. Moreover, hypothermic reconditioning significantly reduced the increase of serum creatinine the first day after transplantation. Similar tendencies were found with systemic urea concentrations. Natrium reabsorption rate in MP and HR groups were not affected after transplantation in contrary to CS group. There was no difference between the three experimental groups for urine formation; all animals had spontaneous urine flow after revascularization. Reconditioning with HMP also decreased innate immunoreactivity (TLR4 and HMGB1) and anti-inflammatory tissue expression (KLF2). MP used alone or preceded by CS also led to improvement of cortical microcirculation.

Stratta et al. presented in 2007 a clinical retrospective analysis over 5 years of intermediates outcomes in ECD kidney transplantations [31]. Among the 141 ECD kidney transplantations, 81% were managed with combined CS and hypothermic MP for 2h and 19% were preserved with CS alone. Mean cold ischemia times were 24,5h for CS + MP and 19h for CS alone. 28% of kidneys preserved with CS + MP has a cold ischemia time superior to 30h whereas none kidney were kept more than 30h when preserved in CS alone. DGF was significantly more frequent when kidneys were preserved in CS (37% vs 11%, P = 0,002). There was no difference regarding PNF, acute rejection, 1-year serum creatinine or 1-year glomerular filtration rate. This clinical analysis clearly demonstrated that the use of hypothermic reconditioning with MP after CS can reduce the incidence of DGF in ECD kidney tranplantation.

Normothermic reconditioning

Hypothermia seems to be a limiting factor of graft preservation particularly in kidneys that have already been damaged by warm ischemia like for DCD. Temperature modulation

offers a way to improve organ preservation, as room temperature conditions maintained graft viability and function in pig models.

With the same manner as hypothermic reconditioning, Bagul et al. compared kidney preservation in CS conditions during 2h or 18h, in HMP during 18h vs. CS during 16h followed by 2h of MP at 37°C [32]. To mimic DCD conditions, preservation was preceded by 10 minutes of warm ischemia. The kidney function was evaluated on an *ex vixo* reperfusion system with autologous blood during 3h. Worse renal function occurred in the 18h CS group. Renal blood flow measured after reperfusion increased when preservation was static and decreased with a 2h normothermic MP period. ATP/ADP ratio, evidence of an aerobic metabolism activity, increased if 2h of normothermic MP succeed to 16h of CS. In the same manner, oxygen consumption after the first hour of reperfusion in the case of normothermic conditioning was higher than in other preservation conditions. Histology showed evidences for positive impact of normothermic reconditioning because of increase of cytoplasmic vacuolation. Tubular dilatation, epithelial flattening, tubular debris, condensed tubular nuclei, red blood cell presence and glomerular shrinkage were similar in all experimental groups.

Hosgood et al. compared pig kidney preservation with HMP during 22h with preservation with MP at 4 °C during 20h and 2h at 37 °C with autologous blood. The warm ischemia period preceded cold preservation was 30 minutes [33]. Sixty minutes after transplantation, lipid peroxydation was reduced significantly in the case of normothermia. Kidney and animal function were followed during 10 days after transplantation but did not show any difference between the two groups. This feasibility study led this research team to realised the first human renal transplantation after *ex vivo* normothermic perfusion [34]. Two kidneys from a ECD (62-year old) was submitted to 30 minutes of warm ischemia followed by 11h of CS and 35min of MP with modified-plasma at 34 °C or 14h of CS. Kidney preserved only in CS had a DGF for 26 days. Even if normothermic reconditioning kidney had a low graft function, receiver did not return to dialysis after transplantation. Cold storage time needs to be standardized and this protocol has to be reproduced in order to validate this preliminary positive experience.

Subnormothermic reconditioning

Always in the quest the right temperature for successful transplantation, Tolboom et al. presented, in 2012, 5 experimental groups of rat liver [35] perfused at 20 °C and 30 °C. Postoperatively, low transaminases suggested a beneficial effect of subnormothermic perfusion, while rising total bilirubin levels suggest inadequate prevention of ischemia- or hypothermia-induced biliary damage [36].

Preconditioning: normothermic extracorporeal perfusion

One other manner to use machine perfusion is organ preconditioning directly on donor (ECD and DCD). The role of normothermic extracorporeal perfusion to avoid ischemia-reperfusion or cold injuries and to allow viability assessment have been reviewed by Vogel [37] and will be developed in the next chapter.

Conclusion

HMP is nowaday well applied on clinical environment but the multitude of protocol, depending on Hospital center, make difficult to access to the reel impact of MP on ischemia reperfusion injuries. However, the few examples reviewed here tip the scale on the side of MP, in the case of kidney. Temperature, MP reconditioning or oxygenated MP were introduced and still are under improvement (Fig. 1). MP also needs to be largely adapted to other organs than lung [38] or pancreas [39]. To conclude, if we took in count the cost effectiveness, MP seems to be a really good option [40].

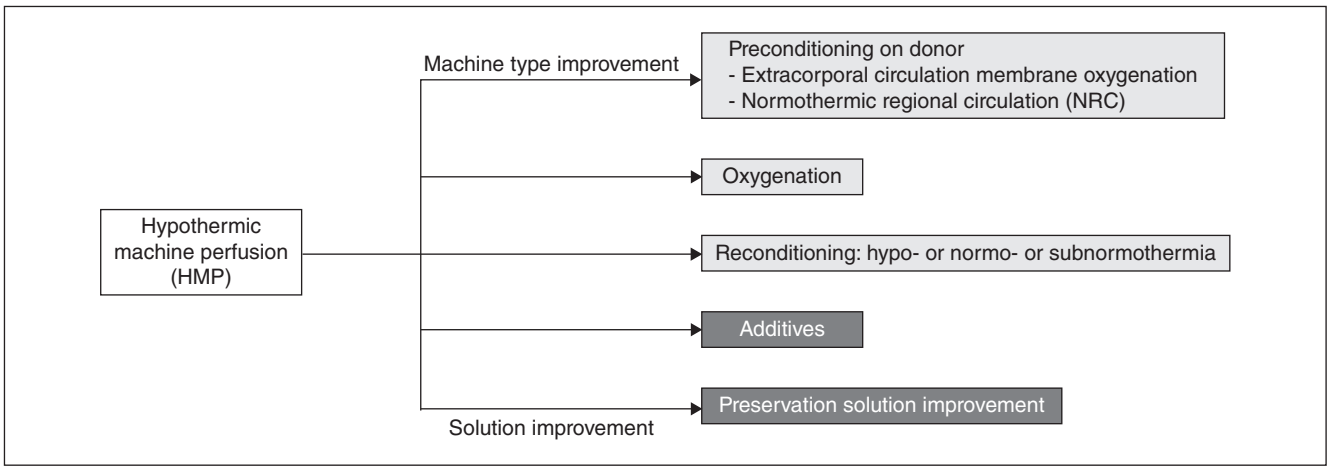

Figure 1. From classical hypothermic machine perfusion already used in clinical environnemt to preservation machine improvement *via* solution or machine improvement.

Disclosure of interest

The authors have no conflicts of interest to declare in relation to this review.

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