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Therapeutic agents for machine perfusion of donor organs

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Abstract

Background. *The urgency of the unmet need for transplant care dictates the necessity to use organs from suboptimal donors. Machine perfusion, which is actively developing at the present time, is designated to solve this problem. The literature presents novel technologies of ex vivo and in vivo machine perfusion of donor organs, which make it possible to*

improve their functions and perform a successful transplant. The most promising areas of research both in animals and in humans may be identified by reviewing the literature sources on this subject.

The objective *was to analyze the world experience of using therapeutic agents in machine perfusion of donor organs*

Material and methods: *sources from 2015 to 2023 found in PubMed, Google Scholar, eLibrary databases*

Conclusion*. The rapid progress in lung, liver and kidney transplantation has made it possible to use grafts from asystolic donors for patients in urgent need of donor organs. In turn, these advances have also prompted the study of potential therapeutic agents that can be used during perfusion.*

Keywords: machine perfusion, ischemic reperfusion injury, suboptimal donor, mesenchymal stem cells, normothermic, hypothermic and perfusion therapy

Conflict of interests Authors declare no conflict of interest

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AFC, alveolar fluid clearance
ALT, alanine aminotransferase
ASOs, antisense oligonucleotides
AST, aspartate aminotransferase
cAMP, cyclic adenosine monophosphate
CHBP, cyclic helix B peptide
CO, carbon monoxide
EMP, extracorporeal machine perfusion

ENP, extracorporeal normothermic perfusion
HMP, hypothermic machine perfusion
IFN, interferon
IL, interleukin
IRI, ischemic reperfusion injury
MP, machine perfusion
MPTAs, machine perfusion therapeutic agents
MSCs, mesenchymal stem cells
NMP, normothermic machine perfusion
PVR, pulmonary vascular resistance
SCP, static cold preservation
SHP, static hypothermic preservation
TNF-α, tumor necrosis factor alpha

Introduction

In 2022, 2252 surgical operations (15.5 per million population) for transplantation of vital organs were performed in the Russian Federation [1], while more than 120 thousand patients were on the waiting list. The shortage of donor organs remains an unresolved problem. The increasing number of patients on the waiting list dictates the need to use organs from suboptimal donors. In this situation, there remains a high probability of potential graft losses, both due to the clinical condition of the donor, predisposing to organ damage, and the inevitable damage to donor organs during static hypothermic preservation (SHP) [2]. In addition, the use of grafts from suboptimal donors is associated with a higher risk of post-transplant complications [3]. In many cases, the organ quality is aggravated by the severe ischemia-reperfusion injury that affects the organs preserved by SHP, the traditional method of graft protection.

In 2010, in literature there appeared the reports on the results of using machine perfusion (MP) of donor organs [4]. In contrast to SHP, AP allows organs to be continuously supplied with oxygen and nutrients throughout the entire preservation period, thereby limiting the period of ischemia. According to A. Petrenko et al. [5], AP has become a promising

alternative for the preservation of kidneys, liver, lungs, heart, and pancreas [5].

Types of machine perfusion

Machine perfusion for organ preservation can be divided into normothermic MP (NMP; at 35.5°–37.5°C) and hypothermic MP (HMP; at 1–8°C). Some studies have also examined subnormothermic MP (at 20°°–35.5°C) [6]. Clinical studies involving NMP have shown promising results in lung, liver and kidney transplantation. Maintaining organs in normothermic conditions reduces the duration of cold ischemia and ensures the delivery of oxygen and nutrients to the organ. In addition, NMP makes it possible to simplify the assessment of the organ status during the perfusion period. Clinical studies of HMP have been conducted primarily in kidney transplantation and some in liver transplantation. In contrast to NMP, HMP is less complex and requires less implementation costs because it requires no oxygen carriers or additives needed to maintain optimal organ metabolism. In addition, HMP allows the measurement of several organ health assessment variables, such as blood flow velocity and mitochondrial oxidative function metabolites.

Both NMP and HMP can act as platforms for repairing damaged organs by adding therapeutic agents to the perfusion solution. Numerous therapeutic agents for MP (MPTAs) have been tested in animal and human models; such as, for example, anti-inflammatory drugs, vasodilators, antibacterial drugs, mesenchymal stem cells (MSCs), gene therapy drugs (siRNA and shRNA, shRNA) and defatting agents. This review will discuss and summarize some of the therapeutic agents used in diseases of the lungs, liver, kidneys, and heart. Although MP has also been studied in pancreas transplantation, no studies of MPTAs have been

published to date. Here we specifically define MPTA as the agent that is added to the perfusion solution during MP ex situ in addition to a standard preservation solution (e.g. Custodiol) with the intention of modifying the graft. We do not consider insulin, heparin, glucose, essential nutrients, and oxygen as MPTAs here.

Perfusion therapy for the lungs

Transplantation is the most effective treatment for end-stage lung disease. Despite the benefits of this life-saving procedure, in a 2017 Report (2017 data from the authors), only 22.6% of recovered lungs were used for transplantation. A high percentage of unused organs may be partly explained by the strict selection criteria for accepting suitable donor lungs. Lung transplant recipients suffer from a high incidence of primary graft failure due to ischemia and reperfusion injury (IRI). Suboptimal lung grafts undergoing SHP are particularly susceptible to IRI due to prolonged cold ischemia time. Recently, numerous clinical studies have demonstrated that suboptimal lung grafts transplanted after extracorporeal normothermic perfusion (ENP) have similar post-transplant outcomes as compared with more intact lung grafts undergoing SHP. While studying the topic of ENP, a wide range of MPTAs have been investigated for further prevention of damage caused by IRI, some of which have even led to successful transplantation of potentially unsuitable lungs. These agents are summarized in Tables 1–3.

Table 1. Stem cell therapy in lung machine perfusion

Study	Therapy	Model	Main effects
P. Mordant et al. [7]	MSCs	Pig	VEGF increase; IL-8 decrease
JW Lee et al. [8]	MSCs	Human	Alveolar fluid clearance increase; endothelial permeability reduction; edema elimination

J.W. Lee et al. [9]	MSCs	Human	Alveolar fluid clearance increase; endothelial permeability reduction; edema elimination; reduction of bacterial load
D.F. McAuley et al. [10]	MSCs	Human	Alveolar fluid clearance increase
M.L. Stone et al. [11]	MSCs – microvesicular	Mouse	Lung elasticity increase; decreased pressure in the pulmonary artery; decrease in the humidity to dryness ratio; decreased neutrophil infiltration
S. Gennai et al. [12]	MSCs – microvesicular	Human	Alveolar fluid clearance increase; increased lung elasticity; pressure in the trachea is restored
J. Park et al. [13]	MSCs – microvesicular	Human	Alveolar fluid clearance increase; decrease in bacterial load
A. Martens et al. [14]	Pluripotent adult progenitor cells	Pig	Decrease in neutrophil count, decreased IL-1 (beta), TNF-α, interferon gamma
S. La Francesca et al. [15]	Pluripotent adult progenitor cells	Human	Reduced inflammation and protein levels in bronchoalveolar fluid

Notes: IL, interleukin; TNF-α, tumor necrosis factor alpha

Table 2. Agents targeting cell receptors during lung machine perfusion

Study	Receptor and action	Model	Major findings
M.L. Stone et al. [16]	Adenosine A2A receptor agonist (ATL 1223)	Mouse	Increased compliance, decreased edema, decreased chemokine CC motif ligand 2 (CCL2), chemokine (C-X-C motif) ligand-1 (CXCL 1) and TNF-α; decrease in the neutrophil count
A. Emaminia et al. [17]	Adenosine A2A receptor agonist (CGS 21680)	Pig	Increased oxygen saturation index; reduction of swelling; decrease in mean airway pressure; decrease in IFN-γ
C.E. Wagner et al. [18]	Adenosine A2A receptor agonist (ATL 1223)	Pig	Increased PaO_2/FiO_2 ratio; decrease in IFN-γ, IL-1β, IL-6, and IL-8
M.E. Huerter et al. [19]	Adenosine A2B receptor antagonist (ATL 802)	Mouse	Increased compliance, decreased pulmonary artery pressure

E.J. Charles, I.L. Kron [20]	Adenosine A2B receptor antagonist (ATL802)	Pig	Increased compliance; decrease in the neutrophil count; decreased IL-12 levels
F. Valenza et al. [21]	β-adrenoreceptor agonist (Salbutamol)	Pig	Increased compliance; decreased pressure in the pulmonary artery; glucose level decrease in the perfusion solution
T. Kondo et al. [22]	β-adrenoreceptor agonist (procaterol)	Dog	Increased compliance; increase in adenosine nucleotide content; decreased pressure in the pulmonary artery; edema reduction
K. Hijiya et al. [23]	β-adrenoreceptor agonist (procaterol)	Dog	Increased compliance; increased oxygenation; promotion; adenosine nucleotide level increase; decreased pulmonary vascular resistance; edema reduction

Note: IFN, interferon

Mesenchymal stem cells and related therapies

Mesenchymal stem cells are multipotent bone marrow cells that secrete paracrine factors that have numerous therapeutic effects. The therapeutic potential of MSCs has been studied in a number of diseases, such as myocardial infarction, sepsis, and diabetes. It was also shown that MSCs in several lung models in vivo reduced the effects of acute lung injury by modulating the inflammatory response. Since then, several laboratories have investigated the protective effects of MSCs as a component of machine perfusate solution during lung preservation. MSCs, MSC-derived microvesicles, and multipotent adult progenitor cells (another bone marrow-derived stem cell) have all been studied in ENP; these agents are summarized in Table. 1.

J.W. Lee et al. [9] first demonstrated the potential of using MSCs as MPTAs in 2009. The authors performed extracorporeal NMP of donor lungs damaged by E. coli endotoxin. Intrapulmonary administration of

allogeneic human MSCs improved alveolar fluid clearance (AFC), pulmonary endothelial permeability, and pulmonary edema when compared with extracorporeal NMP alone. An anti-inflammatory effect was also demonstrated by a slight decrease in neutrophil counts. In addition, the authors were able to show that the anti-inflammatory effects of MSCs were largely accounted for by the secretion of keratinocyte growth factor, since the inhibition of this growth factor reduced the protective effects of MSCs by almost 80%.

In a further project, J.W. Lee et al. [9] used the same model to test donor lungs that were damaged by live E. coli bacteria. Again, anti-inflammatory effects and improvement in AFC were observed in treated lungs. MSCs also reduced the alveolar bacterial load in a dose-dependent manner and increased the phagocytosis activity of alveolar macrophages, thus demonstrating antimicrobial effects. In a followed study, D.F. McAuley et al. [10] tested the therapeutic effects of MSCs using donor lungs not damaged by bacteria. Compared with ENP alone, MSC-treated lungs significantly improved AFC to normal levels after 4 hours of perfusion.

P. Mordant et al. [7] investigated the optimal delivery method and dosage of MSCs using a porcine lung ENP model. They concluded that intravenous delivery of MSCs at a dose of $150x10^6$ cells resulted in the most optimal reduction in the levels of IL-8, a pro-inflammatory cytokine. This dosage was almost 30 times higher than in the studies conducted by J.W. Lee et al. [9] and D.F. McAuley et al. [10] who were able to demonstrate physiological improvements at a lower dosage. However, additional studies using a human lung model are needed to investigate the optimal dosage of MSCs for extracorporeal MP (EMP) of lungs.

MSC-derived microvesicles have also been investigated as potential MPTAs. These microvesicles are round membrane fragments containing biologically active substances, including messenger RNA. Models in vivo showed that these microvesicles have the therapeutic properties similar to MSCs. In addition, microvesicles have a lower risk of causing tumor formation compared to MSCs. M.L. Stone et al. [16] studied the immunomodulatory mechanisms of microvesicles derived from MSCs in mice under EMP conditions. Treated mice showed a significant decrease in the levels of pro-inflammatory cytokines (IL-7, tumor necrosis factor alpha (TNF-α), CXCL1, and high mobility group protein 1 and a significant increase in the levels of anti-inflammatory agents (keratinocyte growth factor, IL-10, and prostaglandin E2)

The therapeutic potential of MSC-derived microvesicles was also tested in two human lung models. S. Gennai et al. [12], using a model of human donor lungs, showed that administering the microvesicles significantly improved AFC and lung elasticity. In a similar study, J. Park et al. [13] administered microvesicles to human lungs damaged by E. coli, resulting in these lungs having significantly less damage, in addition to improved AFC and endothelial permeability, compared to controls.

Multipotent adult progenitor cells are another type of bone marrow-derived stem cell that has attracted interest in the field of MP. Two studies conducted in EMP of lungs showed that these cells can significantly reduce inflammatory markers both in animals, and in humans.

Bronchoalveolar lavage and surfactant replacement

Aspiration of gastric contents can lead to damage to the pulmonary alveolar and capillary network and, in turn, to acute lung injury and aspiration pneumonitis. This acidic damage causes surfactant surfactant

inflammation and dysfunction, which may contribute to a primary lung graft dysfunction. Thus, lung injury from aspiration is a common cause of discarding the graft. Several laboratories have examined the potential of performing bronchoalveolar lavage followed by using exogenous surfactant during EMP to restore the lungs damaged by gastric content aspiration. These studies used two types of exogenous bovine lung extract surfactants, both of which contained primarily surfactant B and C proteins (Curosurf and Bovine pulmonary extract surfactant).

I. Inci et al. [24] used EMP in pigs with lungs damaged by hydrochloric acid and pepsin to simulate aspiration of gastric contents. Lungs that were immediately treated with irrigation and surfactant had higher pulmonary vascular resistance (PVR), better oxygenation, and less edema. However, no significant differences in inflammatory cytokine levels were noted.

In the clinical setting, aspiration injury is usually identified by radiography and histological analysis many hours after its onset. To explain this, K. Hijiya et al. [23] examined lungs damaged by gastric contents 24 hours after aspiration. Again, treated lungs showed better PVR and oxygen saturation. In addition, significantly lower levels of IL-6 were also observed in treated lungs; this additional finding demonstrates that lavage and surfactant administration have a greater protective effect in aspiration-injured lungs and further warm ischemia.

The above results were further confirmed in two porcine lung transplantations. Pig lungs were injured by aspiration of gastric contents followed by 4 and 6 hours of EMP, and these lungs were then transplanted into recipient pigs and reperfused for 4 hours. Washing and administration of surfactant were undertaken immediately before EMP. In both cases, treated lungs showed superior oxygenation, higher compliance, and lower blood levels of IL-1β and IL-6. D. Nakajima et al.

[25] tested the minimum surface tension of the isolated surfactant using a biophysical functional assay and demonstrated that the surfactant from treated lungs had a significantly lower surface tension. Thus, exogenous surfactant administered after bronchoalveolar lavage is a promising MPTA that can be used to treat lungs damaged by aspiration of gastric contents. Future studies using human lung models are needed to confirm its efficacy.

Adenosine receptor agonists and antagonists

Adenosine is naturally released by the body under conditions of cellular stress and has pro-inflammatory and anti-inflammatory effects depending on the effector tissue and receptor type. There are 4 adenosine receptors in the human lungs: A1r, A2AR, A2BR and A3r. Previous animal studies have confirmed the ability of A1r, A2AR and A3r agonists to reduce the manifestation of IRI. Adenosine is one of two major small compounds being investigated as potential MPTAs, the others being β-adrenergic agonists (Table 2). A. Emaminia et al. [17] first explored the potential of using adenosine receptor agonists and antagonists as potential MPTAs during ENP. Pig lungs stored in a refrigerator for less than 14 hours were treated with an A2AR agonist. Improved oxygenation was observed and there was less pulmonary edema and inflammation compared to lungs receiving ENP of lungs alone. In a subsequent study, M.L. Stone et al. [16] used mouse lungs to study the specific changes in gene expression profile that occur when A2AR agonists are added to the perfusate for ENP. The authors noted a significant reduction in inflammatory manifestations, which led to decreased pulmonary edema, decreased levels of inflammatory cytokines, and improved pulmonary function. Research conducted by M.E. Huerter et al. [19] in porcine lung transplantation showed that administration of A2AR agonists during ENP

could improve oxygenation after transplantation. In addition to A2AR agonists, two studies also examined the protective effects of using A2BR antagonists as potential MPTAs. A2BR has both pro-inflammatory and anti-inflammatory effects, and its overall effect on lung injury depends on several factors, such as the injury status and the type of cells involved. Using ENP in mice and pigs, M. E. Huerter et al. [19] and E. J. Charles et al. [20] demonstrated that A2BR antagonists could reduce lung graft injury caused by warm ischemia. The experimental animals had improved lung elasticity and reduced levels of inflammatory markers.

Beta-adrenergic receptor agonists

Two types of β-adrenergic receptor agonists have been tested as MPTAs in porcine and canine models. Salbutamol regulates fluid transport through sodium-dependent mechanisms and can be used as a MPTA to reduce pulmonary edema during lung ENP. F. Valenza et al. [21] showed that salbutamol infusion during pulmonary ENP reduces glucose levels in the perfusate. The glucose concentration shown in the previous study was directly related to the degree of pulmonary edema. In addition, salbutamol also demonstrated a vasodilatory effect leading to increased oxygen saturation. Salbutamol-treated lungs showed lower PVR and compliance compared to controls. It has also been shown that administered by inhalation Procaterol improves AFC via a cAMP-dependent mechanism (cyclic adenosine monophosphate) [24]. F. Chen et al. [26], J. Sakamoto et al. [27] showed in an animal model of lung transplantation that the procaterol administration before preservation could significantly reduce the damage caused by warm ischemia. T. Kondo et al. [22] also examined whether inhaled procaterol during MPTA could have similar protective effects in the lungs of dogs. The treated lungs showed improvements in blood pressure and airway

pressure, in addition to lower PVR and higher lung elasticity. Moreover, total adenosine nucleotide levels were also observed after treatment, indicating good retention of energy substrates.

Fibrinolytic agents

Postmortem microthrombi formation is another major cause of lung dysfunction and rejection, especially in organ explantation from asystolic donors. Fibrinolytic drugs such as urokinase have been shown to have a protective effect when administered to donor lungs after cardiac arrest, leading to the possibility of their use as MPTA. I. Inci et al. [24] performed ENP of the lungs in pigs with the addition of urokinase and recorded a decrease in pulmonary vascular resistance, an improvement in oxygenation, and a decrease in the severity of pulmonary edema. The same group of researchers later used urokinase to treat human donor lungs that had been damaged by pulmonary embolism. After 3-hour urokinase administration, significant improvements in compliance and oxygenation were observed, and the lungs were ultimately transplanted without complications. Likewise T.N. Machuca et al. [28] used another thrombolytic drug, alteplase, to regenerate a pair of human donor lungs that were not initially considered for transplants due to pulmonary embolism. Increased thrombolytic activity and decreased PVR were noted 6 hours after lung ENP, and the lungs were ultimately transplanted without complications.

Antibacterial drugs

Transmission of infection from the donor is a constant threat to the immunocompromised recipient, and ENP may act as a platform for the treatment of lungs infected with bacteria and fungi. A. Andreasson et al. [29] first demonstrated this possibility using meropenem in ENP of 18

lung harvested from marginal donors. After fungal infection was detected in the first three pairs of lungs, the antifungal agent amphotericin B was also added to the perfusate. A significant reduction in bronchoalveolar bacterial and fungal load was observed in the treated lungs, and four infected lungs were reconditioned and transplanted without complications. Later, D. Nakajima et al. [25] explored the possibility of using several antibacterial agents to treat lung rejection in polymicrobial infections. In addition to the significant reduction in bacterial load in bronchoalveolar lavage fluid, numerous inflammatory markers such as TNF-α and IL-1β were also significantly reduced. In turn, the treated lungs also showed better elasticity and oxygenation function compared to controls. A recent study demonstrated the feasibility of using light therapy as an antiviral agent to inactivate hepatitis C virus (HCV) in donor lungs in ENP. Up to 20% of lung donors in the United States test positive for hepatitis C, and many of these donors have a drug overdose as their cause of death, meaning they have young and relatively healthy lungs. In the modified ENP model with lighting device, M. Galasso et al. [30] used photodynamic therapy using the methylene blue activated by red light irradiation to reduce hepatitis C RNA levels by 98% in perfusate and by 91% in lung tissue. Ultraviolet irradiation was also performed in a separate group of donor lungs and demonstrated similar antiviral effects. The results of this study led to a prospective pilot clinical study involving the transplantation of 22 hepatitis C-infected lungs. Half of these lungs were treated with ENP plus ultraviolet irradiation, while the other half received ENP alone. Lungs treated with radiation showed a significantly lower viral load in the recipient's blood during the first week after transplantation.

Gene therapy

The release of proinflammatory cytokines is a major cause of donor lung injury and rejection. The Toronto team conducted several studies in human and pig models to explore the possibility of using interleukin-10 (IL-10) gene therapy to explore the possibility of using interleukin-10 (IL-10) gene therapy in ENP to suppress lung inflammation. Unlike gene therapy in vivo, in which gene delivery must be systemic, gene therapy during pulmonary ENP allows for more isolated delivery with lower dosage and fewer systemic side effects. M. Cypel et al. [31] performed ENP of lungs from a multiorgan donor, which had initially been found unsuitable for subsequent transplantation, using an adenoviral vector encoding human IL-10 (AdhIL-10) for 12 hours. Lungs treated in this way had better oxygenation and lower vascular resistance compared to controls. In addition, a shift from the production of pro-inflammatory to anti-inflammatory cytokines was observed. Later on, J.C. Yeung et al. [32] showed in a porcine lung transplantation model that administration of AdhIL-10 ex situ resulted in improved lung function after transplantation and less inflammation compared with *in situ* delivery *in vivo*, confirming that ENP is an excellent platform for gene transduction. As a following study, T.N. Machuca et al. [28] used a similar porcine model with a 7-day post-transplant period to study the long-term effects of AdhIL-10 therapy. Treated lungs had an improved function after transplantation and less inflammation; and the interferon-γ suppression was demonstrated up to day 7.

Gene modulating drugs

The complex process of IRI activates numerous genes that lead to apoptosis and tissue damage. Therefore, gene silencing agents such as siRNA and shRNA have been investigated in solid organ transplantation

to reduce graft damage. In particular, in lung transplantation, intratracheal delivery of siRNA and shRNA has been shown to reduce IRI in mouse models *in vivo*. These studies targeted pro-apoptotic and pro-inflammatory proteins such as Fas, caspase, myeloid differentiation protein-2, and p38 mitogen-activated protein kinase. Although these results demonstrated the potential of RNA interference in lung transplantation, an intratracheal administration of these drugs in vivo is limited in clinical settings because it requires several hours of tracheal intubation and mechanical ventilation. Lung ENP is a much more practical method of delivering RNA drugs ex vivo and more targeted. Administration of shRNA in ENP using lentiviral vectors has already been explored for suppression of major histocompatibility complex antigens to avoid acute cellular rejection of donor lungs after transplantation. C. Figueiredo et al. [33] showed that a 2-hour lung ENP with the shRNAs targeted at porcine leukocyte antigens resulted in more than 50% suppression of these endothelial antigens. No significant side effects were observed with lentivirus administration. This shRNA delivery method for lung ENP can be applied to targeted anti-apoptotic and anti-inflammatory agents such as those used in the above-mentioned studies *in vivo*.

Other therapeutic agents

Numerous other MPTAs have been tested in mouse and porcine models, but many of these results have not yet been reproduced using transplant models or human donor lungs. Several anti-inflammatory drugs such as methylprednisolone, α1-antitrypsin, and neutrophil cell elastase inhibitors have been shown to make anti-inflammatory effects, improve lung physiology, and reduce inflammatory markers in porcine models of pulmonary ENP. Hydrogen gas, a powerful free radical scavenger, has

been used in lung ENP to improve lung function and reduce inflammation. Sphingosine-1-phosphate, an endothelial barrier regulator, has been shown to reduce endothelial vascular permeability. As studies on lung MP show, the future lies with mesenchymal stem cells. This is evidenced both by numerous research by scientists and by the experimental results. It is likely that this therapy will become more widespread in the near future.

Liver machine perfusion

Liver preservation using EMP is also an emerging field, with numerous recent and ongoing clinical trials demonstrating its effectiveness compared to static cold preservation (SCP) [34]. As the number of recipients registered on the waiting list continues to exceed the number of available donors, the need for the use of marginal livers (steatotic, elderly, and from an asystolic donor) increases. The benefits of EMP are particularly evident in the context of the use of organs from expanded criteria donors, as EMP offers a platform for regenerating these livers prior to transplantation. Additionally, numerous biomarkers in perfusate and bile, such as bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate, have been shown to be associated with IRI and hepatobiliary damage. Several laboratories have already explored the potential of using MPTAs during liver EMP, and these studies can be summarized in Table 3. Almost all studies were conducted under normothermic conditions. Two studies were conducted under subnormothermic conditions, and one study was conducted under hypothermic conditions [35, 36]. The two main categories of MPTAs for the liver are antilipid compounds and vasodilators, both of which have been tested in numerous models of EMP and transplantation in rats and pigs.

Table 3. Main categories of machine perfusion therapy for liver transplantation

Study	Model	Treatment	Treatment category
Q. Liu et al. [37]	Rat	Forskolin, GW7647, scoparone, hypericin, visfatin and GW501516	Degreasing mixture
D. Nagrath et al. [38]	Rat	Forskolin, GW7647, scoparone, hypericin, visfatin and GW501516	
Y. Hara et al. [39]	Rat	Prostaglandin E1	Vasodilator
K. Maida et al. [40]	Rat	Prostaglandin E1	
A. Nassar et al. [41]	Pig	Prostacyclin	
J. Echeverri et al. [42]	Pig	BQ123(endothelin receptor 1 antagonist) and verapamil	
N. Goldaracena et al. [43]	Pig	Antisense oligonucleotide	Gene modulation
A.R. Gillooy et al. [44]	Rat	Small interfering RNA (anti-Fas)	
M.F. Thijssen et al. [45]	Rat	Small interfering RNA (anti-p53)	
N. Goldaracena et al. [46]	Pig	Anti-inflammatory drugs	Other
F. Rigo et al. [47]	Rat	Extracellular vesicles of human liver stem cells	
E.W. Beal et al. [48]	Rat	δ-opioid agonist (enkephalin)	
Y. Yu et al. [49]	Pig	inflammation inhibitor NLRP3	

Defatting agents

Steatosis of the donor liver significantly contributes to the development of primary dysfunction, and as a result, makes the cause of a significant number of liver transplant rejections in recipients. Therefore, a number of authors have studied the possibility of reconditioning a liver with steatosis using EMP. R.W. Jamieson et al. [36] showed that EMP alone could significantly reduce steatosis in pig liver. In this study, the steatosis of porcine livers were induced with streptozocin (a drug that causes hyperglycemia) and a high-fat diet; and these livers were compared with lean livers for over 48 hours of normothermic perfusion

(NP). Fatty liver showed higher production of triglycerides, glucose, and urea in the perfusate during EMP compared with normal liver, reflecting a higher metabolic state. In addition, a significant reduction in lipid deposits was observed upon histological examination [36]. The addition of a defatting mixture during NP has also been studied in a rodent model by D. Nagrath et al. [38]. Livers treated with the anti-lipid drug combination showed a significant decrease in intracellular lipid content and an increase in lipid oxidation and export just 3 hours after irrigation. In addition, an increase in the expression of genes associated with lipid mobilization was also observed in the treated liver [38].

Vasodilators

Damage to the microcirculation is a significant cause of IRI and post-transplant dysfunction in liver transplantation. It is known that liver sinusoidal endothelial cells, compared to hepatocytes, are more prone to IRI when stored under hypothermia. In turn, the use of prostaglandin E1 (PGE1) to improve microcirculation has been studied in several studies. PGE1 is a potent vasodilator that also has antiplatelet and fibrinolytic effects. Y. Hara et al. [39] first used PGE1 as MPTA in a rodent model ex situ under normothermic conditions. Treated rodent livers significantly improved bile production, in addition to reducing levels of liver damage markers: AST and ALT. K. Maida et al. [40], from the same group, later on confirmed these results using a rodent liver transplantation study using a similar setting. The treated rats had significantly higher survival rates, in addition to higher bile production and improved energy storage. Prostacyclin, another natural vasodilator and antiplatelet agent, has also been investigated as a potential MPTA in a porcine model of liver EMP. Similar to PGE1, prostacyclin significantly improved bile production and reduced markers of liver damage compared with EMP alone. Finally, two

additional vasodilators, BQ123 (endothelin receptor agonist) and verapamil (calcium channel blocker), have been studied in liver transplantation in pigs by J. Echeverri et al. [42]. Animals prepared in this manner showed an improved hepatic arterial blood flow and decreased markers of hepatocyte injury during EMP, but no significant differences were observed 3 days after transplantation. The authors attribute the lack of significant differences to the use of a relatively healthy donor liver that was not subject to any thermal ischemic injury. Greater results may be demonstrated using grafts from expanded criteria donors.

Gene modulation drugs

Using gene modulation agents such as antisense oligonucleotides (ASOs) and small interfering RNA (to turn off genes at the RNA level, siRNA) in EMP are especially promising, since it is much more targeted than systemic gene modulation, requires a lower dose and causes fewer side effects. In addition, it does not require a viral transfection, which can negatively affect the immune system. N. Goldaracena et al. [43] first demonstrated the potential of using ASOs to suppress the virulence of hepatitis C virus in a porcine model of liver EMP. ASO targets microRNA-122, the most abundant microRNA in hepatocytes, which is a necessary factor for hepatitis C virus replication. MicroRNA silencing has been shown to significantly reduce hepatitis C virus activity in a model in vitro. Thus, EMP before liver transplantation may potentially prevent reinfection in patients who are hepatitis C positive. A.R. Gillooly et al. [44] were the first to demonstrate the successful use of siRNA during hepatic EMP in rodents, both under normothermic and hypothermic conditions. SiRNA targets the Fas receptor (apoptotic antigen 1), which activation promotes the initiation of a proapoptotic pathway that significantly contributes to the development of IRI. The

same authors used siRNA targeting P53 (a tumor suppressor) to modulate apoptosis in a rat model of EMP. Other potential targets include RelB (transcription factor protein), TNF-α, and proapoptotic proteolytic enzymes, which have already been demonstrated to be protective in rodents when administered intravenously before induction of ischemia.

Other therapeutic agents

Two additional agents were tested in rodents under NP conditions. F. Rigo et al. [47] successfully demonstrated the uptake of extracellular vesicles derived from human liver stem cells during perfusion ex situ. In turn, the treated liver had less histological damage and lower levels of damage markers after 4 hours of perfusion. In another study, enkephalin, a delta-opioid agonist, was used during EMP to reduce injury caused by oxidative stress. The treated liver showed significantly better accumulation of energy substrates and fewer markers of tissue damage.

Therapeutics in conditions of subnormothermia and hypothermia

Q. Liu et al. [37] used a solution for defatting during subnormothermic liver EMP in rodents. Unlike normothermic EMP, subnormothermic EMP requires no temperature control or addition of oxygen carriers, which makes it more practical in clinical settings. However, the time of subnormothermic EMP required for a significant increase in the lipid content in the perfusate was 2 times longer than with normothermic EMP. In addition, no significant changes in intracellular lipid content were observed. In comparison, normothermic EMP appears to be a more effective method of reducing steatosis.

N. Goldaracena et al. [46] used anti-inflammatory drugs to minimize inflammatory damage during subnormothermic EMP in the pig liver.

The authors chose subnormothermic EMP due to the inhibitory effect of low temperature on the Kupffer cells and inflammation, in contrast to NP. Although significantly lower levels of AST and inflammatory cytokines were observed during EMP, no significant differences were observed for 3 days of reperfusion after transplantation. However, significantly lower bilirubin levels were observed in the treated groups after transplantation. Finally, in the hypothermic model of EMP and transplantation. Y. Yu et al. [49] used the leucine-rich nucleotide binding domain mcc950 as an anti-inflammatory MPTA to inhibit the NLRP3 inflammasome. After transplantation, significantly lower levels of markers of inflammation and injury were noted in livers treated in this way.

Vasodilators are the most attractive to use due to their impact on IRI, the main cause of organ rejection. This study, although incomplete (donors with expanded criteria were not used), gives hope for a speedy solution.

Renal machine perfusion therapy

Kidney transplantation is the most effective treatment for end-stage renal disease. The total number of patients receiving hemo- and peritoneal dialysis therapy in 2021 was 60,000 people [2].

Deceased donor kidneys, especially kidneys from an asystolic donor, are more susceptible to a primary dysfunction and delayed graft function. It is because of this circumstance that the use of hypothermic and normothermic EMP has been studied to protect these vulnerable renal grafts for subsequent transplantation. Numerous functional parameters

and biomarkers during normothermic EMP have been demonstrated to be excellent predictors of renal function during perfusion, including renal blood flow and diuresis.

Several biomarkers, such as glutathione S-transferase, have also been studied in normothermic EMP. In addition, MPTAs were studied in normo- and hypothermic EMP; these agents are summarized in Table. 4.

Table 4. Main categories of machine perfusion in kidney transplantation

Study	Model	Therapy	Perfusion temperature
B. Yang et al. [50]	Pig	Erythropoietin	Normothermic
C. Yang et al. [51]	Pig	Cyclic helix B peptide (erythropoietin derivative);	
G.T. Tietjen et al. [52]	Human	CD 31 nanoparticles	
M. Pool et al. [53]	Pig	Mesenchymal stem cells	
L. Brasile et al. [54]	Human	Mesenchymal stem cells	
K. Hamaoui et al. [55]	Pig	Thrombalexin	Hypothermic
M. Gregorini et al. [56]	Rat	Mesenchymal stem cells/Mesenchymal stem cells – extracellular vesicles	
R.N. Bhattacharjee et al. [57]	Pig	CO-releasing molecule 401	

Therapeutics for hypothermic renal perfusion ex situ

Mesenchymal stem cells and its extracellular vesicles known for their broad protective properties have also been tested in a rodent perfusion model. After 40 minutes of warm ischemia, kidneys treated with MSCs or its extracellular vesicles secreted lower levels of ischemic injury markers; and the efferent fluid contained glucose along with higher levels of pyruvate. That indicated an increase in the use of energy substrate compared to the kidneys preserved only by EMP. In addition, treated kidneys also showed upregulation of enzymes related to cellular

energy metabolism and membrane ion transport, potentially explaining the protective mechanism of MSCs during EMP.

Inhalation of carbon monoxide (CO), a vasodilator and anti-inflammatory agent, has been shown to have a protective effect during kidney transplantation in animals. R. N. Bhattacharjee et al. [57] created molecules 401 containing manganese and releasing CO for its targeted delivery (CO-releasing molecules containing manganese, CORM-401). Using a porcine model of EMP, the authors showed that CO-releasing molecules significantly improved the kidney function and decreased histological markers of kidney damage.

Impaired microcirculation due to thrombosis is a hallmark of IRI; in turn, two anticoagulants were studied in porcine models of EMP. A. Sedigh et al. [58] treated explanted kidneys with heparin conjugate for 20 hours of EMP. An improved primary function and lower histological markers of damage were observed in treated kidney grafts. K. Hamaoui et al. [55] studied the effects of thrombalexin, a thrombin inhibitor, in both porcine and human kidney models. The treated porcine kidneys demonstrated significantly better renal blood flow and capillary perfusion parameters. Extracorporeal MP of two human kidneys, with adding thrombalexin to the perfusate, demonstrated similar protective effects, in addition to reducing D-dimer and fibrinogen levels.

Therapeutics for normothermic renal perfusion ex situ

Normothermic EMP occurs at a higher metabolic level, which may allow kidney grafts to more quickly restore energy levels and reduce injury caused by cold ischemia. L. Brasile et al. [54] showed that the addition of MSCs to the perfusate during normothermic EMP of human kidney grafts accelerates the process of their subsequent regeneration. The authors showed that 24-hour perfusion with MSCs significantly

reduced the level of inflammatory cytokines in the kidney graft and significantly increased the accumulation of adenosine triphosphate and various growth factors (endothelial growth factor, fibroblast growth factor-2, and transforming growth factor α). J.M. Sierra Parraga et al. [59] studied the effect of perfusate on the survival and function of MSCs. The perfusate significantly reduced the ability of MSCs to interact with endothelial cells, while increasing the growth of MSCs in number. In addition, the authors showed that perfusion does not affect the secretory profile of MSCs. Since normothermic EMP requires large amounts of MSCs, J.M. Sierra Parraga et al. investigated the effect of the freeze-thaw process on MSCs. In particular, J.M. Sierra Parraga et al. [59] showed that the spontaneous thawing process reduces survival and metabolism, increases oxidative stress and impairs MSC adhesion. M. Pool et al. used fluorescently labeled MSCs to assess the localization and survival of MSCs during normothermic EMP in pigs [53]. After 6 hours of perfusion of 10^7 MSCs, positive MSC staining was detected in small clusters of glomeruli, i.e. these cells reached the renal cortex.

B. Yang et al. [50] explored the potential of using erythropoietin, a hormone with protective paracrine effects in the kidney, as a therapeutic agent in normothermic EMP. In a porcine model of EMP, the addition of erythropoietin significantly suppressed the inflammatory activity (caspase-3 and IL-1β) within 2 hours of perfusion. As a result, an improvement in diuresis was also observed. The same group also examined the renoprotective effects of erythropoietin-derived cyclic helix B peptide (CHBP) in a similar porcine model. Unlike erythropoietin, CHBP did not cause erythropoiesis and its subsequent side effects. Similar to erythropoietin, CHBP improved diuresis, in addition to renal blood flow and oxygen consumption improvement.

G.T. Tietjen et al. [52] recently demonstrated the potential of using antibodies against adhesion molecules (CD31) to enhance the delivery of nanoparticles to endothelial cells in human kidney grafts. Nanoparticle accumulation was increased 5–10-fold by the addition of anti-CD31 antibodies. These nanoparticles can serve as “sustained-release drug stores” and can be used to deliver therapeutic agents specifically to the kidney endothelium. Other authors have also explored the potential of using nanoparticles to deliver antioxidant agents, IRI mediator antagonists, and genetic material to reduce tissue injury during transplantation.

Several other therapeutic agents targeting specific pathways in IRI have also been investigated. Although these agents have not yet been tested in EMP models, they represent promising MPTA agents for the future. Hydrogen sulfate (H_2S) possesses known cytoprotective properties, such as those of reducing oxidative stress and inflammation. Recently, a synthetic donor molecule with extended release of hydrogen sulfate (AP39) was created for use in organ transplantation. Streptokinase has also been shown to improve renal microcirculation when administered intra-arterially. In addition, diannexin (a phosphatidylserine inhibitor), a recombinant molecule derived from the fusion of P-selectin glycoprotein ligand (PSGL) and human IgG1 (an inhibitor of polymorphonuclear leukocyte recruitment), and I5NP (an inhibitor of p53 expression) have been found to reduce inflammation in the kidneys.

Antibodies against adhesion molecules (CD31) are the only study that has made its way to human trials. With this therapy, drugs can accumulate in the kidney endothelium, as a result of which the drugs will act longer than usual. Nanoparticles are also a “landing platform for drugs.” Several studies have examined the potential of administering

agents (antioxidants, IRI mediator antagonists, and genetic material) to reduce tissue damage and achieve successful transplantation.

Conclusion

Rapid progress in lung, liver and kidney transplantation has made it possible to use grafts from asystolic donors for patients in urgent need of organs. In turn, these advances have also prompted the exploration of potential therapeutics that could be used during perfusion. New advances in machine perfusion therapeutic agents are particularly exciting because they offer a number of advantages over systemic treatments. First, their effect requires a smaller dosage of the drug; this is especially important for the drugs, such as siRNA, that are dosed based on weight. Second, machine perfusion therapeutic agents provide a much more targeted method of delivering drugs with potential systemic side effects, such as mesenchymal stem cells and thrombolytic agents. Currently, no machine perfusion therapeutic agents have been tested in randomized clinical trials. However, many promising machine perfusion therapeutic agents, especially in the field of lung transplantation, have been extensively tested in animal and disposed human organ models and are very close to reaching the clinical trial stage. Several therapeutic agents, thrombolytic and antibacterial, have already been used to recondition damaged human grafts and have resulted in successful transplantations. Because successful clinical trials of renal and liver perfusion ex situ have only recently been conducted, most studies of therapeutic perfusion agents in these areas are still at the animal modeling stage. In the future, additional human studies, especially randomized ones, are needed to strengthen the therapeutic potential of therapeutic machine perfusion agents in transplantation of these organs.

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