REVIEW

Ex vivo perfusion in lung transplantation and removal of HCV: the next level

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ABSTRACT

The large gap between high demand and low availability of lungs is still a limiting factor for lung transplantation which leads to important mortality rates on the waiting list. In the last years, with the advent of potent direct-acting antivirals (DAAs), donors carrying active hepatitis C (HCV) infection became an important source in expanding the donor pool. Recent clinical trials exploring different treatment regimens post-transplantation when using HCV-positive abdominal and thoracic organs into HCV-negative recipients have shown encouraging results. Although early data shows no toxicity and similar survival rates when compared to non-HCV organ transplantation, long-term outcomes evaluating the effect of either the transmission of HCV into the recipients or the deliberate use of DAAs to treat the virus remains absent. An important and innovative strategy to overcome this limitation is the possibility of mitigating viral transmission with the use of ex vivo donor organ treatment prior to transplantation. Recent pre-clinical and clinical studies explore the use of ex vivo perfusion and the removal of HCV prior to transplantation with the addition of other innovative therapies, which will be reviewed in this article.

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Introduction

With the rapid rise in mortality from opioid overdose in the last years in the United States and Canada [1,2], many centres have increased their utilization of overdose death donors (ODDs) in solid organ transplantation. A recent study from NYU [3] documents that ODDs comprised as high as 25% of lung transplants in several states across the United States, with a 10-fold increase between 2000 and 2017 and no difference in overall survival when compared to non-ODD transplants. Although these findings were encouraging, almost 10% of organs were ultimately discarded mainly due to the presence of bloodborne infections like hepatitis C (HCV) within the donor. Indeed, transplantation of HCV-positive donor organs into HCV-negative recipients historically has led to as high as a 90% risk of transmission accompanied by poor outcomes [4].

Recent pre-clinical and clinical studies have shown different strategies to reduce or even eliminate viral transmission in the context of HCV-positive donors into HCV-negative recipients. Regarding these strategies, one uses a short regimen of direct-acting antivirals (DAAs) [5] immediately after transplantation as a post-exposure prophylaxis [6], while the other aims to remove HCV completely from the pulmonary graft to

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avoid transmission using normothermic ex vivo lung perfusion (EVLP) and associated therapies [7,8].

This article aims to review recent literature in lung transplantation using HCV-positive donors and, in particular, the use of ex vivo organ perfusion as a strategy to eliminate HCV prior to transplantation.

Lung transplantation and the use of HCV-positive donors in the era of DAAs

Lung transplantation is the definitive procedure for patients with end-stage lung disease [9] and, although its practice has increased significantly in the last decades, the shortage of available organs remains a major problem as waitlist mortality still persists [10]. Recently, there has been increased interest and use of organs previously deemed as unsuitable for transplantation as a way of expanding the donor pool, such as lungs with active HCV infection. Indeed, a study from Mooney *et al.* [11] looking at the estimated impact of HCV-positive lung donors on donor lung supply in the United States shows that the use of these donors would lead to at least an additional 55 transplants per year in the US alone.

Prior to the advent of DAAs, organ transplantation using HCV-positive donors have shown extremely high transmission rates and dismal outcomes [4,12]. With the advent of these potent and pangenotypic DAAs to treat HCV infection, the use of HCV-positive organs has increased markedly in the last few years and has allowed transplantation of donor HCV-positive organs into HCV-negative recipients. Data on clinical trials from kidney transplants using HCV-positive donors and treatment with DAAs early after transplantation show encouraging results [13].

The emergence of thoracic organ transplantation using HCV-positive donors with the use of DAAs was boosted after a successful case report by our group in Toronto, Canada in 2016[14]. Accordingly, successive reports and early outcomes of heart and lung transplant recipients who received HCV-positive donor organs started to take place [15,16], enabling a paradigm shift in the field for thoracic organ transplantation. In early 2019, Woolley *et al.* from Brigham and Women's Hospital published the largest clinical trial-to-date regarding thoracic organ transplants from HCV-infected donors to uninfected recipients [6]. They performed 36 lung transplants and 8 heart transplants using an innovative protocol initiated almost immediately after surgery, where recipients received a 4-week regimen of sofosbuvir plus velpatasvir once daily. With this approach, the authors demonstrated sustained virologic response in all 35 patients at the 6-month follow-up time point. In addition, patients who had been treated for less than 6 months at time of publication had undetectable hepatitis C viral loads. Additionally, the trial showed excellent graft function at 6 and 12 months with no serious adverse events related to the medication administered. Overall, the trial shows encouraging results and has played a significant role in providing more confidence in relationship to usage of HCV-positive donor organs. Nonetheless, it is important to note that data is still not available demonstrating the long-term safety of this approach on parameters such as long-term survival and chronic lung allograft dysfunction. Looking deeper into the data, an interesting finding of the trial to consider is that all of the recipients had HCV viremia early post-transplant which related positively with the amount of viral load from the donor blood. This opens up the idea that reduction of HCV titres prior to the time of transplantation may reduce the incidence of HCV viremia early transplantation.

To this end, another interesting strategy with the objective of avoiding viral transmission is the use of DAAs even before implantation of the allograft into the recipient. A recent clinical trial from our multi-organ transplant group in Toronto proposed an ultra-short course of DAAs plus ezetimibe to prevent viral transmission [17]. HCV-negative recipients received HCV-positive organs (lung, kidney, heart and kidney pancreas) and started treatment 6–12 h before the anticipated implantation of the organ and continued for only 7 days. All 30 transplant recipients met the primary endpoint of undetectable HCV RNA at 12 weeks post-transplantation. This novel treatment regimen is very attractive, but still warrants long-term outcome data and is still dependent on the use of antivirals post-transplantation to treat/mitigate transmission. Thus, if an approach could be put forth to eliminate the virus prior to transplantation (thus avoiding any viral transmission and the use of even more medications post-transplantation), it would truly be a game changer. Specific strategies which focus on pre-transplant organ treatment, such as that enabled by EVLP, may allow for this aim to be achieved. A comparison of these two approaches is shown in Fig. 1.

Ex vivo lung perfusion and its potential to remove HCV prior to transplantation

Within the last 10 years, new technologies such as normothermic EVLP [18] has helped to decrease the gap between high demand and low availability of lungs by

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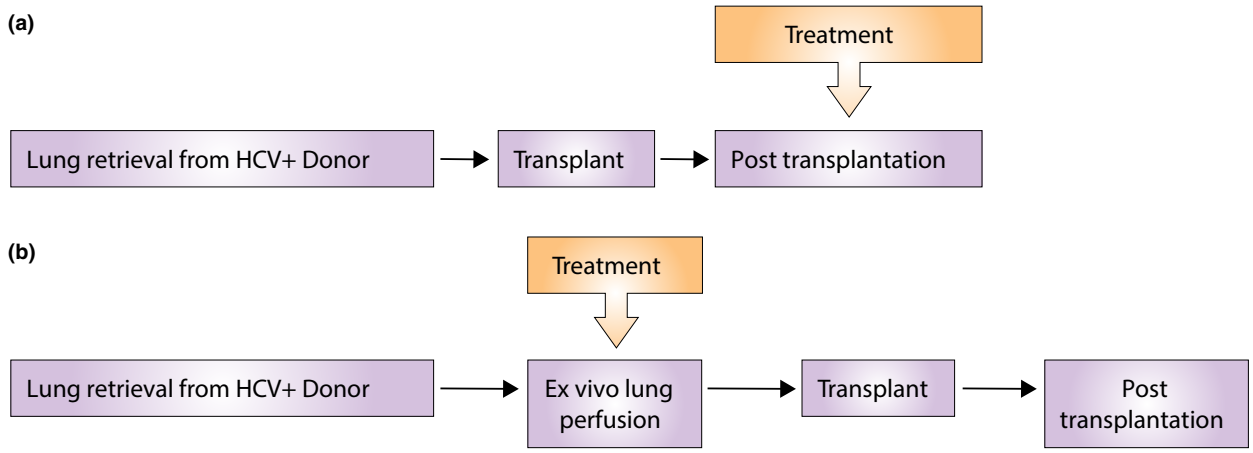

Figure 1 Schematic of different treatment approaches for preventing HCV transmission during lung transplantation. (a) The recipient receives an HCV + organ, and after transplantation, is treated with DAAs. (b) The organ is cleared of HCV during ex vivo lung perfusion and the lungs are subsequently transplanted.

safely expanding the donor pool, almost doubling the number of transplants performed every year [19]. EVLP is a clinical platform used in several transplant centres over the world to assess donor lungs prior to transplantation and is also an excellent platform to treat the grafts under protective physiological conditions [20]. Previous studies have shown that the EVLP technology can be used as a platform to successfully decrease donor lung inflammation [21], deliver cell therapy to attenuate ischaemia-reperfusion injury [22], treat bacterial infections [23] and, more recently, eliminate active viral infection [7]. To date, there are two pre-clinical and one clinical study exploring the potential use of ex vivo perfusion to eliminate HCV prior to transplantation. These studies explore different nonpharmacological approaches, such as an intensive lung wash relying mainly on the mechanical effect of ex vivo perfusion on washing out the virus, and novel technologies such as light-based therapies. The use of light-based therapies to treat infectious diseases is a well-known technology used for blood components sterilization in blood banks prior to blood donation [24]. Recent studies adapted and explored the idea further by applying this concept to whole organ perfusion [7,25]. Table 1 summarizes a list of in vitro, in vivo, ex vivo and clinical studies that reported novel nonpharmacological approaches to eliminate HCV.

EVLP effect on HCV removal from the lung allograft

The isolated effect of EVLP in reducing the HCV viral load in lungs was previously documented by our group in the case report from 2016[14]. After only 6h of perfusion, there was an 80% decrease in HCV levels in lung tissue. The rationale behind this important effect is that there is lack of evidence of the virus infecting or replicating inside lung cells; therefore, a 'washout' effect should be effective in decreasing viral loads. Although true, it was not enough to prevent the recipient from acquiring HCV, indicating the need for other strategies alongside with the perfusion effect.

In a pre-clinical study performed by our group using HCV NAT + human lungs [7], this idea was further explored by performing an intense lung wash in order to washout the virus from the allografts. The protocol consisted of the standard Toronto EVLP method [26] with two modifications: (i) a longer perfusion time of 9 h; and (ii) a complete circuit exchange, where there was replacement of the perfusion solution and the circuit after 3 h of EVLP. When compared to standard EVLP, the circuit exchange strategy was significantly superior in reducing HCV RNA levels in perfusate and lung tissue, reaching 86% and 84% reduction in viral loads, respectively. Accordingly, a safety clinical trial conducted in Toronto [8] which included 22 lung transplants from HCV-positive donors to HCV-negative recipients, evaluated the effect of EVLP on HCV donor-to-recipient transmission with either complete circuit exchange or addition of ultraviolet C (UVC) to the perfusion. Confirming what had been previously observed in the case report from 2016, all the 11 patients that received HCV-positive lungs after EVLP + circuit exchange had viral detection within the first week of

Table 1. Summary of important *in vitro, in vivo*, *ex vivo* and clinical studies using nonpharmacological approaches to treat and/or eliminate hepatitis C.

	Type of study	Strategy used	Type of HCV virus	Approach	Results
Müller-Breitkreutz *et al*, 1998 [32]	*In vitro*	PDT	HCV RNA-positive serum	Fresh human plasma spiked with HCV and treated with 1 μM of MB + fluorescent light	91–97% decrease in HCV RNA levels within 10 min
Steinmann *et al*, 2013 [26]	*In vitro*	UVC and PDT	JFH-1*	Human plasma and platelet concentrates spiked with JFH-1 and treated with 0.8–1.2 μM of MB + red light or UVC at 254 nm, respectively	Complete inactivation of HCV for both treatments. Infectivity of hepatocytes by the blood products post-treatment completely abrogated
Helfritz *et al*, 2018 [24]	*In vitro, Ex vivo* and *In vivo*	MB	HCV RNA-positive serum and JFH-1	1. *In vitro* infectivity assays with 1 μM of MB 2. Porcine kidneys contaminated with HCV during cold flush and treated with 1 μM of MB at 4°C during CSP or HMP 3. *In vivo* infectivity assessment of perfusion solution post-treatment using a human liver-uPA-SCID mouse model	1. Complete inactivation of HCV within 1 hour 2. Reduction of HCV infectivity to background levels and important decrease in HCV RNA copies 3. Mice injected with perfusate after MB treatment were negative for viral RNA and did not establish acute HCV infection; in contrast, mice injected with control perfusate developed viremia as high as 2.57 x 10^5 IU/ml and established acute infection
Galasso *et al*, 2019 [7]	*In vitro* and *ex vivo*	Intense lung wash, UVC and PDT	HCV NAT + human donor lungs and JFH-1	1. In vitro infectivity assays post-treatment of perfusate with UVC at 254 nm or 0.0.1–1 μM of MB + red light 2. EVLP treatment of infected donor lungs with complete circuit exchange, UVC at 254 nm or 1 μM of MB + red light	1. Despite detectable HCV RNA levels at all time points after the different treatments, HCV infectivity was completely abrogated after 150min of UVC irradiation and only 15min of PDT 2. A paired study was performed for each donor lung, consisting of two single-lung EVLPs (control vs. intervention). PDT was the most effective treatment to decrease HCV RNA levels when compared to control (97.9% vs. 69.5% reduction), followed by circuit exchange (85.8% vs. 46.6%). UVC showed no significant reduction compared to control

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Table 1. Continued.

	Type of study	Strategy used	Type of HCV virus	Approach	Results
Cypel *et al*, 2019 [8]	Open-label, single-centre, pilot clinical trial	Intense lung wash and UVC	HCV NAT + human donor lungs	All HCV-positive donor lungs prior to lung transplantation into HCV-negative recipient were treated with 6h of EVLP with 1) complete circuit exchange (n = 11) or 2) UVC at 254 nm (n = 11)	Despite important decrease in HCV RNA copies in perfusate and lung tissue after 6h of circuit exchange or UVC treatment, 100% and 81%, respectively, of recipients developed HCV viraemia. Median time to detection of viraemia was 3 days. All viraemic patients received DAA treatment and had undetectable HCV RNA by the end of treatment. No difference in survival was noted between recipients receiving lungs from HCV-negative vs. HCV-positive donors.

HCV, hepatitis C; PDT, photodynamic therapy; MB, methylene blue; UVC, ultraviolet C light; CSP, cold static preservation; HMP, hypothermic machine perfusion; EVLP, ex vivo lung perfusion; NAT, nucleic acid amplification tests; DAA, direct-acting antiviral.

*HCV cell culture system based on the Japanese Fulminant Hepatitis-1 clone (*JFH-1*), which was derived from a genotype 2a clinical HCV isolate. JFH-1 replicates robustly in human hepatoma cell lines that express viral entry factor CD81, allowing measurements of both HCV RNA levels and HCV infectivity.

transplant regardless of lower HCV levels in perfusate and tissue at the end of EVLP. Although every patient had viraemia after transplantation, the trial did highlight an important effect of EVLP: HCV RNA levels in the recipients were only detectable in two patients at day 1 after transplant, and even at day 2, median viral load was only 61 IU/ml. When compared to the trial by Wooley *et al.* [6], which did not use EVLP, HCV viraemia was detectable on day 1 in 94% of patients with a median viral load of 1800 IU/ml.

The pre-clinical and the clinical studies were important to confirm that EVLP alone has a strong potential in reducing the reservoir of the virus within the lungs, but not enough to eliminate completely, thus not preventing viral transmission to recipient. This most likely happens due to uneven perfusion, where ventilation/perfusion matching may be influenced by lung injuries and perfusion dynamics. Also, virus that is washed out but still circulating through the perfusion solution might not get eliminated with only one complete circuit exchange, which stresses the need for adjuvant strategies during perfusion.

EVLP and light-based therapies on HCV removal from the lung allograft

Antimicrobial effects of light-based therapies

Historically, light-based therapies such as UVC and photodynamic therapy (PDT)[24,27,28] have been used to treat blood components to eliminate different microorganisms prior to blood donation, including HCV, generating a safer process. Ultraviolet irradiation is divided into four distinct areas of spectra: UV (100–200 nm), UVC (200–280 nm), UVB (280–315 nm) and UVA (315–400 nm). UVC light, especially in the range of 250–270 nm, is strongly absorbed by nucleic acids in the microorganisms, and this range is known as the germicidal spectrum [29]. Upon UVC irradiation, DNA and RNA undergo photochemical modification, forming

dimeric photoproducts on bipyridine sites, known as Dewar isomers [30]. The formation of these products is crucial for inactivation of microorganisms since it leads to defects in cell replication and, consequently, cell death. Additionally, even if the nucleic acids still replicate, they produce nonviable binding proteins, which inactivates its infectivity. UVC in the germicidal spectrum has shown effective inactivation of HCV in culture media and human serum [27]. The decay rate of HCV infectivity after UVC irradiation was calculated as 0.067-log/s and 0.041-log/s in culture media and human serum, respectively.

PDT is another light-based therapy used in blood banks for sterilization of blood products, and the use of methylene blue and red-light irradiation also has shown efficacy to eliminate HCV from blood components prior to donation [31]. PDT uses a specific drug, called photosensitizer, which requires light irradiation in a specific wavelength to be activated [32]. Upon activation, the drug changes from a stable state (ground state) to an excited state (singlet state) followed by a decay phase, where there is formation of reactive oxygen species. This induces a sequence of photobiological processes that causes irreversible damage to microorganisms, such as cell death by apoptosis, necrosis and autophagy. *In vitro* data treating HCV-infected human plasma with methylene blue and light irradiation show extensive decrease in viral RNA within 10 min of illumination [33]. Methylene blue poses as an interesting candidate for ex vivo use since it is used in humans for several treatments for more than 100 years, including methemoglobinemia [34].

EVLP as a platform for light-based therapies: pre-clinical and clinical studies

The same pre-clinical trial [7] performed by our group that explored the effect of circuit exchange on the removal of HCV prior to transplantation also evaluated the effect of UVC and PDT added to the perfusion. The authors and collaborators developed a specific illumination device that was attached to the perfusion circuit to be able to deliver either UVC at 254nm or red light at 660 nm to activate methylene blue for PDT. When compared to standard EVLP or EVLP with complete circuit exchange, PDT was the most effective treatment to decrease perfusate and lung tissue HCV RNA levels, with a reduction as high as 98% in perfusate and 91% in lung tissue. The UVC group, on the other hand, did not show any significant decrease compared to control. When exploring further the potential effect of light-based therapies in HCV infectivity *in vitro,* infectivity assessments showed that despite having detectable HCV RNA levels at all time points after light-based therapy exposure, the HCV infectivity of hepatocytes was completely abrogated after only 15 min for methylene blue + red light and 150 min of UVC exposure.

This discrepancy noted between the RNA levels and infectivity of HCV is due to the mechanism of action of light-based therapies. The main challenge with the pre-clinical studies is that quantitative HCV RNA levels as the efficacy endpoint may not predict transmission, since light-based therapies damage and/or fragment the virions, rendering them noninfectious, but the fragments might still be detected by qPCR. A study from Steinmann *et al.* [27] using UVC and PDT to treat blood products infected with HCV has shown that loss of infectivity of the virions happens much before the reduction of HCV RNA levels are detected. Another pre-clinical study evaluating the effect of methylene blue in inactivating HCV in kidneys prior to transplantation corroborates these findings. Helfritz *et al.* [25] explored the use of methylene blue during ex vivo kidney hypothermic machine perfusion in a porcine model infected with human HCV. After 2 h of machine perfusion with the addition of MB, there was still remaining HCV RNA copies in the perfusion solution, but once the solution was injected into a human liver-uPA-SCID mouse model and followed for several weeks, and there was no viral RNA detected. In contrast, when the mice were injected with perfusion without methylene blue treatment or even with the solution used to contaminate the kidneys as a positive control, both developed HCV viraemia. This verifies that only fragments of HCV RNA and no viral particles remained after methylene blue treatment.

A clinical trial conducted in Toronto [8] explored further the effect of light-based therapies during EVLP to clear and mitigate HCV transmission to HCV-negative recipients by adding UVC during the perfusion time. The protocol consisted of standard EVLP with addition of the illumination device to deliver UVC, and a slightly longer perfusion time of 6h. Interestingly, from the 11 patients transplanted after EVLP with the addition of UVC, 2 did not develop viraemia at any time point. Additionally, at the 6-month follow-up period, these 2 patients remained nonviremic, showing complete prevention of transmission. All patients that became viraemic post-transplantation received sofosbuvir plus velpatasvir treatment and all achieved negative HCV PCR within 6 weeks of treatment initiation. Although 81% of the patients from the UVC group

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developed HCV viraemia, the study pointed interesting effects on the kinetics of HCV. For instance, the median viral load doubling time in the recipients in the first week after transplantation was significantly longer in the EVLP plus UVC group than in the EVLP plus circuit exchange group, rendering a median viral load at day 7 significantly lower in the patients from the UVC-treated donor lungs.

UVC exposure during EVLP was inadequate to prevent transmission in all patients most likely because the light is not being applied to the whole organ and only to the perfusate. This suggests that there is still residual blood in lung tissue carrying active HCV which cannot be reached by UVC. UVC irradiation to the organ is not feasible because the light would not penetrate deeply in lung tissue. The solution for this pitfall would be most likely the use of another light-based therapy, such as PDT with methylene blue, as already noticed in the pre-clinical studies comparing infectivity of both approaches [7,25]. The difference here is that MB is added to the perfusate solution and thus has the capacity to infiltrate the lung tissue during the EVLP period, which would be helpful to inactivate any residual HCV in the lungs. Since MB is activated by red light which penetrates better into tissue based on optical properties [35], another potential strategy to enhance the removal of HCV when using PDT would be irradiating the organ itself along with perfusate irradiation. Although this approach has not yet been evaluated in clinical trials, pre-clinical safety studies show no acute [7] or delayed [25] deleterious effects in a porcine EVLP followed by lung transplantation model and a humanized mice infectivity model, respectively, encouraging future innovative safety clinical trials to take place.

Discussion and limitations

The use of ex vivo approaches to prevent transmission of HCV after organ transplantation poses interesting advantages over post-transplant treatment with DAAs, but complete prevention is still not possible with these current strategies. At this time, clinical evidence points to EVLP with or without addition of UVC light as an adjuvant therapy to DAAs post-transplantation, unless the recipient does not become viraemic. Although still not a definitive treatment for eliminating viral transmission in most cases, using EVLP prior to transplantation resulted in a delayed and significantly lower viraemia concentration in HCV-negative recipients [8]. However, further optimization of EVLP therapies is ultimately needed in order to prevent transmission altogether.

Regardless of the use or non-use of EVLP prior to transplantation, these clinical trials are quite recent, and therefore, we are still lacking long-term outcomes to evaluate chronic lung allograft dysfunction related to either HCV in the recipient, deliberated treatment with DAAs or light-based therapies during EVLP. Lastly, one important limitation to the use of EVLP for the removal of HCV is the requirement of having an established EVLP program within the transplant centre.

Concluding remarks

In the past 3 years, there has been accumulating data on transplantation of HCV-positive donors into HCV-negative recipients with encouraging results. Small clinical trials in abdominal and thoracic organs with the use of potent antiviral drugs after transplantation show safety of the procedure, although there is still a gap in knowledge of possible chronic effects of this approach. Hence, complete prevention of viral transmission is an alternative approach to using these HCV-infected organs with some advantages. Ex vivo organ perfusion with the addition of light-based therapies to remove HCV from allografts prior to transplantation and avoid viral transmission is an innovative approach that has shown excellent results in pre-clinical studies and interesting findings in a clinical trial. Finally, there is still space to explore the full potential of these technologies suggesting a safer process in organ donation which could make the use of HCV-positive donors routine in clinical practice for lungs and other organs.

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Conflict of interest

M.C is a co-founder of Perfusix Inc. and XOR Labs Toronto (companies related to ex vivo organ perfusion) and consultant to lung bioengineering. R.V.P.R and A.A have no conflicts of interest.

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