Ex Situ Perfusion of Hearts Donated After Euthanasia: A Promising Contribution to Heart Transplantation

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Background. Organ donation after euthanasia is performed in an increasing number of countries. In this donation after circulatory death procedure, it has not been possible to donate the heart. Recent literature, however, reports positive results of heart donation after circulatory death. Therefore, patients who donate organs following euthanasia might be suitable candidates for heart donation. We want to confirm this assumption by sharing the results of 2 cases of heart donation following euthanasia with ex situ subnormothermic heart preservation. Our aim is to raise awareness of the potential of heart donation following euthanasia for both clinical transplantation and research. Methods. The data of 2 consecutive heart donations following euthanasia were collected prospectively. Informed consent was obtained from the patients themselves for heart donation for research purposes. An acellular oxygenated subnormothermic machine perfusion strategy was used to preserve both donor hearts. Subsequently, the hearts were evaluated on a normothermic perfusion machine using a balloon in the left ventricle. Results. Heart donation following euthanasia was feasible without significant changes in existing retrieval protocols. Duration of machine perfusion preservation was 408 and 432 minutes, for heart 1 and 2, respectively. For heart 1, developed pressure ($P_{dv}$) was 119 mm Hg, maximal rate of pressure rise ($dP/dt_{max}$), and fall ($dP/dt_{min}$) were 1524 mm Hg/s and −1057 mm Hg/s, respectively. For heart 2, $P_{dv}$ was 142 mm Hg, $dP/dt_{max}$ was 1098 mm Hg/s, and $dP/dt_{min}$ was −802 mm Hg/s. Conclusions. Hearts donated following euthanasia are highly valuable for research purposes and can have sufficient quality to be transplanted. With the implementation of ex situ heart perfusion, patients who are to donate their organs following euthanasia should also be able to donate their hearts. The complex combination of euthanasia and heart donation is ethically sound and surgically feasible and can contribute to shortening the heart transplant waiting list.

(Transplantation Direct 2021;7: e676; doi: 10.1097/TXD.0000000000001120. Published online 22 February, 2021.)
the possibility to donate their organs following euthanasia. Although there were 14 patients who donated their organs after euthanasia in the Netherlands in 2019, none of them—in the absence of available adequate preservation and assessment methods—donated their heart, neither for transplantation nor for research purposes. Some were, however, eager to donate their heart. Donation following euthanasia has the potential to become an important source of donor hearts. Contrary to published results on kidneys, lungs, and livers donated following euthanasia, literature on the function of hearts donated following euthanasia is still nonexistent. In the DCD-V donor pool, the agonal phase is usually very short and predictable, as the donor often dies quickly following administration of the euthanasia drugs. This is in strong contrast to DCD-III, in which the agonal phase may extend to over 2 hours, rendering organs unsuitable for donation due to hypoperfusion and ischemia. We therefore expect that DCD-V hearts are subjected to less injury compared with DCD-III hearts.

Still, to optimize DCD-V heart transplantation, improvements of strategies for both preservation and ex situ assessment of the hearts will be required. The common preservation strategy for DBD hearts, cold ischemic storage, is not suitable for DCD hearts, as it fails to prevent cumulative injury to DCD hearts caused by hypoxic circulatory arrest and cold ischemic storage. Circulatory arrest results in warm ischemia, which leads to energy depletion and acidosis. The addition of cold ischemia due to static cold storage has detrimental effects on posttransplantation cardiac function. One recent innovation—ex situ machine perfusion—has proven to be crucial for DCD-III heart preservation. Machine perfusion has been and is being elaborately researched, involving mainly animal models but also human subjects. Ex situ machine perfusion could also facilitate DCD-V heart preservation. Moreover, it could be used to quantify the significance of the injury and the potential for recovery of the heart enabling adequate evaluation of the DCD-V heart.

The aim of this article is to underline the potential of DCD-V heart donation for both clinical heart transplantation and research on human donor hearts. We share the results of 2 DCD-V heart donation cases, with a novel ex situ machine perfusion strategy to preserve and evaluate donor hearts. We will discuss the value of DCD-V donor organs and their potential to contribute to the implementation of innovations in the field of transplantation research in the clinical practice.

**MATERIALS AND METHODS**

**Euthanasia**

In the Netherlands, euthanasia has been legal since 2002. This procedure is performed in accordance with the Dutch euthanasia protocol stipulated by the Royal Dutch Medical Association and the Royal Dutch Pharmacists Association. Euthanasia requests are always reviewed by the treating physician and 1 independent physician, before and independently from the discussion on possible organ donation. Euthanasia is only allowed when the patient is suffering hopelessly and unbearably without any therapeutic options and only after a voluntary and deliberate request. Patients should request organ donation themselves. A national guideline has been developed for the combination of euthanasia and organ donation procedures. Most patients who qualify for organ donation after euthanasia suffer from psychiatric or neurodegenerative diseases.

**Donor Consent**

In DCD-III cases, it is impossible to obtain consent for research from donors themselves. The protocol of the Dutch Transplant Foundation states that if organs are explanted solely for research purposes, the explicit written informed consent from next of kin is required, even if the procurement is part of a procedure in which other organs are explanted for clinical transplantation. Donors’ families may be less motivated to make organs available for research than for transplantation purposes. In donation following euthanasia, however, patients themselves have time to discuss and consider the proposed research use of organs and can decide autonomously whether or not to consent.

**Heart Procurement**

In 2 DCD-V procedures, following induction of a coma using either propofol or thiopental followed by administration of rocuronium, circulatory arrest was awaited. After a no-touch time of 5 minutes to respect the dead donor rule, death was declared, and the donor was transported to the operating theater. A sterno-laparotomy was performed, and the ascending aorta was cross-clamped. The coronary arteries were anterogradely flushed with a 4°C cardioplegic solution (Custodiol HTK solution). The cardioplegic solution was supplemented with lidocaine (500 μmol/L) and adenosine (200 μmol/L). The donor heart was retrieved in a standard fashion, although the aorta was transected at the level of the descending aorta. By doing so, the aortic arch and supra-aortic vessels remained attached to the heart to facilitate cannulation for preservation.

**Machine Perfusion**

**Subnormothermic Preservation**

After retrieval, the donor heart was submerged in preservation solution. The supra-aortic vessels were cannulated, and the distal aorta was sutured closed to facilitate retrograde flow in the aorta, resulting in antegrade coronary perfusion. The perfusion machine was pressure-controlled and produced a pulsatile flow. Target perfusion flow rate was 200 mL/min, with a maximum set pressure of 45 mm Hg. Gas flow was set at 100 mL/min of 100% oxygen. The hearts were continuously perfused using a Perfadex Plus—albumin solution (75 g/L), at 100 mL/min of 100% oxygen. The hearts were continuously perfused using a Perfadex Plus—albumin solution (75 g/L), which was made at the donor hospital site and was supplied with potassium to reach a concentration of 20 mmol/L. The target temperature was 20°C–21°C. After initiation of perfusion, the donor heart was transported to our hospital.

**Evaluation**

A conic balloon was inserted in the left ventricle through the mitral valve annulus for functional evaluation. The heart was connected to a normothermic evaluation perfusion machine. The Heart Assist (Organ Assist BV) consisted of a heater-cooler and a pump unit and perfused the heart using the Langendorff method. The perfusion solution consisted of Perfadex Plus (XVIVO Perfusion AB) with albumin (75 g/L), supplemented with packed red blood cells that had been washed using a cell saver. Target hemoglobin levels were 4.0–4.5 g/dL, and target potassium levels were 3.0–5.5 mmol/L. Electrolytes and blood gases were corrected when necessary.

The normothermic evaluation was initiated at a perfusion pressure of 40 mm Hg at 20°C. Over a time period of 30 minutes, temperature was gradually increased to 37°C. Once
an outflow temperature of 34°C was reached, the hearts were defibrillated if needed and infusion of both insulin (2.25 IU/h) and dobutamine (4 μg/min) was initiated.28 Once at normothermia, the perfusion pressure was increased to 60 mm Hg.

End Points

The timing of administration of rocuronium, circulatory arrest, declaration of death, start of cold cardioplegic flush, start of machine preservation perfusion, and end of the machine preservation perfusion were registered. The heart was weighed after retrieval, after preservation and after normothermic evaluation. Blood gas analyses of the perfusate were performed throughout the evaluation procedure. The intraventricular balloon was connected to a pressure transducer with continuous pressure recording. The volume in the intraventricular balloon was increased and decreased using a syringe with saline to simulate in vivo preload changes. Developed pressure (Pdev) was analyzed, and systolic and diastolic function were assessed as the maximum rate of intraventricular pressure rise (dP/dtmax) and fall (dP/dtdmin), respectively (Figure 1).

Ethics and Informed Consent

Clinical research involving deceased participants does not fall within the scope of the Dutch Medical Research with Human Subjects Law. Therefore, the Medical Ethical Review Board has exempted the study protocol from review (M17.208560), as is customary in the Netherlands. Both patients were informed about the study and were asked to provide informed consent for the use of the heart for research purposes. Heart procurement for research did not interfere with the procurement and transplantation of other organs.

RESULTS

Donor 1

Euthanasia was requested and approved for a patient (55–60 y old) who suffered from Parkinson’s disease for over 20 years. The medical history was negative for cardiac pathology, smoking, and alcohol or drug abuse. A CT of the chest was performed during routine work-up for organ donation, which showed no cardiac abnormalities. Euthanasia was performed in the hospital by patient’s own general practitioner. Circulatory arrest occurred 3 minutes after administration of 1000 mg propofol and 150 mg rocuronium (Table 1). After an additional 6 minutes of no-touch, including a 1-minute delay as a result of delay in communication, the patient was declared dead. Up to the initiation of cold cardioplegic flush, 27 minutes of warm ischemia expired. During the following 48 minutes, dissection, retrieval, and cannula placement took place and subnormothermic ex situ perfusion started. The machine perfusion preservation lasted 6 hours and 48 minutes. While switching the heart from the preservation machine to the evaluation machine and inserting the intraventricular balloon, the heart was submerged in 4°C Custodiol HTK solution for 21 minutes.

Donor 2

Euthanasia was planned for a patient (60–65 y old) who received the diagnosis of amyotrophic lateral sclerosis 2 years earlier. The medical history was negative for other pathologies. The patient had a history of 13 pack years of cigarette smoking but had quit long before the donation procedure. After administering 2000 mg thiopental and rocuronium, circulatory arrest was confirmed after 5 minutes. Following a 5-minute no-touch period, the patient was declared dead (Table 1). Seventeen minutes later, the cardioplegic solution was infused. Machine preservation perfusion was initiated 33 minutes after declaration of death. Total preservation duration was 7 hours and 12 minutes. The switch from preservation to evaluation with insertion of the intraventricular balloon, lasted 16 minutes, with the heart submerged in 4°C Custodiol HTK solution.

Preservation: Oxygenated Machine Perfusion at Room Temperature

The mean perfusion pressure of 45 mm Hg and 40 mm Hg in donor heart 1 and 2, respectively, resulted in a coronary flow of 198 mL/min and 193 mL/min. The mean perfusion temperature was 20°C. The lowest measured temperature was 19°C in heart 1 and 18°C in heart 2. The highest measured temperature was 22°C for both.

Evaluation: Normothermic Machine Perfusion

Mean perfusion pressure was 60 mm Hg and 58 mm Hg in donor heart 1 and 2, respectively. This resulted in a coronary flow of 352 mL/min in heart 1 and 367 mL/min for heart 2.

For both hearts, functional evaluation with the intraventricular balloon started at 1 and 2 hours after initiation of normothermic perfusion. Due to technical difficulties, the normothermic perfusion of heart 1 was extended with half an hour to collect sufficient data. Figure 1 gives an overview on the assessment of functional outcome.

By the end of the evaluation, developed pressure was 119 mm Hg for heart 1, with an intraventricular balloon volume of 30 mL. Maximum rate of pressure rise and fall were 1524 mm Hg/s and −1057 mm Hg/s, respectively. For heart 2 (Figure 1), developed pressure was 142 mm Hg with 50 mL of intraventricular balloon volume. Maximum rate of pressure rise and fall were 1098 mm Hg/s and −802 mm Hg/s, respectively.

DISCUSSION

The description of these 2 cases of heart donation following euthanasia suggests that heart donation in a DCD-V procedure is feasible and does not require modifications of the surgical DCD-V procedure when the heart is retrieved together with other donated organs. Despite prolonged preservation, the subnormothermic preservation strategy showed encouraging results, with both hearts demonstrating satisfactory function evaluations.

Our study is in line with the finding of Van Reeven et al that in DCD-V donors, the agonal phase is very short and predictable.29 Therefore, the donated hearts will be procured rapidly, which will increase the number of effective donation procedures as compared with DCD-III procedures. Approximately 5%–10% of all patients requesting euthanasia might be eligible to donate their organs,28,30 In light of the small total number of heart transplantations performed in the Netherlands, every additional DCD-V donor could have a significant impact on the heart transplant activity in the country.
Suitability of DCD-V Hearts

In this study, cardiac function was assessed by means of an intraventricular balloon. In a comparable setting of for transplantation-rejected human hearts, DCD-III and DBD hearts were preserved using cold storage, the gold standard for DBD hearts. Therefore, these hearts must have been adequately preserved. Similarly to our study, hearts were evaluated using the intraventricular balloon. After 2 hours of reperfusion, a $dP/dt_{\text{max}}$ of 801 mm Hg/s was yielded (compared with 1524 mm Hg/s and 1098 mm Hg/s in our study). A $dP/dt_{\text{min}}$ of −511 mm Hg/s was reported (versus −1057 mm Hg/s and −802 mm Hg/s). It is important to note that a smaller-volume balloon (20 mL versus 30 mL and 50 mL in our study) was used, which implies a lower preload, which could therefore have resulted in lower values, as $dP/dt$ is load-dependent. This may not explain the higher values observed in our study, as our first heart showed greater $dP/dt_{\text{max}}$ and $dP/dt_{\text{min}}$ despite lower volume (30 mL versus 50 mL in our second heart). The same group also performed transplantation studies with porcine DCD hearts, which were subjected to 30 minutes of warm ischemia, and subsequently perfused up until transplantation in a recipient animal. Cardiac function was assessed using a conductance catheter in situ, both before procurement and after transplantation. The values mentioned for $dP/dt_{\text{max}}$ and $dP/dt_{\text{min}}$ in our study were comparable with their baseline values before procurement (1180 mm Hg/s and −829 mm Hg/s) and numerically higher compared with their values after transplantation (723 mm Hg/s and −529 mm Hg/s). The combination of our results in DCD-V and existing literature on heart donation after circulatory arrest (DCD-III) suggests that DCD-V hearts preserved with ex situ heart perfusion may serve as suitable donor hearts.

Although the present study was performed in the preclinical setting, with prolonged preservation times and without subsequent heart transplantation, its results suggest that heart donation is likely to be feasible in euthanasia patients. Importantly, the addition of heart procurement in a DCD-V

**FIGURE 1.** Data retrieved from the evaluation of heart 2 at 120 min of reperfusion. Upper panel: the result of successive volume increments. Intraventricular balloon volumes are specified above each graph. Grids and numbers represent the mean systolic (in red) and diastolic (in blue) pressure for that specific volume. Lower panel: amplification of pressure recording at 50 mL balloon volume. Assessment of developed pressure, contractility, and relaxation are visualized in orange. Specification of grids and numbers are identical to the upper panel. $dP$, difference in pressure; $dt$, difference in time; $dP/dt_{\text{max}}$, maximum rate of intraventricular pressure rise; $dP/dt_{\text{min}}$, maximum rate of intraventricular pressure fall; LV, left ventricle; mm Hg, millimeters of mercury; $P_{\text{dev}}$, developed pressure.
TABLE 1
Timings of successive actions in the DCD-V procedure

|                          | Heart 1 | Heart 2 |
|--------------------------|---------|---------|
| **Agonal phase**         | 3 min   | 5 min   |
| (Administration of rocuronium to circulatory arrest) |         |         |
| **No-touch period**      | 6 min   | 5 min   |
| (Circulatory arrest to declaration of death)         |         |         |
| **Warm ischemic time**   | 27 min  | 27 min  |
| (Administration of rocuronium to start cold cardioplegic flush) |         |         |
| **Cold ischemic times**  |         |         |
| Prepreservation perfusion| 48 min  | 33 min  |
| Postpreservation perfusion| 21 min  | 16 min  |
| Total cold ischemic time | 69 min  | 49 min  |
| **Machine perfusion preservation time** | 6h and 48 min | 7 h and 12 min |

DCD-V: donation after circulatory death after euthanasia.

donor setting did not inflict significant changes to the donation protocol and did not affect the organs procured for transplantation. Parallel to the lung procurement, the cardiac surgeon has to place the aortic cardioplegia cannula, vent the right side of the heart, and flush the coronaries with a cardioplegic preservation solution. Heart retrieval using the direct procurement technique could therefore easily be implemented in current procurement protocols. The euthanasia drugs might have side effects (mainly hypotension) in the donor. However, we do not believe that these substances would impair the heart function in the recipient, because the same substances are used during clinical heart transplantation.31

**Donor Consent for Research**

Organ donation after euthanasia may be a highly valuable resource for both transplantation research and clinical transplantation. Informed consent for research participation can be obtained from the actual donor instead of next of kin, which offers both practical and ethical advantages. Complex and delicate communication with grieving families can be avoided, and the autonomy of the donor can be respected. Even so, the combination of euthanasia and organ donation processes is ethically complex, and the addition of organ donation for research purposes further complicates these parallel processes. It requires careful and extensive information provision, communication and informed consent, as well as planning, logistics, and oversight.

In the 2 cases presented here, the transplant coordinator elaborately informed both patients well in advance about the possibility to donate their hearts for research focused on the development of perfusion strategies. Both donors were highly motivated to participate in the research, had expressed sincere disappointment that their hearts could not be used for clinical transplantation, and viewed the option to donate their hearts to research as a consolation, hoping to indirectly benefit others in the future. To facilitate the clinical implementation of transplantation using donor hearts following euthanasia, it is key that research on human DCD-V hearts continues to improve preservation, develop function assessment, and optimize viability of donor hearts.

**CONCLUSION**

In conclusion, DCD-V donors are highly valuable sources of hearts for research purposes, as these hearts can be procured ethically, with informed consent provided by the donor and without additional harm to the donor. The possibility to perform studies on human hearts is invaluable and indispensable to the development of machine perfusion strategies and will eventually lead to a quicker and better implementation of techniques that improve and enlarge the clinical donor pool. We postulate that heart donation can be easily implemented in the current existing surgical DCD-V donation procedure. Finally, this study suggests that DCD-V donor hearts may be suitable for transplantation, allowing for an increase in the number of heart transplants in countries where euthanasia is legal.

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