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Clamping of the Aortic Arch Vessels During Normothermic Regional Perfusion After Circulatory Death Prevents the Return of Brain Activity in a Porcine Model

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Background. The cerebral effect of clamping following normothermic regional perfusion (NRP) in donation after circulatory death (DCD) remains unknown. We investigated the effect of cerebral reperfusion during NRP and the preventive effect of clamping on brain function in a porcine model. **Methods.** In 16 pigs, intracranial physiological parameters were recorded, including pressure, cerebral blood perfusion (CBF), temperature, and oxygen. Additionally, electroencephalography (EEG) and somatosensory evoked potentials (SSEPs) were used to assess brain function. The animals were cannulated for the heart-lung machine, and baseline measurements were performed before withdrawal from life support. After 8 min of mechanical asystole, the animals were randomly allocated to clamp (n = 8) or nonclamp (n = 8) of the aortic arch vessels. After 30 min of NRP, the animals were monitored for 3 h after weaning (AW). **Results.** Intracranial measurements of CBF, oxygen, and temperature indicated successful occlusion of the arch vessels following NRP and AW in the clamp group versus the nonclamp group. In the clamp group, EEG was isoelectric and SSEPs were absent AW in all pigs. In the nonclamp group, EEG activity was observed in all 8 pigs, whereas SSEPs were observed in 6 of 8 pigs. Additionally, agonal respiratory movements in the form of gasping were observed in 6 of 8 pigs in the nonclamp group. **Conclusions.** Reperfusion of the brain during NRP led to a return of brain activity. Conversely, clamping of the arch vessels halted cerebral circulation, ensuring the permanent cessation of brain function and maintaining the determination of death in DCD.

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INTRODUCTION

Donation after circulatory death (DCD) is an emerging practice and has provided a significant addition to the donor pool in recent years, with several successful programs being developed.[1-7] Especially pertinent is the controlled DCD (cDCD) model. These are donors whose death occur following the clinical decision to withdraw life-sustaining therapies (WLSTs). Organs such as the heart are very susceptible to warm ischemic damage, and cDCD is therefore of particular interest because the process of death can be precisely monitored. WLST is followed by a no-touch (NT) period defined as continuous apnea, loss of circulation, and unresponsiveness before circulatory death is declared. Most countries using DCD recommend an NT period of 5 min, although practices vary from 2 to 10 min.[8]

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F.F.D., N.M., M.P., and H.E. conceived and planned the experiments. F.F.D., N.M., and Z.L.Z. performed the experiments. E.Q. performed analysis of the neurophysiological recordings. F.F.D., N.M., E.Q., S.B., H.E., and M.P. interpreted of the results. Z.L.Z., L.B.I., P.R., and M.E. contributed to the surgical protocol and provided critical feedback. F.F.D. took the lead in writing the article. All authors provided critical feedback and helped shape the research, analysis, and article.

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Normothermic regional perfusion (NRP) by extracorporeal membrane oxygenation has been established as a method to reperfuse transplantable organs with warm oxygenated blood to combat the ischemic damage from circulatory death.[9,10] This method provides an opportunity to optimize and evaluate organ function before transplantation. Before reestablishment of organ perfusion, the main cerebral vessels are occluded as no intervention that may artificially restore cerebral circulation is acceptable.[11-13] Thoracoabdominal NRP (TA-NRP) protocols ensure the cessation of cerebral circulation by positioning a cross-clamp on the aortic arch vessels. Animal studies have provided evidence of some return of brain functions following prolonged periods of cerebral ischemia for as long as 1 h.[14,15] Porcine studies of induced ventricular fibrillation showed limited to good neurological outcomes after 15 min of cardiac arrest.[16,17] These findings support the view that some mammalian neurons survive well beyond common stand-off periods in DCD[18] even if their activity is not congruous with organized brain function.

The determination of death in DCD relies on the permanence principle.[19,20] Clamping ensures the permanent cessation of brain circulation and therefore aims to maintain the determination of death in DCD. There is, however, a lack of knowledge about the effect of cerebral reperfusion on brain function during NRP in DCD and whether clamping of the arch vessels is sufficient to ensure the complete cessation of all cerebral functions.

In this DCD porcine study, we aimed to investigate whether brain activity is restored following resuscitation with NRP if cerebral reperfusion is allowed. Additionally, we investigated whether clamping of the aortic arch vessels successfully prevents the return of cerebral function, thus maintaining the determination of death in DCD.

MATERIALS AND METHODS

Sixteen female Danish Landrace pigs (78–91 kg) underwent circulatory death with 8 min of asystole before resuscitation with NRP. The study was conducted as a prospective randomized intervention-control study and comprised a clamp group (n = 8) and a nonclamp group (n = 8). In the clamp group, the aortic arch vessels were occluded, and in the nonclamp group, a sham operation was performed. Before the intervention, baseline parameters described below were measured for the animals to be their own control.

The study was conducted in accordance with the guidelines given by the Danish Animal Experimentation Inspectorate and was approved by this institution (2018-15-0201-01603).

Surgical Procedure

Anesthesia and Monitoring

Premedication, comprising Zoletil Vet 50 (tiletamine [6.25 mg/mL], benzodiazepine [6.25 mg/mL]), ketamine (12.5 mg/mL), butorphanol (2.5 mg/mL), and xylazine (12.5 mg/mL), was administered with a dosage of 10 mL/kg before transport to the surgical facilities. Anesthesia and analgesia were maintained with propofol (3.5 mg/kg/h) and fentanyl (10 μg/kg/h). Anesthesia was discontinued after circulatory death and resumed because of ethical reasons in cases where electroencephalography (EEG) activity and somatosensory evoked potentials (SSEPs) were observed.

Via the right femoral artery, an intravascular balloon was advanced to the abdominal aorta above the iliac bifurcation to exclude the circulation to the lower extremities while allowing for blood pressure monitoring. Arterial P_{CO_2} and P_{O_2} were maintained at 4.5–6 and 12–16 kPa, respectively. Additionally, mean arterial pressure (MAP) was maintained at >60 mm Hg using an infusion of norepinephrine in dosages of 0.05–0.5 μg/kg/min. Vital parameters including MAP, heart rate, P_{O_2}, lactate, and core temperature were recorded.

Intracranial Monitoring

Four burr holes were fitted with bolts and placed bilaterally to the sagittal suture, targeting the cerebral cortex. A fiber optic probe (NX-BF/OFT/E, Oxford Optronics, United Kingdom), stainless steel electrodes placed in each hemisphere, and a Neurovent-PTO (Raumedic, Germany) probe were inserted into the bolts and secured with flexible sealing caps (for further details see **Supplemental Digital Content, SDC,** http://links.lww.com/TP/C352). Before puncture of the dura mater and insertion of probes, the head was restrained. Because of implantation trauma, baseline measurements were performed 4 h after transcranial insertion of probes.[21]

Assessment of Cerebral Perfusion

Cerebral perfusion was evaluated by measuring cerebral blood perfusion, temperature, and oxygen tension using a fiberoptic probe (Oxford Optronix, United Kingdom). CBF was measured by laser Doppler technique and presented as blood perfusion units. The probe was connected to OxyLite and OxyFlo monitors and data were recorded through a DATAQ Instruments Data Acquisition System and WinDaq Software (DATAQ Instruments, Akron, OH). CBF, oxygen, and temperature measurements were recorded every 4 s. Fluctuations in the raw data were attenuated using a rolling average spanning 1 min for each time point, including baseline, 5 min after asystole (AS + 5), 25 min after NRP (R + 25), and 30, 120, and 180 min after weaning (AW). After insertion, the probe was retracted to alleviate pressure from the tip. For blood perfusion unit, biological zero was observed at AS + 5 and was subtracted from all other values before analysis.[22] Flow measurements that were disturbed from alterations of the probe position were excluded.

A Neurovent-PTO intracranial pressure (ICP) probe was calibrated to zero in room air before insertion into the cerebral parenchyma. Measurements occurred in conjunction with the above-mentioned sampling times.

Assessment of Cerebral Activity

Cortical SSEP in response to stimulation of the median nerve as well as continuous EEG were recorded using stainless steel electrodes inserted into the dura mater in each hemisphere.

SSEP and EEG were recorded at baseline and 30, 120, and 180 min AW from NRP (Figure 1) with recordings at baseline and AW120 being used for direct comparison between the groups. SSEP amplitude and latency were recorded with the first negative and the first positive peak being defined as N1 and P1. In the EEG recordings,

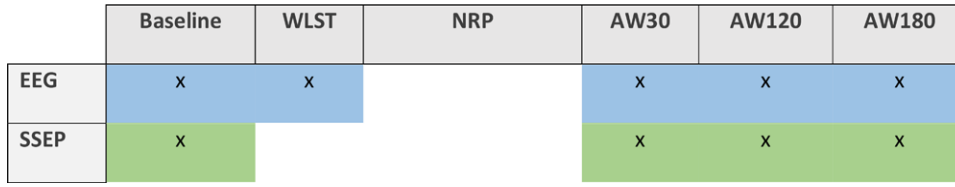

FIGURE 1. Overview of time points for acquisition of cortical electrical activity recordings. AW, after weaning; EEG, electroencephalography; NRP, normothermic regional perfusion; SSEP, somatosensory evoked potential; WLST, withdrawal of life-sustaining therapy.

10-s intervals determined to have minimal artifacts were used for power spectral analysis in the frequency range 0–50 Hz using the fast Fourier transform function of the NicoletOne EEG system (Natus Neuro, USA). Time until isoelectric EEG (ieEEG) from WLST (at least 30 s of ieEEG) was noted. Following recording of SSEP and EEG in the AW period, ventilation was paused briefly (60 s), and the animal was observed for spontaneous breathing effort. Dichotomous qualitative analysis was performed to assess whether brain activity was absent or present. The absence of brain activity was defined by continuous ieEEG (<2 μV) and no-SSEP determined by no reproducible potentials recorded. Conversely, the presence of cortical brain activity was defined by EEG activity (>2 μV) and reproducible SSEP response. All analyses were performed by a neurophysiologist (E.Q.) blinded to group randomization.

Circulatory Death and NRP

After sternotomy and exposure of the heart and major vessels, 40 000 units of heparin were administered to achieve systemic anticoagulation before central cannulation for the NRP circuit.

After recording of baseline parameters, the mechanical ventilation was disconnected, resulting in asphyxiation and circulatory arrest. Asystole was defined as MAP being equal to the central venous pressure after which an 8 min warm ischemic period was observed to mimic the most common NT period (5 min[8]) and a minimum estimated preparation time for NRP (3 min). Before reperfusion, the animals were randomized to either the clamp or nonclamp group. In the clamp group, the arch vessels were occluded, and in the nonclamp group, the arch vessels remained open. In both groups, an abdominal aortic balloon was inflated and pulled distally to the iliac bifurcation before reperfusion. Functional warm ischemic time was defined as the time from saturation of <70% until reperfusion.

Statistics

Normally distributed data are expressed as mean ± standard deviation; nonnormally distributed data are expressed as median and interquartile range. Histograms, Q–Q plots, and residual plots were used to check continuous values for normality. Between-group differences were assessed by *t* tests for normally distributed data and Mann-Whitney U tests for nonnormally distributed data. Repeated measures analysis of variance was used to test for significant difference in means over time. When some values were missing, data were statistically analyzed by a mixed-effects model. $P < 0.05$ was considered statistically significant. Statistical analysis and presentation of data were performed with Graphpad Prism 9.0.1 (GraphPad Software LCC, CA).

RESULTS

Protocol Feasibility and Hemodynamic Data

Sixteen animals were included and successfully resuscitated and weaned from NRP. Time from WLST to asystole was 8.63 ± 1.67 min in the clamp group and 8.88 ± 2.10 min in the nonclamp group ($P = 0.797$). Median functional warm ischemic time was 15.0 (14.5–18.0) min in the clamp group and 16.0 (14.0–16.0) min in the nonclamp group ($P = 0.586$). As per protocol, all animals were weaned from NRP after 30 min of NRP. Parameters, including Pco_2, Po_2, and MAP, were within set limits (Table 1).

TABLE 1.
Physiological parameters

		Baseline	AW30	AW120	AW180
Pco_2 (kPa)	Clamp	5.8 (5.6–6.4)	5.3 (5.2–5.5)	5.2 (4.8–6.0)	5.1 (4.5–5.3)
	Nonclamp	5.8 (5.4–6.5)	5.9 (5.6–6.2)	6.1 (5.7–7.1)	6.6 (6.3–6.9)
Po_2 (kPa)	Clamp	11.5 (10.6–17.1)	13.9 (12.9–16.3)	15.7 (12.8–17.3)	14.2 (13.3–18.1)
	Nonclamp	13.2 (2.0–18.4)	15.1 (11.7–17.3)	13.6 (12.8–15.2)	14.7 (13.1–16.0)
MAP (mm Hg)	Clamp	100.5 (80.5–122.3)	80.5 (69.3–93.8)	95.0 (58.5–116.8)	62.0 (56:0–75.0)
	Nonclamp	79.5 (67.5–107.8)	91.5 (67.8–103.8)	83.5 (67.0–93.3)	80.0 (68.3–89.0)
Heart rate (bpm)	Clamp	66 (54–94)	142 (123–154)	154 (129–191)	150 (144–155)
	Nonclamp	70.0 (61.3–102)	136 (110–140)	115 (108–124)	124 (119–131)
Lactate (mmol/L)	Clamp	2.4 (1.3–2.7)	9.9 (8.2–10.1)	9.4 (6.0–11.1)	8.6 (5.3–10.2)
	Nonclamp	2.3 (0.9–4.3)	9.4 (7.5–12.1)	8.8 (5.7–10.0)	6.9 (4.4–9.2)
Temperature (°C)	Clamp	38.5 (38.0–39.0)	39.1 (38.7–39.3)	39.7 (39.3–40.1)	39.9 (39.4–40.9)
	Nonclamp	38.4 (38.1–38.4)	38.1 (37.5–38.4)	38.6 (37.9–39.4)	39.2 (37.8–39.8)

Data are expressed as median (IQR).
AW, after weaning; IQR, interquartile range; MAP, mean arterial pressure.

Anesthetics

In the clamp group, neither propofol nor fentanyl infusion was reestablished after resuscitation. In the nonclamp group, infusion of propofol and fentanyl was resumed in 6 out 8 pigs because of return of EEG activity and SSEPs. Median total cumulative propofol dose in the clamp group and the nonclamp group was 22.73 (20.88–25.24) mg/kg and 28.20 (23.09–32.11) mg/kg ($P = 0.032$). Median total cumulative fentanyl dose in the clamp group and the nonclamp group was 95 μg/kg (65–117.5) and 135 μg/kg (130–140) ($P = 0.004$).

Cerebral Perfusion

In 1 animal in the clamp group, the probe was not fixated, and data were excluded. Compared with clamp, laser Doppler measurements of CBF increased following resumption of circulation in the nonclamp group ($P = 0.027$) and returned to values comparable with those at baseline (Figure 2). Similarly, cerebral Po_2 measurements increased following resuscitation compared with clamp ($P = 0.004$) and remained elevated throughout the AW period. Compared with nonclamp, temperature decreased in the clamp group in the AW period ($P = 0.016$). Compared with clamp, ICP was elevated following resuscitation and wean from NRP ($P = 0.032$).

Cerebral Activity

At baseline, SSEP recordings showed a median N1–P1 amplitude of 15.90 μV (6.325–26.50) in the clamp group and 12.35 μV (8.625–39.03) in the nonclamp group ($P = 0.950$). Median N1 latency for the clamp and nonclamp groups was 12.10 ms (11.85–15.20) and 13.50 (12.88–18.30) ($P = 0.081$), respectively. At baseline, raw EEG recordings showed continuous EEG activity with median peak frequency of 2.20 (1.28–4.50) Hz in the clamp group and 2.1 (1.33–3.70) Hz in the nonclamp group ($P = 0.82$). Median power for the clamp and nonclamp groups was 1207 μV^2 (670–10 472) and 1814 μV^2 (315–3560) ($P = 0.96$), respectively. Following WLST, median time to ieEEG was 120 s (77.5–120) for the clamp group and 96 s (77.5–117.8) for the nonclamp group ($P = 0.859$).

In the clamp group, no cortical electrical activity returned at any time following resuscitation, weaning from NRP and 3 h of observation (Figure 3). In the nonclamp group, reproducible SSEP response was observed in the AW period in 6 out of 8 animals with median N1–P1 amplitude of 2.65 μV (0.9350–9.775) and median N1 latency of 12.20 ms (11.35–15.73). EEG activity returned in all nonclamp animals with a median peak frequency of 2.35 Hz (2.00–3.70) and power of 1.05 μV^2 (0.50–19.38). Furthermore, EEG recordings contained a peculiar activity of periodic 4–5 Hz complexes, amplitude up to 300 μV, with a repetition interval of 1 in every 8–60 s. Agonal respiration (gasping) was observed in 6 out of 8 animals during brief pauses of ventilation in the nonclamp group, whereas no such movements were observed in the clamp group.

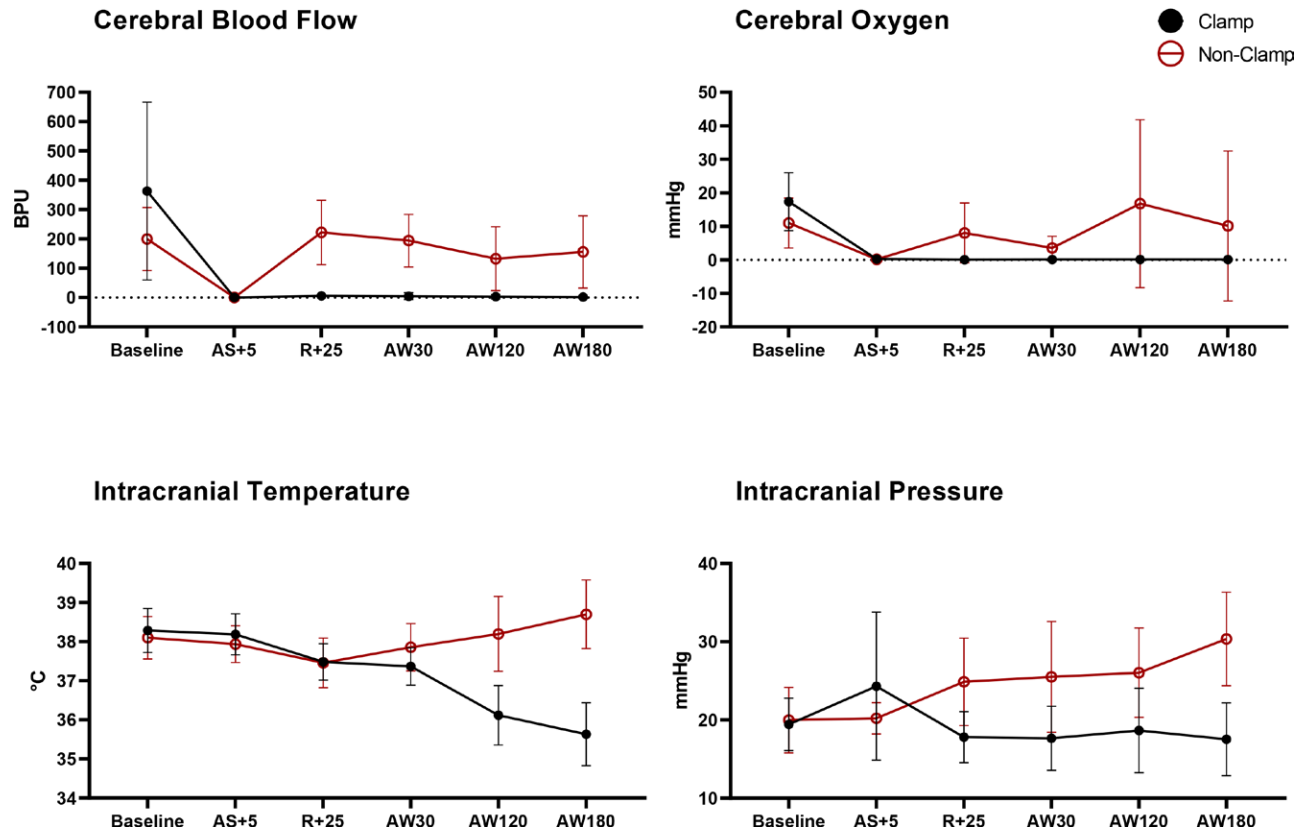

FIGURE 2. Cerebral perfusion. Flow (presented as BPU), oxygen partial pressure, temperature, and ICP as functions of set time points during the experiment. The BPU value at AS + 5 was defined as biological zero and subtracted from all other values. Values are mean 95% CI. AW, after weaning; BPU, blood perfusion unit; CI, confidence interval; ICP, intracranial pressure.

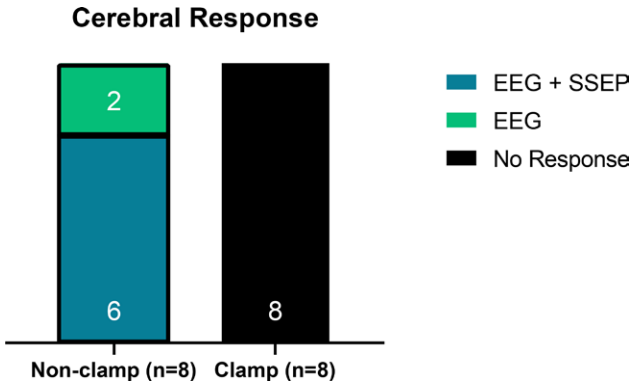

FIGURE 3. Cortical electrical activity determined by EEG and SSEP recorded after resuscitation with normothermic regional perfusion. N = 6 for SSEP in the clamp group. EEG, electroencephalography; SSEP, somatosensory evoked potential.

DISCUSSION

In the present study, we described a novel porcine protocol for the evaluation of donor cerebral perfusion and activity in the context of TA-NRP in DCD. When circulation was restored in the nonclamp group, CBF by laser Doppler and intracranial Po_2 resumed to levels comparable with baseline. Conversely, values did not rise beyond zero levels in the clamp group. ICP increased throughout the experiment when circulation was allowed compared with clamp. EEG activity was observed AW in all animals in the nonclamp group, with most (n = 6) also having reproducible SSEP, whereas ieEEG and no-SSEP response were found in the clamp group. Agonal breathing was observed during brief pauses of ventilation in 6 out of 8 animals in the nonclamp group.

Several drugs, including propofol and fentanyl, have been shown to confound the diagnosis of brain death because of suppression of the EEG.[23] Because of resumption of anesthetics in the nonclamp group, the total dosage of fentanyl and propofol was higher compared with clamp, especially for fentanyl. As such, ieEEG in the clamp group cannot be explained by drug-induced burst suppression. Additionally, no burst suppression was observed on EEG at baseline or AW in the nonclamp group, and physiological parameters were comparable between groups throughout the experiment (Table 1). As per protocol, administration of anesthetics AW in the nonclamp group was an ethical necessity.

Parenchymal cerebral measurements of CBF, Po_2, and temperature indicated cessation of cerebral circulation in the clamp group. Conversely, restoration of circulation in the nonclamp group was indicated by a return of CBF and Po_2 values to levels comparable with baseline. Additionally, ICP increased AW in the nonclamp group, presumably because of the presence of cerebral perfusion pressure and edema following anoxic brain injury. However, probe placement in the subcortical parenchyma cannot perfectly represent whole-brain perfusion. Indeed, other researchers have used similar equipment for the assessment of cerebral oxygenation but supplemented quantification of cerebral perfusion with magnetic resonance imaging.[24] As such, perfusion data and functional brain tests in combination provide a more complete overview of brain physiology.

We observed EEG activity, agonal breathing, and presence of SSEP response when cerebral reperfusion was allowed, indicating resumption of cortical and brain stem function. However, EEG was abnormal with very low power and the SSEP amplitude was markedly reduced. EEG and SSEP are widely accepted electrophysiological tools in the evaluation of brain function and are used to predict neurological outcomes following cardiac arrest.[25,26] Isoelectric EEG and the absence of bilateral N20 peaks (analogous to the N1 peak in the present study) predict poor neurological outcomes early and accurately.[25]

Clinical Implications

Adherence to the dead donor rule is the core principle of organ donation,[27,28] and the determination of death must be undisputable. Following the decision to WLST in DCD, the permanent cessation of cardiorespiratory function will inevitably progress into irreversible cessation of brain function. As such, permanence acts as a surrogate for irreversibility allowing for a timelier declaration of death.[20]

Shemie et al,[29] in collaboration with the World Health Organization, suggested the following definition of human death: "*The permanent loss of capacity for consciousness and all brain stem functions, as a consequence of permanent cessation of circulation or catastrophic brain injury.*" Permanence in the context of DCD, as in everyday clinical practice, depends on 2 principles[28]: (1) the period in which autoresuscitation is possible has passed, and (2) resuscitation will not be attempted following the decision to WLST. In this study, the period of asystole was based upon the most common NT period in countries using DCD, 5 min.[8] Recently, the validity of this period was demonstrated in a prospective study of autoresuscitation where a maximum duration of 4 min and 26 s from WLST to a transient resumption of at least 1 cardiac cycle after pulselessness was found.[30]

Following the permanent cessation of circulation, restoration of circulation to the brain during in vivo reperfusion through NRP would retroactively invalidate the determination of death. As such, no brain circulation may resume after the determination of death.[11-13]

During TA-NRP, the aortic arch vessels are clamped to preclude cerebral circulation. However, collateral circulation between the descending aorta and intercostal arteries to the spinal arteries remains possible. Indeed, drainage of the aortic arch to atmospheric or negative pressure has been proposed to prevent any collateral flow.[19] In the present study, however, neurophysiological assessment of brain function indicated total cessation of cerebral function during TA-NRP without these precautions. Currently, Spanish protocols of TA-NRP in DCD include frontal lobe assessment using bispectral index[7]; however, our results suggest that cerebral monitoring during NRP is redundant when the aortic vessels are clamped. This is particularly true if the proposed methods of circumventing collateral flow are used.

Limitations

As is often the case in large-animal preclinical models, our sample size was small, and our observation period was limited to a few hours. Nevertheless, we were able to produce relevant and consistent results. A more comprehensive overview of the effects of cerebral reperfusion following 8 min of asystole could have been achieved with a longer observation period. However, the focus of our study was to investigate the effects of cerebral reperfusion in donors

after death has been determined by circulatory criteria. As such, any return of brain activity in the short term then seems adequate to demonstrate the need for clamping.

The experiments were performed in a porcine model and clinical translation should be done with caution. Dissimilarities between model and clinical reality include interspecies differences and comorbidities in candidates for cDCD in the intensive care unit. Indeed, we included healthy pigs without preexisting brain damage and therefore did not represent the clinical status of cDCD donors. However, any result suggesting reanimation of brain function during NRP seems adequate to make inferences about the necessity of clamping. Additionally, the porcine brain, heart, and circulatory system are anatomically and physiologically comparable with humans.[31-33]

The number of intracranial monitoring devices was limited, and as such, regional differences in perfusion and electrical activity could have been overlooked. For example, the fiberoptic probe relies on the local tissue environment in the parenchyma that could give rise to interindividual variations in perfusion measurements because of local flow fluctuations. Nevertheless, we were able to demonstrate a clear difference in perfusion between the groups. Additionally, the small size of the porcine brain and the transcranial implantation of electrodes increase the robustness of the neurophysiological recordings.

CONCLUSION

In a porcine model of DCD, reperfusion of the brain during NRP led to a return of brain activity with the presence of EEG, SSEP response, and agonal breathing. Conversely, clamping of the arch vessels halted cerebral circulation and function indicated by the absence of cerebral perfusion parameters, ieEEG and no-SSEPs. These results suggest that clamping is necessary to avoid inadvertently restoring cerebral circulation during NRP. Conversely, clamping of the aortic arch vessels halted cerebral circulation ensuring the permanent loss of capacity for consciousness and all brain stem functions, thus maintaining the determination of death in DCD.

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