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ORIGINAL ARTICLE

A 10 min ''no-touch'' time – is it enough in DCD? A DCD Animal Study

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Summary

Donation after cardiac death (DCD) is under investigation because of the lack of human donor organs. Required times of cardiac arrest vary between 75 s and 27 min until the declaration of the patients' death worldwide. The aim of this study was to investigate brain death in pigs after different times of cardiac arrest with subsequent cardiopulmonary resuscitation (CPR) as a DCD paradigm. DCD was simulated in 20 pigs after direct electrical induction of ventricular fibrillation. The ''no-touch'' time varied from 2 min up to 10 min; then 30 min of CPR were performed. Brain death was determined by established clinical and electrophysiological criteria. In all animals with cardiac arrest of at least 6 min, a persistent loss of brainstem reflexes and no reappearance of bioelectric brain activity occurred. Reappearance of EEG activity was found until 4.5 min of cardiac arrest and subsequent CPR. Brainstem reflexes were detectable until 5 min of cardiac arrest and subsequent CPR. According to our experiments, the suggestion of 10 min of cardiac arrest being equivalent to brain death exceeds the minimum time after which clinical and electrophysiological criteria of brain death are fulfilled. Therefore shorter ''no-touch'' times might be ethically acceptable to reduce warm ischemia time.

Introduction

In order to meet the demands of increasing waiting lists, transplant programs have expanded the range of acceptable donor organs [1,2]. Generally, organ procurement is only permitted when the donor is already dead and the so-called dead donor rule (DDR) is respected, which states that vital organs can only be taken from dead patients and, correlatively, living patients must not be killed by organ retrieval [3–5]. Brain dead donors currently comprise the major part of the organ pool; upon determining irreversible loss of brain function, the patient is declared legally dead and organ donation can be performed [6].

In contrast, organ donation after cardiac death (DCD) is defined as the surgical recovery of organs after the declaration of death based on cessation of cardiopulmonary function [7–9]. While it was the initial form of organ donation prior to the definition of brain death criteria, DCD was excelled by donation from brain dead patients because of improved graft and recipient outcomes resulting from the shorter time of warm ischemia [10].

However, DCD is prohibited by law in some countries such as Germany [11,12]. DCD is considered to be a promising way to increase the number of organs available for transplantation [1,2,13–16]. However, DCD evokes a number of ethical issues that have to be solved in order to be accepted by the society [17,18]. For organ transplantation to be successful, the arrest of circulation and resulting warm ischemic injury must be minimized [20,21]. This time pressure forced the identification of a precise waiting period, which is long enough to ensure the person has died in order to fulfill the DDR but short enough to maintain organ viability for transplantation [8,20].

Several DCD protocols are currently used in different countries. As there exist no common guidelines for ''no-touch'' times which even vary within some countries ranging from 2 to 10 min, recently published data of a survey on DCD activities of some European countries [9] as well as the United States of America and Australia are compiled in Table 1. The British Transplant Society claims an interval of 5 min ''hands-off'' [22] the Maastricht experience 5 min [23] and the Pittsburgh Protocol allows death to be declared 2 min after loss of cardiopulmonary function; [17,24,25] the shortest ''no touch'' time reported in literature was 75 s [26]. However, there is evidence that the time required for irreversible loss of brain function after cessation of circulation is longer than 5 min of cardiorespiratory arrest [27–30]. Coronary and cerebral reperfusion after cardiac arrest can lead to the return of cardiac and brain function during the procurement process hereby not respecting the DDR [4,31,32].

Table 1. Results of a recently published survey on different ''no-touch'' times for some European countries reporting DCD activity [1,16] as well as Australia [59], Canada [60] and the United States of America [61–63] where recommendations for ''no-touch'' times vary between 2 and 10 min. However, there are no guidelines currently available for European countries and therefore ''no-touch'' times vary within countries ranging from 2 to 10 min.

Country	''no-touch'' period (min)
Austria	10
Australia	2
Belgium	5
Canada	5
Czech Republic	10
France	5
Italy	20
Latvia	15
The Netherlands	5
Spain	5
Switzerland	10
United Kingdom	5
United States of America	2–10

Therefore, it has been claimed to wait until the patient fulfils the brain death criteria prior to organ donation [10]. Taking in consideration the wide range of ''no-touch'' times, international guidelines for DCD would be desirable.

The aim of this study was to determine brain death by clinical and electrophysiological criteria after cardiac arrest with varying ''no-touch'' times and subsequent cardiopulmonary resuscitation (CPR) in an animal model in order to explore the scientific basis of the wide range of differing ''no-touch'' times.

Materials and methods

Donation after Cardiac Death (DCD) Model

Animals

Twenty pigs (German Large White, with a body weight of 35 ± 3.2 kg) were used and the experimental design (Fig. 1) was approved by the Ethical Committee for Animal Studies of the University of Veterinary Medicine Vienna and the Austrian Federal Ministry for Science and Research (GZ: BMWF 66.010/0053-II/10b/2009). All experiments were performed in accordance with European and Austrian laws on animal experimentation and the principles stated in the ''Guide for the Care and Use of Laboratory Animals'' published by the National Institute of Health [33]. Pigs were judged to be healthy on the basis of physical examination and were acclimatized at the Medical University of Graz (Biomedical Research) for at least two weeks before each trial. The animals were housed in groups of two to four in solid floor pens on straw bedding and were allowed free access to drinking water and a standard pig diet (PorkoCidKorn F, Garant, Graz, Austria). Environmental temperature was held at 20–26 °C at ambient humidity. Lighting was both natural and artificial with a 12-h on and off cycle (06:00–18:00 h).

Anesthesia

Preanesthetic medication was intramuscular 0.4 mg/kg midazolam (Midazolam ''ERWO'' 5 mg/ml; ERWO Pharma GmbH, Brunn am Gebirge, Austria), 2 mg/kg azaperone (Stresnil® 40 mg/ml; Janssen Pharmaceutica NV, Belgium) and 14 mg/kg ketamine (Ketasol® 10%; Gräub AG, Berne, Switzerland) injected in one syringe 20 minutes before induction of anesthesia with intravenous (IV) 3 mg/kg propofol (Propofol Fresenius 1%; Fresenius Kabi Austria GmbH, Graz, Austria) via an IV cannula placed in the marginal auricular vein. After endotracheal intubation and connection to a circle system (Sulla 808V anesthetic machine with Ventilog; oxygen flow rate 2 l/min, air flow rate 0.5 l/min), pigs were mechanically ventilated. Standardized ventilator settings

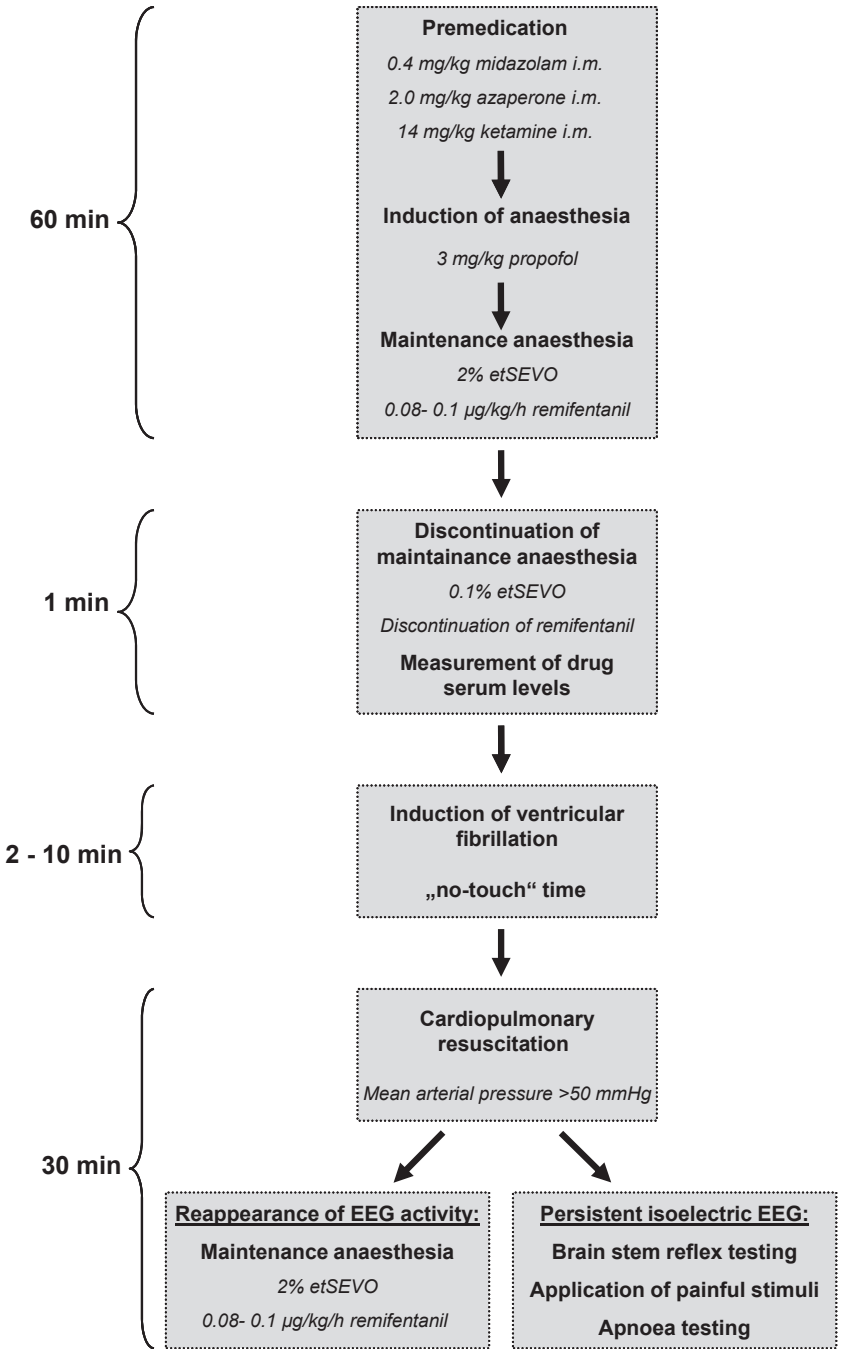

Figure 1 Design of the DCD paradigm in pigs in this study. After premedication and induction of anesthesia, maintenance anesthesia was performed using 2% etSEVO and a remifentanil. 60 min after premedication the animals were randomized into different groups differing in the ''no-touch'' time ranging from 2 to 10 min. Ventricular fibrillation was induced using a 9-V direct current. An isoelectric EEG was detected within the first minute without cardiac output. Then, dependent on the ''no-touch'' time CPR was performed for 30 min according to the standard guidelines achieving a MAP of 50 mmHg. Maintenance anesthesia was reintroduced immediately in all animals where a reappearance of brain bioelectric activity was detected. In animals without brain bioelectric activity, anesthesia remained discontinued and brain death diagnostics including brain stem reflex testing and apnea testing was performed after a total time of CPR of 30 min. Then, all animals were sacrificed and multiorgan donation was performed as described elsewhere. DCD, donation after cardiac death; EEG, electroencephalogram; CPR, cardiopulmonary resuscitation; MAP, mean arterial pressure.

for intermittent positive pressure ventilation (IPPV) were used to maintain eucapnia ($etCO_2$ 35–45 mmHg; tidal volume 8–10 ml/kg; respiratory rate 15–20 breaths/min; volume controlled ventilation mode; positive end-expiratory pressure (PEEP) 2–4 cm H_2O). General anesthesia was maintained with 2% end-tidal sevoflurane (etSEVO) (Sevorane® Abbott Ges.m.b.H, Vienna, Austria) and a continuous rate infusion (CRI) of 0.08–0.1 µg/kg/h remifentanil (Ultiva® 2g; GlaxoSmithKline Pharma GmbH, Vienna, Austria). ELO-MEL isoton solution (Fresenius Kabi Austria GmbH, Graz, Austria) was infused continuously at 10 ml/kg/h IV (Heska Vet-IV Infusion Pump; Heska USA Corporation, Fort Collins, CO, USA). The animals were placed on a heating blanket to maintain normothermia (37.0–39.5 °C). Body temperature was measured continuously using a rectal thermometer. Pulse oximetry was performed at the tail and electrocardiogram (ECG) monitoring was used to observe cardiac function.

Table 2. The ''no-touch'' time (indicated in minutes) and time to isoelectric electroencephalogram (EEG) from the beginning of ventricular fibrillation in each of the animals (indicated in seconds). When no EEG activity reappeared throughout 30 min of continuous recording, brainstem reflexes were tested and painful stimuli applied (n.a. – not applicable). Brain death was confirmed by apnea testing when no EEG activity reappeared and no brainstem reflexes and reaction to painful stimuli were found. Animals in which spontaneous circulation (SC) after CPR reoccurred can be distinguished among the animals which underwent CPR throughout the whole experiment.

Animal	''No-touch'' time (min)	Spontaneous circulation/CPR	Time to isoelectric EEG (s)	Reappearance of EEG activity	Brainstem reflexes	Brain death
DCD IV	2	SC	40	Yes	n.a.	No
DCD VIII	4	SC	22	No	Yes	No
DCD XVI	4	SC	35	No	Yes	No
DCD XVII	4	SC	35	Yes	n.a.	No
DCD XIII	4.5	SC	28	Yes	n.a.	No
DCD XIV	4.5	CPR	32	No	Yes	No
DCD XV	4.5	CPR	22	No	No	Yes
DCD IX	5	CPR	22	No	No	Yes
DCD X	5	CPR	34	No	Yes	No
DCD XI	5	SC	32	No	Yes	No
DCD XII	5	SC	24	No	Yes	No
DCD V	6	CPR	74	No	No	Yes
DCD VI	6	SC	35	No	No	Yes
DCD VII	6	SC	74	No	No	Yes
DCD I	9	SC	24	No	No	Yes
DCD XVIII	9	CPR	27	No	No	Yes
DCD II	10	CPR	46	No	No	Yes
DCD III	10	SC	27	No	No	Yes
DCD XIX	10	CPR	54	No	No	Yes
DCD XX	10	CPR	32	No	No	Yes

DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation.

Arterial blood pressure was measured invasively via a cannula placed in the left femoral artery. A central venous catheter was placed into the left jugular vein to monitor central venous pressure and to have a second venous access for additional drug and infusion therapy. Throughout the experiments arterial blood gas checks were performed in the routine laboratory; according to these results, animals were treated following standard anesthesiologic guidelines [34]. Instrumentation and stabilization phase was finished approximately 60 min after premedication; then, the animals were randomized into experimental groups differing in the ''no-touch'' time as compiled in Table 2. For randomization, sealed envelopes containing the treatment assignments were drawn out of a bowl prior to the induction of ventricular fibrillation for each animal.

Surgical procedure

A subcostal thoracotomy was performed and the pericardium was opened. Maintenance anesthesia was discontinued one minute before ventricular fibrillation was induced by a 9-V direct current. The etSEVO levels were monitored until a decrease to 0.1%; then, mechanical ventilation was stopped. Pigs underwent a ''no-touch'' period ranging from 2 to 10 min (Table 2). After the defined ''no-touch'' period, mechanical and medical resuscitation (CPR) was performed for 30 min according to standard guidelines [35,36]. The aim was to achieve sufficient cardiac output (mean arterial pressure (MAP) 50 mmHg) to enable brain perfusion. When cardiac activity reoccurred during CPR, animals were treated by the anesthesiologist according to standard guidelines for a total time of 30 min [34]. Arterial blood pressure, oxygenation, ECG, body temperature, capnometry, blood glucose and central venous pressure were monitored continuously. Pigs were kept normothermic (37.0–39.5 °C) throughout the experiment and intensive care medication was provided when indicated in order to avoid acidosis, keep electrolytes within normal limits and avoid any metabolic disturbances which would impact on neurologic examinations.

EEG monitoring and brain death diagnostics

Electroencephalograms (EEGs) were obtained by a clinical EEG system (alpha-trace, Vienna, Austria) with needle electrodes positioned as described previously [37]. Briefly, a pair of electrodes (FP1, FP2) was inserted 2.0 cm in front of a reference line connecting both medial eye

borders and 1.0 cm left and right of the midline. Another electrode pair (F7, F7) was positioned 1.0 cm behind the reference line and 3.0 cm left and right of the midline. A third (T7, T8) and fourth (P7, P8) pair of electrodes were inserted 4.0 cm to the left and right of the midline and 2.5 cm and 4.0 cm behind the reference line, respectively. A common average montage was used and ECGs were co-registered. Baseline EEGs were obtained under general anesthesia. EEG recordings were continued throughout interruption of anesthesia, induction of ventricular fibrillation, the ''no-touch'' time, and CPR. With maximum signal amplification, the disappearance of EEG activity (isoelectric EEG) and the eventual reappearance of brain bioelectric activity were noted. With the appearance of an isoelectric EEG, recordings were continued for at least 30 min. When no bioelectric activity reappeared during this time, brainstem reflexes were tested and painful stimuli were applied. In animals with a loss of brainstem reflexes and a lack of reaction to painful stimuli, apnea testing was performed. Animals were disconnected from the ventilator until a pCO_2 >60 mmHg was recorded using arterial blood gas analysis as described above. A lack of spontaneous respiration was regarded confirmatory for brain death [38].

Biochemistry

Blood samples were taken after induction of anesthesia, prior to the induction of ventricular fibrillation, after the ''no-touch'' time as well as after 30 min of CPR. Blood gas analysis was performed every 5 min during CPR. Full blood count, electrolytes, renal and liver function tests were immediately analyzed in the central laboratory. Serum was stored at −80 °C for batch analysis of midazolam with a reversed phase HPLC method [39,40]. The within-day coefficients of variation (CVs) for midazolam were 2.0% and 1.1%, the between-day CVs were 7% and 5.7% at 40 and 200 ng/ml, respectively. According to current national brain death diagnosis guidelines fully reflecting American Academy of Neurology (AAN) practice parameters, midazolam levels have to be below 50 ng/ml in order to fulfill brain dead criteria [38,41,42].

Results

CPR and vital parameters

All animals were declared healthy and did not differ significantly in terms of blood pressure, heart rate, temperature as well as routine laboratory values prior to the experiments (Table 3). Vital parameters were within normal limits; mean arterial blood pressure (MAP) was 73 ± 15 mmHg, mean heart rate (HR) 79 ± 20 beats per minute (bpm) and mean temperature was 39.1 ± 0.3 °C prior to the induction of cardiac fibrillation.

CPR was performed successfully for the whole experimental period according to the standard guidelines in all 20 animals. Eleven animals regained spontaneous circulation (SC) after CPR, whereas nine animals had to be resuscitated throughout the experimental period.

After the different ''no-touch'' times and 5 min of CPR, MAP was 60 ± 43 mmHg, HR 117 ± 45 bpm and the mean temperature was 39.0 ± 0.3 °C. Then, 10 min after the beginning of CPR, pigs showed a MAP of 61 ± 41 mmHg, a HR of 113 ± 41 bpm as well as a body temperature of 38.9 ± 0.23 °C; MAP was 54 ± 36 mmHg, HR 106 ± 32 bpm and mean body temperature 39 ± 0.23 °C after 20 min of CPR respectively. Prior to sacrification, 30 min after the different ''no-touch'' times, animals showed a MAP of 46 ± 16 mmHg, a HR of 104 ± 37 bpm as well as a mean body temperature of 38.8 ± 0.36 °C. Detailed values for vital parameters of all animals during CPR are compiled in Table 4.

Serum levels of midazolam

Mean midazolam levels prior to induction of ventricular fibrillation are 33 ± 10.1 ng/ml ranging from values below 20 ng/ml up to 46 ng/ml. In all animals, midazolam levels were below the threshold of 50 ng/ml. Therefore midazolam levels did not interfere with brain death diagnosis according to our national guidelines fully reflecting AAN practice parameters (Table 2) [38,41,42].

EEG monitoring and brain death diagnostics

Brain death diagnostic criteria are given in Table 3 for all animals. An isoelectric EEG appeared after a mean of 36.0 s (range 22–74 s) following induction of ventricular fibrillation. One animal (DCD IV) underwent a 2-min ''no-touch'' time. During 30 min of CPR, this animal showed reappearance of EEG activity (Fig 2a–c). Among the animals with 4 min ''no-touch'' time ($n = 3$), two did not show reappearance of EEG activity but brainstem reflexes after 30 min of CPR. With 4.5 min of ''no-touch'' time ($n = 3$), one animal did not show reappearance of EEG activity and brainstem reflexes were absent. In the group which underwent 5 min of ''no-touch'' time ($n = 4$), one animal showed neither reappearance of EEG activity nor brainstem reflexes. With longer ''no-touch'' times, 6 min ($n = 3$), 9 min ($n = 2$) and 10 min ($n = 4$), respectively, all animals neither showed reappearance of bioelectric brain activity nor brainstem reflexes (Fig. 2d–f). In all animals with 30 min of isoelectric EEG, loss of brainstem reflexes and lack of reaction to painful stimuli, apnea testing confirmed brain death.

Table 3. Baseline data of the animals used in these experiments.

Animal	MAP (mmHg)	HR (beats/min)	Temperature (°C)	Leuco (G/L)	Ery (G/L)	Hb (g/dl)	Na+ (mmol/l)	K+ (mmol/l)	Crea (mg/dl)	BUN (mg/dl)	tot. Bili (mg/dl)	AP (U/l)	gGT (Ul)	AST (U/l)	ALT (U/l)	LDH (U/l)	Midazolam (ng/ml)
DCD I	65	52	38.7	13.49	5.58	9.3	138	3.9	1.11	11	0.11	108	67	22	38	561	39
DCD II	63	48	39.2	27.4	4.99	8.2	141	3.7	1.57	20	0.07	104	76	13	43	484	26
DCD III	68	75	39.8	22.77	5.19	9	139	3.7	1.08	11	0.1	91	14	23	31	499	< 20
DCD IV	109	120	39.2	15.83	5.6	9.9	140	4.3	1.18	10	0.09	92	54	29	43	611	22
DCD V	83	87	39.5	11.78	4.41	7.6	145	3.6	1.03	20	0.09	108	45	17	51	430	36
DCD VI	67	70	38.9	13.42	4.67	8.1	146	4	0.79	11	0.1	154	44	37	49	497	42
DCD VII	75	66	39	12.5	5.03	8.1	141	4.3	0.77	16	0.09	119	45	31	72	455	40
DCD VIII	105	104	39.5	21.21	5.87	9.6	144	3.8	1.84	13	0.13	76	65	18	30	389	26
DCD IX	75	78	39.3	12.94	6.02	10.8	143	3.8	1.16	12	0.27	126	32	32	51	528	42
DCD X	79	70	39.2	24.04	5.78	9.8	146	4.6	2.18	31	0.08	95	39	151	32	523	43
DCD XI	61	68	39.3	16.13	5.16	9	144	4.4	1.57	13	0.13	118	30	28	37	462	46
DCD XII	65	120	38.8	18.39	6.18	10.2	142	3.7	1.42	21	0.1	143	56	21	42	491	20
DCD XIII	79	74	39.2	12.56	5.91	10.2	144	4	1.4	20	0.04	113	69	33	55	650	44
DCD XIV	52	73	38.7	19.24	6.46	10.9	146	3.7	1.52	17	0.05	218	69	36	57	594	39
DCD XV	60	70	39.2	15.26	6.27	10.4	142	3.4	1.42	20	0.15	157	45	21	37	479	<20
DCD XVI	80	100	38.7	9.06	5.46	9.2	141	4	1.04	30	0.36	167	57	38	41	582	26
DCD XVII	85	97	39.3	15.93	5.07	8.6	140	4	1.41	23	0.09	82	32	29	45	502	<20
DCD XVIII	79	93	38.9	15.78	6.65	11.3	141	4.2	1.27	17	0.18	118	119	33	38	584	<20
DCD XIX	53	60	38.9	13.74	5.92	10.4	142	4.2	1.51	25	0.09	99	60	43	43	501	<20
DCD XX	75	68	39.2	17.63	6.2	9.8	144	3.5	1.2	17	0.09	89	65	22	39	470	<20

Normal values of the pigs used for these experiments are indicated in braces according to the routine laboratory of the University for Veterinary Medicine, Vienna.

DCD, donation after cardiac death; MAP, mean arterial blood pressure (mmHg) (60–70 mmHg in anesthesia); HR, heart rate (beats per minute/bpm) (60–90 bpm in anesthesia); temperature: (27,0–39,5 °C); Leuco, leucocytes (G/L) (11–22 G/L); Ery: erythrocytes (G/L) (5–8 G/L); Hb, hemoglobin (g/dl) [10–16 g/dl); Na^+: sodium (mmol/l) (135–150 mmol/L); K^+: potassium (mmol/L) (7.8–10.9 mmol/L); Crea, creatinine (mg/dl) (1–3 mg/dl); BUN: blood urea nitrogen (mg/dl) (8–24 mg/dl); tot. Bili., total Bilirubin (mg/dl) (0–0.7 mg/dl); AP: alkaline phosphatase (U/l) (9–20 U/l); gGT, gamma-glutamyl transferase (U/l) [10–27 U/l); AST, aspartate aminotransferase (U/l) (0–35 U/l); ALT, alanine-aminotransferase (U/l) (0–40 U/l); LDH, lactate dehydrogenase (U/l) [0–700 U/l); midazolam (ng/ml) [<50 ng/ml to allow brain death diagnostics].

Table 4. Vital parameters of all pigs are documented throughout the experiments. Mean arterial pressure (MAP), heart rate (HR) as well as temperature were documented 5 min, 10 min, 20 min as well as 30 min after the beginning of CPR after the different ''no-touch'' times. All animals lacked massive hypotensive periods interfering with brain death diagnosis throughout the experiments; Body temperature was kept normothermic (37.0–39.5 °C) during the whole experiment.

Animal	MAP 5 min CPR	MAP 10 min CPR	MAP 20 min CPR	MAP 30 min CPR	HR 5 min CPR	HR 10 min CPR	HR 20 min CPR	HR 30 min CPR	Temp 5 min CPR	Temp 10 min CPR	Temp 20 min CPR	Temp 30 min CPR
DCD I	37	40	44	48	112	127	128	137	38.3	38.2	38.2	38
DCD II	30	35	34	40	86	79	60	62	39.1	38.9	39.2	38.4
DCD III	32	44	38	37	180	178	164	170	39.5	39.2	39.1	38.8
DCD IV	86	65	54	67	120	140	120	130	39.2	39.1	39.2	39.2
DCD V	50	38	45	43	200	90	88	70	39.3	39.2	39.3	39
DCD VI	56	63	65	60	150	160	100	98	38.9	38.8	39	38.9
DCD VII	50	45	47	46	82	78	79	83	38.7	38.8	38.9	38.7
DCD VIII	50	55	40	48	45	122	91	95	39.3	39.2	39.3	39.4
DCD IX	38	35	32	31	83	81	78	68	39.2	39.2	39.1	38.9
DCD X	32	30	44	39	90	78	107	130	39.2	39.1	39.2	39
DCD XI	122	153	138	70	170	136	157	143	39.2	39.2	39.3	39.5
DCD XII	157	72	44	46	139	140	150	155	38.6	38.6	38.5	38.6
DCD XII	40	61	56	47	140	120	120	102	39.1	39	39.2	39.1
DCD XIV	37	37	37	36	88	82	83	65	38.6	38.7	38.5	38.5
DCD XV	26	39	35	32	72	75	80	77	39.1	39.2	39.1	39.1
DCD XVI	170	163	63	46	200	180	140	160	39	38.9	39	38.7
DCD XVII	91	140	170	96	130	184	140	133	39.2	39.3	39.2	39.1
DCD XVIII	29	36	34	37	87	93	90	88	38.7	38.6	38.6	38.5
DCD XIX	34	34	37	29	82	50	81	52	38.7	38.7	38.8	38.7
DCD XX	38	38	32	39	81	71	65	67	39.1	39.2	39.2	39

DCD, donation after circulatory death; CPR, cardiopulmonary resuscitation.

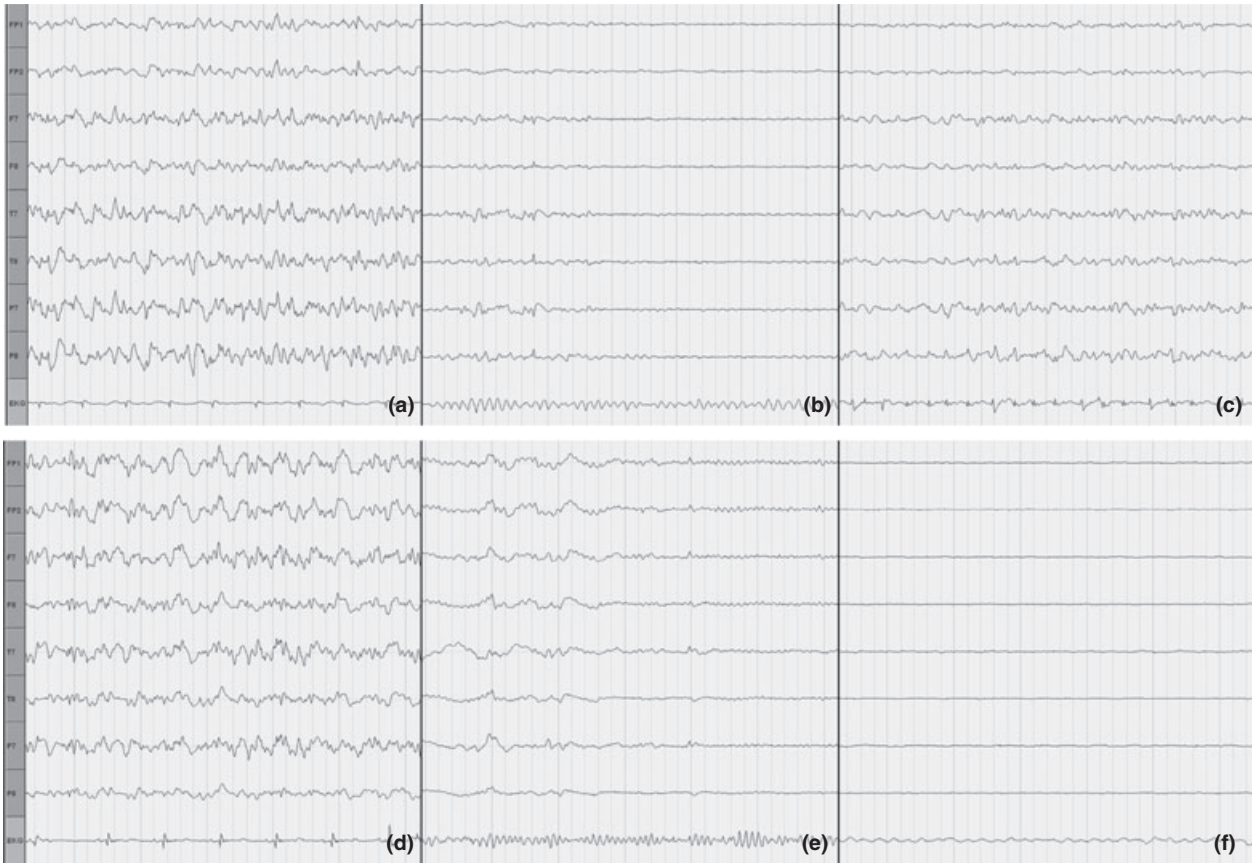

Figure 2 Electroencephalogram (EEG) recording in animal DCD IV 1 min before the induction of ventricular fibrillation (a) and at the beginning of isoelectric EEG at 40 s of cardiac arrest (b). Following 2 min of ''no-touch'' time and subsequent cardiopulmonary resuscitation (CRP), EEG activity reappeared as shown here at 3 min of CPR (c). EEG recording in animal DCD V 1 min before the induction of ventricular fibrillation (d) and at the beginning of isoelectric EEG at 74 s of cardiac arrest (e). Following 6 min of ''no-touch'' time and subsequent CPR, no reappearance of EEG activity was found throughout 30 min of continuous recording (f). See *Materials and Methods* for electrode positions (FP1–P8). Electrocardiogram (ECG) is co-registered. Minimum time interval on *x*-axis is 0.2 s. DCD, donation after cardiac death.

Discussion

DCD is increasingly recognized for organ donation, [16,43] but still discussed controversially. Moreover, DCD is prohibited by law in Germany, the largest country of EUROTRANSPLANT and some other European countries [11,12,16]. Moreover, international guidelines are missing what is reflected by the wide range of ''no-touch'' times currently used in the different countries practicing DCD [40]. This might be because of the lack of exact data on the required ''no-touch'' time as well as ethical and legal issues which have to be taken in consideration [17,19].

In the previous literature, several animal models are described focusing on different DCD protocols. However, none of these studies has included brain death diagnosis [44–46].

Therefore, the aim of this study was to determine brain death by clinical and electrophysiological criteria after cardiac arrest with varying ''no-touch'' times and subsequent cardiopulmonary resuscitation (CPR) in a large animal study to examine the scientific basis of different ''no-touch'' times.

Several DCD protocols [9,16,19,43,47] are currently used in different countries including the protocol of The British Transplant Society (5 min ''hands-off'') [22] the Maastricht experience (5 min ''no-touch'' time) [23] and the Pittsburgh Protocol (2 min ''no-touch'' time). As there exist no common guidelines and the ''no-touch'' times even vary within countries, recently published data on DCD programs are compiled in Table 1 [17,24,25]. The shortest ''no touch'' time reported in literature was 75 s [26]. However, there is still a lack of knowledge about reappearance of bioelectric brain activity when declaring a patient dead according to DCD criteria. There is evidence that the time required for irreversible loss of brain function

after cessation of circulation is longer than 5 min of cardiorespiratory arrest [27–30].

Among the four animals with cardiac arrest for 2 and 4 min, respectively, two pigs showed no reappearance of bioelectric brain activity but brain stem reflexes. Therefore, these animals could not be declared brain dead. In animals with a ''no-touch'' time of 4.5 min, only one pig could be declared brain dead because of an isoelectric EEG as well as the loss of brainstem reflexes and confirmatory apnea testing. Moreover, 5 min of ventricular fibrillation without CPR led to an isoelectric EEG in all animals. However, only one of these animals showed a loss of brainstem reflexes and a lack of reaction to painful stimuli and was declared brain dead upon apnea testing. Animals which suffered from ''no-touch'' times of 6 min or more followed by CPR could be declared brain dead after 30 min of CPR.

According to our study, DCD organ donors according to the Pittsburgh Protocol [17,25] supposedly do not fulfill clinical and electrophysiological criteria of brain death at the time of donation. Regarding the recommendations of the British Transplant Society (5 minutes ''hands-off'') [22], we found that in pigs which suffered from ventricular fibrillation without cardiac output for 5 minutes, all pigs showed an isoelectric EEG for at least 30 min. However, only one of these was declared brain dead upon the loss of brainstem reflexes the lack of reaction to painful stimuli, and confirmatory apnea testing. All animals which were treated 10 min ''no-touch'' time, as suggested by Kootstra and Jacobs as equivalent to brain death [48] fulfilled clinical and electrophysiological brain death criteria.

Since the warm ischemia time should also be as short as possible to avoid hypoxic damage of the organs that should be transplanted, the ideal ''no-touch'' time in our model seems to be somewhere between 5 and 10 min. As humans suffering from cardiac death are usually not completely healthy at the outset and cardiac output also does not cease immediately but they rather suffer from a prolonged period of low cardiac output before cardiac arrest, it can be hypothesized that in humans the duration until brain death occurs, might be even shorter. However, since there are no data available, this cannot be taken for granted. Therefore using the 10 min ''no-touch'' time seems to be safe from an ethical perspective to ensure brain death in all DCD donors before organ retrieval; however, from an organ quality perspective every minute of warm ischemia that can be safely cut down will improve the success of transplantation. This is an important result which can be used in ethical and legal discussions concerning ''no-touch'' time in DCD.

Factors such as hypothermia, lack of brain perfusion because of low cardiac output during CPR, as well as metabolic influences and impact of anesthesia on bioelectric brain activity monitoring were excluded throughout the experiments. While the precise mechanism that inhalant anesthetics exert their general anesthetic effects is not precisely known, they may interfere with functioning of nerve cells in the brain by acting at the lipid matrix of the membrane. Sevoflurane has a very low blood gas partition coefficient (0.6) allowing very rapid anesthesia induction and recovery. This low solubility in blood means that sevoflurane is rapidly removed from the lungs. It is unknown to which proportion sevoflurane is bound to plasma proteins. The majority of sevoflurane is excreted via the lungs, but about 3% is metabolized in the liver [49]. As we observed the etSEVO going down from 2% to 0.1% on the capnograph display after switching off the sevoflurane vaporizer, the vast majority of sevoflurane was considered to be removed from the lungs and, therefore, from the circulation as well.

Remifentanyl is known not to accumulate in the human circulation and the time required to achieve a 50% decrease in plasma concentration after termination of the infusion is independent of cardiovascular circulation [50]; the low doses used as maintenance anesthesia during these experiments prior to induction of ventricular fibrillation therefore may not influence recording of bioelectric brain activity after the ''no-touch'' time and during CPR.

Massive hypotension (MAP <30 mmHg) is known to negatively impact on EEG activity because of the disruption of the autoregulation of blood flow to the brain [51]. However, during our experiments, MAP during CPR as well as MAP of animals which showed a spontaneous circulation after the ''no-touch'' time and short CPR was kept over a mean value of 30 mmHg throughout the all procedures [52]. This is in accordance with a recent study published by Liao *et al.* [53].

Of course the major limitation of this study is the fact that pigs and not humans were used. Any animal model reproduces at best a very limited component of the pathophysiologic spectrum of the human disease state studied. We used healthy pigs as a surrogate for end of life humans. However, this might not be representative for most DCD subjects. In our model, the animals were under controlled ventilation and cardiac output was suddenly stopped by the initiation of ventricular fibrillation. In contrast, human DCD have variable respiratory drive and cardiac output before approaching cardiac death. Presumably most humans approaching cardiac death have a lower physiological reserve as cardiac output slows some time before cardiac arrest. Pig models are regularly used to simulate brain death [54] or cardiovascular disease [55]. The pig model is the preferred large animal model of heart damage because it reflects the pathophysiology of human best. To our knowledge, there are no reports of animal models reflecting an ''end-of-life'' state

with a low physiological reserve in which complete clinical and electrophysiological criteria for brain death diagnosis were applied.

Keeping in mind the limitations, we have chosen our model to match the human situation best. Since our pigs were healthy at the beginning of the experiment, this would most likely bias the result towards a better functional brain reserve.

Previous studies in ''end-of-life'' states in animal models have applied only limited EEG recordings by devices used for measuring depth of anesthesia. In our study, we applied an EEG montage covering the whole porcine brain, and EEGs were recorded by equipment used in clinical routine for brain death diagnosis. Criteria for brain death in our animal model are equivalent to those required for brain death diagnosis in human patients in Austria, fully reflecting AAN practice parameters [38,41,42]. The strength of our animal study is to apply continuous whole brain EEG recordings and subsequent clinical brain death diagnosis in a DCD setting to obtain information about the chronology of brain destruction after complete cessation of circulation.

In summary, 10 min of ''no-touch'' time guarantee that clinical and electrophysiological criteria of brain death are fulfilled in a pig model, as it has also been suggested by [48]. However, after 5 min of ''no-touch'' time also no evidence of electrophysiological brain activity could be found any more, what would ethically allow to shorten the ''no-touch'' time in order to minimize warm ischemia time and therefore probably improve transplantation outcome.

The results of our study suggest that it would be necessary to evaluate the time course of brain damage in human DCD donors in order to establish evidence based guidelines for the management of DCD. To avoid ethical concerns against DCD, the declaration of death has to be based on scientific facts and not on a personal opinion [7–9,21,47,56,57]. Otherwise legitimated opposition will rise in society, especially in religious communities; therefore limiting the acceptance of DCD resulting in a loss of organs being available for transplantation [47,58]. Of course the major limitation of this study is the fact that pigs and not humans were studied; however from our point of view it helps to support the ''no-touch'' period suggested by Kootsra and Jacobs [48] regarding 10 min ''no-touch'' time being equivalent to brain death in DCD organ donation and even to allow shorter ''hands-off'' times as practiced in some other countries.

Authorship

VS, PS and MS: wrote the article, planned the experiments and performed the animal experiments with AP. TS-H and GZ: performed EEG readings. IW and WM: performed anesthesia on the animals. MZ: involved in the planning of the experiments and fund raising. TS and AM: performed routine laboratory analysis. TS-H, VS, MZ and KT: reviewed the article.

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