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Inducibility of ventricular fibrillation during mild therapeutic hypothermia: electrophysiological study in a swine model

Jaroslav Kudlicka[1,2*], Mikulas Mlcek[1], Jan Belohlavek[3], Pavel Hala[1,4], Stanislav Lacko[1], David Janak[1,5], Stepan Havranek[1,3], Jan Malik[2], Tomas Janota[2], Petr Ostadal[4], Petr Neuzil[4] and Otomar Kittnar[1]

Abstract

Introduction: Mild therapeutic hypothermia (MTH) is being used after cardiac arrest for its expected improvement in neurological outcome. Safety of MTH concerning inducibility of malignant arrhythmias has not been satisfactorily demonstrated. This study compares inducibility of ventricular fibrillation (VF) before and after induction of MTH in a whole body swine model and evaluates possible interaction with changing potassium plasma levels.

Methods: The extracorporeal cooling was introduced in fully anesthetized swine ($n = 6$) to provide MTH. Inducibility of VF was studied by programmed ventricular stimulation three times in each animal under the following: during normothermia (NT), after reaching the core temperature of 32°C (HT) and after another 60 minutes of stable hypothermia (HT60). Inducibility of VF, effective refractory period of the ventricles (ERP), QTc interval and potassium plasma levels were measured.

Results: Starting at normothermia of 38.7 (IQR 38.2; 39.8)°C, HT was achieved within 54 (39; 59) minutes and the core temperature was further maintained constant. Overall, the inducibility of VF was 100% (18/18 attempts) at NT, 83% (15/18) after reaching HT ($P = 0.23$) and 39% (7/18) at HT60 ($P = 0.0001$) using the same protocol. Similarly, ERP prolonged from 140 (130; 150) ms at NT to 206 (190; 220) ms when reaching HT ($P < 0.001$) and remained 206 (193; 220) ms at HT60. QTc interval was inversely proportional to the core temperature and extended from 376 (362; 395) at NT to 570 (545; 599) ms at HT. Potassium plasma level changed spontaneously: decreased during cooling from 4.1 (3.9; 4.8) to 3.7 (3.4; 4.1) mmol/L at HT ($P < 0.01$), then began to increase and returned to baseline level at HT60 (4.6 (4.4; 5.0) mmol/L, $P = \text{NS}$).

Conclusions: According to our swine model, MTH does not increase the risk of VF induction by ventricular pacing in healthy hearts. Moreover, when combined with normokalemia, MTH exerts an antiarrhythmic effect despite prolonged QTc interval.

Keywords: Mild therapeutic hypothermia, Ventricular fibrillation, Hypokalemia, Long QT interval

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* Correspondence: jaroslav.kudlicka@vfn.cz
[1] Department of Physiology, First Faculty of Medicine, Charles University in Prague, Albertov 5, Prague 2 128 00, Czech Republic
[2] 3rd Department of Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital, U Nemocnice 2, Prague 2 128 00, Czech Republic
Full list of author information is available at the end of the article

Introduction

Mild therapeutic hypothermia (MTH) is recommended in patients remaining comatose after cardiac arrest caused by ventricular fibrillation (VF) [1-5] and should be considered in those with other presenting rhythms [6-8]. According to animal studies [9-13], early induction of MTH is supposed to be beneficial, but clinical trials did not prove it consistently yet [14-17]. Consequently, earliest possible induction of MTH is emphasized in some recent protocols using nasopharyngeal evaporative cooling and peripheral veno-arterial extracorporeal membrane oxygenation (ECMO) hoping to improve the neurological outcome [18-22]. On the other hand, the occurrence of malignant ventricular tachyarrhythmias during MTH still remains a major cause of death considering predisposing factors such as myocardial ischemia/reperfusion damage, often reported hypokalemia during the cooling phase [23-25], slowing of the heart rate and QT interval prolongation [25-29].

The aim of this experimental study was to assess the safety of MTH in terms of inducibility of malignant ventricular tachyarrhythmias in relation to spontaneous changes of potassium plasma level and QT interval in a whole body pig model. We hypothesized that MTH related hypokalemia together with prolongation of the QT interval might predispose the heart to greater electrical instability and lower VF threshold.

Methods

The experimental protocol was designed to perform electrophysiological (EP) study before and after the induction of MTH using ECMO to provide cooling and hemodynamic support during the stimulation protocol and arrhythmias. All experiments were performed on cross-bred swine (Landrace x White) in an accredited university experimental laboratory by the skilled team of intensive care specialists and laboratory and veterinary technicians. The animals were handled in accordance with the guidelines of research animal use [30]. The protocol was approved by the Charles University First Faculty of Medicine Institutional Animal Care and Use Committee and performed at the Animal Laboratory, Department of Physiology, First Faculty of Medicine, Charles University in Prague in accordance with Act No 246/1992 as amended, Collection of Laws, Czech Republic, that is harmonized with EU Directives 86/609/EEC as amended, 2007/526/ES, 2010/63/EU.

Anesthesia and monitoring

After intramuscular sedation and premedication (azaperone 2–3 mg/kg, ketamine 20 mg/kg all i.m.) the marginal ear vein was cannulated and the pigs were preoxygenated with 100% oxygen via a facial mask. General anesthesia was induced by intravenous bolus of propofol (1–2 mg/kg) and orotracheal intubation was performed. Mechanical ventilation was adjusted by Intellivent-ASV closed-loop system (G5, Hamilton Medical, Bondauz, Switzerland) to maintain normoxia ($SpO_2 > 97\%$, pO_2 100 mmHg) and normocapnia ($EtCO_2$ 38–40 mmHg) respecting the actual metabolic rate. The total intravenous anesthesia was maintained by continuous administration of propofol (6–12 mg/kg/h) and morphine (0.1-0.2 mg/kg/h), pipecuronium boluse (4 mg i.v.) were applied when shivering occurred during MTH. The depth of anesthesia was regularly assessed by the photoreaction and the corneal reflex and adjusted accordingly. Intravenous infusion of Ringer's solution was given to reach and maintain central venous pressure between 6 and 8 mmHg. Anticoagulation was provided by unfractionated heparin bolus (100 IU/kg i.v.) followed by continuous intravenous drip (40–50 IU/kg/h) to maintain target activated clotting time 180–250 seconds (values checked every hour with Hemochron Junior+, International Technidyne Corporation, USA). Sheaths and catheters were inserted to femoral and carotid/jugular vessels as needed. Invasive blood pressure from carotid and pulmonary artery, central venous pressure (TruWave, Edwards Lifesciences, USA), body surface ECG, capnometry and pulse oximetry were continuously monitored by bedside monitor (Life Scope TR, Nihon Kohden, Japan) and trends were recorded. Mixed venous oximetry (SvO_2), continuous cardiac output and pulmonary artery temperature were recorded using Swan-Ganz Combo V cathether (Vigilance monitor, Edwards Lifesciences, USA). A diagnostic decapolar catheter (Response CSL, St. Jude Medical, USA) was inserted under fluoroscopic guidance into the apex of the right ventricle (RV) via jugular vein to provide monitoring of intracardial electrograms and to induce VF. Five bipolar channels were recorded from the apex to base of RV at 3 kHz sampling rate. Heart rate and QT interval were manually measured and QTc was calculated using Bazett's formula (QT interval divided by square root of RR interval). Before starting the protocol, cardiopulmonary bypass was established (Figure 1): an inlet 19 F cannula was inserted via femoral vein into the right atrium and an outlet 17 F cannula into abdominal aorta via femoral artery. The cannulae were connected to ECMO circuit consisting of a blood pump (Levitronix Centrimag, Levitronix, USA) and oxygenator (Quadrox, Maquet, Germany). A custom made cooling system was connected to the oxygenator. The cooling system consisted of a rotary pump and reservoir (volume of 15 L) filled with refrigerated water with ice cubes, which were gradually supplemented to keep the temperature of the coolant between 4 and 6°C. The coolant flow rate was set up to approximately 2 L/min during the cooling phase a consequently was slowed as needed to maintain the core temperature of 32°C. The ECMO circuit was primed with 500 mL of normal saline with 2500 IU of unfractionated

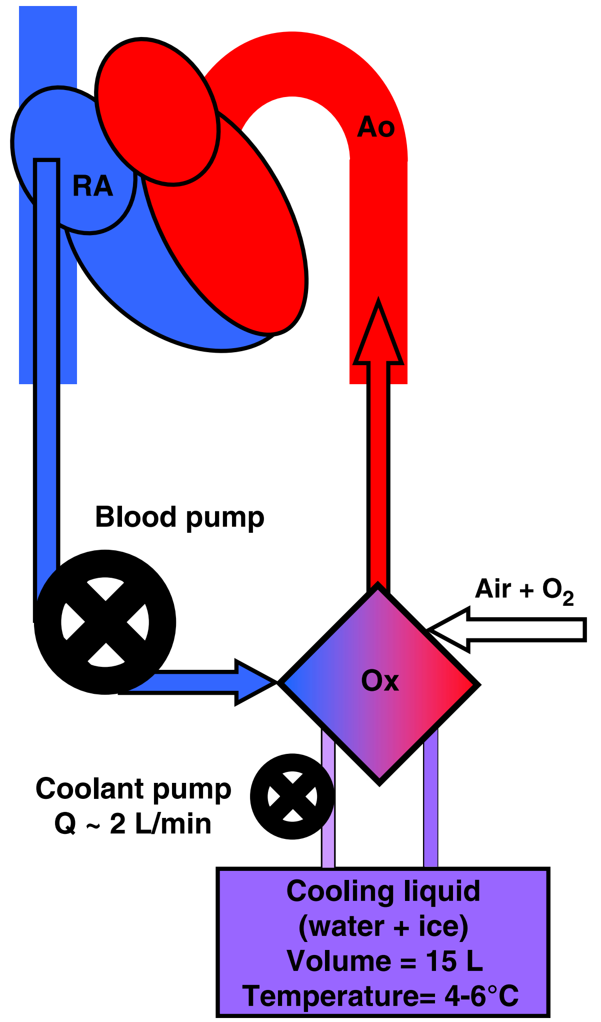

Figure 1 V-A ECMO circuit. Blood is pumped from the right atrium via oxygenator where blood is cooled and gases are exchanged into abdominal aorta. The custom made cooling circuit was assembled from the pump and reservoir filled with refrigerated water and melting ice. RA indicates right atrium; Ox, oxygenator; Ao, abdominal aorta.

heparin. The ECMO blood flow was empirically set at 40 mL/kg/min to provide the cooling and to prevent low cardiac output and hypotension during the stimulation protocol. After the onset of VF, the flow was increased to 80–100 mL/kg/min as needed to keep mean arterial pressure of 60 mmHg until an effective pulsatile sinus rhythm with mean arterial pressure above 60 mmHg was restored. Blood gasses, pH and potassium plasma levels were continuously sampled and recorded in ECMO circuit by a real-time analyzer (CDI 500, Terumo, Japan). At baseline and during the experiment was the real-time analyzer calibrated in 15 minute intervals using a bedside blood analysis system (IRMA TruPoint, International Technidyne Corporation, USA). Despite the expected hypokalemia during MTH, no potassium was replaced during the whole protocol, therefore all changes of potassium plasma levels were spontaneous. Ventilation support, as well as ECMO gas flow, was regularly adjusted to reach the target values (pH 7.4, pO_2 100 mmHg and pCO_2 38-40 mmHg).

Electrophysiological study

VF was induced by programmed ventricular stimulation (PVS, Figure 2B). Briefly, electrical stimuli (12 ms duration, maximal stimulation current 20 mA) were delivered from the apex of RV. Eight basic stimuli (S1) of cycle length 350 or 400 ms according to the heart rate were coupled with up to 4 extrastimuli (S2-S5). The coupling interval of S2 was decreased at 10 ms steps until the ventricular effective refractory period (ERP) was achieved. S2 coupling interval was then set to 10 ms above ERP and analogically S3 or S4 stimuli were delivered until VF or absolute ventricular refractory period (ARP) was reached. There were at least 10 s intervals between pacing sequences. VF was defined as an orderless rhythm without detectable QRS complexes lasting longer than 30 s. Normal SR was restituted by the transcutaneous defibrillation using pads placed in the conventional position sternum - apex (TEC-5521, Nihon Kohden, Japan). Biphasic shocks with rising energy (100-150-200-270 J) were delivered as needed. Another PVS procedure was performed at least 10 minutes after SR restoration in a previous one.

Protocol

EP study began at minimum of 60 minutes after connecting ECMO. After the completion of 3 PVS procedures at the baseline (normothermia, NT), the cooling was started and other 3 PVS procedures were performed after reaching the core temperature of 32°C in pulmonary artery (hypothermia, HT). Finally, 3 PVS procedures were carried out after 60 minutes of stable hypothermia (HT60), Figure 2A.

Statistics

Data are presented as median (interquartile range). Each animal was used as its own control and nonparametric pair Friedman test with Dunn's multiple comparison post test were performed to assess the differences. The linear regression of the dependence of QTc interval on body temperature was performed after passing D'Agostino & Pearson omnibus normality test. The comparison of the VF inducibility was performed by Fisher's exact test. The *P* values < 0.05 were considered significant. Statistical analysis and graphs were performed using Prism 5.0 (GraphPad, La Jolla, CA, USA).

Results

Overall, we used 7 animals (female, 4–5 months old, mean weight 51 ± 2 kg). One of them was excluded from analysis because of frequently reoccurring supraventricular

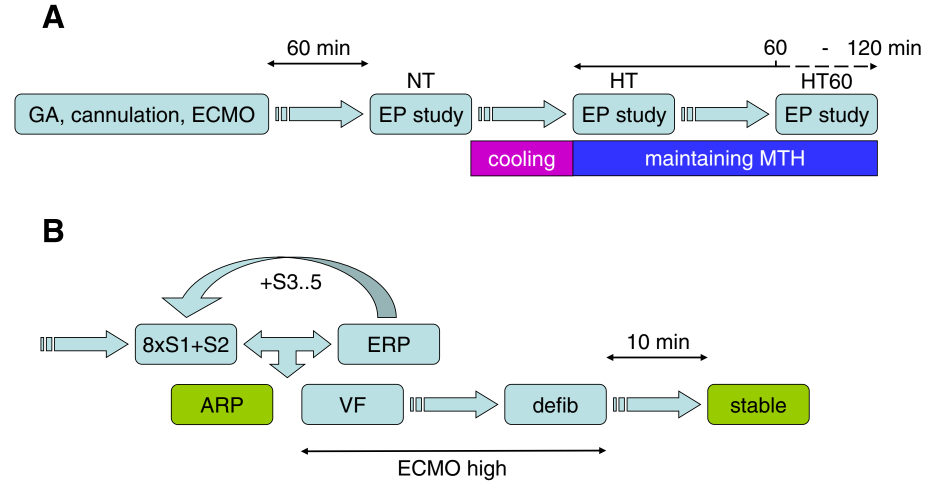

Figure 2 Diagram of study protocol (A) and electrophysiological study (B). GA indicates induction of general anesthesia; ECMO, initiation of ECMO; EP study, electrophysiological study; HT, hypothermia (core temperature of 32°C); 8 × S1, train of eight basic stimuli; S2-5, up to five extrastimuli; ERP, effective refractory period; ARP, absolute refractory period; VF, ventricular fibrillation; defib, defibrillation; steady, steady state; ECMO high, increasing of the ECMO flow to 80-100 mL/kg/min after onset of VF until the sinus rhythm was restored.

tachyarrhythmias at the baseline. Providing ECMO cooling, the core body temperature dropped from normothermia of 38.7 (38.2; 39.8)°C to 32°C during 54 (39; 59) minutes and was further maintained constant at 31.9 (31.8; 32.0)°C, Figure 3. The potassium plasma level decreased along with the body temperature decline from basal 4.1 (3.9; 4.8) mmol/L to 3.7 (3.4; 4.1) mmol/L ($P < 0.01$) when reaching HT. In 3 of 6 animals (50%) dropped plasmatic potassium below 3.5 mmol/L, but none of them reached severe hypokalemia (<3.0 mmol/L). Subsequently, during maintaining of HT the potassium plasma level increased spontaneously again and at HT60 was similar to baseline level (4.6 (4.4; 5.0) mmol/L, P = NS, Figure 4A). Heart rate decreased from 148 (123; 161) at baseline to 85 (82; 100) beats per minute at HT ($P < 0.001$) and remained similar at HT60 (93 (81; 117) beats per minute). The QTc interval was significantly inversely related to the body temperature (−29 ± 2 ms per degree of Celsius, $r^2 = 0.82$, Figure 5) and increased from basal 376 (362; 395) ms to 570 (545; 599) ms at HT. ERP adjusted to S1-S1 cycle length of 350 ms increased from 140 (130; 150) ms at NT to 206 (190; 220) ms when reaching HT ($P < 0.001$) and remained constant at HT60 (206 (193; 220) ms, Figure 4B). Through all the experiments, no spontaneously originating or provoked torsades de pointes (TdP) or heart conduction system disorders were observed. The inducibility of VF at normothermia was 100% (18/18 PVS), 83% (15/18 PVS) when reaching HT, P = NS, and 39% (7/18 PVS) at HT60, $P < 0.0001$, Table 1, Figure 4C.

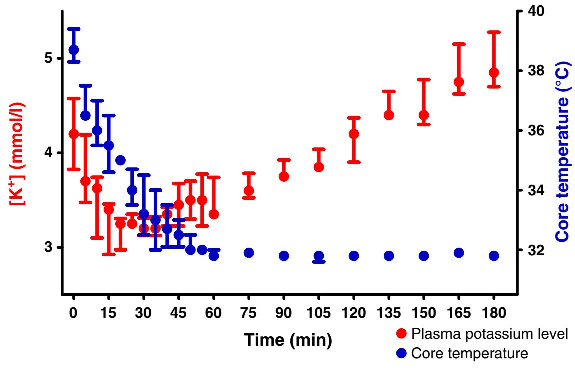

Figure 3 The time dependence of the plasma potassium level and body temperature after the start of the cooling (time = 0). Data are expressed as medians with interquartile ranges.

Discussion

In our experimental study we have proven that MTH is safe in terms of inducibility of malignant ventricular tachyarrhythmias in healthy pig hearts. Despite our hypothesis, the inducibility of VF was not increased both during the spontaneous transient decline of plasmatic potassium level and concurrent significant prolongation of QTc interval. Moreover, inducibility of VF was even significantly lower after normalization of potassium plasma level. Our data show that the potassium plasma level declines during the cooling phase, while during maintenance of MTH potassium plasma level rises spontaneously and ultimately returns to the baseline value.

Regarding dependence of potassium plasma level on body temperature, our data are concordant to clinical trials by Miryozev et al. [25], Soeholm and Kirkegaard [24]. Nevertheless, none of the animals in our experiment reached severe spontaneous hypokalemia (<3.0 mmol/L) which is supposed to be associated with a higher incidence

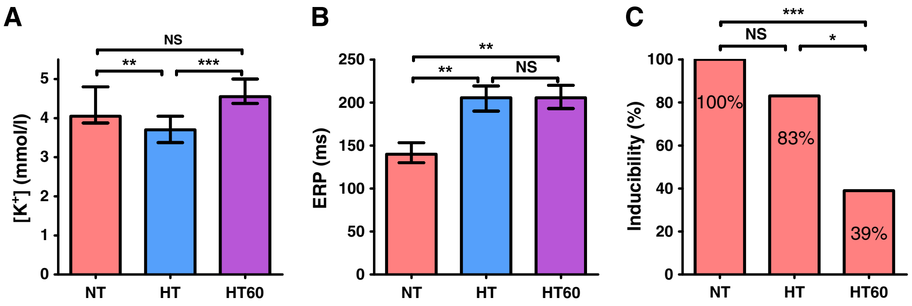

Figure 4 Changes in potassium plasma level (A), effective refractory period (B) and inducibility of ventricular fibrillation (C). Data are expressed as medians with interquartile ranges. NT indicates normothermia; HT, after reaching the core temperature of 32°C; HT60, after 60 minutes of stable hypothermia; NS, statistically non-significant; *P < 0.01; **P < 0.001; ***P < 0.0001.

of VT during cooling phase by the clinical trials. The exact mechanism of potassium plasma level changes remains not fully elucidated. In our study we can exclude the possible effects of drugs such as insulin, cathecholamines or diuretics, as well as significant ischemic damage of tissues or acid–base changes. Except for the suggested temperature related potassium reversible intra and extra cellular redistribution [31], Polderman et al. [32,33] documented hypothermia-induced polyuria caused by transient tubular dysfunction during the cooling phase which disappeared after maintaining MTH and rewarming. Based on our data, despite hypokalemia as a potential physiological phenomenon, maintaining the normokalemia during MTH contributed to electric stabilization of the myocardium. In accordance with other authors [25,27] we assume that close monitoring of potassium plasma level and its timely supplementation during the cooling phase could help decrease the risk of malignant ventricular arrhythmias.

As observed, the prolongation of QTc interval during MTH had not an adverse effect on arrhythmogenesis of ventricular arrhythmias. The QTc interval was inversely related to the body temperature and was independent of potassium plasma changes. Hypothermia related prolongation of QTc interval and decrease of heart rate were previously described in beagle dogs [34] and also in newborns [35]. According to our data the slope of the curve corresponds to the results in newborns well (-29 ms vs. -21 ms per degree of Celsius). Until recently, the occurrence of the long QT interval during MTH was considered to be a negative prognostic factor in terms of higher incidence of TdP or VF [27,36]. However, recent observational studies and meta-analysis did not prove this assumption [37-40]. Furthermore, as reported by Nishiyama et al. in case reports of congenital long QT syndrome [41], the induction of MTH after cardiac arrest did not have a proarrhythmic effect. Despite prolongation of QTc interval to extreme values during MTH, there were no recurrences of TdP documented. Similarly, in our experiments we have not detected any spontaneous or induced TdP taking into account the limitation in the use of healthy pig hearts.

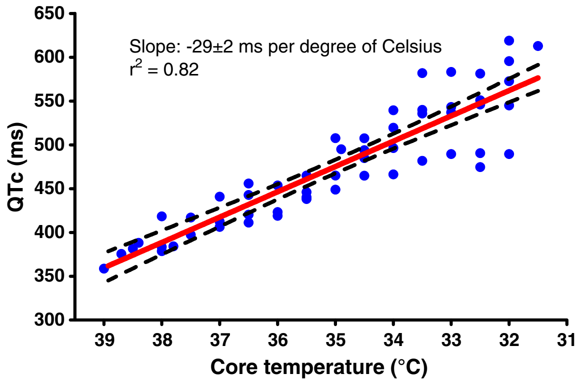

Figure 5 Dependence of QT on body temperature with linear regression. Error bars show 95% of confidence interval.

Decrease in heart rate during MTH might be affected by decreased sympathetic activity as reported by Schwarzl et al. [42], which could also contributed to decreased VF inducibility. According to our previously published data, significant prolongation of ERP was observed as a sign of decreased sympathetic activity after renal denervation [43]. Nevertheless, considering comparable heart rate and ERP at HT and HT60 we can suppose that the tone of autonomic nerve system remained unchanged and could not play a major role in a significant decrease of VF inducibility at HT60.

The influence of profound reduction of myocardial temperature on VF was previously studied in more detail by Chorro et al. [44] in rabbit hearts using high-resolution epicardial mapping and transmural recordings of ventricular activation. They described the antiarrhythmic effect of deep hypothermia (<20°C) by exponential decay of VF dominant frequency, reduction in conduction velocity and subsequently single wave front extinction on activation maps. More recently, Harada et al. [45] proved

Table 1 Characteristics of ventricular fibrillation induction

	NT		HT		HT60	
Animal #	Induction parameter (ms)	Inducibility of VF	Induction parameter (ms)	Inducibility of VF	Induction parameter (ms)	Inducibility of VF
1	400/200/140/110/100	3/3	400/220/130	3/3	400/270/170/160 (ARP)	0/3
2	350/150/110	3/3	350/200/170/160 (ARP)	0/3	350/220/140/130	3/3
3	300/150/90/80	3/3	350/240/140/130	3/3	350/230/160/150 (ARP)	0/3
4	350/140/60	3/3	400/250/180/170	3/3	400/240/180/170/160 (ARP)	0/3
5	350/150/140	3/3	350/220/180	3/3	400/230/220	1/3
6	300/120/60	3/3	400/230/160/150	3/3	400/230/200/190	3/3
Total		100% (18/18)		83% (15/18) NS		39% (7/18)***

Induction parameters represent minimal cycle lengths of basic stimuli and extrastimuli. In each animal and condition was EP protocol made three times. NT indicates normothermia; HT, after reaching the core temperature of 32°C; HT60, after 60 minutes of stable hypothermia. ARP indicates the achievement of absolute refractory period; NS, statistically non-significant; ***$P = 0.0001$.

antiarrhythmic effect of MTH in rabbit hearts. Although both MTH and deep hypothermia caused significant prolongation of action potential duration and significant reduction of conduction velocity, the duration of induced ventricular arrhythmias was significantly lower only in MTH. As they observed by high-resolution optical potential mapping, MTH causes a modification of arrhythmia's spiral wave dynamics (annihilation or exit from the anatomical boundaries) leading to the increase of the chance of arrhythmia self-termination. This corresponds to the results of our study on the pig whole body model.

This study has several translational aspects related to post-resuscitation care. Frequent monitoring and adequate substitution of plasmatic potassium seems to be very important especially during the cooling phase to prevent malignant ventricular arrhythmias. On the other hand, close monitoring of QTc interval and premature termination of MTH due to long QTc appears not to be necessary. There is also potential in treating of incessant ventricular arrhythmias resistant to treatment when MTH could contribute to stabilization of the heart rhythm.

Our study was limited by using healthy animals not considering ischemic-reperfusion injury of the myocardium during the acute myocardial infarction or other predisposition for malignant ventricular arrhythmias. Thus, the translation of the results to humans should be interpreted with caution despite the fact, that our biomodel was represented by a breed repeatedly validated for simulation of human cardiac arrest and resuscitation [46,47]. The number of experimental animals tested is low, however multiple pairwise comparisons in individual animals counterbalances this limitation. The speed of cooling comparing ECMO cardiopulmonary resuscitation protocols [48] was considerably lower. Unlike them, we used a half ECMO flow (40 mL/kg/min) to prevent a hyperkinetic circulation in respect to spontaneous hemodynamics and also the priming the ECMO circuit with the precooled saline could not be applied before. Further, no other ions such as calcium or magnesium have been followed while they might play a significant role. We also did not analyze diuresis and ion excretion in urine. In present study, no potential mapping technics were used to describe exact mechanisms leading to antiarrhythmic effect of MTH preferring the complexity of the whole body biomodel than open-chest or isolated heart techniques.

Conclusions

The induction of MTH in a swine model is safe with respect to the inducibility of malignant ventricular arrhythmias. Potassium plasma level decline during the cooling phase does not impair VF threshold and after achieving spontaneous normokalemia there is a significant effect on the reduction of VF inducibility. Prolongation of QT interval during MTH appears to be a physiological phenomenon reflecting the conduction changes and does not impair the inducibility of VF.

Key messages

- MTH does not increase the inducibility of VF in a pig whole body model.
- Despite prolongation of QT interval, prolonged MTH seems to have an antiarrhythmic effect.

Abbreviations

ARP: Absolute refractory period; ECG: Electrocardiogram; ECMO: Extracorporeal membrane oxygenation; EP: Electrophysiological; ERP: Effective refractory period; $EtCO_2$: End-tidal carbon dioxide; HT: Hypothermia of 32°C; HT60: 60 minutes of stable hypothermia 32°C; MTH: Mild therapeutic hypothermia; NT: Normothermia; pCO_2: Partial pressure of carbon dioxide; pO_2: Partial pressure of oxygen; PVS: Programmed ventricular stimulation; RV: Right ventricle; SpO_2: Peripheral capillary oxygen saturation; SvO_2: Mixed venous oxygen blood saturation; TdP: Torsades de pointes; VF: Ventricular fibrillation.

Competing interests

All authors have no commercial associations that might impose a conflict of interest.

Authors' contributions

JK, MM: made conception and design of the study, performed main procedures of the protocol, data acquisition and statistical analysis, prepared and finalized the manuscript. PH, SL, DJ assisted during the study, cared about ECMO and temperature management. SH participated in a design of EP study and interpretation of EP data. JB, JM, TJ, PO assisted during the study performance and drafted the article. PN and OK participated in a design of the study, contributed to the interpretation of the results, and helped to draft the manuscript. All authors read and approved the final manuscript for publication.

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Author details

[1]Department of Physiology, First Faculty of Medicine, Charles University in Prague, Albertov 5, Prague 2 128 00, Czech Republic. [2]3rd Department of Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital, U Nemocnice 2, Prague 2 128 00, Czech Republic. [3]2nd Department of Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital, U Nemocnice 2, Prague 2 128 00, Czech Republic. [4]Department of Cardiology, Na Homolce Hospital, Roentgenova 2/37, Prague 5 150 30, Czech Republic. [5]2nd Department of Surgery, Cardiovascular Surgery, First Faculty of Medicine, Charles University in Prague and General University Hospital, U Nemocnice 2, Prague 2 128 00, Czech Republic.

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RESEARCH **Open Access**

Renal denervation decreases effective refractory period but not inducibility of ventricular fibrillation in a healthy porcine biomodel: a case control study

Jean-Claude Lubanda[1*†], Jaroslav Kudlicka[2,3†], Mikulas Mlcek[2†], Miroslav Chochola[1†], Petr Neuzil[4†], Ales Linhart[1†] and Otomar Kittnar[2†]

Abstract

Background: Ventricular arrhythmias play an important role in cardiovascular mortality especially in patients with impaired cardiac and autonomic function. The aim of this experimental study was to determine, if renal denervation (RDN) could decrease the inducibility of ventricular fibrillation (VF) in a healthy porcine biomodel.

Methods: Controlled electrophysiological study was performed in 6 biomodels 40 days after RDN (RDN group) and in 6 healthy animals (control group). The inducibility of VF was tested by programmed ventricular stimulation from the apex of right ventricle (8 basal stimuli coupled with up to 4 extrastimuli) always three times in each biomodel using peripheral extracorporeal oxygenation for hemodynamic support. Further, basal heart rate (HR), PQ and QT intervals and effective refractory period of ventricles (ERP) were measured. Technical success of RDN was evaluated by histological examination.

Results: According to histological findings, RDN procedure was successfully performed in all biomodels. Comparing the groups, basal HR was lower in RDN group: 79 (IQR 58; 88) vs. 93 (72; 95) beats per minute ($p = 0.003$); PQ interval was longer in RDN group: 145 (133; 153) vs. 115 (113; 120) ms ($p < 0.0001$) and QTc intervals were comparable: 402 (382; 422) ms in RDN vs. 386 (356; 437) ms in control group ($p = 0.1$). ERP was prolonged significantly in RDN group: 159 (150; 169) vs. 140 (133; 150) ms ($p = 0.001$), but VF inducibility was the same (18/18 vs. 18/18 attempts).

Conclusions: RDN decreased the influence of sympathetic nerve system on the heart conduction system in healthy porcine biomodel. However, the electrophysiological study was not associated with a decrease of VF inducibility after RDN.

Keywords: Renal denervation, Ventricular fibrillation, Electrophysiological study, Biomodel

Background

The occurrence of ventricular arrhythmias is influenced by the activity of the autonomic nervous system [1]. The sympathetic nervous system plays an important role in the onset, maintenance and termination of ventricular arrhythmia. Most typically sympathetic activation enhances ventricular arrhythmia while vagal tone suppresses its occurrence. The onset of ventricular arrhythmias is provoked by shortening the effective ventricular refractory time, being a result of sympathetic stimulation. Therefore modulating the activity of the autonomic nervous system might suppress the incidence of ventricular arrhythmias including ventricular fibrillation and provide a rationale for the reduction of the risk of sudden cardiac death. Moreover, RDN was successfully used to reduce the occurrence of electrical storm in many case reports. Impaired cardiac autonomic control and increased sympathetic tone by physical or emotional stress play an important role in the induction of arrhythmia [2] and increase the risk of sudden death [3]. Moreover, increased sympathetic activity as a result of cardiac

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* Correspondence: jean-claude.lubanda@vfn.cz
†Equal contributors
[1] 2nd Department of Medicine - Department of Cardiovascular Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, U Nemocnice 2, Prague 2 128 00, Czech Republic
Full list of author information is available at the end of the article

dysfunction contributes to the worsening of chronic heart failure and increased mortality [4,5]. Therefore the use of beta adrenergic blocking agents is generally recommended as prophylaxis of malignant ventricular arrhythmias to reduce the risk of sudden cardiac death [6,7]. However, some invasive procedures can modulate the tone of sympathetic nerves. Surgical ablation of the lower part of the left ganglion stellate and the first four thoracic ganglia (left cardiac sympathetic denervation) reduces the rate of cardiac events refractory to therapy in most patients with inherited arrhythmia syndromes, such as long-QT syndrome and cathecholaminergic polymorphic ventricular tachycardia (VT) [8]. The potential effects of renal denervation for the reduction of malignant arrhythmia has been described. And its potential benefit is being discussed in heart failure patients with left ventricle dyssynchrony which is independently associated with the risk of sudden cardiac death [9]. Moreover, catheter-based approach has been developed for renal sympathetic denervation (RDN) using several techniques [10]. The ablation procedure reduces sympathetic activation not just in the kidney but also in the whole body [11-13]. Beyond lowering of blood pressure RDN was shown to reduce the resting heart rate and to prolong PR interval in patients with resistant arterial hypertension [14]. Also in a pig model of atrial fibrillation RDN reduced duration of pacing-induced episodes of the arrhythmia [15]. Moreover, stimulation of left stellate ganglion and rapid atrial pacing in animals after RDN did not increase the inducibility of atrial fibrillation [16]. Similar findings were described in patients after pulmonary vein isolation along with RDN who experienced significantly fewer episodes of atrial fibrillation at follow-up than patients after pulmonary vein isolation alone [17]. Focused on ventricular arrhythmias, Linz et al. [18] reported a significant reduction of spontaneous ventricular extrabeats and ventricular fibrillation (VF) episodes during acute myocardial ischemia and reperfusion in dogs after RDN. According to case reports, a significant decrease of VT/VF was observed in two patients with dilated and hypertrophic cardiomyopathy and in a patient after acute myocardial infarction who all suffered from treatment-resistant electrical storms [19,20]. The objective of this experimental study was to compare changes of electrophysiological parameters and inducibility of VF in a healthy porcine biomodel 40 days after RDN with a control group.

Methods

The experimental protocol was designed to perform the controlled study in 6 biomodels 40 days after RDN (RDN group) and in 6 healthy animals (control group). The electrophysiological (EP) study was performed using catheter based techniques. The peripheral veno-arterial extracorporeal membrane oxygenation (ECMO) was used because of myocardial protection during the ventricular pacing and arrhythmias. An overall scheme of the study is shown in Figure 1.

Cross-bred swine (Landrace x White; 44 ± 3 kg) were used for the experiments in an accredited experimental university laboratory by a skilled team of clinicians and veterinary specialists. The animals were handled in accordance with the guidelines of research animal use [21]. The protocol was approved by the Charles University 1st Medical School Institutional Animal Care and Use Committee and performed at the Animal Laboratory, Institute of Physiology, 1st Medical School, Charles University in Prague in accordance with Act No 246/1992 as amended, Collection of Laws, Czech Republic, that is harmonized with EU Directives 86/609/EEC as amended, 2007/526/ES, 2010/63/EU.

Anesthesia and monitoring

The pigs were sedated by intramuscular application of azaperone (2–3 mg/kg) and ketamine (20 mg/kg). Then the marginal ear vein was cannulated and general anesthesia was induced by intravenous bolus of propofol (1–2 mg/kg). After preoxygenation via facial mask, orotracheal intubation was performed. During the experiment were the biomodels mechanically ventilated using Intellivent-ASV closed-loop system (G5, Hamilton Medical, Bondauz, Switzerland) to maintain normoxia (SpO_2 98%) and normocapnia ($EtCO_2$ 38–40 mmHg) respecting the actual metabolic rate. The total intravenous anesthesia was maintained by continuous administration of propofol (6–12 mg/kg/h) and morphine (0.1-0.2 mg/kg/h). The depth of anesthesia was regularly assessed by the

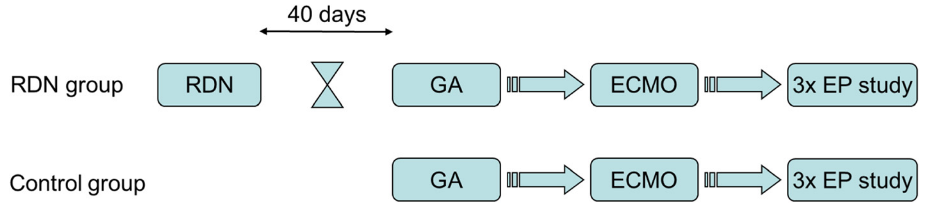

Figure 1 Overall scheme of the study. RDN indicates renal denervation; GA, general anesthesia; ECMO, extracorporeal membrane oxygenation; EP, electrophysiological.

photoreaction and the corneal reflex and adjusted accordingly. Intravenous infusion of Ringer's solution was given to reach and maintain central venous pressure between 6 and 8 mmHg. Anticoagulation was provided by unfractionated heparin bolus (100 IU/kg IV) followed by continuous intravenous drip (40–50 IU/kg/h) to maintain target activated clotting time 180–250 seconds (values checked every hour with Hemochron Junior+, International Technidyne Corporation, Edison, NJ, USA). Sheaths and catheters were inserted to femoral and carotid/jugular vessels as needed. Invasive blood pressure from carotid and pulmonary artery, central venous pressure (TruWave, Edwards Lifesciences, USA), body surface ECG, capnometry and pulse oximetry were continuously monitored by bedside monitor (Life Scope TR, Nihon Kohden, Japan).

Renal denervation

After initiation of general anesthesia and mechanical ventilation an 8 french sheath was placed into the right common femoral artery under ultrasound guidance and consequently another 6 french sheath was introduced into the right common femoral vein. The later was used for the acquisition of samples for biochemical analysis during the procedure. According to the protocol, after preliminary samples were withdrawn, renal angiography was performed and an ablation catheter was then introduced into the renal arteries. In 4 cases, the EnligHTN catheter (St. Jude Medical, USA) was used and in 2 cases we used the Symplicity catheter (Medtronic, USA). The ablation procedure was performed according to the instruction for use of each device in both arteries in each case and the effect of the procedure was controlled by a drop of impedance of at least 10%. At the end of RDN procedure, the femoral sheath was extracted and the groin was surgically sutured. Subsequently, the administration of anesthetics and analgesics was stopped and successful weaning from artificial ventilation was achieved. Then were the animals extubated. For next 40 days, the animals were bred in a certified menagerie.

Cardiopulmonary bypass

Before starting the EP study, cardiopulmonary bypass (CPB) was established: an inlet 19 F cannula was inserted via femoral vein into the right atrium and an outlet 17 F cannula into abdominal aorta via femoral artery. The cannulae were connected to ECMO circuit consisting of a blood pump (Levitronix Centrimag, Levitronix, USA) and oxygenator (Quadrox, Maquet, Germany). The ECMO circuit was primed with 500 mL of saline with 2500 IU of unfractionated heparin. The ECMO blood flow was empirically set at 40 mL/kg/min to prevent low cardiac output and hypotension during the ventricular stimulation. After the onset of VF, the flow was increased to 80-100 mL/kg/min as needed to keep mean arterial pressure of 60 mmHg until an effective pulsatile sinus rhythm with mean arterial pressure above 60 mmHg was restored. Ventilation support, as well as ECMO gas flow, was regularly adjusted to reach the target values (SpO_2 98% and pCO_2 38-40 mmHg).

Electrophysiological study

A diagnostic decapolar catheter (Response CSL, St. Jude Medical, USA) was inserted under fluoroscopic guidance into the apex of the right ventricle (RV) via right jugular vein to provide monitoring of intracardial electrograms and to induce VF. Five bipolar channels were recorded from the apex to base of RV at 3 kHz sampling rate. VF was induced by programmed ventricular stimulation (PVS, Figure 2). Briefly, electrical stimuli (12 ms duration, maximal stimulation current 20 mA) were delivered from the apex of RV. Eight basic stimuli (S1) of cycle length 300, 350 or 400 ms according to the heart rate were coupled with up to 4 extrastimuli (S2-S5). The coupling interval of S2 was decreased at 10 ms steps until the ventricular effective refractory period (ERP) was achieved. S2 coupling interval was then set to 10 ms above ERP and analogically S3 or S4 stimuli were delivered until VF was reached. There were at least 10 s intervals between pacing sequences. VF was defined as an orderless rhythm without detectable QRS complexes lasting longer than 30 s.

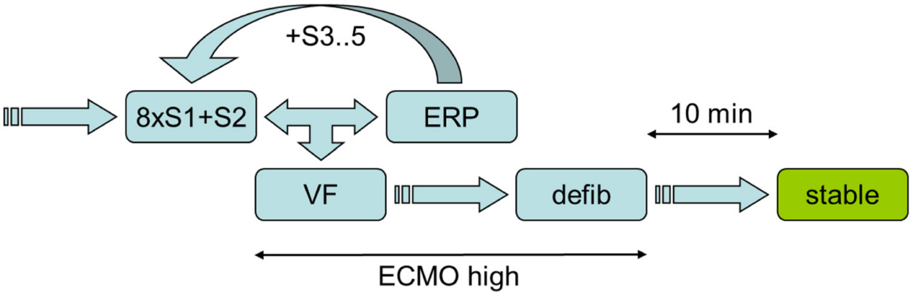

Figure 2 Scheme of electrophysiological study. 8xS1 indicates the train of eight basic stimuli; S2-5, up to five extrastimuli; ERP, effective refractory period; VF, ventricular fibrillation; defib, defibrillation; ECMO high, increasing of the ECMO flow to 80-100 mL/kg/min after onset of VF until the sinus rhythm was restored.

Normal SR was restituted by the transcutaneous defibrillation using pads placed in the conventional position sternum - apex (TEC-5521, Nihon Kohden, Japan). Biphasic shocks with rising energy (150-200-270 J) were delivered as needed. Another PVS procedure was performed at least 10 minutes after SR restoration in a previous one.

Data acquisition protocol

The baseline surface ECG was acquired during 10 minutes at minimum of 60 minutes after general anesthesia initiation. Heart rate (HR), PQ and QT intervals were assessed ten times (in each minute of the ECG record) by offline manual analysis with appropriate time resolution and QTc was calculated using Bazett's formula (QT interval divided by square root of RR interval). Then was CPB established and ECMO connected. EP study began at minimum of 30 minutes after connecting ECMO. PVS procedures were performed three times in each animal. Finally, an autopsy was carried out to obtain samples of renal arteries and macroscopic and histological assessment of the arterial wall and perirenal nerves. All procedures were provided by a skilled pathologist.

Statistics

The sample size was calculated using Medcalc software (Medcalc® Version 12,1.4.0) based on the primary outcome of the study which was the inducibility of VF. The number of attempts to induce VF was estimated according the study by linz et al. [18] where VF occurred in 14% of denervated pigs and in 83% of sham treated pigs. For each experiment, we planned 3 attempts. The minimal required sample size per group for equal samples sizes for alpha = 0.05 and power = 0.80 (beta = 0.20) was 5, meaning 15 attempts per group assuming that the data will be approximately normally distributed. Data are presented as medians (interquartile ranges). The nonparametric unpaired Mann–Whitney test was used to assess the differences. The P values < 0.05 were considered significant. Statistical analysis and graphs were performed using Prism 5.0 (GraphPad, La Jolla, CA, USA).

Results

The electrophysiological parameters and VF inducibility data are summarized in Table 1 and Figure 3. According to histological findings, destruction of perirenal nerves was successful in both arteries in each case regardless of used ablation catheter (Figure 4). No arterial stenosis or endothelial damage was found 40 days after RDN.

ECG changes at baseline

Comparing RDN and control group, basal HR was significantly lower in RDN group: 79 (58; 88) vs. 93 (72; 95) beats per minute, p = 0.003. Accordingly, PQ interval in RDN group was longer: 145 (133; 153) vs. 115 (113; 120) ms, p < 0.0001, and QTc intervals were comparable between RDN and control group: 402 (382; 422) vs. 386 (356; 437) ms, p = 0.1.

Table 1 Comparison of ECG and ventricular fibrillation (VF) induction parameters of RDN and control group

Control group

Animal #	HR (ms)	PQ (ms)	QTc (ms)	Induction parameter S1-S5 (ms)	ERP (ms)	Inducibility of VF
1	89 (88; 91)	114 (113; 117)	361 (357; 364)	400/200/140/110/100	166	3/3
2	94 (93; 94)	118 (116; 118)	359 (356; 360)	350/150/110	140	3/3
3	96 (96; 98)	120 (116; 123)	438 (436; 444)	350/160/120/90	163	3/3
4	72 (72; 73)	113 (113; 113)	368 (367; 368)	350/140/60	130	3/3
5	95 (95; 95)	110 (108; 110)	353 (351; 355)	350/150/140	140	3/3
6	68 (67; 68)	143 (140; 144)	328 (326; 332)	300/120/60	128	3/3
Total	**93 (72; 95)**	**115 (113; 120)**	**386 (356; 437)**		**140 (133; 150)**	**18/18 (100%)**

RDN group

Animal #	HR (ms)	PQ (ms)	QTc (ms)	Induction parameter S1-S5 (ms)	ERP (ms)	Inducibility of VF
1	78 (78; 79)	123 (120; 124)	386 (383; 391)	400/190/170	166	3/3
2	81 (78; 85)	146 (143; 148)	389 (382; 400)	400/170/140	149	3/3
3	88 (87; 89)	149 (145; 153)	472 (466; 475)	350/200/80	190	3/3
4	49 (48; 49)	161 (158; 163)	356 (354; 358)	400/200/160	166	3/3
5	113 (113; 113)	131 (128; 133)	421 (416; 425)	350/180/100	170	3/3
6	58 (58; 59)	153 (150; 155)	405 (402; 407)	350/160/140	150	3/3
Total	**79 (58; 88)****	**145 (133; 153)*****	**402 (382; 422)**^{NS}		**159 (150; 169)****	**18/18 (100%)**

Induction parameters represent minimal cycle lengths of basic stimuli and up to five extrastimuli (S1-5). In each animal was EP protocol made three times. HR indicates heart rate; ERP, effective refractory period of ventricles; VF, ventricular fibrillation; ^{NS},statistically non-significant; **P < 0.01; P ***P < 0.0001.

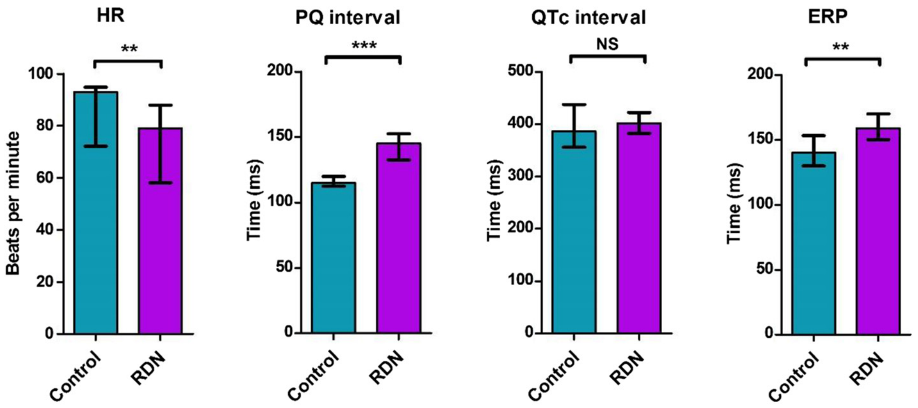

Figure 3 Comparison of electrophysiological parameters of RDN and control group. HR indicates heart rate; ERP, effective refractory period of ventricles; VF, ventricular fibrillation; Control, control group; RDN, RDN group; NS, statistically non-significant; ** P < 0.01; P *** P < 0.0001.

Inducibility of VF

VF was induced using the same protocol in each attempt in all animals of both RDN and Control groups (18/18 vs. 18/18) and also the median number of extrastimuli did not differ. However, ERP was significantly prolonged in RDN group: 159 (150; 169) vs. 140 (133; 150) ms, p = 0.001.

Discussion

In this experimental study we have shown that RDN changed autonomic control of the heart at interval of 40 days. Decreased sympathetic activity was observed as pleiotropic changes in the heart conduction system, e.g. decrease of the rest heart rate, prolongation of atrioventricular conduction and increase of ERP of ventricles.

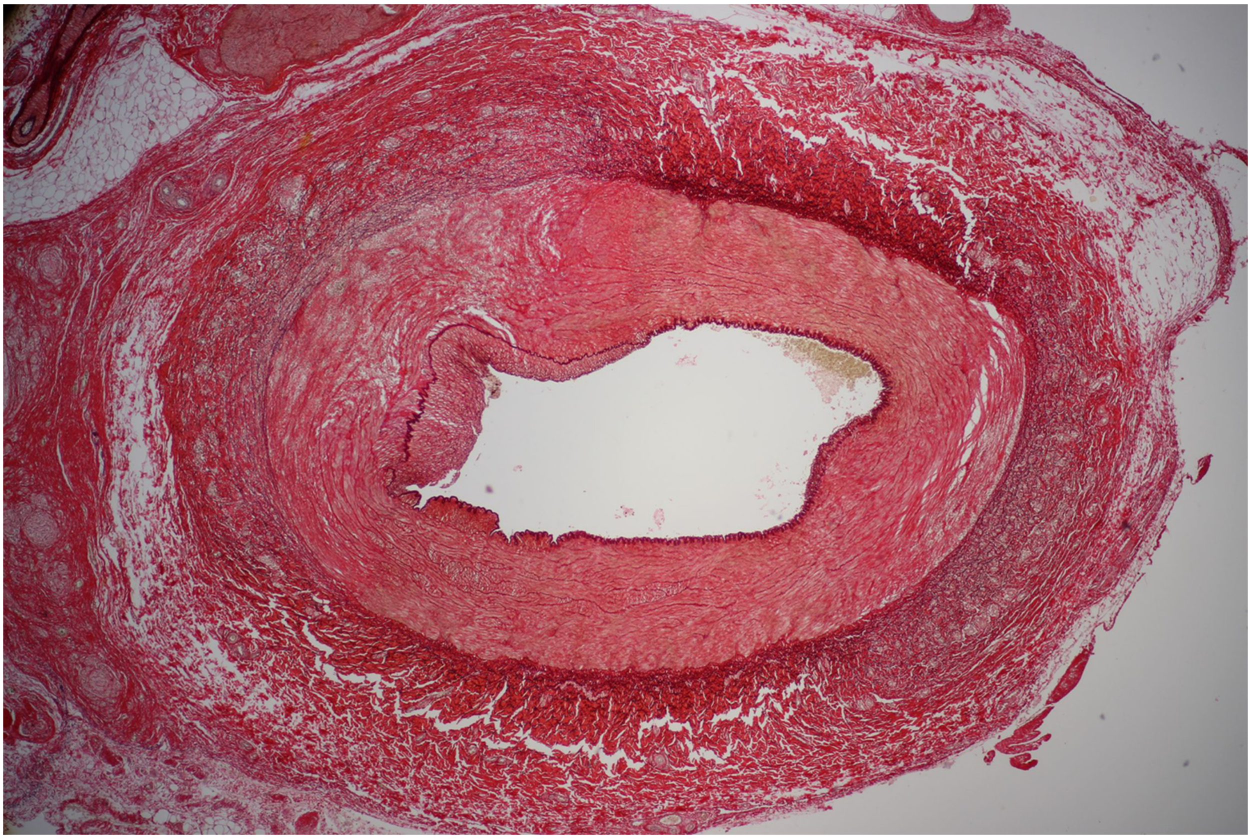

Figure 4 Histological finding of renal artery 40 days after successful renal denervation. In the left portion of the image extensive fibrosis of the intima, the media and fragmentation of the lamina elastica externa and the adventicia depicted.

The prolongation of ERP is a sign of lower ventricular myocardial excitability. By analogy with former experimental studies in dogs, surgical excision of the left stellate ganglion prolonged ERP of 4–7 ms [22] and raised the VF threshold [23]. This indicates that despite ablation of perirenal sympathetic nerves, RDN has a similar effect as the direct destruction of heart-related sympathetic ganglia and nerves. Moreover, the antiarrhythmic effect of RDN seems to be comparable to stimulation of the local parasympathetic system. As described by André et al. [24], stimulation of the vagal nerve on isolated rabbit hearts in vitro prolonged ERP by 13% (vs. 14% in this study) and VF threshold was significantly higher. In vivo data demonstrated the anti-arrhythmic effect of vagal stimulation especially in preventing decrease of VF threshold during the stimulation of the sympathetic nerves [25].

Nevertheless, using the same PVS protocol we did not prove the benefit of RDN in terms of decrease in VF inducibility in this experimental setting (normal porcine heart). According to previously published clinical observational data by Bourke et al. [26], thoracic epidural anesthesia or surgical left cardiac sympathetic denervation allowed to reduce the incidence of ventricular tachycardia (VT) by 68% in 14 patients with structural heart disease refractory to pharmacotherapy and catheter ablation. As mentioned above, Linz et al. [18] reported a significant reduction of spontaneous ventricular extrabeats and VF episodes during acute myocardial ischemia and reperfusion in dogs after RDN. On the other hand, they also discussed that using a beta-blocking agent (atenolol) showed a comparable effect. In case reports by Ukena [19] and Hoffmann [20] concerning patients with dilated/hypertrophic cardiomyopathy or after acute myocardial infarction, RDN was effective in decreasing VT/VF events in addition to excessive antiarrhythmic medical and ablation therapy.

We can hypothesize that the influence of RDN could be more pronounced during the acute stress reaction or in the presence of an arrhythmogenic substrate (e.g. post-infarction scar) than in normal state. Also, our study cannot address the superiority of RDN compared to the use beta-blockers and other antiarrhythmic drugs.

As mentioned above, the study was limited by using healthy animals not considering any predisposition to ventricular arrhythmias like ischemic-reperfusion injury or structural changes of the myocardium. Further, electrophysiological parameters could be influenced by depth of analgosedation, despite the effort to maintain the same depth of anesthesia and similar dosage of propofol and morphine. Also the number of tested experimental animals was low and the electrophysiological study was not performed in the same biomodel before and after RDN because of presumed high morbidity-mortality of biomodels during the 40-day period after the procedure. Thus, the translation of the results to humans should be interpreted with caution despite the fact, that our biomodels were represented by a breed repeatedly validated for simulation of human cardiac arrest and evaluation of VF [27,28].

Conclusions

We can conclude that renal denervation affected the autonomic heart control significantly in favor of decreased tone of the sympathetic nervous system in a healthy porcine biomodel 40 days after RDN procedure. Unfortunately, these changes were not associated with lower ventricular fibrillation inducibility using the same PVS protocol in comparison with the control group.

Abbreviations

CPB: Cardiopulmonary bypass; ECMO: Extracorporal membrane oxygenation; EP: Electrophysiological study; ERP: Effective refractory period; HR: Heart rate; PVS: Programmed ventricular stimulation; RDN: Renal denervation; RV: Right ventricle; VF: Ventricular fibrillation; VT: Ventricular tachycardia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JCL, JK: study conception and design; JCL, JK, MM, MCH, PN, AL: animal experiment conduction, hemodynamic data acquisition, analysis and interpretation; JCL, AL, PN, OK: critical revision and final approval of the manuscript. All authors have read and approved the manuscript for publication.

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Author details

[1]2nd Department of Medicine - Department of Cardiovascular Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, U Nemocnice 2, Prague 2 128 00, Czech Republic. [2]Institute of Physiology, First Faculty of Medicine, Charles University in Prague, Albertov 5, Prague 2 128 00, Czech Republic. [3]3rd Department of Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital, U Nemocnice 2, Prague 2 128 00, Czech Republic. [4]Department of Cardiology, Na Homolce Hospital, Roentgenova 2/37, Prague 5 150 30, Czech Republic.

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