

RAPID COMMUNICATION

Nitrotyrosine and nitrate/nitrite levels in cardiac arrest survivors treated with endovascular hypothermia

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Short Title: Oxidative Stress in Cardiac Arrest Survivors

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SUMMARY

The protective effect of therapeutic hypothermia in cardiac arrest survivors (CAS) has been previously well documented. Animal studies have indicated that attenuation of tissue oxidative stress (OS) may be involved in the mechanisms that lead to the beneficial effect of hypothermia. The extent of OS and nitric oxide (NO) production in adult CAS treated with endovascular hypothermia is, however, unknown.

A total of 11 adult patients who experienced cardiac arrest out of hospital were included in the present study, and all were treated with mild hypothermia using the Thermogard XP (Aelsius, USA) endovascular system. A target core temperature of 33°C was maintained for 24 hours, with a subsequent rewarming rate of 0.15°C per hour, followed by normothermia at 36.8°C. Blood samples for the measurement of nitrotyrosine and nitrate/nitrite levels were drawn at admission and every 6 hours thereafter for two days.

During the hypothermic period, the levels of nitrotyrosine and nitrates/nitrites were comparable with baseline values. During the rewarming period, serum levels of both parameters gradually increased and, during the normothermic period, the levels were significantly higher compared with hypothermic levels (nitrotyrosine, $P < 0.001$; nitrates/nitrites, $P < 0.05$).

In our study, significantly lower levels of nitrotyrosine and nitrates/nitrites were demonstrated during hypothermia compared with levels during the normothermic period in adult CAS. These data suggest that attenuation of OS and NO production may be involved in the protective effect of hypothermia in adult CAS.

Key words: cardiac arrest, hypothermia, nitrotyrosine, nitrates/nitrites, oxidative stress

Cardiopulmonary resuscitation is an essential, life-saving procedure. However, global ischemia during cardiac arrest induces the activation of several pathogenic pathways that potentially lead to fatal damage (ie, ischemia-reperfusion injury). This complex process is generally known as post-cardiac arrest syndrome (PCAS). The clinical impact of PCAS depends on various factors, particularly on the duration of global ischemia, comorbidities and the patient's condition before the cardiac arrest. Management of PCAS is based on the therapy used to treat the primary disease (e.g., myocardial infarction) and the prevention of the recurrence of circulation arrest, with the ultimate goals of hemodynamic stabilization and neuroprotection (Neumar *et al.* 2008; Nolan *et al.* 2008).

Many pathogenic mechanisms are involved in the development of PCAS, with oxidative stress (OS) likely playing an important role. OS is characterized by excessive production of reactive oxygen and nitrogen species (eg, superoxide, hydrogen peroxide, hydroxyl radical, nitric oxide and peroxynitrite) (Dhalla *et al.* 2000b). These very unstable molecules are produced during and after patient resuscitation and can react directly with important cellular structures or form other reactive products (Dhalla *et al.* 2000b). OS results in damage to cellular membranes, mitochondria, endoplasmic reticulum and/or nuclear DNA (Dhalla *et al.* 2000b). Under physiological conditions, the harmful effects of reactive oxygen and nitrogen species are usually prevented by the body's antioxidant defence systems (i.e., superoxide dismutase, catalase, glutathione peroxidase). However, antioxidant capacity to provide protection is insufficient during ischemia-reperfusion and does not effectively protect against cell damage (Dhalla *et al.* 2000b).

Mild hypothermia represents one possible therapeutic approach to prevent ischemia-reperfusion injury. Two randomized trials and their meta-analyses have

demonstrated clear evidence supporting improved outcomes in cardiac arrest survivors treated with mild hypothermia (Bernard *et al.* 2002; Holzer *et al.* 2005; Padosch *et al.* 2002). The fact that hypothermia favorably influences not only a single factor, but rather a variety of simultaneously functioning pathogenic processes, should be considered in the explanation of its beneficial effects.

Several experimental models have demonstrated that hypothermia inhibits the production of free radicals, decreases lipid peroxidation, and protects nuclear DNA from damage and fragmentation caused by reactive oxygen and nitrogen species (Han *et al.* 2002; Jiang *et al.* 2009; Karabiyikoglu *et al.* 2003; Scumpia *et al.* 2004; Stefanutti *et al.* 2005). To date, there is only limited evidence regarding the effect of hypothermia on OS in clinical settings, and studies focusing on OS in adult cardiac arrest survivors are lacking. To explore the hypothesis that hypothermia attenuates OS in cardiac arrest survivors, we designed a pilot study to investigate the extent of OS and nitric oxide (NO) production after cardiac arrest. The objective of our study was, therefore, to assess nitrotyrosine and nitrate/nitrite levels in cardiac arrest survivors treated with mild endovascular hypothermia.

The study was performed in accordance with the Declaration of Helsinki and the study protocol was approved by the Institutional Ethics Committee of the Na Homolce Hospital, Prague, Czech Republic. Surviving patients with favorable neurological outcomes, and family members of deceased subjects or those with unfavorable neurological outcomes provided written informed consent retrospectively. Blood samples drawn from patients who were not willing to participate in the trial (expressed by family members of deceased subjects or those with unfavorable neurological outcomes), were discarded and the clinical data were not used in the analysis.

Patients who experienced cardiac arrest out of hospital, with indications for mild therapeutic hypothermia, were eligible to participate in the present study. Induction of hypothermia was initiated in the ambulance before hospital admission by infusion of ice-cold saline at a rate of 30 mL/kg/hr. Infusion continued after admission and was discontinued when the core temperature reached 34°C. An endovascular cooling method was used in all patients (Thermogard XP, Alsius, USA). A triple-lumen cooling catheter (Icy catheter, Alsius, USA) was introduced into the femoral vein, and a catheter equipped with a temperature sensor was placed into the bladder within 30 minutes of admission. A target temperature of 33°C was set on the Thermogard XP console and maintained for 24 hours. A rewarming rate of 0.15°C per hour was used in all patients, with a final target temperature of 36.8°C; normothermia was subsequently maintained until awakening.

Blood samples for the measurement of nitrotyrosine and nitrate/nitrite levels were drawn at admission and every 6 hours thereafter for a total of 54 hours. The serum was immediately separated by centrifugation at 1500 rpm for five minutes and aliquots were stored at -70°C until measurement. 3-nitrotyrosine (nitrotyrosine) is a marker of peroxynitrite production; peroxynitrite results from the reaction of NO and superoxide (Beckman 1996). Nitrotyrosine levels were assessed with a standard ELISA method using monoclonal antibodies stocked in the hospital laboratory, the details of which are described elsewhere (Fisarkova *et al.* 2004). Nitrates/nitrites are indirect markers of NO production and have been measured in blood serum (Sun *et al.* 2010) using a Sievers chemiluminiscent nitric oxide analyzer (GE Analytical Instruments, USA).

Data were analyzed using a repeated-measures ANOVA test with Newman-Keuls multiple comparisons post-test. *P* values <0.05 were considered to be statistically significant. Values are expressed as mean ± SEM.

Fourteen patients were eligible to participate in the study during the enrollment period. One patient was excluded due to technical problems with blood-sample handling; two patients were excluded because written informed consents could not be obtained; therefore, 11 patients (mean age 58 years) were included in the study, the majority of whom (10 of 11) were male. The mean±SEM core temperature at admission was 34.9±0.2°C (baseline patient characteristics are summarized in Table 1) and all patients reached the target temperature within one hour of admission. Mean arterial blood pressure in all participants was maintained with norepinephrine and dobutamin, with a goal of 65 mmHg to 90 mmHg, none of them needed circulatory support device. All patients who experienced acute myocardial infarction underwent direct percutaneous coronary intervention.

Highly significant differences in nitrotyrosine levels ($P < 0.001$) were found: nitrotyrosine levels during the normothermic period (48 and 54 hours) were significantly higher ($P < 0.05$) compared with nitrotyrosine levels in samples drawn during the hypothermic period (0, 6, 12, 18, and 24 hours) (Figure 1A). Significant differences were observed in nitrate/nitrite levels ($P < 0.05$): the values in samples obtained at normal core temperature (48 and 54 hours) were significantly higher than the values obtained during early hypothermia (6 and 12 hours) ($P < 0.05$) (Figure 1B).

The major finding of our study was the reduction in nitrotyrosine and nitrate/nitrite levels during mild therapeutic hypothermia compared with the normothermic period in cardiac arrest survivors. It has been reported that OS plays an important role in the pathogenesis of ischemia-reperfusion injury (Dhalla *et al.*

2000a), and a rapid rise in markers of OS and NO production can be detected in various acute clinical situations such as trauma, stroke, liver failure or infection (Hayashi *et al.* 1999; Jiang *et al.* 2009). Mild hypothermia was introduced as a therapy for ischemia-reperfusion injury in cardiac arrest survivors after publication of clinical trials proving its effectiveness in improving clinical outcomes (Bernard *et al.* 2002; HACA-Study-Group 2002). Results of the present study have, for the first-time, shown that hypothermia may attenuate OS in these patients, which can at least partly explain its beneficial effect.

Suppression of OS and NO production using hypothermia has previously been described in animal models. Our results are consistent with several other previously published experimental observations. Jiang *et al.* (Jiang *et al.* 2009) described normalization of nitrate/nitrite levels and significant attenuation of NO synthase expression in the brain using hypothermia in a rat model of acute ischemic liver failure. Stefanutti *et al.* (Stefanutti *et al.* 2005) observed that hypothermia suppressed OS in a rat model of intestinal ischemia-reperfusion injury. Inhibition of NO production and myeloperoxidase-mediated damage in the hearts of endotoxemic rats was reported by Scumpia *et al.* (Scumpia *et al.* 2004). Han *et al.* (Han *et al.* 2002) found that hypothermia inhibited NO generation in experimental models of stroke and inflammation. Hypothermia-induced attenuation of NO synthase isoform expression was described by Karabiyikoglu *et al.* (Karabiyikoglu *et al.* 2003) after focal cerebral ischemia in rats. Finally, Lei *et al.* (Lei *et al.* 1994) observed decreased lipid peroxidation and preserved antioxidant defenses as a result of hypothermia in a canine model of resuscitated cardiac arrest.

Supportive clinical evidence of the effect of hypothermia on OS remains insufficient. Our observations are consistent with those of Hayashi *et al.* (Hayashi *et*

al. 1999), who described decreased nitrate/nitrite levels as a result of cerebral cooling to 32°C to 33°C in patients who experienced head injury and intracerebral hemorrhage. Similarly, Wensch *et al.* (Wensch *et al.* 1996) observed a reduction in reactive oxygen species production when mild hypothermia was induced during surgery. Recently, Bayir *et al.* (Bayir *et al.* 2009) published a well-designed study demonstrating the preservation of antioxidant defenses after initiation of therapeutic hypothermia following severe brain injury in infants and children.

Our study has several limitations, including the small number of patients enrolled and, especially, the absence of a control group. We believe, however, that it would have been unethical to arrange for a control group either by delaying the induction of hypothermia or maintaining a temperature other than the recommended temperature of 33°C during hypothermia. It can be speculated that the ischemia-reperfusion reaction that occurs after cardiac arrest, if not mitigated by hypothermia, induces an increase in OS burden comparable with the OS burden previously reported for infection, stroke or trauma (Hayashi *et al.* 1999; Jiang *et al.* 2009). OS and NO production can also be influenced by an imbalance in ionic (e.g, potassium or calcium) homeostasis and lactate acidosis. As per current clinical practice, however, these disturbances were stabilized in all patients during the early hours after admission, likely removing the possibility of significant variations in ionic homeostasis and lactate acidosis. Furthermore, it is unlikely that variations in core temperature would have influenced the levels of the analyzed parameters because the endovascular hypothermia system enables the maintenance of a stable core temperature at a predetermined target value.

In conclusion, our study describes the reduction of OS and NO production during therapeutic endovascular hypothermia in cardiac arrest survivors and,

therefore, provides indirect evidence that hypothermia may influence OS and NO production in these patients. It can be hypothesized that attenuation of OS and NO production is at least partially responsible for the beneficial effect of hypothermia in the clinical outcomes of cardiac arrest survivors.

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FIGURE LEGEND

Figure 1. Levels of nitrotyrosine (Panel A) and nitrates/nitrites (Panel B) in cardiac arrest survivors treated with endovascular hypothermia.

Patients were maintained at a core temperature of 33°C for 24 hours. Rewarming was subsequently performed at a rate of 0.15°C per hour with a target temperature of 36.8°C; normothermia was controlled until awakening. Values are expressed as mean \pm SEM. * P<0.05 for comparisons with values at 0, 6, 12, 18, and 24 hours; # P<0.05 for comparisons with values at 6 and 12 hours.

TABLES

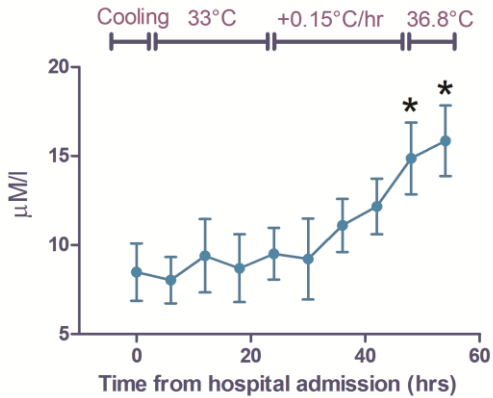
Table 1. Patient characteristics

Patient	First rhythm recorded	Structural heart disease	ROSC (min)	LVEF (%)	Alive (30 days)	CPC (30 days)
1	Asystole	AMI	8	20	Yes	1
2	VFib	AMI	15	40	Yes	3
3	VFib	AMI	17	50	Yes	4
4	VFib	Chronic CHD	12	40	Yes	1
5	VFib	AMI	16	15	Yes	1
6	VFib	Dilated CMP	25	15	No	
7	VFib	AMI	18	35	Yes	1
8	Asystole	None	15	55	Yes	1
9	Asystole	AoS	16	30	Yes	2
10	VFib	Chronic CHD	45	15	No	
11	VFib	None	8	50	Yes	1

ROSC, return of spontaneous circulation; LVEF, left ventricular ejection fraction measured within one hour of admission; CPC, cerebral performance category - CPC 1, conscious and alert with normal function or only slight disability; CPC 2, conscious and alert with moderate disability; CPC 3, conscious with severe disability; CPC 4, comatose or in a persistent vegetative state; and CPC 5, certifiably brain dead or dead by traditional criteria; VFib, ventricular fibrillation; AMI, acute myocardial infarction; CHD, coronary heart disease; CMP, cardiomyopathy; AoS, aortic stenosis.

Figure 1.

A. Nitrotyrosine



B. Nitrates/Nitrites

