EXPERIMENTAL PAPER

Rapid non-invasive external cooling to induce mild therapeutic hypothermia in adult human-sized swine

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Received 21 February 2007; received in revised form 1 July 2007; accepted 10 July 2007

KEYWORDS
Therapeutic hypothermia; Cardiopulmonary resuscitation; Cardiac arrest; Brain temperature; Swine

Summary
\textit{Aim of the study:} Mild therapeutic hypothermia is a promising new therapy for patients resuscitated from cardiac arrest. Early and fast induction of hypothermia seems to be crucial for best results. The aim of the study was to investigate the feasibility and safety of a new surface cooling method using cold metal plates.

\textit{Subjects and methods:} Twelve adult human-sized swine (79 ± 9 kg) were cooled from 38 to 33°C brain temperature. The skin surface was covered with \(-20°C\) metal plates (M), as compared to ice packs, alcohol rubs, and fans used in a control group (C). Each method was tested during spontaneous circulation and, after re-warming, during cardiac arrest. Temperatures were recorded continuously. Data are given as mean ± standard deviation or as median (interquartile range), if not normally distributed. Comparisons between the treatment groups were performed with the independent samples t-test, or the Mann–Whitney rank-sum test.

\textit{Results:} During spontaneous circulation, cooling rates were 9.3 ± 1.4°C/h (M), and 6.1 ± 1.4°C/h (C) (p = 0.003); no skin lesions were observed. During cardiac arrest, cooling rates were 4.1°C/h (1.8–4.8) (M), and 3.7°C/h (3.1–5.3) (C) (p = 0.9); no skin lesions were observed. 

\textit{Conclusion:} Cooling with cold metal plates was an effective method for rapid induction of mild therapeutic hypothermia in adult human-sized swine during spontaneous circulation, without any signs of skin damage. This new surface-cooling device, independent of energy supply during use, should be further investigated.

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Introduction

Mild therapeutic hypothermia is a promising new therapy for patients resuscitated from cardiac arrest, and has been recommended in the latest resuscitation guidelines by the European Resuscitation Council, and the American Heart Association. Therapeutic mild hypothermia might also be beneficial after stroke, traumatic brain injury, and myocardial infarction. To achieve best possible outcome, early and rapid induction of hypothermia is essential. The main challenge in the clinical scenario remains the immediate induction of mild hypothermia.

In recent years, several non-invasive external and invasive cooling strategies for induction of mild hypothermia were investigated. The main disadvantage of invasive blood cooling techniques out-of-hospital. Moreover, invasive cooling methods using a femoral central venous access bear the risk of complications of vascular catheterisation such as thrombosis, infection, and bleeding.

Hypothermia per se presents a relevant but reversible cause of reperfusion injury, and myocardial infarction. To achieve best possible outcome, early and rapid induction of hypothermia was investigated. The main disadvantage of invasive cooling methods using a femoral central venous access bear the risk of complications of vascular catheterisation such as thrombosis, infection, and bleeding.

Methods

The experimental protocol was approved by our institutional animal investigation committee. Animal care and handling were according to National Institutes of Health guidelines and were performed by qualified personnel and supervised by veterinarians. All facilities and transportation comply with the current legal requirements and guidelines. Twelve female swine (Pietrain × German domestic breed, 60–92 kg) were used after a 7-day period of quarantine and observation at the local animal care facility. The animals were fasted 12 h before the experiment with free access to water.

Anaesthesia, preparation, and monitoring

After intramuscular (IM) premedication with midazolam 1 mg/kg, acepromacine 1.75 mg/kg, piritramide 15 mg, and atropine sulphate 0.5 mg, swine were transferred to an operating room. A peripheral venous catheter was placed in an ear vein. Before tracheal intubation (tracheal tube size 8), propofol was given as an initial bolus injection of 200 mg, followed by boluses of 40 mg until intubation was possible. After intubation, swine were ventilated via a respirator (Servo 300, Siemens, Germany) with a tidal volume of 10 ml/kg, positive end-expiratory pressure of 5 cm H₂O, and FiO₂ of 0.3. The respiratory rate was adjusted to achieve a PaCO₂ of 4.7–5.3 kPa, and was kept constant thereafter during cooling.

For anaesthesia throughout the experiment, intravenous (IV) propofol (20 mg/(kg h)), and IM piritramide (60 mg every 2h) were used. For paralysis, rocuronium was given (bolus of 0.6 mg/kg, continuous IV infusion of 0.6 mg/(kg h)). Saline 0.9% was infused at a rate of 5 ml/(kg h). ECG electrodes and a pulse oxymeter were attached for heart rate (HR) and oxygen saturation (SpO₂) monitoring.

For brain temperature (Tbw) measurement, two temperature probes (Biosys, Vienna, Austria) were inserted into the parietal lobes through symmetrical burr holes, centered at 1.0 cm from the sagittal suture and 1.0 cm behind the coronal suture, to a depth of 2.0 cm under the dura mater. Bladder temperature (Tbl) was measured with a Foley catheter (Ruesch Sensor Ch 12, Ruesch, Kernen, Germany). Tympanic (Tty) and oesophageal (Tes) temperatures were measured with a contact thermometer (Mon-a-therm General Purpose Critical Care Temperature Probes, Mallinckrodt Medical Inc., Northampton, UK). An arterial catheter was inserted by Seldinger technique into the left brachial artery for arterial pressure (AP) measurements and blood sampling at baseline and after cooling during spontaneous circulation. A pulmonary artery catheter (Edwards, Irvine, CA) was advanced by Seldinger technique via the right internal jugular vein for measurements of central venous pressure (CVP) and pulmonary artery temperature (Tpa). For re-warming, two venous bypass cannulas (Medtronic®, 17 F, right side: 50 cm, left side: 20 cm) were introduced into both femoral veins via surgical cut-down. After insertion of the cannulae, 10,000 IU of unfractioned heparin were given intravenously, and repeated if necessary by monitoring the activated clotting time.

All monitored haemodynamic and respiratory variables and temperatures were stored in a computerised data management system (VIPDAS, Biosys, Vienna, Austria). Arterial blood gas analysis including acid–base values, electrolytes (PaCO₂, PaO₂, pH, base excess, bicarbonate, K⁺, Na⁺, Ca²⁺, Cl⁻, glucose, lactate) and haemoglobin was performed at the given time points mentioned above with a blood gas analyser (AVL 995 Hb, Roche Diagnostics).

Hypotension was treated with intravenous infusion of hydroxyethyl starch or, if necessary, with continuous infusion of noradrenaline (norepinephrine) to maintain a mean arterial pressure (MAP) of more than 60 mmHg.

Cooling device with cold metal

This external cooling device consisted of multiple metallic cooling plates in different sizes (prototype provided by Emcools, Emergency Medical Cooling Systems AG, Vienna, Austria). These plates were pre-cooled to –20 °C until shortly before the experiment, and kept at this temperature in commercially available isolated cool boxes. Experiments
Rapid non-invasive external cooling to induce mild therapeutic hypothermia in adult human-sized swine were begun with the attachment of the plates to the skin by means of a silicone net, assuring a close contact of the plates to the skin. A body surface area of 0.5—0.6 m² was covered with 15—17 cooling plates each sized 10 cm by 35 cm.

Control group

Control group animals were cooled conventionally with ice packs, alcoholic hand disinfectant, and fans. Twenty kilograms of ice was distributed to 8 packs that were placed on the animals. Simultaneously, alcoholic hand disinfectant was repeatedly sprayed on the abdomen and neck of the animal with two fans directed to the abdomen and the head, to enhance evaporative cooling.

Study protocol

Cooling methods were tested without randomisation, each cooling method in a separate group, first during spontaneous circulation, and after re-warming, during cardiac arrest.

Animals were allowed to stabilise for 1 h after surgical intervention, before baseline measurements were done. Thereafter, the swine were cooled from 38 °C to target 33 °C \(T_{br}\). In the cold metal group, cooling was discontinued at 33.5 °C to prevent temperature-overshooting as observed in pilot experiments. Temperatures and haemodynamics were monitored for 1 h after start of cooling.

Thereafter, the swine were re-warmed via veno-venous bypass linked to a heater—cooler system (Stoeckert Heater—Cooler System, Stoeckert Instruments, Munich, Germany). Additionally, surface warming blankets (Bair Hugger) were used. As soon as \(T_{br}\) of 38.0 °C was restored, a temperature equilibration time of 1 h was started. Thereafter, ventricular fibrillation was induced to test the same cooling method during cardiac arrest no-flow. Temperatures were observed for 1 h after start of cooling.

Statistical analysis

Data are given as mean±standard deviation, or as median and interquartile range, if not normally distributed (Shapiro—Wilks test). Comparisons between the groups were performed with independent samples t-test, or the Mann—Whitney rank-sum test for data not normally distributed. A p-value <0.05 was considered statistically significant. All calculations were performed with SPSS 13.0 for Mac OS X (SPSS Inc., Chicago, U.S.A.).

Results

Cooling was performed in six swine per group (cold metal: 78 ± 8 kg; controls: 79 ± 11 kg; p = 0.8).

Cooling during spontaneous circulation

At baseline, haemodynamic and laboratory data were within physiological range without group differences. One swine in the control group did not reach target \(T_{br}\) of 33 °C within the 60 min observation period. The brain temperature curves during spontaneous circulation are shown in Figure 1A. Calculated cooling rates were 9.3 ± 1.4 °C/h for cold metal cooling, and 6.1 ± 1.4 °C/h for the control group (p = 0.003).

During cooling with cold metal, the maximum temperature gradients to brain temperature were +0.5 °C for oesophagus, followed by pulmonary artery −1.0 °C, tympanum +1.1 °C, and bladder +1.8 °C; maximum temperature gradients to brain temperature in the control group were as follows: oesophagus +0.5 °C, bladder +0.6 °C, tympanum +0.9 °C, and pulmonary artery −1.1 °C (data are not shown, but available on request).

HR and MAP during the 1-h observation period are shown in Figures 2 and 3. HR decreased during cooling in both groups, and was significantly lower at \(T_{br}\) of 34 °C than at baseline in the cold metal group, without differences between groups at baseline or at \(T_{br}\) of 34 °C (Table 1). MAP decreased during cooling in both groups, and was significantly lower at \(T_{br}\) of 34 °C than at baseline in the cold metal group, without differences between groups at baseline or at \(T_{br}\) of 34 °C (Table 1).

Laboratory values were investigated in both groups at baseline, and in the cold metal group after cooling (data are not shown, but available on request). At baseline,
values were mainly within physiological ranges, except elevated PaO2 levels despite a FiO2 of 0.21, and slightly elevated serum lactate. After cooling during spontaneous circulation, in the cold metal cooling group, PaO2 and chloride were increased, while lactate and PaCO2 levels were decreased. However, all values were within normal range except PaO2 and PaCO2, which both resulted from constant ventilatory variables as determined in the Methods section.

None of the swine developed significant haemolysis, arrhythmias, or bleeding. Neither group showed macroscopic signs of skin damage at the end of the observation period.

Comparison of cooling rates between cooling during spontaneous circulation and cooling during cardiac arrest

With cold metal cooling, cooling rates were significantly faster during spontaneous circulation as compared to cardiac arrest (8.9 °C/h [8.3–10.8] versus 4.1 °C/h [1.8–4.8]; p = 0.028). In the control group, differences were not significant (6.7 °C/h [4.8–7.1] versus 3.7 °C/h [3.1–5.3]; p = 0.17) (Figure 4).

Discussion

In this adult human-sized swine study, a new external surface-cooling device proved to be simple and safe for the fast induction of mild therapeutic hypothermia during spontaneous circulation, and to a lesser extent, during cardiac arrest no-flow. A cooling rate of 9.3 ± 1.4 °C/h with externally applied cold metal during spontaneous circulation was considerably faster compared to more invasive cooling methods as veno-venous blood-shunt cooling (8.2 ± 2.8 °C/h), or endovascular cooling (2.6 ± 2.8 °C/h), which were investigated recently in the same large swine model.29 It is essential that mild hypothermia is applied very early after the ischaemic insult to be effective; otherwise, the beneficial effects would be diminished or even abrogated.13–19 Additionally, it can be expected that faster cooling and thereby earlier achievement of target temperature will further improve cerebral outcome. Therefore, new cooling methods should be effective in terms of fast cooling

Table 1 Heart rate and mean arterial pressure during cooling with spontaneous circulation

<table>
<thead>
<tr>
<th>Tbr</th>
<th>HR</th>
<th>p</th>
<th>MAP</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 °C</td>
<td>34 °C</td>
<td>38 °C</td>
<td>34 °C</td>
</tr>
<tr>
<td>metal</td>
<td>96 (78–102)</td>
<td>72 (68–77)</td>
<td>0.028</td>
<td>66 (59–79)</td>
</tr>
<tr>
<td>ctrl</td>
<td>91 (86–110)</td>
<td>69 (65–78)</td>
<td>0.08</td>
<td>69 (60–81)</td>
</tr>
<tr>
<td>p</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
<td>0.08</td>
</tr>
</tbody>
</table>

HR, heart rate; MAP, mean arterial pressure; Tbr, brain temperature; metal, cold metal cooling; ctrl, control group. HR (min⁻¹) and MAP (mm Hg) are given as median and interquartile range (25–75th percentile); between groups analysis (vertical): Mann–Whitney test, between temperatures analysis (horizontal): Wilcoxon Signed Ranks Test.
Figure 4  Cooling rate as a function of cooling method and circulatory state. The box represents the interquartile range. The line across each box indicates the median. The whiskers (L.T) are the highest and lowest values, * cases with values that are between 1.5 and 3 box lengths from either end of the box.

rates and easy to apply already in the pre-clinical setting. The new external cooling method investigated in this study fulfills these requirements. Its major advantage might be the independence from energy supply during use, making it particularly suitable for emergency use outside the hospital. The cooling plates were further developed into cooling pads, filled with a mixture of water and graphite, showing promising results in a very recent preliminary clinical study.37

The external surface cooling methods that were used so far in clinical trials after cardiac arrest showed very slow cooling rates, ranging from 0.3 to 1.5 °C/h.1–3 Recent new devices in surface cooling include water-circulating adhesive pads that have been shown to reduce fever in neuro-intensive care patients,21 and to lower patient temperature at a median cooling rate of 1.2 °C/h in cardiac arrest survivors.22 In the quest for faster cooling rates, more invasive cooling methods have been explored. Intravenous application of 30 ml/kg ice-cooled saline over 30 min has recently been reported to cool core temperature by 1.6 °C,27 but to maintain mild therapeutic hypothermia, an additional cooling method is necessary.38,39 Endovascular cooling devices are inserted via Seldinger technique into a large vein such as the inferior vena cava. Cold fluid is continuously pumped through balloons along the catheter and does not enter the circulating blood. This method has been reported to be safe and delivers cooling rates ranging from 0.9 to 4.5 °C/h.12,23–26 Further invasive techniques include extracorporeal veno-venous cooling with a heat exchanger that cools blood, which is drained from a large vein and re-infused using a roller pump. This technique reportedly delivers cooling rates up to 3.5 °C/h in clinical use,28 and with an optimised technique up to 8.2 °C/h in adult human-sized swine.29 The main disadvantage of invasive cooling methods is the demand for energy supply and for trained medical personnel. These requirements exclude the use of invasive cooling methods early after successful resuscitation outside the hospital.

We also tested the feasibility of external cooling during cardiac arrest no-flow. It was shown in myocytes that injury to ischaemic cells takes place after reperfusion by initiating several cascades leading to cell death, but not during ischaemia itself.40–42 When ischaemic myocytes were made hypothermic before reperfusion, injury to the cells was less, even when the duration of ischaemia was prolonged compared to cells with normothermic reperfusion.43 In a cardiac arrest mice model, induction of moderate hypothermia to 30 °C just before attempted resuscitation, resulted in better 72-h survival as compared to mice, in which induction of hypothermia was delayed to 30 min after start of resuscitation.44 In this study, hypothermia was achieved within 2–3 min by putting an ice water-filled surgical glove on the animal. In large animals or humans, the induction of hypothermia during cardiac arrest might be more challenging. In our study, cooling with cold metal achieved a calculated cooling rate of 4.1 °C/h during cardiac arrest no flow. This cooling rate is substantially faster compared to cooling rates from surface-cooling devices in clinical use during spontaneous circulation,1–3 but no temperature change was observed within the first 10–15 min of cardiac arrest no-flow (Figure 1B). We speculate that starting chest compressions early would further enhance the cooling efficacy of cold metal during cardiac arrest. In dogs, mild hypothermia induced with veno-venous blood-shunt cooling during chest compressions improved outcome compared to normothermic animals.45 If cooling with cold metal can induce hypothermia early during chest compressions, resulting in improved outcome, this needs to be investigated in further studies.

Cooling with cold metal did not cause adverse skin reactions. In a subsequent unpublished study on cooling with cold metal in this animal model, a histopathological analysis of skin sections taken at multiple time points during the first 24 h after cooling revealed no affection of skin integrity to any degree. However, skin thickness and subcutaneous tissue depths are certainly different in humans and swine of similar size, and further experiments are needed to calculate safe time limits before skin freezing might occur.

We measured temperatures at various sites, with brain temperature being the target temperature. In clinical routine, the brain temperature is not available, and the temperature site best reflecting the brain temperature yet has to be found. Several studies have reported suitable surrogates for brain temperature measurement, such as bladder temperature,46 rectal temperature,47 and jugular bulb temperature.48–50 These studies quote temperature gradients smaller than 0.2 °C between jugular bulb, subdural, tympanic membrane, pulmonary artery, and bladder temperature. These findings may be true in steady-state
rapid induction of mild therapeutic hypothermia in adult cooling with cold metal was an effective method for
functions, and survival. Possible long-term effects of this method on skin, organ
validity of the data. We evaluated histological damage to the skin after cooling with cold metal not longer than 24 h; non-randomised study design had no influence on the team members, and the same protocol, we assume that performed within 3 months, in the same lab, with the same metal was performed later. Because all experiments were performed within 3 months, in the same lab, with the same team members, and the same protocol, we assume that the non-randomised study design had no influence on the validity of the data. We evaluated histological damage to the skin after cooling with cold metal not longer than 24 h; only future long-term experiments will allow evaluation of possible long-term effects of this method on skin, organ functions, and survival.

Conclusions
Cooling with cold metal was an effective method for rapid induction of mild therapeutic hypothermia in adult human-sized swine during spontaneous circulation, without any signs of skin damage. This novel external cooling device, independent of energy supply during use, should be further investigated for the use in the out-of-hospital setting.

Conflict of interest
Dr. Behringer is a paid consultant and stock owner of Emcools, Emergency Medical Cooling Systems AG, Vienna, Austria. Both, Dr. Behringer and Dr. Sterz, hold patent rights regarding the reported cooling method invented by Emcools, Emergency Medical Cooling Systems AG. All other authors have no conflict of interest.

Acknowledgments
We acknowledge the inventive talents of Rudolf Faworka who has dedicated his work to the development and refinement of cold metal cooling. Furthermore, we would like to thank Nikolaus Wick (Clinical Institute of Pathology) for his histological contribution, and the nurse staff at the Institute for Biomedical Research for their support.

Funding: The study was made possible through a generous support by a Supplementary Assignment of the Austrian Councils for Development of Research and Technology (BMBWK GZ: 11.100/6-VII/1/2002 3.6.2002). The funding source did not have any involvement in study design, collection, analysis and interpretation of data nor in the writing of the manuscript or in the decision to submit the manuscript for publication.

References

Limitations
There are several limitations in the present study. We used healthy animals without heart or vascular disease, and without a preceding cardiac arrest before cooling during spontaneous circulation, which is in contrast to human cardiac arrest victims. Haemodynamics and the efficacy of external cooling methods might differ after a period of no-flow and low-flow. Group assignment did not follow a randomised order. The cooling device with cold metal was developed during the study of the control group, comparing cooling during spontaneous circulation with cooling during cardiac arrest no-flow; therefore the study with cold metal was performed later. Because all experiments were performed within 3 months, in the same lab, with the same team members, and the same protocol, we assume that the non-randomised study design had no influence on the validity of the data. We evaluated histological damage to the skin after cooling with cold metal not longer than 24 h; only future long-term experiments will allow evaluation of possible long-term effects of this method on skin, organ functions, and survival.
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40. Vanden Hoek TL, Li C, Shao Z, Schumacker PT, Becker LB. Significant levels of oxidants are generated by isolated cardiomyocytes during ischemia prior to reperfusion. J Mol Cell Cardiol 1997;29:2571–83.


