In vivo effects of hypothermia on the microcirculation during extracorporeal circulation

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Abstract

Objective: Induced hypothermia has been shown to be protective during cardiac surgery, but also in traumatic, ischemic, burn, and neurological injury. In previous in vivo animal experiments, we documented increased leukocyte/endothelial (L/E) cell interaction following normothermic extracorporeal blood circulation (ECC). This study was carried out to investigate whether reduced core temperature during ECC affects the damage to the microcirculation as evidenced by leukocyte adherence and edema formation. Methods: Intravital fluorescence microscopy was used on the dorsal skinfold chamber preparation in Syrian golden hamsters. ECC was introduced via a micro-rollerpump (1 ml/min) and a 60 cm silicon tube (1 mm inner diameter) shunted between the carotid artery and the jugular vein after application of 300 IE Heparin/kg per body weight. Experiments were performed in chronically instrumented, awake animals (age 10-14 weeks, weight 65-75 g). Animals of the experimental group were cooled to 18 °C body temperature while ECC, followed by a rewarming period (n = 7), controls experienced ECC under normothermia (37 °C, n = 7). Results: 30 min ECC at 18 °C resulted in a decrease of rolling and adherent leukocytes (stickers) in postcapillary venules after 1, 4 and 8 h compared with the control group (119 ± 46 vs. 274 ± 113 n/mm², P < 0.05, mean ± SD; n = 7 in each group). Functional capillary density was significantly reduced during hypothermia (80 ± 16 vs. 148 ± 16 cm/cm², P < 0.05), but restored after rewarming. In contrast, edema formation was markedly increased during hyperthermia. Conclusions: Hypothermia during ECC significantly reduced L/E cell interaction in the early post-ECC period. Hypothermia markedly reduced microvascular perfusion, but was completely restored upon rewarming. Despite a reduced number of adherent leukocytes, no protection of endothelial barrier function was seen as a consequence of induced hypothermia.

Keywords: Microcirculation; Extracorporeal circulation; Leukocyte/endothelial cell interaction; Hamster; Hypothermia

1. Introduction

Induced hypothermia affords tissue protection in a number of pathophysiological conditions, especially traumatic, ischemic, burn, and neurological injury [1]. The fact that metabolic rate and oxygen consumption are inversely related to the local temperature are the basis for use of hypothermia during cardiopulmonary bypass (CPB). However, the protective mechanisms of cold therapy as well as the hemodynamic changes in the microcirculation in response to hypothermia have to date not been studied in sufficient detail. Local cooling appears to inhibit the inflammatory response after ischemia reperfusion injury [2], the inflammatory response to TNF-alpha [3] and may hence prolong periods with impaired perfusion without damaging the microvasculature [4]. On the other hand, adverse effects on the plasma viscosity during CPB at low temperatures or increased edema formation may theoretically disturb the terminal network of the blood stream.

We have developed a model of extracorporeal blood circulation (ECC) that allows for direct visualization of the microcirculation in response to variations in extracorporeal perfusion modalities. In previous experiments in this model, we demonstrated that exposition of blood to an extracorporeal circuit induces dysregulation in the microcirculatory compartment [5]. We have now used this model to study systemic hypothermia. We hypothesized that hypothermia could prevent the systemic activation of cellular and humoral components during ECC, hence reduces the extent of injury to the microcirculation.

The present study was carried out to determine the extent by which hypothermia interferes with ECC-induced microcirculatory disturbances in the hamster skinfold model.
2. Material and methods

2.1. Model

Experiments were carried out in male Syrian golden hamsters (age 10–14 weeks, weight 65–75 g). The surgical technique has previously been described in detail [6,7]. Briefly, the dorsal skinfold, consisting of two layers of skin and muscle tissue, was fitted with two titanium frames with a 15-mm circular opening surgically installed under Narcoren (60 mg/kg body weight, Pentobarbital, Merial, Hallbergmoos, Germany) anesthesia. Layers of skin muscle were carefully separated from subcutaneous tissue and removed until a thin monolayer of muscle and one layer of intact skin remained. A cover glass held by one frame was placed on the exposed tissue, allowing intravital observation of the microvasculature. The second frame remained open, exposing intact skin.

Catheters were implanted in the jugular vein (polythene tubing 0.40×0.80 mm) and the carotid artery (polythene tubing 0.28×0.61 mm). All experiments were performed after a recovery period of at least 24 h after catheter implantation and 3.6±0.6 days (mean±SD) after chamber implantation. Chambers, catheters and instruments were sterilized before use; surgery was performed under aseptic conditions. Preparations with signs of inflammation, edema, bleeding spots and no flow were excluded from further investigations. Inclusion criteria for systemic parameters were: systemic mean arterial blood pressure (MAPB) greater than 90 mmHg and hematocrit over 45%. All animals received humane care in compliance with the European Convention on Animal Care and the study was approved by the Institutional and Regional Committee for Animal Care.

2.2. Intravital microscopy

Microscopic observations were performed using an intravital microscope (Leica DMLM, Wetzlar, Germany) with a 20×5W 0.40 BD NA objective (Leica Fluotar, Wetzlar, Germany). A 100-W Hg light source was used for epi-illumination. Contrast enhancement for transillumination was accomplished with a blue filter (420 nm), which selectively passes light in the maximum absorption band of hemoglobin, causing red blood cells to appear as dark objects in an otherwise gray background. A heat filter was placed in the light path prior to the condenser. Microscopic images were viewed by a closed circuit video system consisting of a CCD camera (Kappa CF 8/4 NIR, Gleichen, Germany) and a RGB-monitor (Sony, Japan).

Leukocytes were stained with rhodamine 6 (Sigma, St Louis, MO) and classified by fluorescence microscopy according to their interaction with the endothelial lining as adherent, rolling or free flowing cells. Adherent leukocytes were defined in each vessel segment as cells that did not move or detach from the endothelial lining within an observation period of 30 s and are expressed as number of cells per square millimeter (cells/mm²) of vessel surface as calculated from diameter and length (100 μm) of the vessel segment studied. Rolling leukocytes are expressed as percentage of non-adherent leukocytes passing through the observed vessel segment within 30 s.

Red blood cell (RBC) velocity and diameters were measured in five venules per observation chamber using a computer-assisted analysis system (Capimage, Dr Zeintl Ingenieurbuero, Heidelberg, Germany).

Functional capillary density (FCD) was assessed in nine successive microscopic fields by transillumination in a region of approximately 1.3 mm²; the initial field was chosen where microvessels were in focus and there were usually between two and five RBC-perfused capillaries in the field of view. Systematic observations were achieved by displacing the microscopic field of view in three consecutive 450 μm steps in the lateral direction (relative to the observer). The same procedure was repeated after moving the image by one microscopic field in the vertical (333 μm) direction. FCD was evaluated by measuring the length of capillaries that had red blood cell flow. A capillary was defined to be functional if passing RBCs were noted within the entire 20-s observation period.

All intravital microscopic observations (on-line) were recorded and evaluated later (off-line) in order to minimize the duration of the experiment and the time of light exposure to the tissue.

Arterial blood pressure and heart rate were monitored via carotid artery connected to a transducer (Bently, Uden, Holland) with an analog recording system (Hellige, Freiburg, Germany) for continuous measurements.

2.3. Extracorporeal circulation (ECC)

ECC was introduced via a micro-rollerpump (ISM 833A, Ismatec, Glattbrugg-Zürich, Switzerland) and a silastic tube (1 mm inner diameter, length 60 cm, Migge, Heidelberg, Germany) shunted between the carotid artery and the jugular vein. The sterilized extracorporeal circuit was primed with 1 ml Ringer’s solution and a flow rate of 0.7 ml/min was used. Animals tolerate these procedures and show no signs of discomfort or significant changes in blood pressure or heart rate. The expected cardiac output of hamsters is 461±29 ml/min per kg per body weight [8]. The percentage of the cardiac output represented by the ECC is 3% per minute, but within 60 min 800% of the total hamster blood volume (5.25 ml/70 g per body weight) contacted the ECC system.

2.4. Blood analysis

Blood analysis was performed in all animals at BL, 30 min, 4 and 8 h after onset of ECC using 100 μl full blood, obtained from the ECC circuit (COULTER® AcT™ Hematology Analyzer, Beckmann Coulter GmbH, Krefeld, Germany).

2.5. Microvascular permeability

Microvascular permeability was analyzed by quantifying the extravasation of the macromolecular fluorescent marker FITC-Dextran (Mw 150,000) with the computer-assisted microcirculation analysis system (CAPIMAGE) [9]. Measurements were performed 15 min after intravenous injection of the fluorescent marker. In each vessel, gray-levels over
the tissue directly adjacent to the vessel wall \((E_1)\) and over the marginal cell-free plasma layer within the vessel \((E_2)\) were determined over a length of at least 100 \(\mu\)m. Extravasation \((E)\) was then calculated as \(E = E_1/E_2\).

2.6. Induction of hypothermia

For temperature control, a specially designed restrainer-cooling chamber with a surrounding tube system, allowing for temperature control, was developed. In a pilot study, seven hamsters underwent cooling to 18 °C in the restrainer-cooling chamber within 1 h and slowly rewarmed afterwards. The animals were observed for the following 7 days and did not show signs of discomfort or changes in behavior or feeding habits. In the control group, ECC and restrainer-cooling chamber temperature were adjusted to maintain the rectal temperature of the animals at 37 °C, thus preventing temperature changes during the experiment. In the experimental group, the ECC blood and the restrainer-cooling chamber were cooled to 20 °C for 5 min, allowing the awake animal to adapt on the new situation. In a second step, ECC temperature and cooling chamber temperature were lowered to 15 °C until the core temperature of the animals reached 18 °C after 10 min, then to 18 °C. ECC was performed for 30 min at 18 °C followed by a 15 min warming period to base line temperature.

2.7. Experimental protocol

Fourteen animals undergoing 1 h of isovolemic ECC were studied in a randomized way. After intravenous application of rhodamine 6G (2 \(\mu\)g/40 \(\mu\)l saline) and heparin (300 IU/kg body weight in 200 \(\mu\)l Ringer’s solution; Braun AG, Melsungen, Germany) baseline measurements of FCD, vessel diameter, RBC velocity and leukocyte/endothelium interaction were performed. MABP and heart rate were monitored and blood samples were collected from the carotid artery catheter. Follow-up measurements were performed at time points (TP) 30 min, 4 and 8 h. Control group \((n=7)\) received normothermic ECC. In the experimental group \((n=7)\), ECC was performed under hypothermia at 18 °C.

2.8. Statistical analysis

Data were analyzed using SPSS®—statistical analyzing software (SPSS Software, Inc., Chicago, USA) using ANOVA, \(t\)-test or Wilcoxon test with Bonferroni correction. Changes were deemed statistically significant at a level of \(P<0.05\).

3. Results

3.1. Core body temperature

Fifteen minutes after induction of hypothermia the core temperature of the animals reached 18.2 ± 0.9 °C (mean ± SD). This temperature was maintained for 30 min during time of ECC. Rewarming started and the core temperature returned to baseline values within 15 min (Fig. 1).

3.2. Leukocytes in postcapillary venules

Under baseline conditions, the majority of fluorescently labeled leukocytes did not interact with endothelial cells in postcapillary and collecting venules (Fig. 2). In analogy to previous observations [5], ECC under normothermia (control) resulted in a significant increase in rolling and adhesion of leukocytes in postcapillary venules with a maximum for rollers at 4 h and adherent leukocytes at 8 h after onset of ECC \((P<0.05\) vs. Baseline, Fig. 3). In the hypothermic group, the number of rolling and adherent leukocytes was lower than in controls \((P<0.05)\).

3.3. Leukocyte/endothelial cell interaction in arterioles

Under baseline conditions, the majority of leukocytes did not interact with endothelial cells in arterioles (Fig. 4). Leukocyte adhesion increased at TP 8 h in both groups without a statistically significant difference. However, note that the extent of arteriolar adhesion was only a fraction of leukocyte adhesion in venules. It was not possible to detect rolling leukocytes in arterioles.
3.4. Functional capillary density

FCD was unaffected in controls. However, hypothermia reduced FCD after 1 h (P < 0.05; control vs. experimental). FCD completely recovered at TP 8 h (Fig. 5).

3.5. Microvascular permeability

Edema formation was evident in all microvascular compartments after ECC. Compared to baseline levels, extravasation around arterioles was more pronounced at TP 1 h after hypothermic ECC (P < 0.05) and increased in both groups after 4 and 8 h (Fig. 6).

3.6. Microhemodynamic parameters

The diameters of postcapillary venules in postcapillary venules were not significantly affected (Table 1). Red blood cell velocities in postcapillary venules after hypothermic ECC were slightly reduced at 1 h and tended to be slightly increased after 8 h compared to control group. In arterioles, RBC velocity was reduced at TP 1 h, but reached baseline levels in the follow-up period. None of these changes of RBC velocity reached any level of statistical significance.

3.7. Macrohemodynamic parameters

Arterial blood pressure and heart rate were stable and not significantly affected by ECC or hypothermic treatment (data not shown). During the time of the experiments, no states of low pressure were observed.

3.8. Blood analysis

Analysis of hemoglobin and hematocrit in full blood decreased throughout the experiment in all animals, irrespective of body temperature, but these changes were not statistically significant.

3.9. Anticoagulation

Application of 300 IU/kg per body weight heparin resulted in a prolongation of partial thromboplastine time (PTT) to more than 300 s at the end of ECC compared to a baseline level of 26.5 ± 5.9 s (n = 3, not shown).

4. Discussion

The principal findings of this study are that (1) ECC and systemic hypothermia to 18 degree centigrade significantly reduced functional capillary density (FCD) in the microcirculation within 1 h. (2) ECC induced rolling and adhesion of activated leukocytes in postcapillary venules and arterioles was significantly reduced, suggesting a protective effect of hypothermia. (3) In contrast during early hypothermia edema formation was markedly enhanced.
Administration of hypothermia is a mainstay of clinical supportive treatment for a variety of traumatic, neurological and ischemic conditions [1,10]. Tissue metabolic rate and oxygen consumption are inversely related to local temperature [11], but the exact nature of the protective mechanisms is incompletely understood. We were interested in the effects of systemic hypothermia on the ECC-induced systemic inflammatory reaction. Blood contact with an artificial surface (CPB, ECMO, hemodilution) induces activation of numerous humoral and cellular mediators and results in a systemic inflammatory reaction. In turn this leads to the activation of neutrophils, which upregulate adhesion molecules and damage tissue after adhesion to endothelial cells [12]. Neutrophils play a pivotal role because of their ability to release oxygen-derived free radicals and proteolytic enzymes with subsequent tissue damage [13]. In previous studies, using the same animal model, we demonstrated in vivo that ECC induces Leukocyte/Endothelial (L/E) cell interaction and that the intensity of induction was ECC time dependent and related to the production of oxygen-derived free radicals [5,14]. The cooling device, especially designed for the present study, allows a combined cooling process, internally via ECC system and, the major effect, externally via the cooling chamber, thus giving control over the systemic temperature of the animal, as established in pilot experiments.

Our finding that the systemic inflammatory response after ECC was inhibited by hypothermia, as expressed by reduced L/E interaction, is in accordance with results from Thorlacius and co-workers [4], who described a protective effect of local cooling after ischemia/reperfusion to 8 °C. In an experimental study in pigs by Qing and co-workers [15] the systemic application of moderate hypothermia (28 °C) during ECC showed better results compared to deeper hypothermia to 20 °C as evidenced by reduced TNF-alpha production and histological determination of organ damage. The inhibition of the inflammatory response results in a reduction of leukocyte migration ability, which is thought to mediate the protective effect of hypothermia on tissue microcirculation [16].

Inasmuch as oxygen radicals are involved in the upregulation of adhesion molecules during ECC [12,17], the observed reduction in ECC-induced L/E cell adhesion may, at least in part, result from the inhibition of the release of oxidants by activated leukocytes under hypothermia as described by Frohlich and co-workers [18]. This is also supported by the clinical observation that along with myocardial ischemia, coagulopathy, bleeding, and metabolic disorders, mild hypothermia predisposes patients to immunodepression and sepsis. In a series of patients undergoing colorectal surgery, perioperative mild hypothermia increased the incidence of wound infections [19]. In addition, temperature elevation has been shown to enhance several parameters of immune function, including antibody production, T-cell activation, neutrophile and macrophage function [20,21]. It may be well that this effect is reversed under hypothermia and that the tissue protective effect is due to a decreased metabolism of the above-mentioned cells.

Our findings of reduced FCD and increased extravasation early after hypothermia strengthen the hypothesis, that negative effects of deeper hypothermia are evident, opposing the oxygen saving effects due to a restricted microvascular perfusion. However, the extent, severity and time of hypothermia need to be carefully tailored to the clinical condition at stake and no undifferentiated standard recommendation can be derived for the mode of hypothermia under various conditions.

In cardio-thoracic surgery, edema formation is frequently seen in patients undergoing ECMO. In its extreme form, the capillary leak syndrome leads to massive edema and may even lead to organ failure [22]. An intriguing finding of the present study is hence the significantly aggravated occurrence of a microvascular leak after hypothermic ECC. Since macro-hemodynamic parameters were stable throughout the experiment in both control and experimental groups, tissue edema is likely not secondary to mechanically impaired tissue perfusion and/or drainage. The most reasonable explanation for the development of edema during inflammation is L/E cell interaction within the postcapillary segments of the microcirculation that may cause tissue damage [13]. However, hypothermia may induce vasoconstriction, thus decreasing hydrostatic and filtration pressure in the microcirculation, as suggested by Meeusen and co-workers [23]. Even though we were not able to detect any vasoconstriction in arterioles or changes in the flow on the level of the investigated vessels.

### Table 1

<table>
<thead>
<tr>
<th>Parameter (μm) venous</th>
<th>Experiment</th>
<th>Baseline</th>
<th>1 h</th>
<th>4 h</th>
<th>8 h</th>
</tr>
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<tr>
<td>Diameter</td>
<td>venous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 °C BT</td>
<td>37 °C BT</td>
<td>41.3 ± 5.2</td>
<td>40.3 ± 5.4</td>
<td>41.9 ± 5.4</td>
<td>41.1 ± 5.0</td>
</tr>
<tr>
<td>18 °C BT</td>
<td>18 °C BT</td>
<td>43.6 ± 7.0</td>
<td>41.3 ± 7.1</td>
<td>43.5 ± 7.8</td>
<td>43.6 ± 7.3</td>
</tr>
<tr>
<td>Diameter (μm) arterial</td>
<td>arterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 °C BT</td>
<td>37 °C BT</td>
<td>40.6 ± 6.5</td>
<td>38.9 ± 5.9</td>
<td>39.3 ± 6.2</td>
<td>40.2 ± 6.2</td>
</tr>
<tr>
<td>18 °C BT</td>
<td>18 °C BT</td>
<td>41.8 ± 6.5</td>
<td>34.7 ± 6.7</td>
<td>40.5 ± 4.9</td>
<td>41.2 ± 6.2</td>
</tr>
<tr>
<td>RBC velocity (mm/s)</td>
<td>venous</td>
<td>0.98 ± 0.32</td>
<td>0.95 ± 0.37</td>
<td>0.97 ± 0.40</td>
<td>1.02 ± 0.32</td>
</tr>
<tr>
<td>arterial</td>
<td>arterial</td>
<td>0.98 ± 0.24</td>
<td>0.87 ± 0.33</td>
<td>1.10 ± 0.43</td>
<td>1.24 ± 0.53</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>venous</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
</tr>
<tr>
<td>arterial</td>
<td>arterial</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>venous</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
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<tr>
<td>arterial</td>
<td>arterial</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
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</table>

Microvessel diameter and red blood cell velocity were assessed in venules and arterioles. Hemoglobin and hematocrit were taken from full blood. Control group (n=7 empty bars) received normothermia. In the experimental group (n=7 solid bars), hypothermia to 18 °C was applied. Data are mean ± SD.
(diameter 20–60 μm), this does not exclude vasconstriction at another level of the microvasculature as a reason for the reduced capillary perfusion. In previous experiments, we demonstrated a decrease in FCD under conditions of hyperoxia, assuming a vasconstriction/shunt proximal to the capillary network [14], and Tsai and co-workers were able to demonstrate vasconstriction of arterioles to explain this phenomenon. Of interest, they did not detect a reduction in oxygen delivery in the microcirculation using the Pd-phosphorescence quenching method [24], suggesting a compensatory mechanism, which is regulated upstream of the capillary level. Suggesting that such a regulatory mechanism ‘controls’ the amount of necessary oxygen in the terminal network, reduction of FCD might be a compensation in a hypothermic tissue, with temporarily reduced oxygen demand. This may prevent oxygen delivery in excess and save energy for the organism. This hypothesis is also supported by the fact that FCD is reduced only in the early phase after hypothermic ECC and rapidly returns to baseline values after discontinuation of hypothermia. Since macrohemodynamic parameters of all animals were stable during the time of the experiments, we do not expect to consider changes of capillary perfusion pressure and endothelial cell swelling, as described in states of low flow or shock [25] to be operative under the conditions of our experiment. Even though we did not specifically measure the width of red cell perfused capillaries, our subjective impression is that no such capillary compromise was present during or following hypothermia.

The moderate increase in RBC velocity, observed in both experimental groups, is most likely due to the decrease of hemoglobin and hematocrit values. Since the tube system is primed with Ringer’s solution, there is a minor isovolemic hemodilutional effect after starting the ECC, as it is present in the clinical setting of CPB, ECMO or dialysis.

A limitation of the study is the fact that no oxygenator, heater, cooler or cardiotomy suction devices are used in our experiments. In addition, effects of hypothermia without ECC on the microcirculation were not addressed; therefore, it is not possible to analyze the mechanistic effects of hypothermia separately. Yet, the standardized model of ECC in the awake hamster allows to investigate effects of blood contact with a foreign surface on the microcirculation under different temperatures.

In summary, the present observation is hard in that hypothermia induced protection from systemic inflammation (as evidenced by reduced leukocyte endothelial cell interaction) does not abrogate the energy and oxygen-sparing effects of hypothermia (as evidenced by reduced functional capillary density in the microcirculation), and that the increased breakdown of endothelial barrier function (as evidenced by increased macromolecular leakage) despite reduced systemic inflammation suggests that the particular pathophysiological conditions of ECC under hypothermia necessitate a thorough analysis of potential protection and also adverse effects of any further manipulation. A better understanding of the microcirculation under hypothermia and the knowledge of the ideal temperature may provide the basis for effective therapeutic interventions, before these are entered into clinical practice.

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References


