Effects of Hematocrit on Cerebral Microcirculation and Tissue Oxygenation During Deep Hypothermic Bypass

Lennart F. Duebener, MD; Takahiko Sakamoto, MD; Shin-ichi Hatsuoka, MD; Christof Stamm, MD; David Zurakowski, PhD; B. Vollmar, MD; Michael D. Menger, MD; Hans-Joachim Schäfers, MD; Richard A. Jonas, MD

Background—One rationale for hemodilution during hypothermic cardiopulmonary bypass (CPB) has been improved microcirculation. However, the optimal degree of hemodilution remains unclear. We therefore studied cerebral microcirculation and tissue oxygenation in a new intravital microscopic model at 3 different hematocrit (Hct) values.

Methods and Results—Three groups of 5 piglets with a cranial window over the parietal cortex underwent cooling at Hct of 10%, 20%, or 30%, followed by 1-hour deep hypothermic circulatory arrest (DHCA) and rewarming on CPB. For assessment of functional capillary density (FCD), plasma was labeled with fluorescein-isothiocyanate-dextran. Rhodamine-stained leukocytes were observed in postcapillary venules with analysis for adhesion and rolling. NADH, a natural intracellular fluorophore that increases during ischemia, was measured densitometrically during bypass and DHCA. FCD did not significantly differ from baseline during cooling in any group. However, during early reperfusion (5 minutes) after DHCA, the FCD was significantly higher in the Hct 30% group than in the Hct 10% group. Leukocyte adherence decreased in all groups during CPB and was only moderately increased at the end of the experiment. However, severe hemodilution (Hct 10%) was associated with a significantly greater number of rolling leukocytes relative to Hct 30%.

Conclusions—Higher Hct does not impair cerebral microcirculation and reduces white cell/endothelial activation after deep hypothermic bypass and circulatory arrest. Severe hemodilution (Hct 10%) results in evidence of inadequate cerebral tissue oxygenation during the cooling phase of CPB. This study suggests that Hct of 30% is preferable relative to lower Hct values during hypothermic CPB, particularly if DHCA is used. (Circulation. 2001;104[suppl I]:I-260-I-264.)

Key Words: microcirculation ▪ endothelium ▪ cardiopulmonary bypass ▪ ischemia ▪ reperfusion ▪ hemoglobin

The optimal hematocrit (Hct) in cardiac surgery, including cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA), remains controversial. Cardiac surgical textbooks suggest that hemodilution is important in minimizing microcirculatory disturbances, which are said to occur because of increased blood viscosity and adverse rheologic effects during CPB at low temperatures. However, at some point, the reduced oxygen-carrying capacity of very dilute blood must overwhelm the usefulness of its reduced viscosity. Few studies have directly examined the microcirculation during deep hypothermic bypass, so it is unclear what degree of hemodilution, if indeed any, should be used. Not surprisingly, many hemodilution protocols have evolved at cardiac surgical centers. We developed a novel experimental model for direct visualization of the effects of different Hct values on cerebral microcirculation and tissue oxygenation during normothermic and hypothermic CPB and tissue hypoxia during DHCA.

We used intravital microscopy with fluorescent labeling of plasma for visualization of cerebral microcirculation. Tissue oxygenation was evaluated by monitoring NADH fluorescence, which increases during ischemia.

Methods

Animals
Fifteen 5- to 6-week-old Yorkshire piglets (Parsons Farm) with a mean body weight of 9.3±1.1 kg were divided into 3 groups (Hct 10%, Hct 20%, and Hct 30%). All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institutes of Health (NIH publication No. 86-23, revised in 1985).

Surgical Preparation
After premedication with an intramuscular injection of ketamine (20 mg/kg) and xylazine (4 mg/kg), the piglets were intubated and ventilated with 30% oxygen at a respiratory rate between 10 to
18/min to achieve an arterial PCO$_2$ of 35 to 40 mm Hg. After induction with fentanyl (25 μg/kg IV), anesthesia was maintained with continuous intravenous infusion of fentanyl (25 μg · kg$^{-1}$ · h$^{-1}$), midazolam (0.2 mg · kg$^{-1}$ · h$^{-1}$), and pancuronium (0.2 mg · kg$^{-1}$ · h$^{-1}$). Temperature probes were placed into the esophagus and rectum. Catheters were inserted through the left femoral artery and vein into the thoracic aorta and vena cava. After the administration of heparin (300 IU/kg IV) and a right anterolateral thoracotomy in the third intercostal space, the bypass cannulas were placed into the right femoral artery (8F Bio-Medicus cannula) and the right atrium (24F Harvey cannula).

Then the piglets were positioned prone in a stereotactic frame, and a cranial window (10 × 20 mm) was created over the parietal cerebral cortex with a drill. After sealing of the bone edges with bone wax and incision of the dura, the surface (pial) vessels were visualized. The cranial window was closed with a coverslip; a new coverslip was used for each experiment.

**CPB Management**

The prime of the CPB circuit (including a hardshell reservoir, Minimax membrane oxygenator, and 40-μm arterial filter) consisted of 800 mL blood only (Hct 30% group) from an adult donor pig drawn on the morning of the experiment, 400 mL blood and 400 mL Plasmalyte A crystalloid solution (Hct 20% group), or 800 mL Plasmalyte A solution (Hct 10% group). Methylprednisolone (25 mg/kg), cephalzin (25 mg/kg), 10 mL sodium bicarbonate 7.4%, and furosemide (0.25 mg/kg) were added to the prime before the start of CPB and on reperfusion. CPB flow was set at 100 mL · kg$^{-1}$ · min$^{-1}$. The pH stat strategy was used (sweep gas 95% O$_2$/5% CO$_2$).

After baseline recordings of the cerebral microcirculation, the piglets were placed on normothermic CPB (esophageal temperature 37°C) for 10 minutes. Then the piglets underwent 40 minutes of cooling on CPB to 15°C. After 60 minutes of DHCA, the piglets were rewarmed over 40 minutes to 37°C. The hearts were defibrillated at a temperature of 33°C. Ten minutes before weaning from CPB, ventilation was restarted with 100% oxygen. The piglets were studied for 120 minutes after weaning. The esophageal temperature was maintained at >35°C with a heat lamp.

**Intravital Microscopy**

The microscope system that was used was an MZFL III epifluorescence microscope (Leica Microsystem Systems Ltd) on a surgical stand (Wild-Heerbrugg) equipped with a rapid filter exchanger that included 3 sets of filters: a blue filter set (450- to 490-nm excitation/515-nm emission wavelength) for visualization of NADH autofluorescence, a UV filter set (340- to 380-nm excitation/515-nm emission wavelength) for visualization of fluorescein fluorescence, and a green filter set (536- to 556-nm excitation/590-nm emission wavelength) for documentation of NAD fluorescence. A Scion LG-3 frame grabber card (Scion Corporation) and a professional S-VHS videocassette recorder (Panasonic AG 7350). A Scion LG-3 frame grabber card (Scion Corporation) and a computer-assisted image analysis system (NIH Image) were used for subsequent offline analysis of microcirculatory parameters.

**Measurements**

**Macrohemodynamics and Biochemical Parameters**

The arterial pressure was monitored continuously throughout the experiments and recorded every 5 minutes. Hemoglobin, Hct, glucose, lactate, Po$_2$, PCO$_2$, and pH were measured in arterial and venous blood every 10 minutes on CPB during the cooling and rewarming phase of the experiments and every 30 minutes before and after CPB with a blood gas analyzer (Stat Profile 5; Nova). Systemic white blood cell counts were obtained at baseline, at the end of cooling, at the end of rewarming, and 120 minutes after weaning from CPB.

**Microcirculation**

For assessment of functional capillary density (FCD), defined as total length of erythrocyte-perfused capillaries per observation area, the plasma was labeled with 1 mL fluorescein-isothiocyanate-dextran 5% (150 kDa; Sigma Chemical Co) before each measurement. For assessment of leukocyte–endothelial cell interaction, circulating leukocytes were stained with 1 to 2 mL rhodamine 6G 0.2% (Sigma) and observed for adherence (number of adherent cells per 100-μm vessel length) and rolling (number of rollers/100-μm vessel length per minute) in postcapillary venules. For measurements of NADH fluorescence, the automatic brightness and contrast control of the video camera was disabled and the optical densities were evaluated offline densitometrically. Measurements of the microcirculatory parameters were performed at baseline; at 10 minutes of normothermic CPB; at 20 minutes of cooling; at the end of cooling; at 5, 10, and 40 minutes of reperfusion; and at 120 minutes after weaning from CPB. In addition to these time points, NADH fluorescence was recorded every 15 minutes during circulatory arrest.

**Statistics**

Continuous variables are expressed as mean ± SD. The Kolmogorov-Smirnov test indicated that the variables followed a normal distribution. Therefore, repeated-measures ANOVA was used to evaluate changes over time and to compare rates of change between the groups. Paired t tests were used for specific time point comparisons within an experimental group. One-way ANOVA followed by post hoc Bonferroni t tests were used to detect statistical differences between the experimental groups at fixed time points. All reported P values are 2-tailed. The software package used for statistical analysis was SPSS 10.0 for Windows (SPSS Inc).

**Results**

There were no statistically significant differences at baseline (before CPB) among the 3 experimental groups regarding Hct, blood gases, mean arterial pressure (MAP), systemic leukocyte count, whole body lactate, FCD, number of rolling or adherent leukocytes, and tissue oxygenation as measured by NADH fluorescence.

**Hct Groups**

With the priming protocol, the 3 different Hct values on CPB during cooling were successfully achieved in the 3 groups (Figure 1). After rewarming, the Hct in the Hct 10% group was increased through hemofiltration (Hct 10% group) or blood transfusions (Hct 20% group). B indicates baseline; N, normothermic CPB; and Rewarm, rewarming.
in the Hct 20% group, blood transfusions were used to increase the Hct.

**Macrohemodynamics**

In the severe hemodilution group (Hct 10%), the MAP (Figure 2) was significantly lower during normothermic CPB (37±11 versus 83±7 mm Hg, P<0.001) and cooling (at the end of cooling: 31±6 versus 63±12 mm Hg, P=0.001) and in the early (5 minutes) reperfusion phase (33±7 versus 50±9 mm Hg, P=0.009) relative to Hct 30%. At the end of rewarming, no significant group differences were detected in MAP (Hct 10% 92±15 mm Hg, Hct 20% 80.6±21 mm Hg, Hct 30% 94±12 mm Hg; P=0.83, F test in ANOVA).

**Microcirculatory Parameters**

**FCD Values**

FCD did not significantly change from baseline to normothermic CPB or to the end of cooling in any experimental group. There were no significant differences between the groups at the end of cooling (93±4% Hct 30%, 94±6% Hct 20%, 93±7.6 Hct 10%; Hct 10% versus 30%, P=0.84). Even in the Hct 30% group, microvascular perfusion on CPB was preserved at low temperature. Thus, there was no evidence of red or white cell plugging in the cerebral microvasculature or viscosity-impaired flow with a higher Hct (Hct 30%) at deep hypothermia.

In the early reperfusion phase, however, contrary to expectations, FCD was significantly higher (P=0.002) in the Hct 30% group (76±12%) than in the Hct 10% group (40±14%), although the differences between Hct 10% and Hct 20% and between Hct 20% and Hct 30% were not significant (Figure 3). The recovery of microvascular perfusion after DHCA was slower in the Hct 10% group than in the Hct 30% group but was complete. At 40 minutes of rewarming, the FCD did not statistically differ from baseline values in any group.

**White Cell Counts and Leukocyte–Endothelial Cell Interactions**

The number of systemic circulating leukocytes as measured by white blood cell counts was not significantly different at baseline (before CPB) among the 3 groups (15.0±5.7 versus 16.7±3.9 versus 16.5±6.6×1000/μL for Hct 10%, 20%, and 30%, respectively). At the end of cooling, the number of circulating leukocytes decreased to 4.3±3.3 (Hct 10%) versus 8.1±3.7 (Hct 20%) versus 7.5±3.3 (Hct 30%)×1000/μL. Two hours after weaning, the white blood cell count reached 17.3±6.7 (Hct 10%) versus 15.3±4.0 (Hct 20%) versus 17.0±8.4 (Hct 30%)×1000/μL.

At baseline, the number of adherent leukocytes to postcapillary cerebral venules (length 100 μm) for 20 seconds was 4.0±1.0 versus 3.8±1.0 versus 4.2±1.1 in the Hct 10%, 20%, and 30% groups, respectively. At the end of cooling, the number of adherent leukocytes decreased to 1.6±0.9 versus 2.2±0.8 versus 2.4±0.7 in the Hct 10%, 20%, and 30% groups, respectively. Two hours after weaning, the leukocyte adherence increased to 4.6±1.6 (Hct 10%) versus 4.2±1.2 (Hct 20%) versus 4.8±0.8 (Hct 30%). None of these differences between the groups at either time point was statistically significant.

**Figure 2.** MAP was significantly less during normothermic bypass, whole cooling phase, and first 5 minutes of reperfusion in Hct 10% group relative to Hct 30% group. B indicates baseline; N, normothermic CPB; and Rewarm, rewarming. *P<0.05, Hct 10% vs Hct 30%.

**Figure 3.** Pial vessels and plasma labeled with fluorescein-isothiocyanate-dextran. A, Baseline Hct 30% group. B, 5 Minutes of reperfusion, Hct 30% group. C, Baseline, Hct 10% group. D, 5 Minutes of reperfusion, Hct 10% group. Microvascular and capillary density was significantly less in Hct 10% group at 5 minutes of reperfusion relative to Hct 30% group. White bars indicate 100 μm.

**Figure 4.** Number of rolling leukocytes in postcapillary venules. *Significantly greater number of rolling leukocytes 120 minutes after weaning from bypass (P=0.01, Hct 10% versus Hct 30%). Rewarm indicates rewarming.
Figure 5. Tissue oxygenation is significantly less (greater NADH fluorescence) during cooling and circulatory arrest in the Hct 10% group. *P<0.05, Hct 10% vs Hct 30%. Rewarm indicates rewarming.

At baseline, there were 4.0±1.2 versus 4.3±1.0 versus 5.2±1.5 rolling leukocytes in postcapillary venules (100 μm) per 60 sec in the Hct 10%, 20%, and 30% groups, respectively. Two hours after weaning, there was a significant increase (P=0.01) in the number of rolling leukocytes (“rollers”) in the Hct 10% group (24.3±8.6) relative to Hct 30% (7.0±2.7) (Figure 4).

**NADH Fluorescence**

At the end of 10 minutes of normothermic bypass, NADH fluorescence had decreased to 94±4% (Hct 30%) and 98±5% (Hct 20%) of baseline values but increased to 105±4% (Hct 10%), indicating a decrease in tissue oxygenation in the severe hemodilution group (P<0.001, Hct 30% versus Hct 10%) (Figure 5). At the end of the cooling phase, NADH fluorescence reached 98±3% (Hct 30%) versus 103±4% (Hct 20%) versus 110±9% (Hct 10%) of baseline values (P=0.015, Hct 30% versus Hct 10%). At the end of DHCA, it increased to 119±6% (Hct 30%) versus 126±5% (Hct 20%) versus 144±2% (Hct 10%) of baseline levels (P=0.002, Hct 30% versus Hct 10%). NADH fluorescence was still significantly higher (P=0.008) after 5 minutes of reperfusion in the Hct 10% group (118±4%) relative to the Hct 30% group (106±7%). Other intergroup differences were not significant (Hct 10% versus Hct 20%, Hct 20% versus Hct 30%). However, reperfusion restored the metabolic state of brain tissue in all experimental groups.

**Biochemical Parameters: Lactate**

There was a tendency to higher whole body lactate levels throughout the experiment, probably reflecting a larger increase in anaerobic metabolism in the Hct 10% group relative to the 2 other groups with lesser degrees of hemodilution. At 250 minutes (90 minutes after weaning) and 280 minutes (120 minutes after weaning), lactate was significantly higher in the Hct 10% group relative to the Hct 30% group (P<0.01 for both).

**Discussion**

Few, if any, previous studies have used intravital microscopy for direct visualization of cerebral microcirculation, leukocyte–endothelial cell interaction, and tissue oxygenation before, during, and after hypothermic CPB and circulatory arrest. Previous reports have described the use of laser Doppler9 and microspheres10,11 for the study of brain circulation and microcirculation. Near infrared spectroscopy has been used to evaluate brain tissue oxygenation.12,13 However, because of the indirect nature of these techniques, they do not allow for visualization of perfusion in individual microvascular segments or measurement of tissue oxygenation at high spatial resolution.

With intravital microscopy, microvascular (including capillary) blood flow can be directly visualized and analyzed after labeling of plasma or red blood cells with fluorescent dyes.14 Intravital fluorescence microscopy seems especially appropriate for the study of organs like the brain where blood vessels lie on the organ surface. Thus, there is no direct surgical trauma to the tissue that might induce artificial microcirculatory disturbances via the surgical preparation.

One of the major findings of the present study is that cerebral capillary flow is maintained even in the high Hct (Hct 30%) group during hypothermic CPB. This is in contrast to the traditional cardiac surgery dogma that hemodilution is necessary to counteract the viscosity increase of blood during deep hypothermic CPB. However, this is well in keeping with experimental and clinical studies from our institution and elsewhere that have demonstrated an improved neurological outcome with higher Hct on CPB.15,16

An intriguing finding of the present study is the significantly lower FCD in the early reperfusion phase after 60 minutes of DHCA in the severe hemodilution (Hct 10%) group relative to the Hct 30% group. The mechanism of this phenomenon is unclear and needs further elucidation. Although it is possible that the lower perfusion pressure at this time point could have been the cause of the reduced FCD in the Hct 10% group, it has been our observation and the observation of others that cerebral blood flow is consistently increased by hemodilution despite the reduced perfusion pressure that results from the fall in viscosity.17 We believe that a more likely explanation for the lower FCD is the occurrence of hypoxic injury to endothelial cells during cooling and circulatory arrest in the severe hemodilution group and that this is responsible for delayed microvascular reperfusion. There are literature reports that hypoxia in a nonbypass setting results in a decrease in endothelial NO synthase expression.18 An hypoxic decrease in NO production by the endothelium could explain the observed microvascular vasoconstriction in the early reperfusion phase after DHCA.

A surprising finding of our study was the modest degree of white cell activation during and after CPB. In fact, the number of adherent leukocytes in cerebral venules actually decreased during the cooling phase of the experiment. Two hours after weaning, the leukocyte adherence was only slightly increased compared with baseline. These findings correlated well with the systemic white blood counts. The sensitivity of the method for the study of leukocyte–endothelial cell interaction with rhodamine labeling of circulating leukocytes was confirmed in a subsequent study using lipopolysaccharide, which showed a marked increase in leukocyte adherence to endothelium.
Reasons for the only moderate leukocyte response to CPB might include the administration of a high dose of steroids (25 mg/kg methylprednisolone into the prime before the start of CPB and on reperfusion). Other explanations include the protective effect of the low temperature. 20  

As with any experimental model, this intravital microscopic model of cerebral microcirculation has its limitations. Due to the epi-illumination technique, only the superficial layer of brain tissue and microvessels can be visualized. This raises the question of whether the observed changes reflect global alterations of cerebral microcirculation. The MAP was not controlled in this model to avoid direct effects of vasopressor agents on the cerebral microcirculation. Furthermore, in contrast to clinical practice in most centers, in this experimental model, animals underwent 10 minutes of normothermic bypass before cooling to achieve stable baseline conditions.

In conclusion, we present a novel model for simultaneous study of the cerebral microcirculation, tissue oxygenation, and leukocyte–endothelial cell interactions. This intravital microscopic study on the effects of different degrees of hemodilution on cerebral microcirculation revealed, in contrast to traditional cardiac surgery dogma, a maintenance of cerebral capillary flow during and after hypothermic bypass in the high Hct group (Hct 30%). It further showed slow microvascular reperfusion in the severe hemodilution (Hct 10%) group in the early reperfusion phase after DHCA. Further studies are necessary to evaluate the cellular mechanism of this observation.

In summary, this study shows the superiority of a higher Hct (30%) relative to lower Hct values for cerebral tissue oxygenation during cardiac operations, including deep hypothermic bypass and circulatory arrest.

References


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