Deleterious effects of oxygen during extracorporeal circulation for the microcirculation in vivo

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Abstract

Objective: Clinical complications arising from extracorporeal circulation (ECC) have been linked to disturbances in the microcirculation. Hyperoxia, a mainstay of supportive treatment, is clinically used for a variety of pathological states. In previous in vivo animal experiments we found increased leukocyte/endothelial (L/E) cell interaction following ECC due to oxygen derived free radicals. This study was carried out to investigate the link between arterial pO2 during ECC and the potential damage to the microcirculation, supposedly caused by oxygen derived radicals.

Methods: Intravital fluorescence microscopy was used on the dorsal skinfold chamber preparation in syrian golden hamsters. ECC was introduced via a micro-rollerpump (0.7 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/bw. Experiments were performed in chronically instrumented, awake animals (age: 10–14 weeks, weight: 65–75 g). Control inspired room air, experimental group 1 inspired 100% oxygen, group 2 received 100% oxygen and 2000 IE of Heparin i.v. (n = 7/group), that releases endothelial bound superoxide dismutase, a natural scavenger of oxygen derived free radicals in the hamster.

Results: Normobaric inhalation of 100% oxygen increased arterial pO2 from 64 ± 8.1 mmHg to 512 ± 124 mmHg (P, 0.05 vs. baseline). ECC under 100% oxygen reduced functional capillary density (FCD) to 70% of baseline values 8 h after ECC (P, 0.05). Adherent leukocytes in postcapillary venules and arterioles increased significantly (P, 0.05). 2000 IE Heparin prevented the reduction in FCD and decreased the number of adherent leukocytes.

Conclusions: Reduction in FCD, increased leukocyte adherence to the microvascular endothelium of postcapillary venules and arterioles under hyperoxia compared to ECC under room air conditions, demonstrates harmful effects of oxygen during ECC in vivo. A high dose of Heparin enhances functional capillary density, thus attenuating the microvascular dysfunction/damage in the period after ECC.

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1. Introduction

Although there is some acceptance that oxygen supplementation is beneficial to augment tissue oxygenation when used in states of anemia or ischemia due to occlusive vessel disease [3,10], no evidence exists that a positive clinical outcome is warranted. One possible reason is vasoconstriction, caused by hyperoxia, thus lowering tissue perfusion as demonstrated extensively in vitro (isolated vessels) and in vivo [5,6]. Recently, using intravitalmicroscopy, Tsai et al. found a decrease of functional capillary density during hyperoxia [24]. In the context of cardiopulmonary bypass (CPB) less information is available about potential effects of hyperoxemia to improve tissue oxygenation, however, adverse events have been reported [14].

We hypothesized that hyperoxia could aggravate extracorporeal circulation (ECC) induced injury to the microcirculation due to increased generation of reactive oxygen species (ROS), which may be partially responsible for the systemic activation of cellular and humoral components during cardiopulmonary bypass (CPB), extracorporeal membrane oxygenation (ECMO) or hemodialysis [9]. This inflammatory response is represented by clinical complications from moderate disturbances of organ functions ranging from increased body temperature...
or leukocytosis to severe dysfunction of single organs up to multi-organ failure [11]. Neutrophils play a pivotal role because of their ability to release oxygen derived free radicals and proteolytic enzymes with subsequent tissue damage [12], followed by upregulation of leukocyte adhesion molecules (CD11/CD18) and expression of adhesion molecules on endothelium. In previous in vivo experiments we demonstrated that exposition of blood to an extracorporeal circuit induces dysregulation in the microcirculatory compartment [15]. Up to date no information is available concerning arterial pO₂ during ECC in regard to a probable damage of the microcirculation.

The present study was carried out to determine the extent by which hyperoxic blood may interfere with ECC induced microcirculatory disturbances in the unanaesthetized hamster skinfold model and if formation of oxygen free radicals is involved.

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 [8]. Briefly, the dorsal skin fold, 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 wt., Pentobarbital, Merial, Hallbergmoos, Germany) anaesthesia. 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. Five 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 (MABP) 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 × SW 0.40 BD NA objective (Leica Flutar, 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 monitor (Sony, Japan).

Leukocytes were stained with rhodamine 6 G (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 5 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 between two and five capillaries with RBC flow in the field of view. Systematic observations were achieved by displacing the microscopic field of view in three consecutive steps in the lateral direction (relative to the observer). Each step had the extension of the area seen on the monitor, being 436 μm long when referred to the tissue. 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 a 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.
2.7. Statistical analysis

Data were analysed using SPSS®—statistical analysing software (SPSS Software Inc. Chicago, USA) using ANOVA, t-test or Wilcoxon test with Bonferroni correction. Changes were deemed statistically significant for \( P < 0.05 \).
group significantly different (Table 1). At tp 4 h RBC velocity in arterioles was higher, when compared to control ($P < 0.05$).

### 3.4. Functional capillary density

FCD was unchanged in controls. 100% oxygen (Group 1) reduced FCD to lowest levels after 8 h ($P = 0.011$ bl vs. tp, $P = 0.029$ control vs. group I at tp 8 h). In group 2 FCD recovered at tp 8 h (Fig. 4).

### 3.5. Macrohemodynamic parameters

Arterial blood pressure and heart rate were stable and not significantly affected by ECC or treatment. During the time of the experiment no states of low pressure were observed.

### 3.6. Blood analysis

Analysis of partial oxygen pressures ($pO_2$) in arterial blood demonstrated stable conditions throughout the experiment in all groups. Under baseline conditions during room air breathing (control) $pO_2$ was $64 \pm 8.1$ mmHg compared to $512 \pm 124$ mmHg (Group 1 means $\pm$ SD, $P < 0.05$ vs. baseline) and $502 \pm 116$ mmHg (Group 2; means $\pm$ SD, $P < 0.05$ vs. baseline).

### 3.7. Anticoagulation

Application of 300 as well as 2000 IU/kg/body wt. heparin resulted in a prolongation of partial thromboplastin time (PTT) to more than 300 s at the end of ECC compared to a baseline level of $26.5 \pm 5.9$ s ($n = 3$).

### 4. Discussion

The principal findings of this study are that (1) normobaric inspiration of 100% oxygen significantly reduced functional capillary density (FCD) and (2) increased rolling and sticking in postcapillary venules and arterioles. (3) Treatment with high dose heparin reduced L/E cell interaction and prevented severe reduction of FCD.

Administration of high concentrations of oxygen (hyperoxia) is a mainstay of clinical supportive treatment for a variety of pathologies. However, hyperoxia, by generating systemic reactive oxygen species (ROS), can exacerbate organ failure by causing cellular injury and damage solid organs like the kidneys or the brain [21,25]. Effects of hyperoxia on the lung include pulmonary edema, inflammation, and respiratory failure [2]. In a clinical study with 19 patients undergoing CPB for coronary heart disease hyperoxic reoxygenation was associated with increased cardiac Malondialdehyde (MDA) levels immediately after aortic declamping, emphasizing the importance of the adequate oxygenation level [1]. During surgical correction of congenital cardiac defects, the use of hyperoxic CPB might be harmful for the hypoxic immature heart [13].

In previous studies we demonstrated in vivo that ECC induces L/E cell interaction and that the intensity of induction was ECC time dependent and related to the production of oxygen derived free radicals [15]. The consequences for the microcirculation are demonstrated in our study. The finding that FCD is reduced after normobaric hyperoxia is in accordance with a report from others, who found a reduced capillary flow in the pig brain [23]. Tsai et al. explained this phenomenon in hamsters with hyperoxia (hypoxia) is a mainstay of clinical supportive treatment for a variety of pathologies. However, hyperoxia, by generating systemic reactive oxygen species (ROS), can exacerbate organ failure by causing cellular injury and damage solid organs like the kidneys or the brain [21,25]. Effects of hyperoxia on the lung include pulmonary edema, inflammation, and respiratory failure [2]. In a clinical study with 19 patients undergoing CPB for coronary heart disease hyperoxic reoxygenation was associated with increased cardiac Malondialdehyde (MDA) levels immediately after aortic declamping, emphasizing the importance of the adequate oxygenation level [1]. During surgical correction of congenital cardiac defects, the use of hyperoxic CPB might be harmful for the hypoxic immature heart [13].

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vasoconstrictor, was found to be elevated under hyperoxic CPB [22]. Therefore, vasoconstriction could be the reason for reduced FCD in our experiments. However, L/E cell interaction within capillary segments of the microcirculation can reduce FCD due to capillary plugging or endothelial cell swelling after a provocation like ischemia/reperfusion. In our experiments hemodynamic parameters were stable throughout the entire experiment, thus tissue edema should not have developed due to mechanically severed perfusion. Compared to ischemia/reperfusion experiments using the skinfold chamber model of hamsters [20], the number of interacting white cells was lower in our setup, therefore we did not expect a significant effect caused by capillary plugging, which was never observed. ECC in our model seems to trigger the inflammatory response on a lower level, without rendering the tissue ischemic or even necrotic. The increase in RBC velocity in group 1 is in good agreement with results from Reber et al. [22], who found increased pulmonary shunting in patients undergoing hyperoxia during CBP. It is assumed that an increased RBC velocity under constant vessel diameters increases blood flow, consequently microvascular perfusion on the capillary level should increase. In our experiments FCD decreased under hyperoxia suggesting a significant shunt proximal to the capillary network.

Heparin in the dose of 2000 IU/kg body wt. has been shown to release high amounts of endothelium bound SOD into the systemic circulation in rodents [16]. In the case of the hamster baseline values of 60–80 IU/ml can be increased to 500 IU/ml plasma by iv. injection of 2000 IU/kg body wt. heparin. Side effects like bleedings were not observed. The authors are aware that this fact makes it difficult to transfer individual drug doses and their actions among animal species or from animal species to humans. Yet our model allows to study pathophysiological and pharmacological mechanisms in respect to their specific individual actions in a standardized way.

The fact that FCD is reduced not only early after ECC but also 8 h after ECC supports the concept that hyperoxic ECC sets the stage for a self-amplificatory series of events, resulting in aggravation of tissue damage inflicted by ECC per se [15,19] as demonstrated by reduction of FCD in group I. Considering the half live of SOD (after heparin injection 50 min compared to 7 min when SOD was injected iv. [17]), recovery of FCD in group 2 at time point 8 h might be a result of inhibition of the above described events.

In our experiments inhibition of rolling was more pronounced compared to sticking, suggesting that heparin itself, via a selectin blocking mechanism, prevents rolling of leukocytes [18]. The time course of leukocyte activation, with a peak 8 h after ECC, suggests a delayed mechanism like generation and release of proteolytic enzymes and oxygen-derived radicals during and after ECC by activated...
neutrophils. This inflammatory response exacerbates under hypoxia, leading to a significant increase of leukocyte activation compared to control. The fact that leukocyte adhesion is reduced not only early after ECC but also 8 h after ECC again supports the above mentioned concept of aggravation of tissue damage inflicted by ECC. Inasmuch as oxygen radicals are responsible to induce upregulation of adhesion molecules during ECC [4], the observed reduction in ECC-induced L/E cell adhesion may, at least in part, result from inhibition of the action of the oxidants in our experiments. Under hyperoxic ECC Heparin (SOD release) reduces the inflammatory response. However, not to a level as under ECC alone in the absence of hypoxia, suggesting an overload of ROS under hypoxia, thus overcharging the scavenging capacity of SOD.

A serious limitation of the study is the fact that no oxygenator, heater, cooler or cardiotomy suction devices are used in our experiments. 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 levels of oxygenation. Reduction in FCD, increase in leukocyte accumulation and adherence to the microvascular endothelium of postcapillary venules and arterioles were major components of the inflammatory response to hypoxia. Disturbances in the microcirculation under hypoxia are supposed to result from the harmful action of oxygen during ECC in vivo. A better understanding of the microcirculation under hypoxia may provide the basis for effective therapeutic interventions.

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Appendix A. Conference discussion

Dr L. Von Segesser (Lausanne, Switzerland): One of your statements was that the red cell length column was shorter when there was higher oxygen if they carried more oxygen?

Dr Kamler: Yes, that’s correct.

Dr Von Segesser: Could it be that there was less need for red cells with oxygen if they carried more oxygen?
Dr Kamler: This is possible. We didn’t measure the tissue oxygenation in the tissue because we couldn’t do this at the time. It’s possible with a special method. But probably this is a compensatory mechanism.

Dr R. Poston (Baltimore, MD, USA): Tissue PO2 was going to be my question, too. Because leukocytes function better at a higher tissue PO2, that might be the reason why you saw an increased sticking and rolling. ECMO, per se, may not necessarily have been the cause of that observation. One way to help tease that out would be to identify some kind of pathophysiological mechanism attributable to ECMO like increased selectin or ICAM in the tissue. Have you considered doing that?

Dr Kamler: No, we haven’t done this in the experiment.

Dr H. Vohra (Essex, UK): On the basis of your experiments, it seems that you’re suggesting that heparin is an antioxidant.

Dr Kamler: No, not heparin itself.

It’s known, this is published from Karlsson and Marklund in 1988, that this high-dose heparin releases endothelial-bound superoxide dismutase, which is the scavenger. And it’s possible to measure the SOD level in hamster blood, which is very high. This is only valid for the hamster and not for other rodents or other animals and not for humans as well.

Dr Vohra: That was my next question. Would it be clinically relevant in the humans? Because at the end of the cardiopulmonary bypass, we do use heparin.

Dr Kamler: Not to humans.

Dr Vohra: So on the basis of your study, how can we extrapolate these results to humans?

Dr Kamler: Well, it’s just a warning not to use high amounts of oxygen. It is not possible to transfer the SOD releasing effect of heparin to humans, but our finding of increased production of reactive oxygen species during ECC und high oxygen is transferable to humans.