Excessive negative venous line pressures and increased arterial air bubble counts during miniaturized cardiopulmonary bypass: an experimental study comparing miniaturized with conventional perfusion systems

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Abstract

OBJECTIVES: Miniaturized cardiopulmonary bypass (MCPB) is increasingly used in cardiac surgery, because it can lower clinically significant complications such as systemic inflammatory response, haemolysis and high transfusion requirements. A limitation of MCPB is the risk of excessive negative pressure in the venous line during volume depletion, probably leading to gaseous microembolism.

METHODS: In an experimental study with 24 pigs, we compared conventional open cardiopulmonary bypass (CCPB group, n = 11) with MCPB (n = 13). The same pump and identical tubing materials were used in both groups. Primary endpoints were pressure values in the venous line and the right atrium as well as the amount of air bubbles >500 µm. Secondary endpoints were biochemical parameters of systemic inflammatory response, ischaemia, haemodilution and haemolysis.

RESULTS: Nearly 20% of venous pressure values were below −150 mmHg and approximately 10% of the right atrial pressure values were below −100 mmHg in the MCPB group, during the experiment. No such low values were observed in the CCPB group. In addition, the number of large arterial air bubbles was higher in the MCPB group compared with the CCPB group (mean ± standard deviation [SD]: 13 444 ± 5709 vs 0.9 ± 0.6, respectively; P < 0.001). Bubble volume was also significantly larger during MCPB compared with CCPB (mean ± SD: 1522 ± 654 vs 4.1 ± 2.5 µl, respectively; P < 0.001). Blood levels of interleukin-6, free haemoglobin and creatine kinase were significantly higher in the CCPB group compared with the MCPB group.

CONCLUSIONS: Despite the benefits of MCPB regarding systemic inflammatory response and haemolysis, this technique is associated with excessive negative venous line pressures and a significant increase in the number and volume of arterial air bubbles compared with CCPB. Mini-perfusion systems and the management of MCPB require further refinements to avoid such adverse effects.

Keywords: Cardiopulmonary bypass · Gaseous microembolism · Systemic inflammatory response · Haemolysis · Haemodilution

INTRODUCTION

The use of cardiopulmonary bypass (CPB) in cardiac surgery has expanded rapidly during recent decades. Despite many advances related to the membrane oxygenator, heparin-coating etc., the technology is still associated with limitations that can cause clinically significant organ dysfunction. CPB can trigger the systemic inflammatory response, which includes activation of complement, macrophages, neutrophils and cytokines [1]. Additional adverse effects of CPB are neurological and bleeding complications [2, 3]. Blood coming into contact with large foreign surfaces, reperfusion injury as well as the use of priming solution are possible causes of the unfavourable effects of CPB mentioned above [4]. Since the 1990s, many efforts have aimed at developing miniaturized cardiopulmonary bypass (MCPB) systems with low-priming volume and centrifugal pumps. The reduced foreign surface contact area and the elimination of a blood/air interface by omitting the venous reservoir allowed decreasing the inflammatory response with MCPB [5]. Moreover, lower priming volume limits the amount of haemodilution [5]. Although MCPB is now frequently used, its limitations have been inadequately addressed. The risk of air bubble formation is one of those disadvantages [6], which may result in neuropsychological deficits such as strokes [7, 8]. Omission of the venous reservoir in the MCPB may change the capability of the circuit of handling air, thereby lowering its performance in terms of minimizing gaseous microemboli [9]. Another factor is spontaneous formation of microbubbles in the extracorporeal circuit (ECC), which is associated with excessive...
negative pressures in the venous limb of the circuit [10]. Excessive subzero pressures may be transmitted to the right atrium and eventually cause air aspiration [11]. With the reservoir removed in minimized systems, the perfusionist cannot compensate insufficient venous return by adding reservoir blood as usually done in the conventional CPB (CCPB). Compared with the CCPB, MCPB requires a very particular volume-management strategy, adding new challenges in the clinical application of CPB [11]. Although the MCPB systems usually have similar construction, many different types were described in the literature and used worldwide [5, 12]. In the present study, we use a closed perfusion system without a reservoir and without an air removal device on the venous side. The study was performed to investigate the effects of MCPB and CCPB on venous line and right atrial pressures (RAPs). In addition, the effect of pressure differences on the formation of air bubbles was evaluated in the two-perfusion systems. Both parameters, such as pressures and air bubble formation, play an important role in the safety management of MCPB systems.

**MATERIALS AND METHODS**

**Animals**

The study was carried out on 24 healthy pigs weighing 55–65 kg. The animals were kept in accordance with the German national standards on laboratory animal welfare. All experiments performed in this study were approved by the Local Ethics Committee on the Animal Research of the Federal State of Thuringia and conducted at the animal laboratory at the Friedrich Schiller University Jena, Germany. The study was supported by the German Heart Foundation.

**Anaesthesia protocol**

The animals were premedicated with intramuscular ketamine (10 mg/kg body weight [BW]) and midazolam (0.5 mg/kg BW) and subsequently endotracheally intubated. Inhalation anaesthesia was maintained with 1–2% isoflurane. After an initial intravenous dose of fentanyl (0.1 mg) and pancuronium (1 mg), additional doses were administered as needed. Volume-controlled ventilation was maintained at a frequency of 14/min, a tidal volume of 6–8 ml/kg BW and a positive end-expiratory pressure of 5 mbar. The inspired oxygen fraction was kept constant at 0.5. A right carotid artery catheter was inserted to monitor blood pressure and blood gases. In addition, a central venous catheter was inserted through the right external jugular vein. At the end of the experiments, all animals were euthanized with a potassium chloride overdose while in deep anaesthesia. This conforms to the German regulations on animal studies.

**Surgical technique**

All surgical procedures were carried out under sterile conditions. After instituting the haemodynamic monitoring, a sternotomy was performed. A 300 IU/kg BW dose of heparin was administered. CPB was prepared by inserting a 40- to 32-F dual-stage venous cannula (Sorin Group, Munich, Germany) through the right atrial appendage and a 21-F aortic cannula (Maquet Cardiopulmonary AG, Hirrlingen, Germany) into the aorta ascendens. Normothermic CPB was commenced and maintained for 3 h to achieve equal bypass times in all experiments. Continuous positive airway pressure was maintained at 5 mbar during CPB.

Two hours after the initiation of CPB, diastolic cardiac arrest was achieved and maintained by clamping the ascending aorta and by instilling intermittent doses of antegrade warm blood cardioplegia (Calafiore). In all pigs, the left internal mammary artery was anastomosed to the left anterior descending coronary artery. The cross-clamp time was approximately 30 min. After removal of the aortic clamp and after 10 min of reperfusion, the animals were weaned from CPB. Subsequently, the cannulas were removed and the heparin effect was reversed with a matched protamine dose. This was followed by 15 min of post-CPB observation.

**Study design**

The animals were allocated to either extracorporeal perfusion with CCPB or extracorporeal perfusion with MCPB. At each operation day, one or two animals were randomly operated on with the same technique. The study duration was from May 2007 until June 2009. We lost one animal in the CCPB group due to ventricle fibrillation after introduction of anaesthesia. At the end of the study, data assessment was possible in 13 animals in the MCPB group and 10 in the CCPB group. Primary endpoints of the study were pressure values and the amount of air bubbles >500 µm. Secondary endpoints were biochemical parameters.

**Perfusion systems**

The MCPB circuit is a fully heparinized closed CPB system with a high-performance hollow fibre membrane oxygenator (HILITE® 7000, MEDOS Medizintechnik AG, Stolberg, Germany) with a flow rates of up to 7 l/min. The surface area for gas exchange is 1.9 m², and the priming volume is 275 ml. In addition, a centrifugal pump (DELTASTREAM DP2; MEDOS Medizintechnik AG) was used. A driving console (DELTASTREAM Driving Console, MEDOS Medizintechnik AG) provides control, adjustment and surveillance of pump function. Automatic pump speed regulator function of the device was not used in this study. Pump priming volume is 17 ml, pump speed 100–10 000 rpm and flow capacity 0–8 l/min. We used the same heparin-coated arterial filter (SENTRY, Sorin Group) with a minimum priming volume and a simple debubbling system in all experiments. Phosphorylcholine (PC)-coated tubing (PVC Tubing, Sorin Group) was used because of its high haemo-compatible. PC coating considerably lowers platelet activation and inflammatory response [13]. The tubing length was <200 cm. This setup with a total priming volume of approximately 450 ml (275 ml oxygenator, 17 ml arterial line filter, approximately 142 ml tubing) provides for a small blood/foreign surface contact area and for low haemodilution. The absence of a cardiotomy reservoir limits the artificial surface/blood contact area and the air/blood contact area. The standard CPB system included a venous cardiotomy reservoir (HILITE, MEDOS Medizintechnik AG), which allows air/blood contact. A separate roller pump was used for cardiotomy suction (S III Encore, Stöckert, Sorin Group). Tubing length was approximately 250 cm. All other equipment (oxygenator, centrifugal pump, arterial filter and tubing) was identical in both groups. The cardiotomy reservoir was primed with ringer solution (500 ml), 6% hydroxyethyl starch (500 ml), mannitol (250 ml) and heparin (300 IU/kg BW). The composition of the priming volume...
was the same in both groups. There was a standard perfusion protocol for both groups with target arterial pressures of 50–60 mmHg and CPB flows of 65–75 ml/kg/min BW.

Study procedures

During CPB, RAP was monitored by a catheter (LAP 1751, Maquet Cardiopulmonary AG). Additionally, venous line pressure was measured. All pressure values were digitized into 250 ms intervals using a modified analog-to-digital converter and special software developed by the Department of Medical Technology at the University of Jena.

Microbubbles were measured and analysed with the BCC200 system (GAMPT GmbH, Zapfendorf, Germany), which is certified for clinical use. This system not only detects and counts microbubbles, but also determines their size (20 to >500 µm) and volume, displaying the data in a histogram. The venous and arterial lines of the ECC were monitored with two independent sensors. The venous sensor was placed on the venous line directly before entering the perfusion system in order to detect the bubbles in the venous blood emerging from the right atrium. For the arterial sensor, we chose a position on the arterial line after the arterial filter in order to detect the air bubbles in the arterial blood entering the aorta.

We also collected blood samples before CPB (t0), and 10 min (t1), 60 min (t2) and 120 min (t3) after commencing CPB. An additional sample was collected immediately after CPB termination (t4). The following parameters were measured: blood gases, blood entering the aorta.

Statistics

Approximately 28 000 arterial and venous pressure values were assessed per animal. It was of particular interest to assess the percentage of very low negative pressures in the right atrium and the venous line, rather than only the presentation of mean pressure values. Therefore, values were categorized into seven (right atrium values) and five (venous line values) pressure subgroups. The percentage of observations in each pressure group was assessed. For the statistical analysis, these values were treated as continuous variables. Since only bubbles >400 µm may be of clinical importance with respect to embolic complications [14], we only counted bubbles with a large size (>500 µm). The pressure value percentages in each category and the air bubble numbers and volumes were analysed using the unpaired t-test. A two-factor repeated measures analysis of variance was used to assess time effects and to analyse time × treatment (type of perfusion system) interaction effects on all dependent biochemical variables. Since several biochemical parameters such as leucocytes, IL-6 and bilirubin were not normally distributed, all biochemical were a logarithmically transformed to achieve almost normally distributed data. All continuous variables were expressed as mean and standard deviation. A P-value of <0.05 was considered significant. We used the statistical software package PASW, version 18 (Chicago, IL, USA), to perform the analyses.

RESULTS

Primary endpoints

The percentage of venous line and RAPs in each pressure category is presented in Table 1. As opposed to the CCPB group, the pressure values in the venous line and in right atrium included excessive negative values during MCPB: Nearly 20% of venous pressure values were below −150 mmHg and approximately 30% of the RAP values were below −30 mmHg in the MCPB group. 9.5% of them were below −100 mmHg. No such low values were observed in the CCPB group. The low pressure values in the MCPB group were accompanied by a much higher number of arterial and venous air bubbles >500 µm in the miniaturized system compared with the conventional system (Fig. 1). In detail, the number of air bubbles in the arterial line was 13 444 ± 5 709 vs 0.9 ± 0.6 (P < 0.001) and 16 640 ± 16 070 vs 530 ± 626 (P < 0.001) in the venous line. In addition, the volume of the arterial and venous bubbles was much higher during MCPB than CCPB (Fig. 2).

Arterial air bubbles during MCPB and CCPB had a volume of 13 444 ± 5 709 vs 0.9 ± 0.6 (P < 0.001) in the miniaturized system compared with the conventional system (Fig. 1). In detail, the number of air bubbles in the arterial line was 13 444 ± 5 709 vs 0.9 ± 0.6 (P < 0.001) and 16 640 ± 16 070 vs 530 ± 626 (P < 0.001) in the venous line. In addition, the volume of the arterial and venous bubbles was much higher during MCPB than CCPB (Fig. 2).
bubbles had a volume of 1683 ± 1322 µl during MCPB vs 50 ± 72 µl during CCPB (P < 0.001).

Secondary endpoints

The time courses of the measured biochemical parameters are illustrated in Figs 3–5. During the procedure, haemoglobin and haematocrit values decreased significantly in both study groups. In contrast, free haemoglobin values increased markedly. This increase was more pronounced in the CCPB group than in the MCPB group. In addition, there was a time-dependent decrease in leucocyte counts, whereas IL-6 concentrations increased during the study. These changes were more pronounced in the CCPB group than in the MCPB group. Moreover, there was a distinct increase in biochemical indicators of cardiac ischaemia, such as lactate dehydrogenase, CK and troponin I, which was more pronounced in the CCPB group than in the MCPB group for LDH and CK, but not for troponin I. Similarly, total bilirubin concentrations increased in both groups until the end of the surgical procedure. This time course was less pronounced in the MCPB group than in the CCPB group.

DISCUSSION

This study demonstrates that, despite the benefits of MCPB regarding systemic inflammatory response and haemolysis, this technique is also associated with excessive negative venous line pressures and a huge increase in the number and volume of arterial air bubbles compared with CCPB. To the best of our knowledge, this is the first study that provides continuous recordings of venous lines as well as RAPs and the occurrence of air bubbles in two different types of CPB.

CCPB continues to be the gold standard for most cardiac surgical procedures despite its well-known adverse systemic effects, such as inflammatory response syndrome, haemodilution and haemolysis [1, 15, 16]. Miniaturized perfusion systems were developed with the aim of avoiding some important adverse effects associated with CCPB. Miniaturized systems are characterized by markedly reduced foreign surface areas and lower priming volumes [12]. However, the lack of a venous reservoir and reduced priming volumes in MCPB systems may lead to less efficient air handling than in conventional systems [6]. Additionally, it has been assumed that negative pressure in the venous line facilitates entrainment of air around the venous cannula, possibly increasing

Figure 2: Mean volume of air bubbles >500 µm in the arterial and venous lines according to the study group. ***P < 0.001 MCPB vs CCPB group.

Figure 3: Time course of haemoglobin (A), haematocrit (B) and free haemoglobin (C). There were significant time effects for haemoglobin (P < 0.001), haematocrit (P < 0.001) and free haemoglobin (P < 0.001). Significant time × treatment effects were observed for free haemoglobin (P < 0.001), but not for haemoglobin (P = 0.378) and haematocrit (P = 0.341).
This assumption has clearly been supported in our study, demonstrating that the extreme negative pressure values in the venous line were also associated with more large arterial air bubbles. In line with our results, Norman et al. [9] detected, at all blood flow rates in the arterial line of a low prime perfusion system, 8–10 times more gaseous microemboli compared with CCPB. In the clinical setting, minimized systems are characterized by active drainage of the venous return through suction applied by a pump. Certain conditions, such as poor backflow, can result in excessive negative pressures and cause spontaneous formation of microbubbles in the venous line.

The large differences in the number of arterial bubbles between CCPB and MCPB may have clinical consequences by causing postoperative neurological events, such as stroke or transitory psychotic events.

Figure 4: Time course of leucocytes (A), IL-6 (B) and bilirubin (C). There were significant time effects for leucocytes ($P < 0.001$), IL-6 ($P < 0.001$) and bilirubin ($P < 0.001$). Significant time × treatment effects were observed for leucocytes ($P = 0.014$), IL-6 ($P < 0.001$) and bilirubin ($P < 0.001$).

Figure 5: Time course of lactate dehydrogenase (A), CK (B) and troponin I (C). There were significant time effects for lactate dehydrogenase ($P < 0.001$), CK ($P < 0.001$) and troponin I ($P < 0.001$). Significant time × treatment effects were observed for lactate dehydrogenase ($P = 0.022$), CK ($P = 0.003$), but not for troponin I ($P = 0.077$).
syndrome [7]. Helps et al. [14] reported in an experimental study in rabbits that arterial air emboli >25 μl caused only transient changes in the cortical somatosensory evoked response, whereas bubbles >400 μl in size caused prolonged adverse effects. It is well known that there are many sources of microembolus during CPB, including the surgical field, improper priming of the venous line and loose purge-string sutures around the venous cannula [9]. In addition, bubbles can be due to cavitation, cardiotomy suction and insufficient volume in venous reservoirs. Our study demonstrates that excessive negative pressure in the venous line plays an important role in the production of microemboli. This problem can probably be prevented by the administration of additional volume. Specific MCPP pressure and volume monitoring and careful volume management can prevent the formation of gaseous emboli secondary to excessive negative pressures in the venous line. The number of gaseous emboli due to CPB has been lowered by the use of arterial line filters and by improvements in oxygenator and venous reservoir designs [18]. In contrast to our results, other studies have shown that the number of gaseous emboli entering the patient with minimized CPB systems is lower compared with CCPB [6, 19].

Roosenhoff et al. [20] observed a 97% reduction in bubble counts using a bubble trap on the venous side. This confirms the idea that deairing is strongly needed during MCPP to avoid gaseous microembolism.

For MCPP systems, it has been shown that they reduce systemic inflammatory response and haemolysis compared with CCPB. This is particularly due to less foreign surfaces and avoidance of blood-air contact within the MCPP system [5, 21]. Proinflammatory cytokines, such as IL-6, increase in response to many major surgical procedures as well as after CPB [5]. In line with earlier findings [5, 22], the IL-6 kinetic in our study indicates that MCPP reduces the inflammatory response. Moreover, lower free haemoglobin levels indicate less haemolysis in the MCPP group compared with the CCPB group. Generally, the phenomenon of haemolysis during CPB is related to mechanical cell damage, positive as well as negative pressure, blood contact with non-endothelial surfaces, blood-air contact and shear stress [16]. Keeping the tubing as short as possible will reduce priming volume, pressure gradient and blood trauma and thus, may have caused less haemolysis in the MCPP group in our study. We noticed slightly more haemolysis with CCPB compared with MCPP. This could be related to cardiotomy suction. We also observed higher CK values and a tendency towards higher troponin I values in the CCPB group than in the MCPP group. Data indicate more cardiac damage during CCPB compared with MCPP, thereby confirming the results obtained by Immer et al. [23]. Several factors such as more severe haemodilution during CCPB may explain those differences. Another factor that may help in the interpretation of the results is the residual perfusion of the arrested heart during MCPP due to less unloading [23].

In summary, mini-perfusion systems are associated with excessive negative venous line pressures and a significant increase in the number and volume of arterial air bubbles compared with CCPB. Consequently, the systems and the management of MCPP require further refinements to avoid such adverse effects.

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