The embolic potential of liquid fat in pericardial suction blood, and its elimination

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Diffuse brain damage is a complex problem in cardiac surgery postoperatively. Liquid fat from recycled pericardial suction blood (PSB) is an embolic source. PSB can be discarded, but the recycling can be life saving, and methods have been developed to remove the fat. Blood washing by centrifugation is suggested to be the most effective method. In retained PSB, fat also separates without centrifugation, which is a novel and simple approach. Alternatively, inline fat filtration is easily accomplished but its effectiveness has been questioned. The present study aimed to investigate this phenomenon. Fat was heat extracted from retrieved pericardial fat tissue of coronary artery bypass graft (CABG) patients (n = 6), and was mixed, 1.25%, with postoperative mediastinal-shed blood. The mixture was filtered using a LipiGuard SB at constant flow rate. The filtration was scaled down to 3 mL and performed under temperature control, 37°C, 20°C and 10°C. At these temperatures fat removal was 46.9 ± 6.1%, 61.5 ± 7.0% and 76.8 ± 5.0%, respectively, with a statistical difference of P < 0.001. The improved fat removal at low temperature dramatically increased filtration pressures (P < 0.001) and caused haemolysis (P < 0.018). It is concluded that fat filtration is technically difficult. Cooling of blood increases fat extraction, but with negative side effects due to filter occlusion. Perfusion (2003) 18, 69–74.

Introduction

Brain damage after cardiac surgery is a disaster to the patient and may ruin an otherwise successful operation. Despite extensive research,1–3 the exact mechanisms are not fully understood. In general terms, the clinical pattern can be subdivided into stroke versus diffuse brain damage (DBD). From its time of occurrence, brain damage is either peroperative or postoperative. Stroke is well defined, such as by clinical scores,4 and is reported in various frequencies of around 2.5%, of which about one-third of cases are peroperative.5,6 The variability in reported frequencies may reflect stroke definitions and study design, e.g., prospective studies may gain higher rates than retrospective analyses.

DBD is more complex than stroke and covers a wide range, from discrete loss of memory7 to psychiatric forms of delirium.8 There are no exact definitions of DBD after cardiac operations, a fact that illustrates the challenge of identifying possible mechanisms. Nevertheless, with a reported frequency within a range of between 20% and 80%,7,8 DBD is a major clinical problem. The character and duration of DBD vary and may in part depend on pre-existing factors.8,9

The nature of stroke and its clinical pattern are fairly well understood in terms of an interrupted cerebral oxygenation.4 In the peroperative situation, several mechanisms can be identified, such as surgical factors, anaesthesia and the management of cardiopulmonary bypass (CPB).5,10 Of recent interest is the problem with aortic clamping.11,12 Manipulation of the aorta has been found to dislodge particulate matter from the intima. Limited use of aortic clamping is a surgical method with suggested benefits, although conflicting results are reported.13 For similar reasons, the intra-aortic filter was developed with ambitions to remove particles arising from aortic manipulation.14 Aortic clamping is usually discussed in relation to stroke and macroparticles. However, we demonstrated recently that clamping also produced microscopic particles of significant magnitude.15 It may thus be speculated that aortic clamping not only contributes to stroke but also to DBD.

The best described mechanism behind DBD appears to be cerebral microembolization of liquid fat from recycled pericardial suction blood (PSB).16,17 Wound fat accumulates in the pericardium, together with blood, which is sucked back to the cardiopulmonary circuit and is expelled into the aortic

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circulation. Fat emboli give rise to small capillary arterial dilations (SCADs). Fat in PSB deteriorates the in vitro capillary flow properties of plasma. From this knowledge, PSB fat should be avoided. To discard PSB is perhaps the simplest approach, which would result in about a half litre of blood loss in routine coronary bypass surgery. However, PSB varies in volume, and autotransfusion can be life saving. Among available PSB fat reduction methods, ‘cell-saver’ centrifugation is considered to be the most effective. At present there are two principally different methods to wash blood. The continuous plasmapheresis approach was found to eliminate 100% of the fat, whereas the more conventional centrifugation bowl removed 85%.

Human liquid fat has a density of 0.9 g/mL and separates from blood even without centrifugation. Therefore, fat spontaneously separates while PSB is collected during CPB. If the top 20% fraction is retained, about 65–80% of the fat could be removed after a 10-minute incubation. Fat removal involved both spontaneous density separation and fat-to-surface adsorption and was found to be temperature dependant. Human fat hardened at about 10°C, which affected its biophysical characteristics during separation.

Fat removal by means of an inline filter is perhaps the most easily adopted method by traditional cardiopulmonary technology. However, the elimination of liquid fat by filtration has been found difficult to do and there are conflicting opinions about its efficacy. The aim of the present study was to investigate this issue further, to measure the fat-removal capacity of a commercial filter under optimal in vitro conditions. Further, a tested hypothesis was that fat filtration is temperature dependant and that the efficiency may increase with raised fat viscosity at low temperature.

**Methods**

**Patients**

This study was approved by the Ethics Committee of the Umeå University Hospital. Patients signed a written consent to allow collection of discarded fat tissue or mediastinal blood. Excess pericardial tissue, removed to gain access to the left internal mammary artery, was collected from six male patients undergoing routine coronary bypass surgery. Their average age was 67.1 ± 5.0 years. Shed mediastinal blood was collected the day after surgery from six routine cardiac patients, 64.7 ± 3.4 years of age, three females and three men. The blood was used as a vehicle in fat-filtration experiments.

**Fat extraction and biophysical characteristics**

The collected fat tissue was frozen at −18°C until processing. Liquid fat was extracted with heat, at 220°C, to simulate the melting of fat by surgical use of diathermia. The extraction time varied with the amount of fat tissue, 914 ± 85 seconds. The liquid fat was allowed to reach room temperature followed by particle separation by sedimentation and refreezing.

**Blood and fat mixture**

Experiments were carried out at 37°C, 20°C and 10°C and the results are based on a total of 18 filtrations. Mediastinal blood was prefilttered through a standard transfusion set with a 200-µm screen filter (Mediplast AB, Malmö, Sweden). A sample of blood was collected in a 20-mL syringe and the blood volume was determined as weight, with correction for blood density, on average 7.63 ± 0.06 mL. Heparin, 50 IE/mL, was added to the defibrinated drain blood to ensure full anticoagulation. A three-way stopcock was connected to the syringe, and while positioned on a digital balance, a small volume of liquid human fat was deposited inside the Luer connector to yield a target volume concentration of 1.25%, with correction for density. The fat was gently aspirated into the syringe for mixing.

**Measurement of fat concentration and experimental steps prior to filtration**

A novel system was developed to measure small amounts of fat in blood. Standard 150-mm Pasteur pipettes (SN110601, Tamro MedLab Möllndal, Sweden) were calibrated for volume versus length, from tip to bottom. The volume displacement was divided in two parts, the tapered pipette tip and its cylindrical barrel, and transformed mathematically into polynomial equations. Within the same lot number of pipettes, the volume-to-length variation was negligible.

The bottom end of the pipette was sealed using silicon rubber. The weight of each pipette was determined to allow exact measurement of injected blood volume by weight with correction for density. The pipette was filled through its wide end by means of a needle penetrating the silicon plug. Immediately following mixing of a blood–fat sample, a fraction of the mixture was injected into the pipette, representing the control sample prior to filtration.

**Filtration system**

A commercially available fat-removal filter was used (LipiGuard SB; Pall Biomedical, New York, NY, USA). The filter was cut out from its plastic shell to unfold the active filter medium. The supporting
screen net was removed from each side of the filter and did not contribute to fat removal. Circular 13-mm pieces of the filter were punched out to fit a standard plastic Swinney membrane holder (Poretics Corp., Livermore, CA, USA). The syringe with the blood–fat mixture was mounted in a syringe pump (Pilote C; Fresenius Vial S.A., Brezins, France). Blood was pumped towards the filter using a 0.9/200 mm diameter/length polypropylene tube (Codan GmbH, Lensahn, Germany) and a 1.6/160 mm diameter/length stainless steel cannula (Secalon T; Viggo-Spectramed, Swindon, UK). The tubing, cannula and filter unit were lowered into a water bath for temperature control. The blood output from the filter was directly collected into a second Pasteur pipette via a needle from the filter holder through the bottom silicon plug of the pipette. This sample represented the blood postfiltration. The entire system was, thus, built up around the filter and the delivering syringe with the blood–fat mixture. This was done in order to avoid artefacts from lost fat, such as in the transfer of blood between syringes and test tubes. The pump was positioned vertically to ensure that no fat remained inside the system due to density separation. The flow rate was 50 mL/hour and on average 2.87 ± 0.10 mL was filtered. Only small amounts of blood remained unfiltered with this design.

The filtration pressure was determined via the syringe pump. The pump had a built-in strain gauge that was calibrated against known pressures. At lower temperatures, the fat occluded the filter prior to full-volume filtration (3 mL). The pressure limit was 1011 mmHg.

**Measurement of fat concentration**

A total of 36 blood samples were processed. A special design support tube for the Pasteur pipette was made to fit a swing-out rotor of a Sigma 4K15 centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). This was done so that the pipette would withstand the centrifugal force at 2000 g, (21°C, five minutes). The pipette was centrifuged upside down.

The two pipettes from each experiment, control and postfiltration, were centrifuged in two steps. The initial centrifugation was done in order to condense plasma foam. After topping up with NaCl (9 g/L), the pipette was recentrifuged, during which time the fat collected in the narrow tip of the pipette. With this method small amounts of fat were amplified in relation to the blood volume of the pipette barrel to yield a resolution of about 0.05%.

**Statistical methods**

Numerical data were consistent and appeared normally distributed. Mean values ± SEM are given throughout. Differences between experiments were evaluated using repeated measures ANOVA. Post-hoc analysis was performed using Duncan’s multiple range test. In addition, because of the limited number of observations, nonparametric Mann–Whitney U-test and Wilcoxon matched pairs were also used. All tests were two tailed and alpha was set to 0.05.

**Results**

**Method evaluation**

The determination of small fractions of fat was reproducible, with a measured concentration of 1.42 ± 0.06% (n = 18 prefiltration blood samples). This was slightly more than the target concentration of added fat, 1.25%. The volume fraction of erythrocytes in drain blood was 15.1 ± 1.1% (n = 18).

**Fat removal rate versus temperature**

After filtration at 37°C, the blood contained on average 53.1 ± 6.1% of the input fat with a significant removal (p < 0.001). The removal showed an increased efficiency with lowered temperature (p = 0.001), a phenomenon that is illustrated in Figure 1. At 10°C, 23.2 ± 5.0% of the fat remained after filtration. Statistical post hoc analysis revealed significance between all groups (p = 0.029 to p < 0.001).

**Filtration pressure versus temperature**

The filtration pressure increased as a function of filtration volume, with a positive slope (Figure 2). However, the filtration pressure also increased with lower temperature (ANOVA, p < 0.001) versus that at 37°C, which became significant for 10°C (post hoc analysis, p = 0.003). At 20°C and 10°C filter occlusion occurred prior to full-volume filtration. This was most pronounced at 10°C, at which temperature...
only one of the six samples went through prior to the cut-off pressure (Figure 2).

**Filtration-induced haemolysis**

The experimental model worked well without significant haemolysis at 37°C. The recorded haematocrit postfiltration was 98.7 ± 2.0% of the value prior to filtration. However, at the two lower temperatures, the postfiltration haematocrit was significantly depressed (p = 0.018) due to haemolysis (Figure 3).

**Discussion**

Cardiac surgery is a challenge, not only in terms of operational skills, but also from a perspective of industrial technology. Despite extensive development in surgical technology, the problem with brain damage remains. Different mechanisms have been identified from which new surgical methods and technology have been developed. The complexity is increased by the interference from predisposing factors in individual patients. The present study focused on one of these mechanisms, in terms of fat microembolization from recycled PSB.

Cerebral embolization of fat droplets from recycled PSB has been described in several well-designed experimental and clinical studies. Further, the in vitro consequence of fat contamination on the capillary flow function of PSB was found to be devastating. However, the extrapolation of experimental findings to clinical outcomes remains complex, in part due to the variable nature of DBD and the lack of standardized methods to measure it.

Fat is difficult to work with under experimental conditions. In brief, fat may escape detection or become concentrated due to experimental artefacts. Fat separates fast from blood by spontaneous density separation and, further, fat adheres to surfaces in a temperature-dependent way. These phenomena were described in a previous study. In the present study, these difficulties were considered in the experimental design. The direct transfer of the blood-fat mixture to the modified Pasteur pipettes worked well and helped to avoid these problems. The pipettes were centrifuged upside down by which small fractions of fat concentrated in the
pipette tip to amplify the resolution of measurement. This novel method was found simple and gave visible and reproducible readings of small fractions of fat. With this technique, clinically relevant fat concentrations could be tested, rather than using excessive fat in order to gain resolution. Excessive fat, such as 20%, is described in other studies. This is of particular importance in experiments dealing with fat filtration because filters become saturated in a way that may affect the results and clinical interpretations. Further, in a previous study we described that the common use of soya oil as a reference fat differed substantially from that of liquid human pericardial fat. The main difference related to their biophysical properties versus temperature. Human liquid fat hardens at about 10°C, a fact that is emphasized in the results of the present study. Other methods exist to measure small amounts of fat, such as by different chemical approaches.

The ambition of the present experimental study was to investigate whether the efficiency of fat filtration could be improved by increasing the fat viscosity at low blood temperature. This theory was strongly confirmed. Commercially available fat-removal filters have been questioned for their efficacy. There are discrepancies in reported fat-removal rates between industry and independent scientists. The present study in part fills this gap, in that we demonstrate variable outputs depending on input parameters, e.g., temperature. Other contributing factors relate to the type of tested fat, accuracy of fat measurements, used volume fractions of fat versus flow rates and whether the study was conducted under optimal in vitro conditions or in clinical practice.

Under optimal in vitro conditions, the LipiGuard SB removed about 50% of the added fat at 37°C. This removal was increased to about 80% if the fat viscosity and surface properties were altered by lowering the temperature to 10°C. This encouraging finding was, however, paralleled by negative consequences. In fact, the fat adsorption by the filter became too efficient, which produced filter occlusion and haemolysis at the lower temperatures. Haemolysis may in part also reflect the increased erythrocyte fragility in cold blood. In the present experimental design, the downscaled filtration corresponded to a volume of 630 mL PSB through the intact LipiGuard SB unit. This volume is within the suggested span of PSB volume load according to the manufacturer’s recommendations.

The results from the present study are limited to the in vitro situation. The fat removal rate is likely to vary depending on experimental modifications. Further, the present report is based on a rather small number of blood samples, although the results were very consistent and gave highly significant statistical outputs.

It is concluded that the efficiency of fat removal by filtration depends on temperature. About 50% of the added fat was retained by the filter at 37°C, a rate that increased to about 80% at 10°C. The effective fat absorption at low temperature caused filter occlusion and haemolysis. However, these findings may open new perspectives to how fat can be removed from PSB.

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