Stepwise improvement of cardiopulmonary bypass for neonates and infants

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chapter 1

INTRODUCTION

The components of the cardiopulmonary bypass system, the prime volume and the techniques of perfusion are believed to have a significant impact on postoperative morbidity and even mortality in pediatric heart surgery. Complete repair of congenital heart defects is increasingly performed in neonates and small infants with a weight of ranging between two and five kilogram. The consequences of CPB are more pronounced because of the immaturity of their organs and the discrepancy between prime volume of the CPB system and patient blood volume. CPB results in a systemic inflammatory response syndrome (SIRS) [1]. SIRS is a nonspecific inflammation process that can lead to capillary leakage. Capillary leakage results in an extravascular fluid accumulation. When the extravascular fluid accumulation is severe the interstitial edema that occurs can lead to end organ dysfunction [1, 2]. The CPB system is seen as the main activator of the inflammatory response. However, several other factors play a role in the activation of the inflammatory response such as surgical trauma, thrombin activation, ischemia-reperfusion injury and blood-air contact [3-5]. The degree of hemodilution is another factor [6].

Miniaturization of all components of the bypass system leads to lower prime volumes, resulting in a reduced hemodilution factor [2, 5]. The extent of the dilution factor contributes to the capillary leakage due to complement activation. Böning et al. showed that a large prime volume leads to an increase in IL-6 production and tumor necrosis factor-α [6]. A Low prime volume results in less use of donor blood during and after CPB as the dilution factor affects postoperative blood loss ass well [7]. The disadvantages of donor blood are well known: the risk of virus and prion transfer, depression of the immune system, delayed haemolysis and the metabolic load, low pH, high glucose and potassium concentrations, of stored blood. It should be mentioned that also in fresh red blood cells (< 5 days) a low pH and a high glucose concentration is found [8]. This metabolic overload can be avoided by washing the donor red cells using a cell saver. Swindell et al. showed that washing of donor blood reduces potassium and lactate loads during CPB [9].

The aim of this thesis was to investigate several techniques or adaptations that were developed to reduce the deleterious effects of CPB in neonates and infants.

Ultrafiltration (UF) is widely used during and after CPB to reduce the total body water increase, to reduce the need for donor blood and to remove some inflammatory mediators [10, 11, 12]. There has to be a minimal volume in the venous reservoir to for optimal bloodflow; this limits the efficiency of UF performed during CPB. [10]. Zero-balanced ultrafiltration (Z-BUF) or dilutional ultrafiltration (DCUF) is developed especially to remove some proinflammatory mediators during CPB; fluid is added to the venous reservoir continuously [11, 13].
Modified ultrafiltration (MUF) is a technique described for the first time in 1991 by Naik and Elliott [10]. MUF was especially developed to diminish the effects of hemodilution thereby increasing hemoglobin and hematocrit values without the use of donor blood. MUF permits the return of the concentrated residual volume of the CPB system. MUF is performed after cessation of CPB, but before the administration of protamin. There are several techniques to perform MUF, arterial-venous, venous-venous or venous-arterial [14]. The arterial-venous method has the preference above the other techniques because warm oxygenized blood is presented to the lungs. This results immediately to a decrease in pulmonary vascular resistance and stabilizes hemodynamic conditions during the MUF procedure [15]. Other reported effects of MUF are an immediate rise in systolic blood pressure, improved ventricular function, as well as a decreased need for blood transfusion due to an increase of hematocrit and a decrease of postoperative bleeding [16].
We studied a group of 198 patients retrospectively on the effects of MUF on donor blood use and postoperative blood loss. We investigated whether MUF was able to reduce the use of donor blood and the influence of MUF on postoperative chest drain loss. This study is presented in chapter 2.

In chapter 3 and 4 we describe the anti-oxidative capacity of the CPB prime used for neonates. Pyles et al. described a decrease of the antioxidant capacity of plasma after CPB in children [17]. It has been reported that neonates and infants have a poor antioxidative capacity and a low iron binding capacity [18]. Transfusion of a relatively

small volume of fluid with a low antioxidant capacity decreases the ability of the plasma to catabolize reactive oxygen species [19]. Because of the relative large prime volume of the bypass system compared to the circulating volume of the patient, the composition of the prime therefore may play an important role in increasing the anti-oxidative capacity and thereby preventing reactive oxygen species (ROS) formation. ROS activates nuclear factor – κB, which is an important protein in the regulation of the acute phase response of inflammation. Nuclear factor – κB stimulates the production of, among others, IL-1, IL-6, and tumor necrosis factor-α [20]. We compared in vitro two different prime compositions, one prime solution based on human albumin and second prime solution based on fresh frozen plasma. Of both primes the total antioxidant capacity, as well as that of selected individual antioxidants was measured. We also measured the release of the important prooxidants non-protein bound iron and Hb/haem in both primes.

The CPB system is believed to be the main activator of the SIRS. Coatings of the different components of the bypass system are developed to reduce the contact activation between blood and the surface of the bypass system. Several coatings are commercially available: human albumin coating, heparin coatings, trillium coating and phosphorylcholine coating. A lot of controversies are found in the literature concerning coating of CPB systems. It is difficult to compare the different studies because of the differences in methods and composition of the study groups. In several studies children with a bodyweight of less then 5 kilogram are compared to children weighing more than 15 kilograms. Furthermore different coatings are used and the measured parameters are numerous. All over it appears that most coatings preserve platelets but do not completely inhibit the inflammatory response [21]. This is due to the fact that the CPB system is only one of the many triggers of the inflammatory response. Children undergoing major heart surgery without CPB compared with children undergoing heart surgery with the use of CPB are showing a simulair SIRS reaction [4]. The results of our prospective blind randomized study comparing uncoated to PHISIO® coated CPB systems in neonates and infants with very strict inclusion criteria on bodyweight, cyanoses and syndromes are described in chapter 5.

There is lack of information as to the interaction between the coating of the CPB system and medication. Mehta and colleagues describe the loss of several medications during time in an in vitro extracorporeal membrane oxygenation circuit [22].
The use of corticosteroids in pediatric cardiac surgery is controversial. Corticosteroids are sometimes used in pediatric cardiac surgery to reduce the pro-inflammatory mediators. The timing of administration seems to be important [23]. When corticosteroids are given before start the of CPB and during CPB, the concentration of proinflammatory cytokines has been reported to decrease [24]. For this reason corticosteroids are added to the prime in the same amount as the quantity that is given intravenously to the patient. We investigated whether dexamethasone concentration decreased during recirculation of the prime in an in vitro setting both with coated and with uncoated systems. The results are described in chapter 6.

Many of the questions that are described in this introduction section are also discussed in chapter 7 (review article).

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chapter 2

Modified Ultrafiltration After Cardiopulmonary Bypass in Pediatric Cardiac Surgery

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Ann Thorac Surg 1997;64:521-5

Abstract

Background. Cardiopulmonary bypass in children results in considerable water retention, especially in neonates and small infants. Dilution of plasma proteins increases water loss into the extravascular compartments. Excessive total body water may prolong ventilatory support and may contribute to a prolongation of intensive care convalescence. After discontinuation of cardiopulmonary
bypass, modified ultrafiltration can be used to withdraw plasma water from the total circulating volume.

Methods. This retrospective study included 198 pediatric patients who underwent cardiac operations in the period from September 1991 to November 1994. Two groups were compared: 99 patients without ultrafiltration and 99 patients receiving modified ultrafiltration. The following indices were analyzed: cardiopulmonary bypass prime volume, transfused blood volume during and after the operation, postoperative chest drain loss, and hemoglobin and hematocrit levels before, during, and after the procedure.

Results. Modified ultrafiltration resulted in a significant increase in hemoglobin and hematocrit levels and a significantly lower amount of transfused blood. Mean postoperative chest drain loss was significantly less in the patients who underwent modified ultrafiltration.

Conclusions. Modified ultrafiltration decreases blood transfusion requirements and chest drain loss after pediatric cardiac surgical procedures.

Introduction

Cardiopulmonary bypass (CPB) with hypothermia and hemodilution results in an increase of total body water. Water retention is especially important in neonates and infants. The ratio of prime volume to patient blood volume is greater in smaller patients. Hemodilution increases tissue perfusion during CPB and allows the use of hypothermia, which is needed to protect against ischemic organ damage during periods of low flow and circulatory arrest [1]. Hemodilution also reduces donor blood requirements during CPB. Dilution of plasma proteins increases water loss into the extravascular compartment and postoperative blood loss as a result of clotting disturbances [2]. Cardiopulmonary bypass itself produces an important inflammatory response, and this "whole body inflammatory response" is especially large in children [3]. One of the consequences of this inflammatory reaction is increased capillary leakage. Higher capillary permeability accounts for an increase in total body water, especially in the extracellular interstitial compartment. All of these factors may have negative consequences in the postoperative course. Renal immaturity together with decreased cardiac output further delays the return to normal body water content. Intravenous diuretics and inotropic agents frequently are necessary in the younger age group to reduce the increase in total body water. If diuresis is inadequate, peritoneal dialysis may be needed temporarily. To avoid an excessive increase in body water while aiming to reduce the use of blood products as much as possible, synthetic large molecular substances are added to the CPB prime to increase the colloid osmotic pressure. Conventional ultrafiltration during CPB to reduce excess water is limited by the need to maintain a minimum level in the venous CPB reservoir. This is especially true in pediatric CPB because lower prime volumes are used. Naik and Elliott [4] could not demonstrate a significant effect of conventional ultrafiltration on reduction of the total body water increase and donor blood requirements. Using ultrafiltration immediately after the cessation of CPB (modified ultrafiltration, MUF) they were able to diminish significantly the total body water increase and donor blood requirements [4]. Another advantage of MUF over conventional ultrafiltration is its ability to return the contents of the CPB circuit to the patient in a concentrated form.

In this study, we compared two groups of 99 patients each who underwent pediatric cardiac operations in our institution. In one group we used MUF; in the other group

ultrafiltration was not used. The technique of MUF is described and its effects are evaluated on total donor blood use, postoperative chest drain blood loss, and preoperative, perioperative, and postoperative hemoglobin and hematocrit levels.

Material and Methods

The study included 198 patients who underwent cardiac operations with CPB from September 1991 to November1994. Two longitudinal cohorts were studied. The first cohort had procedures between September 1991 and July 1993, at which point MUF was introduced and used in all pediatric patients operated on thereafter. The second cohort consisted of patients operated on from July 1993 to November 1994. In the first 99 patients, no ultrafiltration was used; MUF was used in the second group of 99 patients. The two groups were comparable in sex, age, and body weight, as well as the duration of CPB. Preoperative values of hemoglobin and hematocrit were comparable (Table 1).

Table 1. Patient Characteristics[a]

Characteristic	No UF	Modified UF	p Value[b]
Sex (male/female)	50/49	55/44	...
Mean age (mo)	33.6 ± 3.94	31.8 ± 3.52	0.73
Mean weight (kg)	11.5 ± 0.75	11.1 ± 0.65	0.67
Mean CPB time (min)	114 ± 7.25	125 ± 7.30	0.26
Mean preoperative hemoglobin (mmol/L)	8.0 ± 0.13	8.1 ± 0.14	0.54
Mean preoperative hematocrit (%)	39 ± 1	39 ± 1	0.47

[a]Data are presented as n or mean ± SEM. [b]Student's t test or χ^2 test. CPB = cardiopulmonary bypass; MUF = modified ultrafiltration; UF = ultrafiltration.

Diagnoses did not differ between the groups (Table 2). Methods of surgery and anesthetic techniques essentially did not change during the study period. No changes in perfusion techniques concerning prime solutions or blood transfusion policy occurred in our unit from September 1991 to November 1994. Aprotinin (Bayer AG, Germany) is not used in

our institution for pediatric cardiac operations. Conventional ultrafiltration also was not used in our unit for these procedures.

Table 2. Patient Diagnoses

Diagnosis	No UF	MUF
ASD	18	14
VSD	29	21
AVSD	8	11
Tetralogy of Fallot	17	19
TAPVC	3	3
PA + IVS	...	2
Aortic stenosis	3	5
Aortic insufficiency (± VSD)	...	2
Mitral stenosis	...	1
Mitral insufficiency	5	4
Monoventricular malformation	1	5
TGA/DORV	9	5
VSD + CoAo	...	1
VSD + IAA	1	...
AVSD + CoAo	1	1
Miscellaneous	4	5

ASD = atrial septal defect; AVSD = atrioventricular septal defect; CoAo = coarctation of the aorta; DORV = double outlet right ventricle; IAA = interrupted aortic arch; MUF = modified ultrafiltration; PA + WS = pulmonary atresia with intact ventricular septum;
TAPVC = total anomalous pulmonary venous connection; TGA = transposition of the great arteries; UF = ultrafiltration; VSD = ventricular septal defect.

A Dideco (Dideco, Mirandola, Italy) 701 oxygenator was used for patients with a weight up to 14 kg. For patients with a body weight between 14 and 35 kg, a Dideco 702 oxygenator was used. The prime volume of the CPB circuit with the Dideco 701 oxygenator was 650 mL, whereas the prime volume of the system with the Dideco 702 oxygenator was 750 mL. The calculated volume of red blood cells needed for CPB circuit priming was deduced from the following formula:

$$TBV = Ht_2 \times (C_v + CPB_v) - (Ht_1 \times C_v) / Ht_{tbv}$$

where C_v = circulating volume (weight [kg] × 80 mL); CPB_v = prime volume in the CPB circuit (mL); Ht_1 = preoperative hematocrit; Ht_2 = target hematocrit during CPB; Ht_{TBV} = hematocrit of transfused blood; and TBV = transfused blood volume (mL). The pH was corrected with 10 mL $NaHCO_3$ 8.4% for every 250 mL of red blood cell volume. One hundred milliliters of human albumin 20% solution (CLB, Amsterdam, the Netherlands) was added, using Ringer's solution to complete the CPB priming volume. Mannitol was substituted in a dose of 0.5 g per kilogram of body weight.

A Minntech Hemocor HPH hemoconcentrator (Minntech Corporation, Minneapolis, MN) was used for MUF. In the patients with a body weight up to 14 kg, we used the Minntech HPH 400 ultrafilter with a prime volume of 27 mL. In the group of patients with a body weight from 14 to 35 kg, the Minntech HPH 600 ultrafilter with a prime volume of 43 mL was used, as the higher circulating volume in these patients necessitated a filter with a higher filtration rate. The molecular cutoff weight of both ultrafilters is 65,000 D.

The ultrafilter was primed together with the rest of the CPB circuit. The arterial cannula was connected to the inlet of the ultrafilter while the venous cannula was in connection with the outlet of the ultrafilter. During CPB, the inlet of the ultrafilter is partially clamped to allow limited flow through the filter (Fig 1). In this setup, conventional ultrafiltration is possible. Immediately after discontinuation of CPB, both cannulas remain in situ, the inlet is completely opened, and blood flows from the patient through the arterial cannula with the aid of a roller pump through the ultrafilter and back to the patient through the venous cannula (Fig 2). Our method of MUF leaves the arterial and one venous cannula in situ, whereas other groups replace the venous cannula for the purpose of MUF [4]. During MUF, the remainder of the volume in the CPB circuit is gradually remitted to the patient after having been concentrated by passage through the ultrafilter. The outlet of the ultrafilter may be partially clamped to allow a higher transmembrane pressure and a higher ultrafiltration rate. No vacuum suction is used. The volume loss in the venous reservoir is replaced first by the blood remaining in the venous tubing and later by added Ringer's solution. Modified ultrafiltration is finished when the residual fluid in the CPB circuit is almost completely replaced by clear Ringer's solution. Because the CPB system always remains fully primed, CPB can be restarted at any moment.

The following variables were noted and compared in both groups: CPB prime volume, red blood cell transfusion volume (total amount and the volumes transfused during and after the operation), hemoglobin and hematocrit levels (before, during, and after CPB as well as immediately after MUF and, for both groups, at 4 hours after arrival of the patient to the intensive care unit), and volume of postoperative chest drain loss.

Statistical analysis of the compared variables was with the Student's t test for all variables with the exception of the sex differences between the two cohorts, for which the χ^2 test was used. A p value of 0.05 or less was regarded as statistically significant (A. H. Zwinderman, PhD, Department of Medical Statistics, Leiden University).

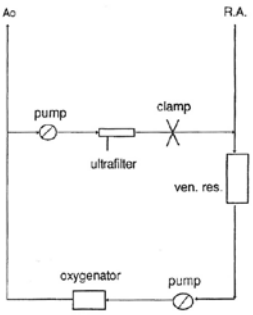

Fig 1. Placement and blood flow of the ultrafilter in the circuit during cardiopulmonary bypass. (Ao = aorta; R.A. = right atrium; ven. res. = venous reservoir.)

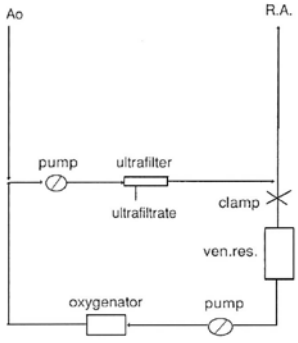

Fig 2. Placement and blood flow of the ultrafilter during modified ultrafiltration. (Ao = aorta, R.A. = right atrium; ven.res. = venous reservoir.)

Results

Mean CPB prime volumes were larger in the MUF group, with a mean (± standard error of the mean [SEM]) volume of 707 ± 7.89 mL in the group without ultrafiltration and 809 ± 8.64 mL ($p < 0.01$) in the group with MUF. This difference is attributed exclusively to the volume needed to fill the ultrafilter and the extra tubing required for MUF.

Table 3. Red Blood Cell Transfusion[a]

Transfusion	No UF	MUF	p Value[b]
Mean RBC volume transfused during CPB (mL)	173 ± 10.41	157 ± 10.52	0.26
Mean RBC volume transfused after CPB (mL)	147 ± 13.43	109 ± 9.17	<0.05
Mean total RBC volume transfused (mL)	318 ± 16.78	267 ± 11.89	<0.05

[a]Data are presented as mean ± SEM. [b]Student's t test.

CPB = cardiopulmonary bypass; MUF = modified ultrafiltration; RBC = red blood cell; UF = ultrafiltration.

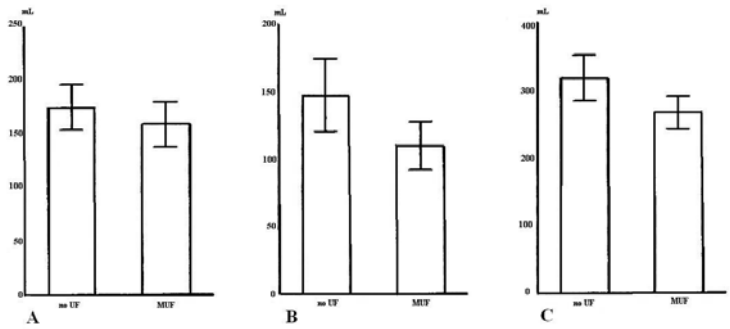

Fig 3. (A) Red blood cell volume transfused during cardiopulmonary bypass. (B) Red blood cell volume transfused after cardiopulmonary bypass. (C) Total transfused red blood cell volume. (MUF = modified ultrafiltration; UF = ultrafiltration.)

No difference was found between the groups for the red blood cell volume supplied during the operation. However, postoperative red blood cell transfusion was significantly less frequent in the group receiving MUF. The total of perioperative red blood cell transfusions was also lower in the group with MUF (Table 3; Fig 3).

Mean postoperative chest drain loss was significantly less in the patients who underwent MUF: 20.1 ± 1.57 versus 29.1 ± 3.67 mL/kg ($p < 0.05$).

Preoperative hemoglobin and hematocrit levels did not differ between the groups (see Table 1). During CPB, the mean values of hemoglobin and hematocrit were lower for the group having MUF ($p < 0.05$). After discontinuation of CPB, the hemoglobin and hematocrit levels did not change in the group in which MUF was not used. Red blood cell transfusions were used in the early postoperative period to increase the hemoglobin concentration to a target level of 7.0 mmol/L in all patients. Modified ultrafiltration resulted in a significant rise of hemoglobin and hematocrit levels ($p < 0.001$). Early red blood cell transfusion could therefore be avoided in most patients in the MUF group. Hemoglobin and hematocrit values 4 hours after arrival to the intensive care unit were not different for both groups, as a result of blood transfusion in the group of patients without MUF (Table 4; Fig 4).

Table 4. Hemoglobin and Hematocrit Values During and After the Operation[a]

Measurement	No UF	MUF	p Value[b]
Mean hemoglobin during CPB (mmol/L)	5.2 ± 0.09	4.9 ± 0.06	<0.05
Mean hematocrit during CPB (%)	25 ± 5	24 ± 3	<0.05
Mean hemoglobin after CPB/MUF (mmol/L)[c]	5.2 ± 0.09	6.7 ± 0.10	<0.001
Mean hematocrit after CPB/MUF (%)[c]	25 ± 5	33 ± 5	<0.001
Mean hemoglobin 4 h after arrival at ICU (mmol/L)	6.8 ± 0.12	6.6 ± 0.09	0.24
Mean hematocrit 4 h after arrival at ICU (%)	33 ± 6	32 ± 5	0.20

[a]Data are presented as mean ± SEM. [b]Student's t test. [c]In the group with no ultrafiltration, values were measured after discontinuation of CPB; in the group with MUF, values were measured after MUF.
CPB = cardiopulmonary bypass; ICU = intensive care unit; MUF = modified ultrafiltration; UF = ultrafiltration.

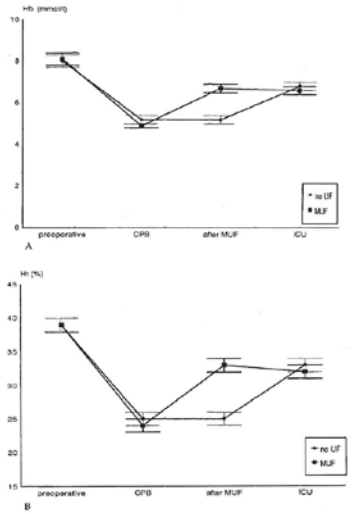

Fig 4. (A) Course of hemoglobin (Hb) levels. (B) Course of hematocrit (Ht) levels. (CPB = cardiopulmonary bypass; ICU = intensive care unit; MUF = modified ultrafiltration; UF = ultrafiltration.) Hb (mmol/l)

Discussion

The findings that the use of MUF after cessation of CPB increases hemoglobin and hematocrit levels and reduces postoperative chest drain loss and blood transfusion requirements have been described by Naik and Elliott [4]. Our study confirms these results and encourages us to continue using the MUF technique.

Modified ultrafiltration removes water from the circulating volume, leading to an immediate increase in hemoglobin and hematocrit levels. Removing water from the circulation gives us the opportunity to return almost entirely the volume of the CPB circuit to the patient. The need for blood transfusion decreases significantly, as does postoperative blood loss.

In many patients, mean blood pressures increased during MUF. This has also been observed by others [4, 5]. The rise in blood viscosity due to water loss may be responsible for this blood pressure increase. Another explanation may be found in the removal of vasoreactive substances by MUF. Inflammatory mediators such as interleukins, tumor necrosis factor, and activated complement components, many of them having cardiodepressive characteristics, are reported to be removed by ultrafiltration [6, 7].

We did not measure total body water content, but others have reported significant decreases in total body water using MUF [5]. The problem of excess water is especially important after neonatal cardiac procedures. In the pre-MUF period (before July 1993), we observed this problem much more often than in the patients in whom MUF was used (after July 1993).

Modified ultrafiltration is a useful tool to combat water retention after pediatric cardiac operations. It diminishes the need for blood transfusion by both removing excess water and returning all CPB blood to the patient in a concentrated form. It also decreases postoperative chest drain loss. Of course, the use of MUF does not reduce the need for further efforts to limit the CPB prime volume to diminish water overload while at the same time trying to restrict the use of blood and blood products as much as possible. Our CPB prime volumes for pediatric cardiac operations have been reduced substantially in recent years. Up to 6 kg body weight, a Dideco Liliput oxygenator is now used with a total prime volume of 350 mL, including the ultrafilter and extra tubing needed for MUF. In the group of patients with a body weight of 6 to 14 kg, a Dideco 701 oxygenator is used with a total CPB prime volume of 650 mL, and in the group of patients with a body weight of 14 to 29 kg, a Dideco 702 is used with a total CPB prime volume of 750 mL.

We studied retrospectively two comparable cohorts of patients and found significantly lower blood transfusion requirements and chest drain blood loss after MUF. Our perioperative protocols did not change during the period of the study. We believe that despite the shortcoming of not being prospective and randomized, this study clearly demonstrates the beneficial effects of MUF in a pediatric cardiac operative population.

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chapter 3

Prime solutions for cardiopulmonary bypass in neonates: Antioxidant capacity of prime based on albumin or fresh frozen plasma

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Abstract

Background. Oxidative damage and inflammation are believed to play an important role in postoperative complications after cardiopulmonary bypass. During bypass, a prime solution with a high antioxidant capacity may reduce the oxidative damage and inflammation. We investigated total antioxidant capacity and individual scavengers during the preparation of 2 different prime solutions.

Methods. The prime solutions were prepared with either pasteurized human albumin or fresh frozen plasma. The total antioxidant capacity was measured with the total radical antioxidant parameter assay and with the ferric-reducing ability of plasma assay. The individual scavengers vitamin C, sulfhydryl groups, uric acid, and total protein were measured before, during, and after the prime preparation. Malondialdehyde was measured as a parameter for lipid peroxidation.

Results. Neither prime solution showed a total radical antioxidant parameter value. The ferric-reducing ability of plasma value of prime solutions was lower than that of undiluted human albumin or fresh frozen plasma. Addition of mannitol did not increase the ferric-reducing ability of plasma value. Vitamin C was only found in the fresh frozen plasma prime. Both prime solutions contained sulfhydryl groups and uric acid in low concentrations. During ultrafiltration, low-molecular-weight antioxidants were lost into the ultrafiltrate.

Conclusions. We showed that prime solutions based on either albumin or fresh frozen plasma had very low antioxidant capacity and that ultrafiltration of the prime solution further lowers this capacity. A prime solution with a low antioxidant capacity may increase oxidative stress in neonates undergoing cardiopulmonary bypass.

Introduction

Oxidative damage and inflammation are believed to be important causes of morbidity related to cardiopulmonary bypass (CPB), which is high, especially in small infants [1]. During CPB, prooxidative substances, such as nonprotein-bound iron, are released while the plasma antioxidant capacity decreases, resulting in excess accumulation of reactive oxygen species [2]. This may be especially important in neonates who, compared with more mature patients, already have low plasma iron-binding capacity and poor antioxidant defenses, which decrease even further after CPB [3, 4]. This may cause oxidative stress, direct tissue damage, and multiorgan failure. Moreover, oxidative stress may upregulate the inflammatory response and initiate a vicious oxidative circle [1, 5]. Enhancing the antioxidant capacity of patients during CPB could limit the direct oxidative tissue damage and modulate the undesired inflammatory response.

The prime solution of the CPB system can substantially affect the plasma antioxidant capacity because of the high ratio between prime and circulating volume of neonatal patients. As previously reported, even transfusion of a relatively small volume of fluid with a low antioxidant capacity decreases the ability of plasma of neonates to catabolize reactive oxygen species [6]. Thus, a large prime volume with a low antioxidant capacity may dramatically decrease the antioxidant capacity of neonates undergoing CPB. On the other hand, a supplementation of the prime solution with antioxidants could decrease the negative effect of the dilution and limit the oxidative stress during CPB [7-9]. Albumin-based prime solution, which is routinely used in our institution, may have a lower antioxidant capacity than an alternative prime solution based on fresh frozen plasma (FFP). Ultrafiltration during prime preparation could change the antioxidant status of prime solution by means of removal of water-soluble antioxidants or prooxidative substances, such as nonprotein-bound iron. Therefore, we investigated the total antioxidant capacity, as well as that of selected individual antioxidants, during the preparation of these two different prime solutions.

Materials and Methods

This in vitro study was approved by the Scientific Committees of the Department of Pediatrics and the Department of Thoracic Surgery. Preserved packed red blood cells

(RBCs; stored for <5 days) and FFP were delivered by our blood bank. RBCs were preserved and stored in saline, adenosine, glucose, and mannitol (SAGM) solution. FFP contains citrate, which is used as an anticoagulant during donor blood preparation. Informed consent of the donors was obtained. Twenty percent human albumin solution was obtained from CLB (Amsterdam, The Netherlands). This is a plasma-derived product prepared by means of ethanol fractionation and pasteurization (10 hours at 60°C). It contains mainly albumin (95%), but other proteins are also present, such as prealbumin and haptoglobin.

Prime Composition and Preparation

Prime composition and preparation are shown in Figure 1. Two different prime solutions on the basis of either albumin or FFP were prepared, each on 5 separate occasions.

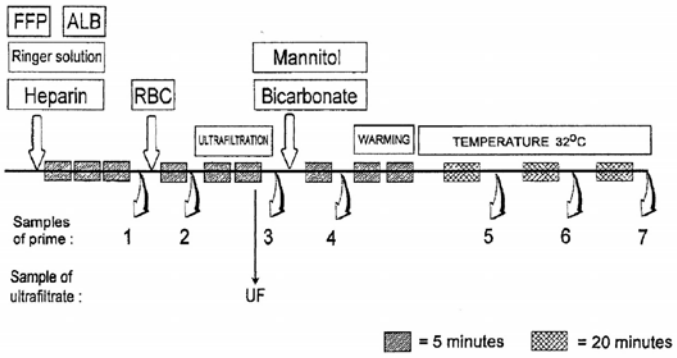

Figure 1. Composition, preparation, and sampling of the primes. *1*, Clear prime; *2*, RBC prime before ultrafiltration; *3*, RBC prime after ultrafiltration; *4*, RBC prime after mannitol and bicarbonate; *5*, *6*, and *7*, RBC prime after 20, 40, and 60 minutes, respectively, at a temperature of 32°C. Sample of ultrafiltrate was collected at the end of ultrafiltration.

At room temperature, the cardiotomy reservoir of a Dideco Lilliput 901 CPB system (Dideco, Mirandola, Italy) was filled with 500 mL of Ringer's solution with 1500 IU of

heparin and either 100 mL of 20% human albumin (ALB prime) or 100 mL of FFP (FFP prime). The oxygenator was filled, and after 15 minutes of circulation, 100 mL of packed RBCs were added to the clear ALB or FFP prime. After 5 minutes of circulation of this RBC prime, ultrafiltration was performed with a Minntech Hemocor HPH 400 (Minntech Corp, Minneapolis, Minn) to reduce the prime volume to 350 mL. Then 1.0 g of mannitol and 4.0 mL of 8.4% sodium bicarbonate were added. Five minutes later, the temperature of the prime was increased to 32°C for 60 minutes. (In a clinical situation, this decreases the temperature difference between prime and patient.) The flow of the prime and the air flow (fraction of inspired oxygen = 0.21) was 0.50 L/min. The flow through the ultrafilter was 0.20 L/min, with a constant pressure of 75 mmHg.

Samples

Samples of undiluted albumin solution and FFP were collected. Samples (3 mL) of the prime at various stages of its preparation and one sample of ultrafiltrate were collected, protected from light, immediately cooled, and transported to the laboratory to be centrifuged (4°C for 5 minutes at 2000 rpm). For measurements of vitamin C, the samples were stabilized with metaphosphoric acid and deferoxamine mesylate (Desferal). The samples were frozen until analysis (–80°C under argon). Preliminary studies showed that values did not change during storage [3].

Laboratory Measurements

The total antioxidant capacity was measured with two different methods. The total radical antioxidant parameter (TRAP) assay measures the ability of the investigated sample to inhibit peroxidation of the target lipid (linoleic acid) induced by peroxyl radicals. This lipid peroxidation is measured by means of oxygen consumption with an oxygen electrode. Extent of the inhibition, if any, is quantified by using Trolox (water-soluble analog of vitamin E) as a calibrator [6, 10]. The ferric-reducing ability of plasma (FRAP) assay measures the capacity of the sample to reduce ferric ion (Fe^{3+}) to the ferrous form (Fe^{2+}). This reduction can be measured by means of spectrophotometry because ferrous ions bind to tripyridyltriazin to form a blue-colored complex. The results of the assay are

quantitated by use of a solution containing ferrous ions in a known concentration as a calibrator [11].

Individual Antioxidants

Vitamin C was measured with high-performance liquid chromatography, as previously described [12-14]. This method measures total ascorbic acid and its oxidized form, and reduced ascorbic acid is calculated by subtraction. Knowing the concentration of vitamin C and the exact (corrected for sampling) volume of prime, we calculated the total amount of vitamin C in the prime. Sulfhydryl groups were determined by spectrophotometry, as previously described [15]. Uric acid, total protein, and albumin were measured by means of an automatic analyzer (Hitachi 747; Roche Diagnostics GMBH, Mannheim, Germany).

Lipid Peroxidation Product

Malonyldialdehyde (MDA) was measured by means of high-performance liquid chromatography, as adapted and modified from the previous study [16].

Statistics

All results are reported as means ± standard deviation (SD). Differences between oxidized/total ratio of vitamin C before and after FFP prime preparation were tested by using the *t* test for paired samples. Differences between means of the amount of vitamin C present in the prime in different samples were measured by 1-way analysis of variance. Differences between the amount of vitamin C present in FFP prime before and after ultrafiltration were tested by using a paired *t* test. Correlation between free hemoglobin/heme concentration and oxidized/total vitamin C ratio was tested with the Pearson method by the 2-tailed test of significance.

Results

The samples from one FFP prime preparation became hemolytic during centrifugation. After box-plot analysis, we excluded the results of these samples from FRAP and vitamin C statistic analysis.

Total Antioxidant Capacity

ALB prime. The TRAP assay detected no antioxidant capacity in either undiluted albumin solution or in the samples of the prime. The FRAP value in the undiluted albumin solution was 1813.7 µmol/L. In the clear prime, the FRAP value was 651.6 ± 190.8 µmol/L and increased during the procedure, reaching a value of 901.2 ± 255.9 µmol/L. The ultrafiltrate had a FRAP value of 542.5 ± 225.3 µmol/L (Figure 2).

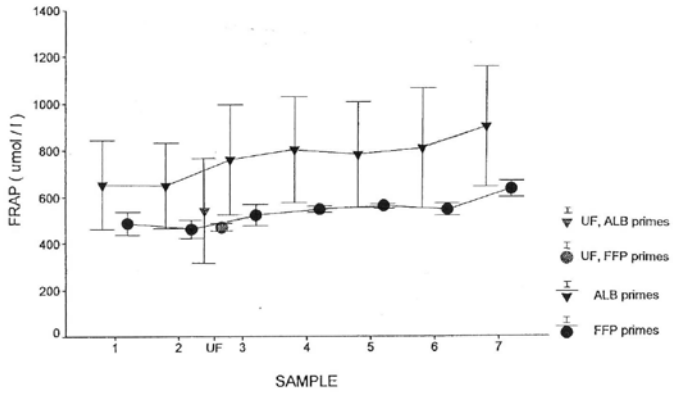

Figure 2. Measurement of FRAP assay (mean ± SD; circulation 6 excluded). Sampling is described in the legend for Figure 1. *UF*, Ultrafiltration.

FFP prime. The TRAP value of undiluted FFP was 752.75 ± 40.1 µmol/L; however, the assay detected no antioxidant capacity in the samples of the prime. The FRAP value in the undiluted FFP was 2841.5 ± 703.2 µmol/L. In the clear prime the FRAP value was 486.9 ± 49.8 µmol/L and increased during the procedure, reaching a value of 635.3 ± 36.4 µmol/L. The ultrafiltrate had a FRAP value of 472.1 ± 17.4 µmol/L (Figure 2). Although there was a difference in FRAP values between the ALB and the FFP primes after the preparation, this difference was not significant ($P = .08$).

Vitamin C

ALB prime. No vitamin C was detected.

FFP prime. FFP prime contained vitamin C (Figure 3,*A*-*C*). Its mean concentration in the clear prime was 8.86 ± 3.15 µmol/L, ranging from 6.07 to 11.80 µmol/L and decreasing to 4.64 ± 1.27 µmol/L after 20 minutes of circulation of the prime at 32°C and to 2.78 ± 0.37 µmol/L after completion of the procedure (analysis of variance, $P = .0035$).

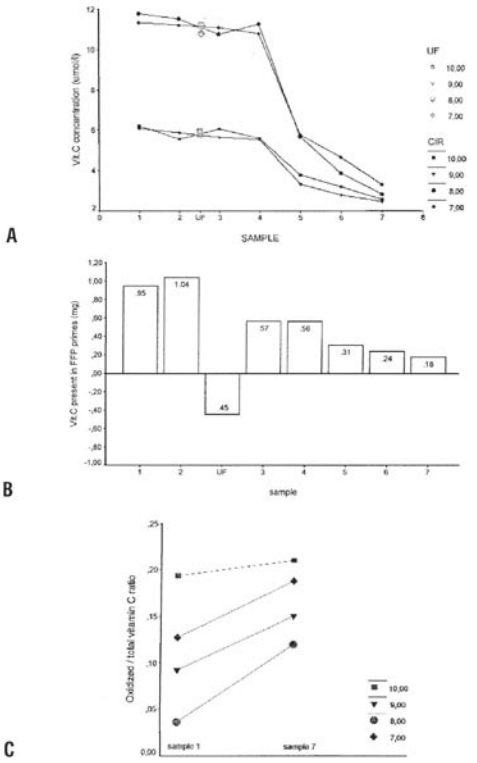

Figure 3. A, Individual vitamin C concentrations (in micromoles per liter) during the preparation of FFP prime (circulation 6 excluded). Sampling is described in the legend for Figure 1. Notice the large variability in concentrations between the different circulations *(CIR)* and especially the concentrations in the ultrafiltrates *(UF)*. B, The total load (mean) of vitamin C in milligrams during the preparation of the FFP primes (circulation 6 excluded). Sampling is described in the legend for Figure 1. Notice the loss of vitamin C during the whole preparation (paired *t* test: $P = .017$ for sample 1 and sample 7; before and after the preparation, respectively) and especially during ultrafiltration (paired *t* test: $P = .019$ for sample 2 and sample 3; before and after ultrafiltration, respectively). C, Measurements of oxidized/total ratio vitamin C (circulation 6 excluded). Sampling is described in the legend for Figure 1 (paired *t* test: $P = .006$ for sample 1 and sample 7).

In the ultrafiltrates, vitamin C was found in the same concentration as in the primes. Because of this, the total amount of vitamin C in the prime decreased during ultrafiltration from 1.04 ± 0.40 mg to 0.57 ± 0.20 mg. During the circulation of the prime at 32°C, the total amount of vitamin C decreased even further to 0.18 ± 0.03 mg after completion of the procedure. The oxidized/total vitamin C ratio increased from 0.11 ±

0.07 in the clear prime to 0.17 ± 0.04 after completion of the procedure. The ratio was positively correlated with free hemoglobin/heme concentrations in the primes (r = 0.88-0.83 and 0.83-0.69, respectively). Interestingly, in the hemolytic samples the ratio increased to 0.49 after the preparation and was strongly correlated with concentrations of heme (r = 0.89 and P = .003, data not shown).

Sulfhydryl Groups

Sulfyhdryl data are shown in Figure 4.

ALB prime. Clear prime contained sulfhydryl groups at a concentration of 203.4 ± 0.9 μmol/L. This concentration did not change after addition of RBCs but increased after ultrafiltration to 315.8 ± 10.9 μmol/L and remained stable until completion of the preparation. Ultrafiltrate contained no sulfhydryl group. Calculated sulfhydryl/total protein ratio was 4.74 ± 0.20 in clear prime and 5.53 ± 0.77 after completion of prime preparation.

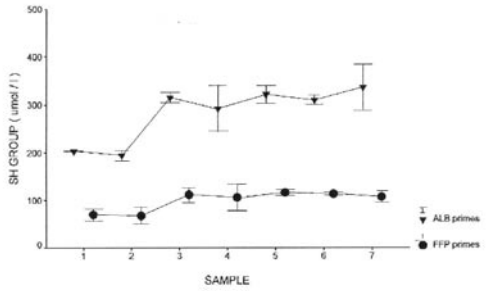

Figure 4. Measurements of sulfhydryl *(SH)* groups (mean ± SD). Sampling is described in the legend for Figure 1. No sulfhydryl groups were present in the ultrafiltrate.

FFP prime. Clear prime contained sulfhydryl groups at a concentration of 70.1 ± 12.2 μmol/L. This concentration did not change after adding RBCs but increased slightly after ultrafiltration to 111.8 ± 15.2 μmol/L and remained stable until completion of the preparation. The ultrafiltrate contained no sulfhydryl group. Calculated sulfhydryl/total protein ratio was 5.70 ± 1.19 in clear prime and 5.77 ± 0.64 after completion of prime preparation.

Uric Acid

ALB prime. Clear prime contained no uric acid. After adding RBCs, uric acid was detectable at a concentration of 0.01 ± less than 0.001 mmol/L (mean ± SD) and increased to 0.02 ± 0.01 mmol/L at the end of preparation. In ultrafiltrate, uric acid was found at a concentration of 0.01 ± 0.01 mmol/L.

FFP prime. Clear prime contained uric acid at a concentration of 0.04 ± less than 0.001 mmol/L and remained stable until the temperature increased and then increased to 0.06 ± 0.01 mmol/L after completion of prime preparation. In the ultrafiltrate, uric acid was found in the concentration of 0.04 ± less than 0.001 mmol/L.

Total Protein and Albumin

ALB prime. Clear prime contained 42 ± 1.8 g/L protein (consisting of albumin, although we did not exclude traces of other proteins). Addition of RBCs slightly decreased the concentration of protein, and ultrafiltration increased it to 62 ± 2 g/L. After that, the protein content remained stable.

FFP prime. Clear prime contained 12 ± 0.9 g/L protein. Adding RBCs slightly decreased the concentration of protein, and ultrafiltration increased it to 18.4 ± 0.9 g/L. After that, the protein content remained stable. Protein in all FFP samples consisted of 55% to 60% albumin.

Malonyldialdehyde

ALB prime. Clear prime contained MDA at a concentration of 0.43 ± 0.05 μmol/L. This concentration increased slightly after adding RBCs (0.47 ± 0.1 μmol/L) and much more after ultrafiltration to 0.66 ± 0.08 μmol/L and slightly decreased during circulation at 32°C, reaching 0.62 ± 0.1 μmol/L after completion of the preparation. The ultrafiltrate contained MDA at a concentration of 0.13 ± 0.02 μmol/L.

FFP prime. Clear prime contained MDA at a concentration of 0.19 ± 0.07 μmol/L. This concentration increased slightly after adding RBCs to 0.28 ± 0.02 μmol/L and after ultrafiltration to 0.31 ± 0.05 μmol/L and remained stable until completion of the preparation. Ultrafiltrate contained MDA at a concentration of 0.12 ± 0.04 μmol/L.

Discussion

CPB was reported to completely deplete the total antioxidant capacity in neonates [4]. However, a possible effect of the composition of prime solution and its dilution effect on plasma of patients was not discussed. In our experience, even transfusion of a relatively small volume of pasteurized plasma protein solution, a plasma-derived product with no TRAP value, can decrease the antioxidant capacity of plasma of neonates [6].

Table 1. Plasma concentrations of the various plasma antioxidants compared with the concentrations in the prime solutions after the prime preparation procedure

	Term babies	Adults	ALB prime	FFP prime
Uric acid (μmol/L)	317 ± 66	285 ± 55	0.02 ± 0.01	0.06 ± 0.01
Vitamin C (μmol/L)	130 ± 55	62 ± 16	ND	2.78 ± 0.37
Sulfhydryl groups (μmol/L)	422 ± 80	496 ± 57	315.8 ± 10.9	111.8 ± 15.2

Data are presented as means ± standard deviation. Data for term babies and adults are adapted from reference[28]. *FFP,* fresh frozen plasma; *ND*, not detectable.

The ratio between the prime volume and the circulating volume of neonates is much higher than during transfusion. As a result, prime solution with a low antioxidant capacity could substantially decrease the ability of these patients to metabolize reactive oxygen species. We therefore studied the antioxidant capacity of two different prime solutions. We showed that both ALB and FFP prime solutions have a low total antioxidant capacity. TRAP assay revealed no antioxidant capacity in ALB prime, reflecting the fact that human albumin solution has no TRAP value. This is probably because of aggressive processing of donor plasma causing damage or loss of antioxidants during manufacturing of this plasma-derived product. The TRAP value of FFP prime was also undetectable, probably as a result of dilution of donor plasma, which had a normal TRAP value. The FRAP value of human albumin solution was originally much lower than that of FFP. However, the FRAP value of the clear ALB prime was, surprisingly, slightly higher than that of FFP prime. This suggests a different effect of dilution of human albumin solution

and FFP or an interaction with heparin. Heparin is believed to have an antioxidant activity; however, its exact mechanism is not clear [17]. Moreover, measurement of total antioxidant capacity can be influenced by heparinization of samples, giving higher apparent results [18]. In FFP prime this effect of heparin can be lower because of its binding to antithrombin III in donor plasma. In ALB prime, unbound heparin can possibly increase the results of the FRAP assay. Relatively high FRAP values of ultrafiltrates of both primes suggest that this assay mainly measures the effect of low-molecular-weight antioxidants. Interestingly, the hemolytic samples had very high FRAP values. Mannitol, which is widely used as an important hydroxyl radical scavenger, did not improve the total antioxidant capacity of the prime solutions [19,20].

For further insight into the antioxidant properties of the prime solutions, we also investigated a few selected antioxidants during the prime preparation. Vitamin C, an important secondary antioxidant, was not detected in ALB prime, probably because of its loss during the production process of albumin solution. There was a large variability in vitamin C concentrations between the different preparations of the FFP prime, probably because of donor variability. These differences became small after the prime preparation. The concentrations remained stable after adding RBCs, bicarbonate, and mannitol but decreased sharply during circulation at 32°C. RBCs rapidly take up oxidized vitamin C and slowly release the reduced form of vitamin C [21]. This active uptake of vitamin C may explain the decrease of concentration of total vitamin C. Concomitantly, with a decreasing concentration of vitamin C, its oxidized/total ratio increased, suggesting a rise in oxidation or less effective recycling of oxidized vitamin C. Oxidation of vitamin C can be explained by interaction with pro-oxidative free hemoglobin/ heme present in the prime. Glutathione plays a crucial role in the recycling of oxidized vitamin C [22]. The ability of RBCs to maintain glutathione is diminished during the prime preparation (unpublished data). This can result in decreased recycling of vitamin C and increasing of its oxidized/total ratio in the prime solution. Ultrafiltration did not change the concentration of vitamin C; however, its total amount in the prime decreased because of loss into the ultrafiltrate.

Uric acid is the most important antioxidant and contributor to the TRAP value of human plasma [6, 10]. Both primes contained very low concentrations of uric acid. However, it

was much higher in FFP prime than in ALB prime. Uric acid in FFP prime originated from donor plasma, and its low concentration is an effect of its dilution. Clear ALB prime contained no uric acid, and RBCs added to the prime contributed to its minimal concentration thereafter. The concentration of uric acid in ultrafiltrate was the same as in the primes, indicating that this small molecule was (similarly to vitamin C) freely filtered out during ultrafiltration.

Proteins used to maintain the colloid osmotic pressure during CPB (especially albumin) can contribute to the antioxidant capacity of the primes. Albumin binds pro-oxidative heme and transition metals, whereas its sulfhydryl and, as recently reported, hydroxyl groups can act secondarily (eg, scavengers and antioxidants) [23, 24]. Moreover, human albumin solution also contains some haptoglobin, which binds potentially pro-oxidative hemoglobin [25]. ALB prime has higher protein (albumin) contents than FFP prime, which is a logical consequence of their composition. High albumin concentration in ALB prime could improve its antioxidant capacity [23]. Plasma sulfhydryl groups are mainly present in the cysteine components of proteins and in low concentrations in glutathione. However, the sulfhydryl/protein ratio was lower in ALB prime than in FFP prime, and we found free heme and nonprotein-bound iron in ALB prime (unpublished data). This can be explained by oxidation of sulfhydryl groups as a result of plasma processing during the production of albumin solution.

MDA is a product of lipid peroxidation but can also attack proteins and DNA [26]. It was present in both clear primes in very low concentrations because of the effect of dilution of donor plasma and albumin solution. Concentrations of MDA in ultrafiltrate were much lower than in the primes, indicating that MDA, despite its small molecular size, was not effectively ultrafiltered. MDA can bind to proteins, which probably prevents its ultrafiltration.

We saw hemolysis during centrifugation in the samples from FFP prime. Interestingly, when the same RBCs were used for ALB prime, no hemolysis was seen. It is possible that these particular RBCs were more susceptible for factors that could be stronger in FFP prime than in ALB prime. No TRAP value, lower FRAP value, the presence of vitamin C, a lower concentration of protein/albumin, and sulfhydryl groups in the FFP prime can be explanations for the hemolysis.

We used ultrafiltration during the preparation of the primes in an attempt to decrease the metabolic load from preserved RBCs and to reduce the prime volume [27]. In this study we showed that ultrafiltration also removes important low-molecular-weight antioxidants, such as vitamin C and uric acid. Their concentrations were not changed; however, the total amount of these antioxidants in the prime decreases. This implies that ultrafiltration during and after CPB may reduce the amount of important low-molecular-weight antioxidants in the circulation of the patient. However, we showed that despite the loss of these low-molecular-weight antioxidants, the total antioxidant capacity of the prime is not decreased after ultrafiltration.

In conclusion, we showed that both ALB prime and FFP prime had no TRAP value. FRAP values were much lower than the values measured in the undiluted human albumin solution or in FFP prime. Mannitol did not improve the antioxidant capacity of the primes. During ultrafiltration, low-molecular-weight antioxidants were lost into the ultrafiltrate. Ultrafiltration was not able to decrease the concentration of MDA in the primes. Composition and antioxidant capacity of prime may substantially affect antioxidant capacity of neonates undergoing CPB. To emphasize the marked dilutional changes in antioxidant concentration that could occur, we compare the normal plasma antioxidant concentrations in terms of babies and adults with the concentrations found in prepared primes (Table 1) [28]. If ultrafiltration is used during the prime preparation, attention has to be paid to supplementation of antioxidants to the prime after the use of ultrafiltration.

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chapter 4

Increasing the antioxidative capacity of neonatal cardiopulmonary bypass prime solution: an in vitro study

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Abstract

Background. Inflammation and oxidative damage are believed to play an important role in the postoperative complications after cardiopulmonary bypass (CPB) in neonates. During the preparation of the prime, red blood cells (RBCs) release non-protein-bound iron (NPBI) and free haemoglobin/haem (Hb/haem). The presence of these prooxidants in the prime solution may increase oxidative stress in neonates undergoing CPB. The solution used as the basis of the prime solution may influence the degree of this oxidative stress.

Methods. We investigated the NPBI and the Hb/haem binding capacities of two different prime solutions: a prime based on pasteurized human albumin and a prime based on fresh frozen plasma. The presence of NPBI and free Hb/haem were measured during and after the preparation of the prime solution.

Results. Only in the albumin prime was NPBI detectable. However, in both primes, the concentrations of free Hb/haem increased.

Conclusion. Thus, to reduce the prooxidative effects of NPBI and free Hb/haem, RBCs should be added to the prime at the last possible moment. Adding fresh frozen plasma should be considered, as this would result in no detectable NPBI in the prime solution.

Introduction

It is well known that neonates have poor antioxidative and iron binding capacities compared with infants and adults [1]. Pyles and coworkers demonstrated that after cardiopulmonary bypass (CPB) in children, the antioxidant capacity is diminished [2]. Other studies showed that the iron binding capacity during and after CPB decreases due to the effect of haemodilution [3, 4]. Preserved red blood cells (RBCs) are often added to the CPB prime solution to reverse the effect of haemodilution. Shear stress during the prime procedure and during CPB may cause haemolysis of RBCs and non-protein-bound iron (NPBI), free haemoglobin and haem (Hb/haem) are released [5]. Mumby showed that, especially in neonates, an iron overload occurred [6]. NPBI is normally not present in plasma. The antioxidative proteins, ceruloplasmin and transferrin, respectively, oxidize and bind iron, thereby offering considerable protection against oxygen radicals generated by iron [7]. Iron, when reduced and not protein bound, acts as a prooxidant, converting hydrogen peroxide to the highly reactive hydroxyl radical [8, 9]. Also free Hb/haem acts as a prooxidant. Free Hb/haem is bound by haptoglobin and haemopexin. Free Hb/haem stimulates lipid peroxidation [10]. Haem induces the expression of adhesion molecules in vascular endothelial cells and can potentiate the oxidative damage of these endothelial cells, caused by activated leucocytes [11, 13]. The prime solution of the CPB system can substantially affect the plasma antioxidant capacity of neonatal patients because of the relatively high prime and circulating volume ratio in this age group. We have recently demonstrated in vitro that even transfusion of a relatively small volume of fluid with a low antioxidant capacity decreases the ability of plasma of neonates to catabolize reactive oxygen species [14].

We investigated the release of NPBI and Hb/haem during the preparation of the prime solution. We prepared the prime solutions using either pasteurized human albumin solution, as routinely used in our institution, or fresh frozen plasma (FFP). It has been reported that FFP, obtained from adult donors, has a higher iron binding capacity than human albumin solution [15].

Materials and methods

Five units of preserved, leukocyte-depleted packed RBCs, stored less than five days, as well as five units of FFP were delivered by our bloodbank (Sanquin, Leiden, The Netherlands). RBCs are preserved and stored in a solution consisting of saline, adenosine, glucose and mannitol (SAGM). FFP contains citrate, which is used as anticoagulant during donor blood preparation. Informed consent of the donors was obtained. Human albumin 20% Cealb® solution was obtained from CLB (Amsterdam, The Netherlands). This is a plasma-derived product prepared by ethanol fractionation and pasteurization (10 hours at 608C). It contains mainly albumin (95%), but other proteins are also present, such as prealbumin and haptoglobin.

Prime composition and preparation (Figure 1)

The two different prime solutions, based on either albumin or FFP, were prepared on 10 separate occasions, imitating the typical clinical procedure for preparing the prime for neonatal CPB used in our hospital. At room temperature, the cardiotomy reservoir of a Dideco Lilliput 901 cardiopulmonary bypass system (Dideco, Mirandola, Italy) was filled with 500 ml of Ringer's solution, with 1500 IU of heparin and either 100 ml human albumin 20% (ALB prime) or 100 ml fresh frozen plasma (FFP prime). The oxygenator was filled and, after 15 min of circulation, 100 ml of packed RBCs were added to the 'clear' ALB prime or FFP prime. After 5 min of circulation of this 'RBC prime', ultrafiltration was performed with a Minntech Hemocor HPH 400 (Minntech Corp., Minneapolis, MN, USA) to reduce prime volume to a minimum needed prime volume of 350 ml. Then, 1.0 g of mannitol and 4.0 ml of sodium bicarbonate 8.4% were added. Five min later, the temperature was increased to 328C for 60 min (in the clinical setting, we would increase the temperature of the prime to avoid a temperature difference between prime and patient). During the whole preparation, the flow of the prime and the air flow (FiO2 0.21) was 0.50 LPM. The flow through the ultrafilter was 0.20 LPM with a constant pressure of 75 mmHg.

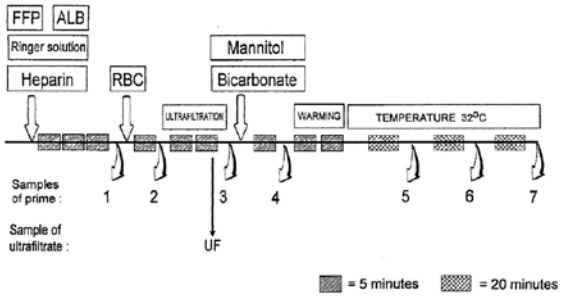

Figure 1. Composition, preparation and sampling of the primes. Samples of prime: 1. Clear prime; 2. RBC prime before ultra.ltration; 3. RBC prime after ultra.ltration; 4. RBC prime after mannitol and bicarbonate; 5, 6 and 7. RBC prime after 20, 40 and 60 min at a temperature of 328C, respectively. Sample of ultrafiltrate (UF) was collected at the end of ultrafiltration.

Samples (Figure 1)

Samples (3 ml) of the prime at various stages of its preparation and one sample of ultrafiltrate were collected, protected from light, immediately cooled, transported to the laboratory and centrifuged (4^0C, 5 min, 2000 rpm). The supernatant was frozen till analysis (- 80^0C, under argon). Preliminary studies showed that values did not change during storage [1].

Laboratory measurements

The bleomycin assay was used to measure nonhaem NPBI, as has been described elsewhere [16]. In brief, the assay system contained DNA, bleomycin and the investigated sample. In the presence of ascorbate, bleomycin causes DNA degradation if NPBI is present in the sample. The extent of this degradation is proportional to the amount of free iron. The products of the DNA degradation form adducts with thiobarbituric acid, which were measured at 532 nm with a spectrophotometer (Perkin-Elmer Corp., Norwalk, CT, USA). Haem iron does not participate in this reaction nor interfere with the test if its concentration is less than 46.5 mmol/L, i.e., if the sample is not visibly pink [17].

Free Hb/haem was measured as previously described [18]. Briefly, glacial acetic acid added to the sample breaks down free haemoglobin to haem, which accelerates oxidation

of tetramethylbenzidine (TMB) in the presence of hydrogen peroxide. This oxidation is proportional to the quantity of haem present in the sample. Oxidized TMB was measured at 600 nm. Transferrin and ceruloplasmin were measured in our routine clinical chemistry laboratory using the Hitachi 911 (Hitachi Ltd., Tokyo, Japan).

Statistics

All results are reported as mean value ± standard deviation (SD). Differences in NPBI and Hb/haem between prime solutions during and after the procedure (within ALB prime or FFP prime group) were tested using the paired Student's t -test. A p value < 0.05 was considered significant.

Results

NPBI (Figure 2a,b)

NPBI levels were measured in the samples of clear prime, after completion of the preparation and also in the ultrafiltrate. The FFP prime contained no detectable NPBI at any time. In the ALB prime, NPBI was always detected and the concentrations increased after completion of the preparation (13.3 ± 1.8 versus 26.0 ± 8.6 mmol/L). The ultrafiltrate samples, obtained during the ultrafiltration of ALB prime, also contained NPBI (8.0 ± 0.8 mmol/L).

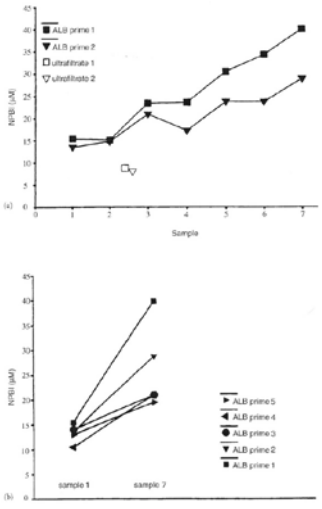

Figure 2. (a) Measurements of NPBI during preparation of two selected ALB primes. Sampling as described in Figure 1. Notice measurements in samples of ultrafiltrate. (b) Individual measurements of NPBI in clear ALB primes (sample 1) versus the same ALB primes after the preparation (sample 7). Paired t -test: p = 0.019.

Hb/haem (Figure 3a,b)

Both clear primes contained free Hb/haem in micromolar concentrations. These concentrations increased after adding the preserved RBCs. During ultrafiltration, no Hb/haem was filtered out during ALB prime preparation and only a small amount during FFP prime preparation, resulting in an increase of the Hb/haem concentration. Circulation of both primes at 32^0C further increased free Hb/haem concentration. There was a correlation between Hb/haem and free iron in the ALB prime ($r= 0.79$, $p<0.001$).

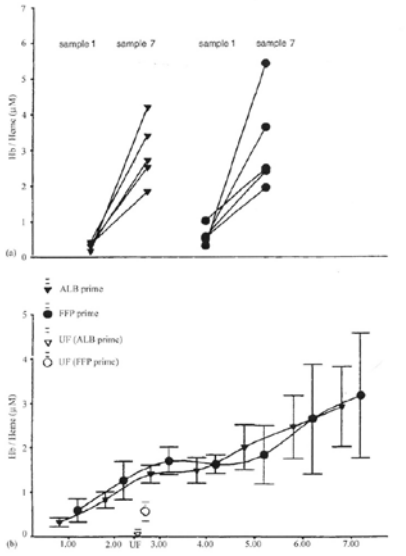

Figure 3. (a) Individual measurements of free Hb/haem in clear primes (sample 1) and in primes after preparation (sample 7). Paired t - test: p = 0.004 for ALB prime and p = 0.021 for FFP prime. (b) Measurements of free Hb/haem (mean SD) during preparation of ALB and FFP primes. Sampling as described in Figure 1. Notice measurements in samples of ultrafiltrate.

Transferin and ceruloplasmin

The concentrations of both transferrin and ceruloplasmin were below the detectable range.

Discussion

We showed that the ALB prime contained NPBI, whereas the FFP prime did not. The time course of changes in NPBI levels was very similar to that of free Hb/haem levels and there was a strong correlation between these measurements in the ALB prime. Both NPBI and free Hb/haem probably originated from RBCs as a result of haemolysis.

In the FFP prime, NPBI was not detected, which suggests that FFP binds NPBI by intact transferrin and ceruloplasmin activity [1]. This antioxidative activity was present despite the fact that the dilution of plasma in FFP prime resulted in the concentrations of both transferrin and ceruloplasmin being below the detectable range of our routine clinical chemistry laboratory. The concentration of NPBI in the ALB prime was high and may have a prooxidant effect [22]. NPBI converts hydrogen peroxide into a highly reactive hydroxyl radical, which can react with lipids, proteins or DNA. This oxidative damage is believed to play a role in the pathogenesis of many neonatal diseases [23]. Mumby and coworkers demonstrated that neonates especially showed an iron overload after CPB [6]. Also, the effect of severe iron loading of transferrin and the presence of NPBI in acute dysfunction of the right ventricle after repair of tetralogy of Fallot has been reported [4]. Both clear primes contained potentially prooxidative free Hb/haem in micromolar concentrations, which is probably related to the damage to the RBCs when they are initially separated from the plasma during the production of albumin and FFP. The concentration of free Hb/haem increased after adding RBCs, after ultrafiltration and during circulation at 32^0C, suggesting that the stressed haemolysing RBCs contributed to this increase. Since no free Hb/haem was filtered out in the ALB prime, and only in a small amount in the FFP prime, the concentration of free Hb/haem increased after ultrafiltration due to fluid removal. The absence of Hb/haem in the ultrafiltrate of the ALB prime can be explained by its binding to albumin [11]. This probably did not occur in the FFP prime because of its minimal haptoglobin and haemopexin levels and a much lower concentration of albumin. The haem concentration in the prime was low, but additional haem release due to haemolysis during and after CPB could result in a larger haem load [5]. Haem induces the expression of adhesion molecules in vascular endothelial cells and can potentiate their oxidative damage caused by activated leucocytes [12, 13]. This process may play a role in hyperactivation of leucocytes after CPB [19]. Haem also diminishes the structural stability of the membrane of RBCs undergoing shear stress [20]. This can further increase the release of prooxidative Hb/haem or NPBI during CPB [5]. On the other hand, after long-term exposure, haem upregulates expression of haem oxygenase enzyme (H0-1), which protects cells against oxidative stress [21]. We used ultrafiltration during the preparation of the primes in an

attempt to decrease the metabolic load from preserved RBCs and to reduce the prime volume [24]. We studied the ability of ultrafiltration to remove prooxidants from the primes. The concentration of Hb/haem after ultrafiltration increased due to fluid removal. This mechanism may also have contributed to the increase in NPBI after ultrafiltration of the ALB prime since the concentration of NPBI was low in the ultrafiltrate. Another explanation of the increasing levels of Hb/haem and NPBI after ultrafiltration is the release of these prooxidants caused by shear stress-induced damage of RBCs during the ultrafiltration procedure.

In summary, this study showed that, during and after the prime preparation procedure, RBCs were a source of prooxidative NPBI and free Hb/haem, which were not filtered out during the ultrafiltration. FFP is able, even after strong dilution, to bind prooxidative iron, in contrast to albumin where NPBI remained detectable. In an attempt to reduce the prooxidative capacity of the prime solution, we suggest adding the fresh, stored RBCs just prior to bypass, as this will decrease the stress affecting RBCs during the preparation of the prime. An in vivo study should determine the advantage of the FFP prime results in less iron overload during and after CPB in neonates.

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chapter 5

Phosphorylcholine Coating of Bypass Systems Used for Young Infants Does Not Attenuate the Inflammatory Response

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Abstract

Background. Contact of blood with the artificial surfaces of the cardiopulmonary bypass (CPB) system is considered to be a main cause of complement activation. Improving the biocompatibility of the system by reduction of contact activation of blood elements and thereby producing less inflammatory response is evidently desired, especially for neonates and infants who are more susceptible to the deleterious effects of CPB. A phosphorylcholine coating, PHISIO®, is designed to mimic the natural interfaces of blood. The aim of this study is to compare the influence of a phosphorylcholine-coated CPB system versus an uncoated CPB system on complement activation and clinical outcomes.

Methods. In this prospective, randomized, blind, one center study, 28 neonates and infants with a bodyweight between 3 and 6 kg who were undergoing cardiopulmonary bypass were divided in two groups, the phosphorylcholine group and the control group. Thirteen patients were assigned to the phosphorylcholine group and 15 patients to the control group. Patients with Down syndrome, prematurity, cyanosis, or reoperation were excluded. Complement factor C3b/c, human neutrophil elastase (HNE), interleukin-6, and C-reactive protein were measured before, during, and after CPB. Duration of intensive care stay, ventilation time, highest body temperature, and inotropic medication were the clinical variables.

Results. No significant differences were found between the groups for complement factor C3b/c, HNE, interleukin-6, or C-reactive protein during and after CPB. No clinical differences were observed between the groups.

Conclusions. Phosphorylcholine coating does not attenuate the complement activation during CPB in neonates and infants.

Introduction

Seghaye and colleagues reported that the fullterm neonate shows significant complement activation and leukocytes stimulation when undergoing cardiopulmonary bypass (CPB) [1]. Complement activation and leukocytes stimulation may result in postoperative organ dysfunction. Contact of blood with the nonbiologic surfaces of the CPB system has been designated as the main cause of complement activation. Improving the biocompatibility of CPB systems by means of less contact activation of blood elements and thereby less inflammatory response is evidently desirable [2].

A phosphorylcholine coating, PHISIO® (Dideco, Mirandola, Italy), is designed to mimic natural cellular surfaces and thereby to avoid recognition by the blood as foreign material. The outer cell surface is composed of predominantly phosphorylcholine polar groups that largely contribute to the nonthrombogenic properties exhibited by blood cells. Because of the hydrophilic character of the polar head group, there is less protein adsorption [3]. Furthermore, phosphorylcholine is a regular component of the outer cell membrane of all human cells [4]. In this prospective, randomized, blind, one-center study, we aimed to compare the effects of phosphorylcholine coating versus noncoating of the CPB systems on complement activation, its effect on leukocytes stimulation, and clinical outcomes in neonates and infants with a bodyweight between 3 and 6 kg. For this purpose, we measured C3b/c, a stable complement activation product, human neutrophil elastase (HNE), as a marker of neutrophils degranulation [1], the cytokine interleukin-6 (IL-6), which is a good predictor of clinical outcome [5], and C-reactive protein (CRP), an acute phase protein, as a marker for inflammatory response. Clinical variables were intensive care unit stay, ventilation time, the highest body temperature in the first 24 postoperative hours, and use of inotropic medication.

Material and Methods

Patients

Twenty-eight patients with a bodyweight between 3 and 6 kg who were undergoing surgical repair of their congenital heart defects with the use of CPB and moderate (28^{o}C) hypothermia were included in this prospective, randomized, blind study. The Medical and

Ethical Committee of our institution approved the study in January 2002. Written informed consent from parents or guardians was obtained for all patients.
Patients were randomly assigned into the phosphorylcholine-coated group (PC group; n= 13) or into the noncoated control group (NC group; n=15). Patients with Down syndrome, other syndromes or chromosomal abnormalities, prematurity, cyanosis (defined as oxygen saturation lower than 75%), use of circulatory arrest, and cardiac reoperation were excluded from this study. Postoperatively, inotropic support was used if necessary. No steroids, aprotinin, and other medication that might affect the inflammatory response were used throughout the study.

Anesthesia

All patients were premedicated with oral midazolam (0.5 mg/kg). Anesthesia was induced with sevoflurane and continued with midazolam (0.10 mg · kg-1 · h-1), sufentanil (0.04 ug · kg-1 · min-1), and pancuronium (0.15 mg/kg).

Cardiopulmonary Bypass

Before aortic cannulation, heparin (300 IU/kg) was given, and the activated clotting time was maintained above 480 seconds during CPB. For all patients, a Dideco Lilliput D901 (Dideco, Mirandola, Italy) closed system was used, with the difference of the phosphorylcholine coating. The coating was from arterial canula to venous canula, from "tip to tip." A Stockert roller pump (Stockert Instrumente GMBH, Munich, Germany) was used, inducing a nonpulsatile flow. The priming solution of 270 mL consisted of 100 mL fresh frozen plasma, 50 mL 20%, w/v, human albumin (Sanquin, Amsterdam, Netherlands), Mannitol (0.5 g/kg), Ringers' solution, and packed red blood cells if necessary to maintain a hematocrit of 25%. The CPB flow was maintained at 2.4 $L \cdot m^{-2}$. min^{-1} at 37^0C and 1.6 $L \cdot m^{-2} \cdot min^{-1}$ at 25^0C nasopharyngeal. The alpha-stat method was used for blood gas management. For myocardial protection, St Thomas cardioplegia solution was given and repeated every 30 minutes. Ultrafiltration, conventional or modified, was not used throughout the study.

Samples

Samples of whole blood (2 mL) were collected in tubes containing 10 mmol/L ethylenediamine tetraacetic acid, 10 mmol/L benzamidine, and 100 mg/mL soy bean trypsin inhibitor, final concentrations. Sample moments were after induction of anesthesia but before sternotomy (baseline), 10 minutes after start of CPB, 5 minutes before end of CPB, and 15 minutes and 6 hours after protamine administration. Samples were centrifuged (3,000 rpm, 10 minutes) and stored at -70^0C until analysis.

Laboratory Measurements

Plasma levels of activated C3 were measured with enzyme-linked immunosorbent assay (ELISA) in which specific monoclonal antibody against a neoepitope on activated C3 was used to catch the activation fragments, and biotinlayed polyclonal sheep antibodies against C3 to detect bound complement fragments [6]. As the assay does not discriminate between C3b, C3bi, or C3c, the activation products detected in the assay are further referred to as C3b/c. Results were expressed as nmol/L C3b/c, referring to an in-house standard with known levels of activation products. Elastase-α1-antitrypsin complexes were measured with an ELISA in which antielastase antibodies were coated onto an ELISA plate, and bound complexes detected with biotinylated monoclonal antibody against complexed-α1-antitrypsin. Purified human neutrophili elastase added to pooled plasma was used as a standard [6]. Results were expressed as ng/mL elastase (HNE). Interleukin 6 and CRP were measured with sandwich-type ELISAs (CLB; Department Immune Reagents, Amsterdam, Netherlands).

Collection of Clinical Data

Data on intensive care unit stay, ventilation time, highest body temperature in the first 24 hours, and use of inotropic medication were collected in a retrograde fashion.

Statistical Analyses

Statistical analyses were performed using the statistical computing package SPSS12.01 (SPSS, Chicago, Illinois). Patients' data were compared with unpaired t test. Data

corresponding to cytokines values were not normally distributed. After logarithmic transformation of the raw data, we used repeated measures analysis of variance with the Greenhouse-Geisser correction to test for differences within the groups throughout the five time periods and between the two groups. Clinical data were also not normally distributed and were analyzed with the Mann-Whitney U test. Raw data are expressed as mean ± SD values. All *p* values less than 0.05 were considered statistically significant.

Results

There was no difference between the two groups in patients' age (p=0.80) bodyweight (p=0.97), and diagnoses. The CPB time (p=0.60) and aortic cross clamp time (p=0.94) showed no differences between the groups (Table 1). Four patients in the PC group and 3 patients in the NC group were preoperatively treated with prostaglandin E1 (PGE1). No deaths or major complications occurred in either group.

Table 1. Demographic and CPB Data

	PC group		NC group		
	mean	SD	mean	SD	*p value*
Number of patients	13		15		
Demographic data					
Age (days)[a]	79.4	84.6	87.5	80.4	0.80
Body weight (kg)[a]	4.2	0.98	4.2	0.96	0.97
Diagnosis					
AVSD	0		3		
VSD	5		2		
TAPVC	1		2		
Truncus arteriosis	0		1		
TGA	5		5		
TOF	0		1		
DORV	1		1		
ASD	1		0		
CPB data					
CPB time (min)[a]	117.5	48.6	108.6	38.0	0.60
Clamp time (min)[a]	68.9	32.1	69.7	26.3	0.94

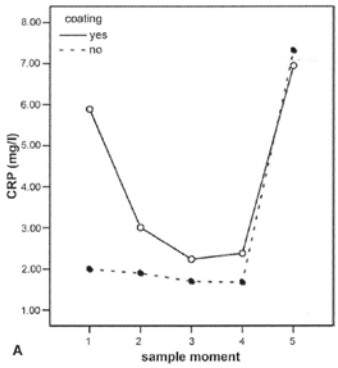
[a] Values expressed as mean ASD = atrial septal defect; AVSD = atrioventricular septum defect; CPB = cardiopulmonary bypass; DORV = double outlet right ventricle; min = minutes; NC = noncoated control group; PC = phosphorylcholine-coated group; *p* value = student *t* test; TAPVC = totally abnormal pulmonary connection; TGA = transposition of the great arteries; TOF = tetralogy of Fallot; VSD = ventricular septal defect.

There was a significant rise in complement factor C3b/c for both groups, but there was no significant difference between the groups. The C3b/c increased rapidly in both groups after the start of CPB and reached the highest point after the protamine administration. Six hours after CPB, complement factor C3b/c values had almost returned to baseline in both groups (Table 2). The HNE peaked at the end of the CPB procedure. The values decreased rapidly and had almost returned to baseline after 6 hours. Also, for HNE, there was a significant time difference in the groups, but over time, both groups showed equal behavior (Table 2). Interleukin-6 started to increase slightly during CPB and rose rapidly after the CPB procedure and protamine administration. After 6 hours, IL-6 was still increased (Table 2).

Table 2 laboratory measurement results

	group	t=1	*p* value	t=2	*p* value	t=3	*p* value	t=4	*p* value	t=5	*p value*
Complement factor C3b/c (nmol/L) (SD)	PC	49.1 (36.28)	0.15	94.08(42.10)	0.82	184.62(57.94)	0.26	269.46(126.02)	0.46	80.31(52.79)	0.12
	NC	32.47(12.12)		93.07(43.63)		164.47(64.70)		234.40(98.32)		52.47(32.40)	
Human neutrophil elastase (ng/mL) (SD)	PC	33.23(23.80)	0.58	29.23(12.95)	0.76	150.15(91.46)	0.48	130.62(69.42)	0.53	96.23(70.59)	0.09
	NC	43.86(79.03)		37.60(54.95)		137.20(109.50)		124.07(101.82)		62.73(25.87)	
Interleukin-6 (pg/mL) (SD)	PC	5.91 (2.49)	0.56	6.07 (3.16)	0.77	15.85 (14.56)	0.16	32.95 (24.09)	0.36	247.84(230.43)	0.48
	NC	5.42 (1.14)		6.16 (2.46)		25.67 (19.66)		40.29 (21.75)		231.71(117.56)	
C-Reactive protein (mg/L) (SD)	PC	0.95 (1.58)	0.22	1.04 (0.96)	0.25	0.74 (0.57)	0.36	0.81 (0.82)	0.26	6.25 (2.69)	0.78
	NC	1.54 (4.21)		1.31 (3.01)		1.34 (2.97)		1.38 (3.29)		6.39 (4.06)	

The mean baseline of CRP of the PC group is higher (5.8 $\pm$ 8.8 mg/L versus 2.0 $\pm$ 4.1 mg/L) compared with that of the control group. When the patients who received preoperative PGE1 were left out, no difference was observed anymore between the groups at baseline point (0.9 $\pm$ 1.5 mg/L versus 1.5 $\pm$ 4.2 mg/L). The CRP level did not increase during the CPB procedure but rapidly increased after the protamine administration. No significant differences were found between the groups (Fig 1A and B; Table 2).



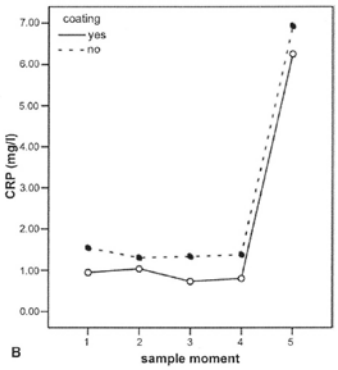


Fig 1. (A) Values of C-reactive protein (CRP), with patients who used preoperative prostaglandin E1 included for statistical analysis. (Solid line = with phosphorylcholine coating; dashed line = no coating.) (B) Values of C-reactive protein (CRP), with patients who used preoperative prostaglandin E1 excluded from statistical analysis.
(Solid line = with phosphorylcholine coating; dashed line = no coating.)

The mean length of stay in the pediatric intensive care unit was not different between the two groups. Also ventilation time, body temperature, and inotropic dosage were not significantly different in both groups (Table 3).

Discussion

The present study was performed to compare the effects of CPB on the inflammatory response and clinical outcomes of a phosphorylcholine-coated CPB system versus an uncoated CPB system in pediatric patients with a bodyweight of 3 to 6 kg. We were not able to show any differences between the two groups. Yu and colleagues showed an improved biocompatibility during an in vitro study with reduced protein adsorption and complement activation [3]. The results during in vivo studies are controversial. De Somer and colleagues showed lower complement activation and a better preservation of platelets

in a study with 10 patients [7]. Böning and coworkers demonstrated that phosphorylcholine coating or heparin coating of the CPB system used for pediatric surgery causes the same biologic effects [8]. They also demonstrated that the group with a larger prime volume shows higher values of IL-6 and higher tumor necrosis factor-α [8]. Horten and coworkers were not able to show any differences between a heparin-coated system and an uncoated system with a study population of 200 patients [9]. Interestingly, Tárnok and colleagues demonstrated that children undergoing major cardiovascular surgery without CPB show almost the same complement activation as the children undergoing major cardiovascular surgery with the use of CPB [10]. These findings are also reported in adults [11, 12]. The inflammatory response after CPB is not only initiated by the artificial surface of the CPB system, although it is seen by many as being the main cause [13]. The response is also triggered by the gas-blood interfaces, ischemia-reperfusion injury, and other proinflammatory stimuli [10, 11]. The duration of the CPB procedure and the condition of the patient play roles in the extent of the inflammation after CPB [14]. It is also known that suction and retransfusion of mediastinal shed blood contribute to the inflammatory response [15]. In our study, suction and retransfusion was performed equally in both groups. The pediatric population is a widely spread population regarding to the differences in age, bodyweight, diagnoses, and syndromes. To achieve a homogeneous group of patients for this study, we used strong exclusion criteria.

Radical oxygen species (ROS) activates nuclear factor- κB (NF-κB), which is an important protein in the regulation of the acute phase response of inflammation. Nuclear factor-κB stimulates the production of, among others, IL-1, IL-6, and tumor necrosis factor-α [16]. Down syndrome is a genetic disorder associated with ROS, and patients with Down syndrome show a wide range of defects regarding either specific or nonspecific immunity [17, 18]. Body and blood values of cyanotic patients are severely affected by the chronic hypoxia, and uncontrolled reoxygenation is associated with ROS [19]. Circulatory arrest with deep hypothermia gives a higher postoperative leukocyte count, probably due to a higher β-adrenergic stimulation [20]. Complement levels correlate with gestational age, particularly in the late prenatal period. Preterm infants have lower complement activity and complement component levels than full-term infants

[21]. Also, neutrophil counts are lower in preterm infants [22]. Patients indicated for reoperations were excluded because of the expected longer surgery time.
In conclusion, phosphorylcholine coating does not attenuate the complement activation during CPB, nor does it have an influence on clinical outcomes. As mentioned above, the inflammation after CPB is not only triggered by the artificial surface but is dependent upon many factors. Coating alone will not affect the inflammation as desired. Other agents should be considered for this purpose, such as ultrafiltration during or after CPB and the use of corticosteroids and protease inhibitors.

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chapter 6

The use of dexamethasone in coated and uncoated cardiopulmonary bypass systems. An In Vitro study

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Submitted

Abstract

Background: Corticosteroids are used in cardiac surgery to reduce pro-inflammatory mediators in pediatric cardiac surgery. Information about the interaction between the phosphorylcholine coating and corticosteroids is lacking. Phosphorylcholine coating is designed to improve the biocompatibility of the cardiopulmonary bypass (CPB) system and thereby to attenuate the activation of blood elements and to reduce the inflammatory response.

Methods: In this in vitro study 20 separate CPB pump runs (4 groups) with and without dexamethasone and with and without coating were prepared. Measurements were done to compare the effects of adding dexamethasone to phosphorylcholine coated CPB systems versus uncoated systems with the use of whole blood. The changes in concentration of dexamethasone itself in the two different systems were investigated and the production of the cytokines IL-6, IL-8 and IL-10 as well.

Results: Changes in dexamethasone concentrations were significant throughout the study period within each group. Coating did not significantly affect dexamethasone distribution throughout the study. Dexamethasone was also found in the ultrafiltrate. No differences in interleukin production were found between the groups except for the group with coating without dexamethasone, IL-8 increased significantly in this group.

Conclusions: We showed that there is no interaction between dexamethasone and the phosphorylcholine coating. We also showed that coated CPB systems do not affect significantly the production of interleukins in an in vitro model of CPB except for IL-8 which significantly increased in de coated group without dexamethasone. Our study confirms that an in vitro model is unable to trigger the production of IL-10.

Introduction

The use of CPB is associated with a systemic inflammatory response that can lead to organ injury and postoperative morbidity.[1] CPB is a potent stimulus for the release of pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α) and interleukin (IL) 1, 6 and 8.

Corticosteroids have been used in cardiac surgery for the last 30 years. Dexamethasone is a long-acting glucocorticoid with powerful anti-inflammatory and immunosuppressive effects. Dexamethasone is increasingly used in cardiac surgery. Several groups have reported that steroids given before the start of CPB, reduce pro-inflammatory mediators in adult and pediatric cardiac surgery alike.[2,3] Dexamethasone is strongly bound to proteins. No information exists about the effect of the pump prime and subsequent (modified) ultrafiltration on distribution and elimination of the free dexamethasone. There is also lack of information as to the interaction between the phosphorylcholine coating and dexamethasone.

Improving the biocompatibility of CPB systems with a coating is believed to attenuate the activation of blood elements thereby decreasing the inflammatory response. The phosphorylcholine coating PHISIO® (Dideco, Mirandola, Italy), is designed to mimic natural cellular surfaces. Phosphorylcholine is a regular component of the outer cell membrane of all human cells.[4]

In a previous study we have compared a phosphorylcholine coated cardiopulmonary bypass system to an uncoated system in pediatric patients undergoing cardiac surgery. The use of this coated system did not affect the parameters measured, such as C3b/c, Elastase HNE, Il-6 and CRP.[5]

The aims of the present in vitro study were twofold. First to measure the changes in concentration of dexamethasone in the two different systems, phosphorylcholine coated and uncoated CPB systems and due to the fact that the CPB system is not the only activator of the inflammatory response we also measure inflammatory cytokines in both systems.

Material and Methods

Ten units of fresh whole blood were delivered by our blood bank (Sanquin, Leiden, The Netherlands) and used for 20 separate in vitro experimental circulations. Five hundred ml of whole blood was collected in a bag containing citrate-phosphate-dextrose (70 ml) as anticoagulant and cooled directly to 22^0C. Written informed consent of the blood donors was obtained. Every blood bag was used for 2 circulations (200 ml for each run), in an uncoated system and in a coated system. Four experimental circulation groups were studied, 5 uncoated systems without dexamethasone (UND), 5 coated systems without dexamethasone (CND), 5 uncoated systems with dexamethasone (UWD) and 5 coated systems with dexamethasone (CWD).

Bypass system and prime procedure

For all circulations a Dideco Lilliput D901® (Dideco, Mirandola, Italy) with integrated soft shell venous reservoir was used. The PHISIO® coated system were completely coated 'from tip to tip'. A Stockert® SIII rollerpump (Stockert Instrumente Gmbh, Munich, Germany) was used inducing nonpulsatile flow. At the end of the circulation ultrafiltration was performed with a Jostra BC 20 plus® (Maquet CP, Hirrlingen, Germany) imitating modified ultrafiltration.

All systems were primed with 100 ml 20% human albumin (Octapharma, Vienna, Austria) 200 ml Ringers' solution, 5 ml 20% mannitol (Baxter BV NL, Utrecht, The Netherlands) and 1000 IE Heparin (LEO Pharma, Breda, The Netherlands) imitating the standard prime composition used in our institution. In the dexamethasone groups 5 mg dexamethasone (Pharmacy, Leiden University Medical Center (LUMC), The Netherlands) was added to the prime. Doses of mannitol and dexamethasone were estimated for a patient weight of 5 kg. After de-airing and 15 minutes circulating at 32^0C, 200 ml whole blood was added to each system. During the circulation the flow was kept at 0,60 liters per minute (l/min) with a pressure between 25 – 30 mmHg. The flow through the ultrafilter was kept at 0,10 l/min during the circulation and 0,15 l/min with a pressure of 50 mmHg during ultrafiltration.

Samples (table 1)

After priming and de-airing the prime was warmed to 32^0 C. Ten minutes later 5 mg of dexamethasone was added to the clear prime in the dexamethasone group and 5 minutes after that sample 1 was collected. Five minutes after adding the blood sample 2 was collected and the prime was cooled to 28^0 C. After 50 minutes circulation at 28^0 C sample 3 was collected and the prime was rewarmed to 37^0 C. Fifteen minutes later sample four was collected and ultrafiltration was started for 10 minutes. After ultrafiltration sample 5

Table 1. sample measurements

Measurement/ Sample no	IL-6	IL-8	IL-10	Dexamethasone
1				x
2	x	x	x	x
3	x	x	x	x
4	x	x	x	x
5	x	x	x	x
ultrafiltrate	x	x	x	x

and a sample of the ultrafiltrate were collected. Al samples were collected in EDTA (BD, San Jose, CA, USA). protected from light, immediately cooled, transported to the laboratory and centrifuged (15 min, 4^0C, 1550 g). The plasma was aliquoted and stored at -80^0C.

Laboratory measurements (table 1)

Dexamethasone concentrations were measured by radioimmunoassay (IgG Corporation Nashville, TN, USA) following a slightly modified protocol.[6] Interleukins IL-6, 8, and 10 were measured using a commercial enzyme immunoassay (PeliKine®, Sanquin Reagents

(Sanquin, Amsterdam, Netherlands)). All assays were performed according to the manufacturers instructions.

Statistical analysis

Data were analysed with the statistical package SPSS v11.5 (SPSS Software Inc., Chicago, IL, USA). Data are summarized as mean ± Standard Deviation (SD) unless stated otherwise. Dexamethasone concentrations as well as changes in IL-8 were analyzed with repeated ANOVA measures using the Greenhouse-Geisser correction. Repeated measures analysis for dexamethasone concentrations were tested against and without values in the ultrafiltrate. As IL-8 values were not normally distributed, these data were first subjected to a natural logarithmic transformation before analysis by repeated ANOVA measures. $P < 0,05$ was considered statistically as significant.

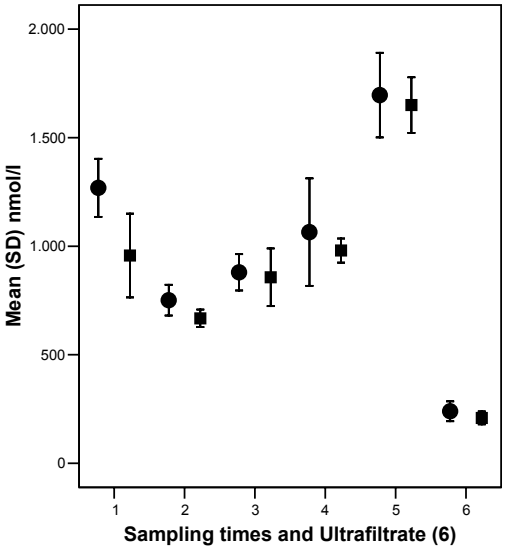

Figure 1. Changes in dexamethasone concentrations at different sampling times and in the ultrafiltrate. Uncoated (●) and coated (■) bypass circuit. Values expressed as mean (SD).

Results

Dexamethasone

Changes in dexamethasone concentrations throughout the study period are presented in relative (Table 2) and absolute (Table 3) values, and as a graphic representation in figure 2. After addition of 5 mg of dexamethasone, 7 to 10% of the total dose was present as free fraction in the prime. There was an absolute reduction of free dexamethasone following the addition of blood to the prime. This trend was reversed after cooling (0.34 mg in NWD and 0.33 mg in CWD) with a further increase after rewarming (0.42 mg in NWD and 0.38 mg in CWD). Absolute concentrations of dexamethasone increased further after ultrafiltration. Changes in dexamethasone concentrations were significant throughout the study period within each group. Coating did not significantly affect dexamethasone disposition throughout the study. Dexamethasone was also found in the ultrafiltrate (approximately 2% of the total dose).

Table 2: Dexamethasone concentrations in nmol/l

	1	2	3	4	5	UF
UWD*	1269 (149)	751 (79)	879 (93)	1065 (276)	1696 (218)	239 (51)
CWD*	957 (215)	667 (45)	857 (148)	980 (143)	1650 (143)	209 (33)

Changes in dexamethasone concentrations at different sampling times and in the ultrafiltrate (UF). Values expressed as mean (SD) in nmol/l. * Statistical significance within groups.

Table 3: Dexamethasone concentrations in mg

	1	2	3	4	5	UF
UWD*	0,49 (0,06)	0,29 (0,03)	0,34 (0,03)	0,42 (0,10)	0,66 (0,08)	0,09 (0,02)
CWD*	0,37 (0,08)	0,26 (0,02)	0,33 (0,06)	0,38 (0,02)	0,64 (0,05)	0,08 (0,01)

Changes in dexamethasone concentrations at different sampling times and in the ultrafiltrate (UF). Values expressed as mean (SD) in mg. * Statistical significance within groups.

Interleukines

Interleukins IL-6 and IL-10 were not detectable. IL-8 was only detectable at the sample moments 3, 4 and 5 and in some ultrafiltration (UF) samples (Table 4). Overall no significant differences were observed except in the group of coated systems without dexamethasone (CND). This group showed a significantly higher IL-8 production. The concentration of IL-8 increased after ultrafiltration. IL-8 was also found in the ultrafiltrate if the concentration was high (> 20 pg/ml) in the prime before ultrafiltration.

Table 4: IL-8 Changes

Sample moment	UND	CND	UWD	CWD
3	0,80 (0,70)	1,95 (2,00)	ND	1,82 (1,18)
4	2,38 (1,36)	9,70 (8,20)†	5,30 (2,70)	5,70 (3,60)
5	11,00 (4,50)	24,10 (20,40) †	11,30 (6,70)	16,30 (6,90)

Changes in IL-8 according to combinations of coated/uncoated plus/minus dexamethasone. UND (uncoated without dexamethasone), CND (coated without dexamethasone), UWD (uncoated with dexamethasone) and CWD (coated with dexamethasone). ND not detectable. Values expressed as mean (SD) in pg/ml. † Denotes statistical significance between groups.

Discussion

In the present study we measured changes of dexamethasone concentrations in the prime in coated and uncoated CPB systems as well as the effect of dexamethasone and coating on the production of cytokines in both phosphorylcholine coated and uncoated systems. To our knowledge these interactions have not been previously investigated.

In pediatric cardiac surgery the use of steroids has not been investigated to the same extent as in adults, and its use is as controversial. Nevertheless steroids are used widely in pediatric cardiac surgery and its use remains controversial.[7] There is evidence in the literature that steroids given before the start of CPB reduce the pro-inflammatory mediators in adult and pediatric cardiac surgery alike.[2,8] Bronicki and colleagues showed in a prospective randomized study, that dexamethasone administered prior to CPB led to a reduction in the post bypass inflammatory response as assessed by cytokine levels.[2]

Butler et al investigated the level of cytokines during CPB and the effect of intraoperative methylprednisolone at a dose of 10 mg/kg in a pediatric group.[8] IL-6 concentrations were higher and peaked earlier in the group without steroids. It has also been proven that dexamethasone decreases the pro- to anti-inflammatory cytokine ratios during adult cardiac surgery.[9] At this moment no consensus exists on which steroid and which doses should be used. It has also been shown that steroids reduce the production of C-reactive protein without any effect on the release of protein S100B and Von Willebrand factor.[10] The concentration of proinflammatory cytokines decreases when steroids are administered before CPB starts.[8] The reduction is even more remarkable if steroids are given before and during CPB.[11] Oxygen delivery and cardiac output increased more rapidly when steroids were used in an animal model.[12] Even the timing of the administration seems to be relevant.[13]

Drug disposition of hydrocortisone in a in vitro model of ECMO (Extracorporeal membrane oxygenation) has been evaluated recently.[14] The authors recorded more than 20% loss after 30 minutes in a crystalloid-prime circuit. Drug disposition can be affected by CPB, this subject has been reviewed elsewhere.[15] We were not able to find any study assessing the effect of CPB on concentrations of dexamethasone either in vivo or in vitro. In the present study the reduction of free dexamethasone after the addition of whole blood to the prime may be explained by dilution and by binding to proteins in the blood product. We have no explanation as to why the concentration of free dexamethasone increased after cooling and continued to rise after rewarming. Hemoconcentration after modified ultrafiltration explains only partly the further rise of free dexamethasone in absolute values. Of interest is that nearly 2% of the total dose of dexamethasone is lost in the ultrafiltrate fluid.

Coating of the cardiopulmonary bypass has been developed to reduce systemic inflammation during cardiopulmonary bypass. We were unable to show any differences between the uncoated and the coated systems except for IL-8 which was significantly higher in the coated group without dexamethasone. IL-8 was found only in 3 of the 5 sample times. In vitro studies performed by other groups showed significant differences and improvement of biocompatibility for the coated groups. [16,17]

Previous studies have shown that isolated CPB induces an increase in IL-8 [16]. It is not surprising that in our study IL-10 was undetected. Previous investigators have shown that an in vitro model is unable to generate anti-inflammatory cytokines.[18]

The fact that IL-6 was undetected in our study may be due to differences in methodology. Two hundred ml's of whole blood were added to the prime. It can be speculated that this amount of blood was not enough to generate the production of IL-6. Blood added to the prime had been preserved with sodium citrate. Flower and colleagues found that levels of IL-6 were significantly lower when sodium citrate was used although De Jongh and colleagues could not reproduce their findings.[19,20] IL-8 was regularly detectable in 3 of the 5 sample moments (3, 4 and 5). There were no significant differences except for the coated group without dexamethasone. Increases in IL-8 after ultrafiltration can be partly explained by hemoconcentration: not all the UF samples contained IL-8. IL-8 is produced by activated neutrophils, monocytes and T-cells. Fung et al. found that the IL-8 production was dependant on temperature.[21] At lower temperatures the levels did not increase. In our study, after rewarming of the prime to 37^{o}C, we measured an increase of Il-8 in all groups which was more pronounced in the coated group without dexamethasone. Gormley and colleagues measured IL-8 in an "adult" in vitro model of CPB with a control group and after addition of methylprednisolone to the prime.[22] These investigators found peaks of more than 35 pg/ml of IL-8 in the control group. The addition of methylprednisolone reduced the IL-8 concentration in the samples significantly.

In a previous study we compared a phosphorylcholine coated cardiopulmonary bypass system with an uncoated system in pediatric patients undergoing cardiac surgery.[5] The use of phosphorylcholine coated system did not affect C3b/c, elastase HNE, Il-6 and CRP. Horton and co-workers also were not able to show any differences between a heparin coated system and an uncoated system in a study population of 200 patients.[23]

In conclusion our study shows that there is no interaction between the phosphorylcholine coating and dexamethasone and a small part of dexamethasone is lost into the ultrafiltrate. We also showed that in the group with PHISIO® coating the production of IL-8 was increased significantly, IL-6 was not detected. Our study confirms that an in

vitro model is unable to trigger the production of IL-10. We document for the first time dexamethasone disposition in a CPB circuit.

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chapter 7

Coated *versus* Noncoated Circuits in Pediatric Cardiopulmonary Bypass

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A systemic inflammatory response after pediatric cardiac surgery is not uncommon. The cardiopulmonary bypass (CPB) circuit is seen as the main activator of the inflammatory response. For that reason, several coatings have been developed to create a more biocompatible CPB circuit. At this moment, several coatings are commercially available: human albumin coating, Duraflo II and Carmeda coatings (both based on heparin), phosphorylcholine PHISIO coating (which mimics the outer cell membrane), polymethoxyethylacrylate coating, and the biopassive surface Trillium coating.

Do these coatings result in less inflammatory response after pediatric cardiac surgery? In reviewing the literature on coating of pediatric CPB circuits, a lot of controversies are found. *In vitro* studies show significantly better biocompatibility of coated circuits than of noncoated circuits [1-3]. The outcomes of *in vivo* or clinical studies are less convincing; many times only a few parameters show improvement [4–7]. Some of the problems found in reviewing studies on coatings are: different methodologies, lack of proper control groups, differences between the patients concerning age and bodyweight, and different prime volumes in different groups. Furthermore, measured parameters are numerous, and sample moments show large variability (table 1).

In the adult population, many more studies on coatings have been published. Here, we find more evidence for beneficial effects of coatings, but it is not clear whether the results of these studies can simply be extrapolated to the practice of pediatric cardiac surgery [13].

Several factors may cause an inflammatory response. The artificial surface of the CPB circuit is an important activator, but other activators are probably as important: ischemia-reperfusion, surgical trauma (especially that of cardiovascular surgery), endotoxemia, and blood–air contact [14]. Patients that have cardiovascular operations without the use of CPB have been reported to show also signs of serious inflammatory response [15–17]. Although CPB is not the only causative factor for an inflammatory response, coating of the CPB circuit is expected to result in improved biocompatibility with less activation of the complement system, the contact system, as well as a reduced activation of leucocytes and platelets.

To what should we thus adhere? *In vitro* studies provide the most reliable information because of their controlled environment and similar outcomes. The most important for

clinical studies is a homogeneous patient group, especially in the pediatric population where a wide spread in age, bodyweight, and diagnosis is commonly encountered.

Table 1. Pediatric In Vivo Studies: Randomized and Prospective

Authors	Year	N	Measurements
Schreurs et al. [8]	1998	19	Less platelet activation/ complement activation similar
Jensen et al. [9]	2004	40	Fibrinolysis reduced/ inflammatory parameters not measured
Grossi et al. [10]	2000	23	Less C3a, IL-8/ similar C5a, IL-6
Olsson et al. [11]	2000	19	Less C3a, C5b-9, and IL-6
Ashraf et al. [12]	1997	21	Less C5b-9, elastase, (IL-6)/ similar IL-8
Horton et al. [4]	1999	200	Similar IL-6, IL-8, and platelets

Our group recently performed a prospective, randomized, and blinded clinical study in 28 neonates and small infants to compare complement activation and leucocytes stimulation of phosphorylcholine coated and noncoated CPB circuits. Strict inclusion criteria were used: bodyweight between 3 and 6 kg, no syndromal anomalies such as Down syndrome, no severe cyanosis (oxygen saturation < 75%), no circulation arrest, no reoperations, and no premature patients. All patients received the same protocolized way of anesthesia and CPB management; none of the patients received aprotinin or other drugs that might interfere with the inflammatory response. No ultrafiltration, conventional or modified, was used in the study patients. No differences were observed between the two groups for the changes of complement factor C3b/c, elastase HNE, interleukin-6, and C-reactive protein before, during, and after CPB until 6 hours postoperatively. We concluded that phosphorylcholine coating does not result in a reduction of inflammatory response (data not shown).

Summarizing the review of the literature and our study, we conclude that an improved biocompatibility by coating is most reliably demonstrated by *in vitro* studies, whereas clinical studies in the pediatric population do not show equally convincing beneficial effects. More strictly protocolized randomized prospective clinical studies will be needed to gather evidence for positive effects of CPB circuit coating in pediatric cardiac surgery.

In the meantime, we should realize that coating alone cannot lead to an adequate reduction of inflammatory response. Other agents should be considered for this purpose, such as ultrafiltration during and/or after CPB, corticosteroids and protease inhibitors, and the use of monoclonal antibodies [18]. Efforts to further miniaturize the pediatric CPB-circuit will also be helpful.

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SUMMARY

Cardiopulmonary bypass (CPB) is a technique that makes open heart surgery possible. When CPB is used the heart can be stopped while the blood circulation, oxygen delivery and carbon dioxide removal are guaranteed. In the last decades CPB has become much safer but still causes a systemic inflammation reaction (SIRS). SIRS may cause morbidity and, when severe, even mortality. SIRS reaction is worse in neonates and infants due to the immaturity of organs and the unfavourable ratio of CPB prime volume to patient circulating volume. This thesis focuses on different techniques that have been developed to decrease the deleterious effects of CPB in pediatric cardiac surgery.

In **chapter 2** we describe the technique of modified ultrafiltration (MUF). This technique, described for the first time in 1991 by Naik and Elliott, is designed to reverse the hemodilution created during CPB. After the cessation of CPB excess water is removed by using an ultrafilter thus providing a higher hematocrit at the end of the procedure. We compared in a retrospective way 2 groups of 99 patients each, in one group we used MUF and in the other group ultrafiltration was not used. We concluded that modified ultrafiltration decreases blood transfusion requirements and chest drain loss after pediatric cardiac surgery.

The ratio between CPB prime solution and circulating blood volume is highest in the neonatal patient. It has been reported that neonates have a poor antioxidative and iron binding capacity. During CPB, prooxidative substances, such as nonprotein-bound iron, are released while the plasma antioxidant capacity decreases, resulting in excess accumulation of deze afkorting voluit opschrijven!Radical Oxygen Species. In **chapter 3 and 4** we describe the antioxidative capacity of CPB prime based on human albumin or fresh frozen plasma. We have demonstrated that a prime based on fresh frozen plasma has higher antioxidative and iron, Hb/Heam binding capacities.

Contact of blood with the non-biological surfaces of the CPB system has been designated as the main cause of complement activation. Improving the biocompatibility of CPB

systems by means of decreasing the contact activation of blood elements and thereby attenuating the inflammatory response is evidently desired, and for this reason several coatings have been developed. In **chapter 5** we investigated the PHISIO® coating in neonatal CPB. We were not able to show any differences between a coated and an uncoated CPB system in this prospective randomized study.

In **chapter 6** we describe the results of the use of dexamethasone in both coated and uncoated CPB systems. Information is lacking about the interaction of medication and the CPB prime or the coating of a CPB system. We did not observe any interaction between dexamethasone and the PHISIO® coating, but have observed that in the group with PHISIO® coating without dexamethasone the production of IL-8 was significantly increased.

In literature many controversies are found on the topic of CPB coatings. It is difficult to compare these studies due to different patient groups, differences in measured parameters and lack of proper control groups. **Chapter 7** reviews the literature on the use of CPB coatings both in vitro and in vivo.

SAMENVATTING

Cardiopulmonale bypass (CPB) is een techniek die open hart chirurgie mogelijk maakt. Wanneer CPB wordt gebruikt kan de hartactie worden gestopt terwijl de bloedcirculatie en zuurstof afgifte gewaarborgd blijft. Ondanks dat in de laatste decennia CPB een veiliger procedure is geworden blijft CPB een algehele onstekingsreactie veroorzaken. Deze ontstekingsreactie kan morbiditeit en in sommige gevallen zelfs mortaliteit tot gevolg hebben. De ontstekingsreactie is ernstiger bij neonaten en zuigelingen omdat hun organen nog niet volgroeid zijn en omdat de ratio CPB prime volume tot circulerende volume hier het minst gunstig is. Dit proefschrift richt zich op de verschillende technieken die zijn ontwikkeld om de nadelige gevolgen van pediatrische CPB te verminderen.

In **hoofdstuk 2** wordt de techniek van gemodificeerde ultrafiltratie (MUF) beschreven. Deze techniek werd voor het eerst beschreven in 1991 door Naik en Elliott en is ontworpen om de hemodilutie die ontstaat door het gebruik van de hart-longmachine te verminderen (REF). Wanneer de hart-longmachine is gestopt wordt het teveel aan water onttrokken door middel van een hemofilter. Wij vergeleken in een retrospectief onderzoek twee groepen van 99 patientjes ieder waarbij in de ene groep wel MUF werd gebruikt en in de andere groep niet. Wij vonden dat met MUF de hoeveelheid gebruikt donorbloed significant minder was terwijl het postoperatieve bloedverlies ook afnam.

Het verschil in het prime volume van de hart-longmachine en het bloedvolume van het kind is groot. We weten ook dat neonaten en pasgeborenen een lage anti-oxidatieve capaciteit hebben en een lage capaciteit om vrij ijzer te binden. Tijdens het gebruik van de hart-longmachine worden pro-oxidatieve stoffen zoals vrij ijzer gevormd terwijl de capaciteit in het plasma om dit te binden verminderd. Hierdoor ontstaan er reactieve oxidatie deeltjes. In **hoofdstuk 3 en 4** beschrijven wij de anti-oxidatieve capaciteit van twee verschillende samenstellingen van de prime, één gebaseerd op menselijke albumine en één gebaseerd op fresh frozen plasma. Wij toonden aan dat de CPB prime gebaseerd

op fresh frozen plasma een hogere anti-oxidatieve capaciteit had en beter in staat was om vrij ijzer te binden.

Het contact van bloed met het lichaamsvreemde oppervlak van de hart-longmachine wordt gezien als de belangrijkste activator van de algehele onstekingsreactie. Deze reactie zou verminderd kunnen worden als het bloed de hart-longmachine niet meer als lichaamsvreemd herkent. Hiertoe worden de kunstlong en de slangen van de hart-longmachine bekleed met een minder lichaamsvreemde stof, dit wordt coating genoemd. In **hoofdstuk 5** bestudeerden wij de PHISIO® coating in neonatale CPB. Wij waren niet in staat om aan te tonen dat er verschil was in de mate van ontstekingsreactie tussen de gecoate hart-longmachine en de ongecoate in deze prospectieve en gerandomiseerde studie.

In **hoofdstuk 6** beschrijven we de resultaten van het onderzoek naar de interactie van dexamethason en de coating van het CPB systeem. Er is weinig bekend over de interactie tussen medicijnen en de CPB prime of met de coating van het systeem. We vonden geen interactie tussen dexamethason en de PHISIO® coating, maar we vonden wel in de groep met PHISIO® coating zonder dexamethason een verhoogde IL-8 productie.

In de literatuur bestaan er veel verschillen tussen de verschillende studies over het onderwerp CPB coatings. Het is lastig om deze studies te vergelijken door de verschillen in patiëntengroepen, verschil in de gemeten parameters en het gemis aan een goede controle groep. **Hoofdstuk 7** in een literatuur review over het gebruik van coating in zowel in vitro als in vivo onderzoeken.

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CURRICULUM VITAE

Anjo Martzen Draaisma is geboren op 5 maart 1962 te Rotterdam. Na het behalen van zijn HAVO diploma, behaald in 1986 aan de Rotterdamse Avondscholen Gemeenschap te Rotterdam, behaalde hij zijn HLO getuigschrift richting medische microbiologie in 1991 aan de Hogeschool Rotterdam en Omstreken te Delft. Hierna kreeg hij een aanstelling als klinisch perfusionist in opleiding op de afdeling klinische perfusie te Leiden. Hij studeerde in 1996 af met het onderzoek "gemodificeerde ultrafiltratie na cardiopulmonale bypass bij kinderen, een retrospectief onderzoek"aan de afdeling Specialistische Opleidingen van het Academisch Ziekenhuis Leiden. Hierna werd hij geregistreerd als erkend klinisch perfusionist in het NeSECC register. Zijn promotie onderzoek heeft geheel plaatsgevonden op de afdeling klinische perfusie te Leiden in samenwerking met onder andere de afdeling neonatologie en het laboratorium klinische chemie. Tot op heden werkt hij als senior klinisch perfusionist op de afdeling klinische perfusie met als specialisatie CPB tijdens kinderhart chirurgie. Hiernaast is hij ook als docent perfusie technologie verbonden aan de specialistische opleidingen van het Leids Universitair Medisch Centrum.