CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

REGIONAL GENERATION OF FREE OXYGEN RADICALS DURING CARDIOPULMONARY BYPASS IN CHILDREN

Studies on free radical generation during cardiopulmonary bypass have focused mainly on the heart and the lungs. However, low pumping pressure, nonpulsatile perfusion, and hypothermia affect the entire circulation, resulting in decreased splanchnic blood flow, increased intestinal permeability, and endotoxemia. To evaluate regional phenomena, we studied 16 children undergoing cardiopulmonary bypass. Free radical production, granulocyte activation, and hypoxanthine metabolism were assessed separately in the circulations drained by the inferior and superior venae cavae, as well as in the oxygenator. Three minutes after the onset of cardiopulmonary bypass, significant gradients between the inferior vena cava and the arterial line of the oxygenator existed in malondialdehyde ($+0.60 \pm 0.12 \mu\text{mol/L}$), lactoferrin ($+18.21 \pm 7.65 \mu\text{g/L}$), myeloperoxidase ($+53.75 \pm 16.50 \mu\text{g/L}$), hypoxanthine ($-0.62 \pm 0.15 \mu\text{mol/L}$), and urate ($+8.87 \pm 4.03 \mu\text{mol/L}$). These gradients decreased in parallel with decreasing body temperature. Except for a transient gradient in malondialdehyde at 3 minutes after the onset of cardiopulmonary bypass ($+0.23 \pm 0.08 \mu\text{mol/L}$), no changes were detected between the superior vena cava and the arterial line. In the oxygenator, granulocyte activation was observed only after aortic declamping. We conclude that during cardiopulmonary bypass, significant free radical generation, granulocyte activation, hypoxanthine elimination, and urate production take place in the region drained by the inferior vena cava. In the oxygenator, granulocyte activation occurs only after aortic declamping. (J THORAC CARDIOVASC SURG 1995;110:768-73)

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Increased free radical activity during cardiopulmonary bypass (CPB) has been associated with myocardial and pulmonary dysfunction.¹ CPB activates

the complement system, which leads to granulocyte activation and free radical production.² During ischemia, the enzyme xanthine dehydrogenase is converted to xanthine oxidase, in which form it generates free radicals as by-products during metabolism of hypoxanthine and xanthine to urate.³

Interest in free radical activity during cardiac operations has focused on the heart and the lungs, because they are exposed to ischemia and subsequent reperfusion during CPB. However, low pumping pressure, nonpulsatile perfusion, and hypothermia cause considerable hemodynamic changes in the entire circulation. During hypothermic perfusion in animal models, cerebral, renal, and splanchnic flows diminish and femoral artery flow increases.⁴ Recent studies in human beings show a 46% reduction

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Table I. Patient data

Number	16
Sex	
Male	9
Female	7
Age (yr)	2.99 (0.49-4.71)
Weight (kg)	11.6 (5.8-19.0)
Diagnosis	
Ventricular septal defect	5
Tetralogy of Fallot	3
Univentricular heart	3
Aortic valve insufficiency	2
Transposition of great arteries	1
Mitral valve insufficiency	1
Pulmonary valve stenosis	1
Crossclamping time (min)	65 (27-117)
Duration of CPB (min)	99 (53-164)

in gastric mucosal blood flow during CPB.⁵ Splanchnic hypoperfusion may cause increased intestinal permeability and endotoxemia.^{5,6} If free radicals play a role in these changes, increased free radical activity may be measurable in venous return from the splanchnic region.

This study was conducted to evaluate regional changes in free radical activity and its two potential sources, granulocyte activation and xanthine metabolism, during CPB in children.

Methods

Patients. We studied 16 children undergoing CPB for elective repair of congenital heart defects (Table I). All the patients were in stable condition during the induction and preinduction phases.

Anesthesia and operation. Patients were premedicated with flunitrazepam (0.1 mg/10 kg p.o.). Anesthesia was induced with fentanyl, thiopentone sodium or ketamine, and pancuronium and maintained with a high dose of fentanyl (50–100 µg/kg). Isoflurane was administered if needed. CPB was established with the use of a Masterflo 34 (<15 kg) or a Masterflo 51 (>15 kg) pediatric hollow-fiber membrane oxygenator and a Midicard 20µ cardiomy reservoir (Dideco S.p.A., Mirandola, Italy). A roller pump (Gambro, Lund, Sweden) was used with a standard flow of 2.4 L/min per square meter (at 37° C), which was then reduced to match the requirements according to the degree of hypothermia. Circulatory arrest was used in one patient; postarrest values for this patient were excluded. Barbiturates or steroids to protect the brain were not given.

A prime solution comprising albumin and fresh whole blood was used to adjust the hematocrit value to 25%. During rewarming, hemofiltration (Ultraflux 400 filter, Fresenius, Germany) was used consistently and uniformly to increase the hematocrit value to at least 30%. To achieve this, we added fresh whole blood, if needed. Mannitol (15%) was administered as an infusion of 1

Fig. 1. Mixed venous concentrations of malondialdehyde (MDA), hypoxanthine (HX), urate (UR), lactoferrin (LF), and myeloperoxidase (MPO). Preoperative values corrected for hemodilution during CPB are depicted with dots. †*p* < 0.05, ††*p* < 0.01, and †††*p* < 0.001 versus 0 minutes; **p* < 0.05 and ***p* < 0.01 versus 40 minutes after the onset of CPB (Wilcoxon test).

ml/kg per hour from the beginning of the perfusion. The protein inhibitor aprotinin (30,000 IU/kg) was added in the priming solution, and the same amount also was infused for 1 hour after the induction of anesthesia. Infusion of aprotinin (8000 IU/kg per hour) was continued throughout CPB. For myocardial preservation, cold

Table II. Differences in parameters between IVC or SVC and the arterial line of the oxygenator (A)

Parameter	Time after onset of CPB	Differences	
		IVC-A	SVC-A
MDA	3 min	0.60 ± 0.12**	0.23 ± 0.08*
(μmol/L)	20 min	0.36 ± 0.13*	0.06 ± 0.09
HX	3 min	-0.62 ± 0.15**	0.06 ± 0.09
(μmol/L)	20 min	-0.24 ± 0.66	0.18 ± 0.44
UR	3 min	8.87 ± 4.03*	2.00 ± 2.31
(μmol/L)	20 min	9.93 ± 5.79**	5.00 ± 6.87
LF	3 min	18.21 ± 7.65*	0.51 ± 5.45
(μg/L)	20 min	0.94 ± 42.63	-1.46 ± 16.33
MPO	3 min	53.75 ± 16.50**	2.60 ± 11.23
(μg/L)	20 min	17.63 ± 51.91	16.00 ± 27.19

CPB, cardiopulmonary bypass; MDA, malondialdehyde; HX, hypoxanthine; UR, urate; LF, lactoferrin; MPO, myeloperoxidase.

* $p < 0.05$; ** $p < 0.01$.

(+4° C) blood cardioplegic solution (30 ml/kg) was used initially and repeated (10 ml/kg) every 20 minutes. On the decision of the surgeon, the last cardioplegic solution (300 ml/m²), containing 2 mmol/100 ml aspartate and 2 mmol/100 ml glutamate ("hot shot"), was infused immediately before aortic declamping. None of the patients received allopurinol. Whole body temperature was assessed by measuring mixed venous temperature.

Sample collection. Blood samples were collected after induction of anesthesia but before the operation began; at 3, 20, and 40 minutes after the onset of CPB; and at 2, 7, and 12 minutes after aortic declamping. Blood samples were obtained simultaneously from radial arterial and central venous catheters before CPB and from the superior (SVC) and inferior (IVC) venae cavae and from the arterial and venous lines (mixed venous value) of the oxygenator during CPB. Samples specifically from the IVC were obtained by means of a side line on the IVC cannula, and samples from the SVC were obtained through a central venous catheter, the tip of which was placed in the SVC. The CPB cannulation and snaring of the vessel proximally prevented blood in the SVC from mixing with blood from the IVC. The samples collected in tubes containing ethylenediaminetetraacetic acid were immediately centrifuged for 5 minutes at 1000 g. An aliquot of 150 μl of plasma for malondialdehyde measurement was immediately added to 15 μl of deferoxamine 100 mg/ml (Desferal, Ciba-Geigy, Basel, Switzerland). The samples were stored at -70° C.

Assays. Hypoxanthine was quantified with high-pressure liquid chromatography, urate by a Hitachi 705E device (Hitachi Ltd., Tokyo, Japan) by an enzymatic method, lactoferrin and myeloperoxidase by radioimmunoassay, and malondialdehyde by measurement of thiobarbituric acid-reactive material.⁷⁻¹⁰

Statistical analysis. Plasma concentrations were calculated both corrected and uncorrected for hematocrit value. Within CPB, hemodilution did not have a significant effect on results. Therefore results during CPB are presented only uncorrected. Pre-CPB values are given both uncorrected and corrected to correspond with the

hemodilution of the first sample during CPB. In statistical analysis, the two-tailed Wilcoxon test and analysis of variance (ANOVA) were used. A p value less than 0.05 was considered significant. Patient data are presented as median and range. Results are presented either as individual values or as mean ± standard error of the mean.

Ethics. The study was approved by the ethics committee of the Children's Hospital, University of Helsinki, and informed consent was obtained from the parents.

Results

Malondialdehyde. After the onset of CPB, there was an apparent decrease in mixed venous malondialdehyde concentration compared with preoperative values, but after correction for hemodilution during CPB, this difference disappeared (Fig. 1, A). At 3 minutes after the onset of CPB, malondialdehyde concentration was higher in the SVC than in the arterial line of the oxygenator (Table II). At 3 and 20 minutes after the onset of CPB, malondialdehyde concentration was higher in the IVC than in the arterial line of the oxygenator (Table II and Fig. 2, B). This difference diminished gradually with decreasing body temperature (Fig. 2, B). No differences could be detected between the arterial and venous lines of the oxygenator.

Hypoxanthine. At 3 minutes after the onset of CPB, mixed venous hypoxanthine concentration was already increased, and it became elevated further after aortic declamping (Fig. 1, B). During the entire period of CPB, there were no differences in hypoxanthine concentration between the SVC and the arterial line of the oxygenator nor between the arterial and venous lines of the oxygenator (Table II). At 3 minutes after the onset of CPB, hypoxanthine concentration was significantly lower in the IVC than in the arterial line of the oxygenator (Table II and Fig. 2, C). Significant differences in the same direction were also observed 2, 7, and 12 minutes after aortic declamping ($p = 0.0174$, $p = 0.0022$, and $p = 0.0024$, respectively).

Urate. Compared with preoperative values, the mixed venous concentration of urate decreased during CPB, but not if corrected for hemodilution (Fig. 1, C). No differences in urate concentration were observed between the SVC and the arterial line of the oxygenator, nor between the arterial and venous lines of the oxygenator (see Table II). Urate content was higher in the IVC than in the arterial line of the oxygenator at 3 and 20 minutes after the onset of CPB (Table II and Fig. 2, C) and at 2 minutes after aortic declamping ($p = 0.0025$).

Fig. 2. Mixed venous temperature (V-TEMP) and differences in plasma concentrations of malondialdehyde (MDA), hypoxanthine (HX), urate (UR), lactoferrin (LF), and myeloperoxidase (MPO) between the IVC and the arterial line of the oxygenator. † $p < 0.05$ and †† $p < 0.01$ for IVC versus A (Wilcoxon test).

Lactoferrin and myeloperoxidase. Mixed venous concentrations of both lactoferrin and myeloperoxidase increased significantly during CPB (Fig. 1, D and E). Further increase of lactoferrin concentration occurred after aortic declamping (Fig. 1, D). No differences in lactoferrin or myeloperoxidase concentration were observed between the SVC and the

Fig. 3. Plasma lactoferrin (LF) and myeloperoxidase (MPO) concentration differences between arterial and venous lines of the oxygenator. † $p < 0.05$ and †† $p < 0.01$ for arterial versus venous line (Wilcoxon test).

arterial line of the oxygenator (Table II). At 3 minutes after the onset of CPB, both lactoferrin and myeloperoxidase were higher in the IVC than in the arterial line of the oxygenator (Table II and Fig. 2, D and E). This difference diminished gradually with decreasing body temperature (Fig. 2, D and E). Both lactoferrin and myeloperoxidase concentrations were higher in the arterial than in the venous line of the oxygenator after aortic declamping, but not during crossclamping (Fig. 3).

Discussion

This study demonstrates increased granulocyte activation, hypoxanthine metabolism, and free radical generation during CPB in the circulation drained by the IVC. The magnitudes of these phenomena decrease in parallel with decreasing body temperature.

We did not detect any increase in lipid peroxidation, measured as malondialdehyde concentration, during the entire CPB period. This is consistent with a recent study.¹¹ Increased malondialdehyde production after aortic declamping has been demonstrated in blood samples obtained from the left atrium, that is, in blood drained specifically from the pulmonary circulation.¹² Those results may not be

compared with the mixed venous concentrations measured in this study. Although no systemic increase in malondialdehyde was detected in this study, a transient increase was found at the start of CPB in both the SVC and the IVC.

We found a very rapid increase in hypoxanthine as early as 3 minutes after the onset of CPB. The most probable explanation is cellular hypoxia caused by hypoperfusion.¹³ Despite seemingly adequate central hemodynamics and oxygenation during cardiac operations, peripheral tissue perfusion is restricted, as suggested by an increase in arterial concentration of lactate after the onset of CPB.¹⁴ During reperfusion after aortic declamping, we observed a further increase in hypoxanthine, probably reflecting washout of hypoxanthine accumulated in ischemic myocardial and pulmonary tissues. The finding is in accordance with earlier observations of hypoxanthine liberation after transient cardiac ischemia, during reperfusion in cardiac operations, and during heart-lung transplantation.¹⁵⁻¹⁷

Xanthine oxidase/dehydrogenase activities vary between organs. In this study, at 3 minutes after the onset of CPB and at 2 minutes after aortic declamping, hypoxanthine decreased and urate increased in the IVC but not in the SVC. This result is compatible with high xanthine oxidase/dehydrogenase activity in splanchnic organs but low or no activity in skeletal muscle and brain.^{18,19}

CPB leads to rapid intravascular granulocyte activation, presumably caused by complement activation through the alternative pathway when plasma contacts the unphysiologic surfaces of the oxygenator.^{2,20} However, granulocytic degranulation, indicative of granulocyte activation, has not previously been measured in the oxygenator. We found a rapid increase in lactoferrin and myeloperoxidase concentrations from the beginning of CPB. At the beginning of CPB, degranulation was localized not to the oxygenator but to the circulation drained by the IVC. Only after aortic declamping did significant degranulation take place in the oxygenator, and it was temporally associated with further increase in mixed venous plasma lactoferrin. The finding is in accordance with data showing a release of several leukocyte-activating factors only after aortic declamping.^{21,22}

On the basis of this study, the precise origin of increased malondialdehyde or degranulation proteins in the IVC cannot be determined. In a canine model, a decrease in body temperature decreases splanchnic and renal perfusion but increases per-

fusion through the femoral artery.⁴ Decreased splanchnic perfusion during hypothermia has recently been documented also in human beings.⁵ In our patients, hypoxanthine concentration decreased and urate concentration increased in the IVC 3 minutes after the onset of perfusion, while hypothermia still was relatively mild. As body temperature decreased, these differences disappeared. This may reflect a shift of perfusion from splanchnic organs containing xanthine oxidase/dehydrogenase to lower extremities in which the enzyme activity is low.¹⁸ This mechanism could also account for the changes in myeloperoxidase, lactoferrin, and malondialdehyde concentrations in the IVC in relation to decreasing mixed venous temperature. Endotoxemia caused by intestinal damage has been associated with CPB, and intestinal permeability increases 3 hours after elective coronary artery bypass grafting.^{5,6} Our results suggest that increased splanchnic granulocyte activation and free radical activity during CPB may have pathophysiologic relevance to the intestinal damage associated with cardiac operations.

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