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Original Article

In Vitro Recovery of Sufentanil, Midazolam, Propofol, and Methylprednisolone in Pediatric Cardiopulmonary Bypass Systems

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Objectives: To evaluate in vitro drug recovery in cardiopulmonary bypass (CPB) systems used for pediatric cardiac surgery.
Design: Observational in vitro study.
Setting: Single-center university hospital.
Participants: In vitro CPB systems used for pediatric cardiac surgery.
Interventions: Three full neonatal, infant, and pediatric CPB systems were primed according to hospital protocol and kept running for 6 hours. Midazolam, propofol, sufentanil, and methylprednisolone were added to the venous side of the systems in doses commonly used for induction of general anesthesia. Blood samples were taken from the postoxygenator side of the circuit immediately after injection of the drugs and after 2, 5, 7, 10, 30, 60, 180, and 300 minutes.
Measurements and Main Results: Linear mixed model analyses were performed to assess the relationship between log-transformed drug concentration (dependent variable) and type of CPB system and sample time point (independent variables). The mean percentage of drug recovery after 60 and 180 minutes compared with T1 was 41.7% (95% confidence interval [CI] 35.9-47.4) and 23.0% (95% CI 9.2-36.8) for sufentanil, 87.3% (95% CI 64.9-109.7) and 82.0% (95% CI 64.6-99.4) for midazolam, 41.3% (95% CI 15.5-67.2) and 25.0% (95% CI 4.7-45.3) for propofol, and 119.3% (95% CI 101.89-136.78) and 162.0% (95% CI 114.09-209.91) for methylprednisolone, respectively.
Conclusions: The present in vitro experiment with neonatal, infant, and pediatric CPB systems shows a variable recovery of routinely used drugs with significant differences between drugs, but not between system categories (with the exception of propofol). The decreased recovery of mainly sufentanil and propofol could lead to suboptimal dosing of patients during cardiac surgery with CPB.

Key Words: cardiopulmonary bypass; in vitro; midazolam; sufentanil; propofol; methylprednisolone

Cardiopulmonary bypass systems were made available free of charge by Terumo Europe NV, Leuven, Belgium, and Sorin Group, Mirandola, Italy.
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CARDIOPULMONARY BYPASS (CPB) is necessary to facilitate most cardiac surgery in children. The effect of CPB on in vivo drug concentrations in patients can be profound and is attributed to hemodilution, altered hemodynamic status, hypothermia, systemic inflammation, changes in acid-base status, exclusion of the lungs from the circulation, and hemofiltration.[1] Furthermore, the plastic components of the CPB system themselves have been shown to absorb drugs.[2-7]

In the authors' institution, multiple experiments have been performed to determine in vitro drug recovery in extracorporeal membrane oxygenation (ECMO) systems.[8,9] Drug recovery has been defined as the concentration of drug present in the priming fluid after a certain amount of time has passed since addition of the drug to the CPB system.[8,9] Previous publications have used the term absorption to indicate the decrease in concentration of drug in the priming fluid. In the authors' institution, recovery has been deemed a more precise definition because not all drug is actually absorbed by the system components. Drugs also are subject to spontaneous degradation, for example, providing an altogether different reason for a decrease in drug concentration than absorption of drug to components of the CPB system.[8] There is a lack of data in the literature concerning pediatric CPB systems. As part of the authors' CPB PHARM study, which aims to measure and model drug concentrations during CPB for pediatric cardiac surgery (registered at the Netherlands Trial Register [NTR3579]), the in vitro experiments described herein were undertaken. The ultimate goal is to incorporate these data into in vivo population pharmacologic models.

Methods

The present study was conducted at the Department of Cardiothoracic Surgery of a tertiary teaching hospital. No human participants were involved in the study, so the need for medical ethical review board approval was waived according to Dutch law.

Soon to be expired CPB systems were made available free of charge by Terumo Europe NV, Leuven, Belgium, and Sorin Group, Mirandola, Italy. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. There was no role for Terumo Europe NV or Sorin Group in the design of the study, collection, analysis, and interpretation of data; writing of the report; or the decision to submit the report for publication.

CPB Systems

Table 1 shows the composition of the different CPB systems used. All systems contained a hollow-fiber membrane oxygenator with a polymethylpentene membrane. For the neonatal and pediatric systems an arterial filter was integrated in the oxygenator, and for the infant system a stand-alone arterial filter was used. Silicone and polyvinylchloride (PVC) tubing with different lengths and diameters were used in the neonatal and infant roller-pump systems. In the pediatric system a centrifugal pump was used, and the silicone tubing was discarded. Tubing was made continuous via a one fourth-to-one fourth or a one fourth-to-three eighths connection piece. A venous reservoir completed the systems. Terumo components of the systems were coated with X-coating (poly[2-methoxyethylacrylate]), which is a nonheparin biocompatible polymer with hydrophilic and hydrophobic properties. Sorin components of the systems were coated with P.h.i.s.i.o. (Sorin) coating, which is a nonheparin, biomimetic layer consisting of a phosphorylcholine polymer.

All systems were placed on a conventional mast-mounted, remote pump head console (Stöcker S5 Perfusion System; Sorin Group) with a specific pediatric configuration.

Three full systems were assembled for each category (neonatal, infant, and pediatric) and primed according to hospital-based protocol (Table 2). Priming fluid contained fresh frozen plasma and Gelofusine (B. Braun, Melsungen, Germany). Red blood cells were added to the priming to achieve a hematocrit of 28%. Recently expired red blood cells and fresh frozen plasma obtained from the authors' local blood bank were used for priming. The priming fluid was completed with human albumin (Sanquin Plasma Products BV, Amsterdam, The Netherlands) and 2 to 5 mL sodium bicarbonate 8.4% (Fresenius Kabi Nederland BV, Zeist, The Netherlands). Heparin was added to the system according to hospital protocol to prevent clotting.

The CPB systems were kept running for 6 hours. This is the maximum runtime with guaranteed quality by the manufacturers. The temperature was maintained at 36°C., pCO2, and pH were measured with an iStat handheld device (Abbot BV, Hoofddorp, The Netherlands) and maintained within physiological ranges by titration of sweep gas flow, gas composition, and addition of sodium bicarbonate 8.4% if needed.

A flow rate of 0.5 L/min was maintained for the neonatal circuits, 1.5 L/min for the infant circuits, and 3 L/min for the pediatric circuits. Postmembrane pressures were kept at 100 mmHg by adapting the resistance using the venous clamp.

Drug Administration

For the neonatal system a standardized body weight of 5 kg was used for drug amount calculations, 15 kg for the infant system, and 30 kg for the pediatric system were used. Drugs were added to the venous reservoir via a manifold sample port in a dose that normally would be used for the induction of general anesthesia according to the following authors' institution's guidelines: midazolam (1 mg/mL; Actavis Group PTC ehf, Hafnarfjördur, Iceland) 0.2 mg/kg; propofol (10 mg/mL; Fresenius Kabi Nederland BV) 2 mg/kg; sufentanil (50 μg/mL; Hameln Pharma Plus GmbH, Hameln, Germany) 2 μg/kg; and methylprednisolone (100 mg/mL; Pfizer BV, Capelle a/d IJssel, The Netherlands) 30 mg/kg. Drugs were injected in the same order for all systems. Between administration of each drug and after administration of the last drug, the sample port was flushed with 2 mL of 0.9% saline solution to prevent crystallization or pooling of drug. Midazolam, propofol, sufentanil, and methylprednisolone were used because these drugs are commonly used for pediatric cardiac anesthesia in the authors' institution.

Table 1
CPB Systems

	Oxygenator	Reservoir	Arterial filter	Venous Filter Cardiotomy	Defoaming Sponge	Silicone Tubing	PVC Tubing	Priming Volume
Neonatal roller	Capiox FX05 (Terumo Europe NV, Leuven, Belgium) Hollow fiber Polycarbonate housing, polypropylene membrane 0.5 m^2 Priming volume 43 mL X-coating	Open, hard shell polycarbonate Minimum capacity 15 mL Maximum capacity 1,000 mL	Integrated polyester screen type Surface area 130 cm^2 Pore size 32 μm	Polyester screen type Pore size 47 μm	Polyurethane	Sorin Kids neonate set, custom made, (Sorin Group, Mirandola, Italy) Diameter $^1/_4$ inch, length 1.10 m, 0.02 m^2 contact surface area, P.h.i.s.i.o. coating	Sorin Kids neonate set, custom made (Sorin) Diameter $^1/_4$ inch, length 2.95 m, 0.069 m^2 contact surface area, P.h.i.s.i.o. coating	230 mL
Infant roller	Sorin Kids D101 (Sorin) Hollow fiber Polycarbonate housing, polypropylene membrane 0.61 m^2 Priming volume 87 mL P.h.i.s.i.o. coating	Open hardshell, polycarbonate Minimum capacity 30 mL Maximum capacity 1,500 mL	Sorin Kids D131 stand-alone arterial filter Polycarbonate housing, phosphoryl-chloride screen type membrane Surface area 27 cm^2 Pore size 40 μm Priming volume 28 mL	Polyester Pore size 51 μm	Polyurethane	Sorin Kids, custom made Diameter $^1/_4$ inch, length 1.05 m, 0.02 m^2 contact surface area P.h.i.s.i.o. coating	Sorin Kids neonate set, custom made Arterial part diameter $^1/_4$ inch, length 1.88 m Venous part diameter $^3/_8$ inch, length 1.51 m Total 0.08 m^2 contact surface area P.h.i.s.i.o. coating	420 mL
Pediatric centrifugal Revolution (Sorin) Pump casing polycarbonate Priming volume 57 mL	Capiox FX15 (Terumo Europe) Hollow fiber Polycarbonate housing, polypropylene membrane 1.5 m^2 Priming volume 144 mL X-coating	Open hardshell polycarbonate Minimum capacity 70 or 200 mL Maximum capacity 3,000 or 4,000 mL	Integrated polyester screen type Surface area 360 cm^2 Pore size 32 μm	Polyester screen type Pore size 47 μm	Polyurethane	None	Sorin Kids Pediatric set, custom made Diameter $^3/_8$ inch, length 4.87 m, 0.15 m^2 contact surface area P.h.i.s.i.o. coating	700 mL

Table 2
Priming Fluid Composition

	Neonatal	Infant	Pediatric
Priming volume (mL)	263	430	683
RBC (mL)	135	235	365
FFP (mL)	30	40	50
Gelofusine (mL)	30	40	50
Albumin 20% (mL)	40	50	100
Mannitol 15% (mL)	20	50	100
NAHCO3 8.4% (mL)	8	15	18
Heparin (mL)	0.4	0.5	1
Flow (L/min)	0.5	1.5	3.5
Temperature (˚C)	36	36	36
Line pressure (mmHg)	100	100	100

Abbreviations: FFP, fresh frozen plasma; NAHCO3, Sodium bicarbonate; RBC, red blood cells.

Samples

Four-milliliter blood samples were taken from the arterial (post oxygenator) side of the circuit via a manifold sample port in a polypropylene (PLP) ethylenediaminetetraacetate tube (7.2 mg) (BD Vacutainer, BD Life Sciences, Plymouth, UK). Samples were taken immediately after injection of the drugs (T1) and after 2, 5, 7, 10, 30, 60, 180, and 300 minutes.

Samples were stored at 4˚C until processing. After centrifugation (10 min at 3,600 rpm), the supernatant serum was transferred to PLP cryogenic vials with PLP screw caps (Sarstedt Aktiengesellschaft & Co, Nümbrecht, Germany) and stored at −80˚C until analysis.

Assay Methods

Drug concentrations for sufentanil, midazolam, propofol, and methylprednisolone were measured using liquid chromatography mass spectrometry. Methods were validated according to US Food and Drug Administration guidelines for bioanalytical method validation.[10] All analyses included quality control samples, as is required for Food and Drug Administration analyses, and were performed in International Organization for Standardization- and Good Clinical Practice-certified laboratories by a certified research technician.

Drug concentrations for sufentanil were measured using a Thermo TSQ Vantage triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA) at the pharmacy laboratory of the Erasmus Medical Center. Drug concentrations for midazolam were measured using a Quattro Premier mass spectrometer (Waters Corp, Milford, MA) at the pharmacy laboratory of the Erasmus Medical Center. Propofol was measured using a Thermo TSQ Quantiva triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific) at the pharmacy laboratory of the University Medical Center in Groningen, the Netherlands. Drug concentrations for methylprednisolone were measured using a SCIEX Triplequad 6500+ mass spectrometer (AB SCIEX, Concord, Ontario, Canada) and Analyst software, Version 1.7 (AB SCIEX) at the Institute for Biomedical and Pharmaceutical Research in Nürnberg-Heroldsberg, Germany.

The lower limit of quantification was 0.25 μg/L for sufentanil, 2.0 μg/L for midazolam, 100.0 μg/L for propofol, and 10 μg/L for methylprednisolone. The upper limit of quantification (ULOQ) was 50.0 μg/L for sufentanil, 2,400 μg/L for midazolam, unknown for propofol, and 30100 μg/L for methylprednisolone.

Statistical Analysis

The relationship between log-transformed drug concentration (dependent variable) and type of CPB system and sample time point (independent variables) was assessed with linear mixed model analyses. Linear mixed model analyses were used because a correlation can be expected between repeated measurements of the same variable (ie, drug concentration) in the same subject (ie, individual CPB systems). Both independent variables were treated as categorical variables, and a 2-way interaction effect between the type of CPB system and sample time point was included in the model. To correct for within-system correlations between time points, a random intercept was used and we assumed a first-order autoregressive error covariance matrix. This model specification was chosen by comparing values of the Akaike information criterion between different structures for the random effects and the error covariance matrix.

For each time point and each CPB system, the difference between the predicted log-transformed estimated marginal means[11] at T1 and the predicted log-transformed concentration at the different time points was calculated, as was the 95% confidence interval (CI) of this difference. Finally, this difference and the 95% CI were exponentiated to obtain the percentage drug recovery (the percentage of drug still present in the priming fluid) for T2 to T300. The maximum expected concentration in case of perfect mixture of drug (MEC) was calculated by dividing the amount of drug added to the CPB systems by the total priming volume used because it was unclear whether mixing of drug with the priming fluid would be complete at T1.

Spearman correlations were calculated to assess the relationship between drug recovery at 60 and 180 minutes and log P, protein binding, and pKa among the 4 drugs (ie, with a sample size of $n = 4$ drugs). For each drug, the recovery used for the calculation of this correlation was based on the estimated marginal means of the linear mixed model.

Statistical analyses were performed using SPSS Statistics for Windows, Version 24 (IBM Corp, Armonk, NY). All statistical tests used a 2-sided significance level of 0.05.

Results

No technical problems were encountered during the experiments. A total of 81 samples (27 for each CPB system category) were analyzed. No loss of drug samples occurred.

Fig 1 shows predicted drug recovery (based on the estimated marginal means of the linear mixed models) versus time for propofol for each CPB system category. There was a sharp and significant decline in recovery of propofol compared with T1 in all the system categories in the first 60 minutes, to 41.3%

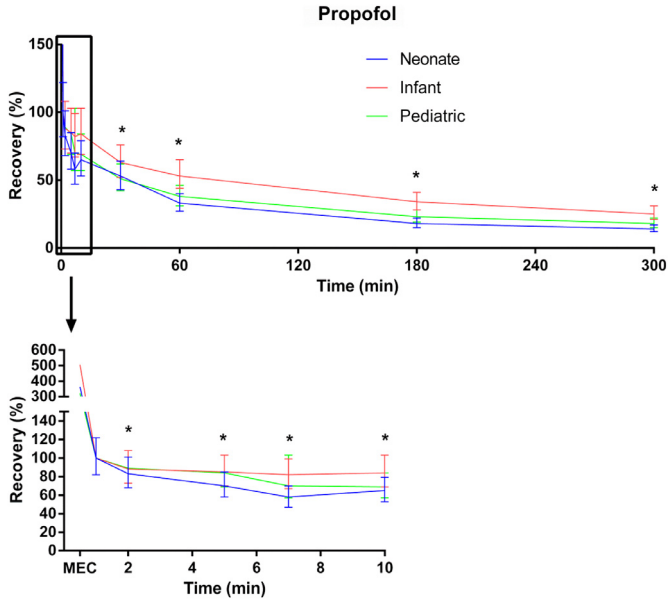

Fig 1. Drug recovery versus time based on estimated marginal means for propofol, for each cardiopulmonary bypass system category, expressed as means and 95% confidence interval based on the linear mixed models.

MEC, maximum expected concentration in case of perfect mixture of drug.
*p < 0.002.

(95% CI 15.5-67.2), meaning that approximately 59% of the added drug was lost from the circulating prime fluid at that time. After that, recovery continued to decrease slightly but significantly throughout the study period to 25% (95% CI 4.7-45.3) after 180 minutes and 19% (95% CI 5.2-32.8) after 300 minutes. Based on the interaction effects in the mixed models, there was a significant difference in the pattern of recovery over time among systems for propofol ($p < 0.001$). Recovery was the greatest in the infant system, followed by the pediatric system. The neonatal systems appear to absorb the largest amount of propofol to their system components.

Fig 2 shows predicted drug recovery (based on the estimated marginal means of the linear mixed models) versus time for sufentanil, midazolam, and methylprednisolone. Because there was no significant interaction effect between the type of CPB system and sample time point for sufentanil ($p = 0.111$), midazolam ($p = 0.213$), or methylprednisolone ($p = 0.829$), a single graph was used to depict decrease of drug recovery over time for all 3 systems. The pattern of decline in recovery of sufentanil shows stable drug concentrations in the first 7 minutes, with a significant decline compared with T1 from T10 onward. Drug recovery was 41.7% (95% CI 35.9-47.4) at 60 minutes. After 60 minutes, recovery continued to decrease slightly but significantly throughout the study period. For sufentanil, recovery was 23.0% (95% CI 9.2-36.8) after 180 minutes and 15% (95% CI 1.3-31.3) after 300 minutes.

For midazolam, there also was stable drug recovery in the first 7 minutes. The decline in drug recovery compared with T1 reached significance at T10 and from T60 forward. Drug recovery was 87.3% (95% CI 64.9-109.7) after 60 minutes and 82.0% (95% CI 64.6-99.4) after 180 minutes. For methylprednisolone, there was stable recovery of drug in the first 30 minutes. After that, recovery increased significantly compared with T1 to values much higher than 100%.

No significant correlation between log P and percentage recovery of the 4 drugs at 180 minutes (ρ −0.324; $p = 0.304$) was found. In addition, a decreased recovery of highly protein-bound drugs ($\rho = -0.822$; $p = 0.007$) was found. The third factor that correlated to percentage recovery at 180 minutes in the present study was pKa ($\rho = 0.822$; $p = 0.007$).

Discussion

These in vitro experiments investigated drug recovery in 3 different pediatric CPB systems used in the authors' center. Decrease of drug concentration in the circulating prime fluid for propofol and sufentanil was fast in the first 60 minutes. Because 60 minutes is a relatively common bypass time in pediatric cardiac surgery, this period is clinically very relevant. The decreasing speed of reduction in drug concentration after 60 minutes may be an indication of near complete saturation of binding places on the different components of the CPB systems. It is, however, unknown whether there is a finite amount of binding places and if complete saturation of these binding places is possible. Hammaren et al.[12] and Myers et al.[13] have shown that for propofol there appears to be no maximum saturation of binding places in complete adult CPB systems, even at very high propofol concentrations. Binding of propofol may be concentration dependent.[12] In contrast, complete saturation of oxygenator membrane fragments has been shown for fentanyl in in vitro studies.[5]

In the present study, midazolam recovery was remarkably large in both centrifugal and roller-pump systems. Unfortunately, there are no in vitro studies of pediatric CPB systems with which to compare the present study's results. An ECMO study performed in the authors' hospital showed a recovery pattern similar to the present experiment in systems with a centrifugal pump.[8] In roller-pump systems there was just 7.5% recovery after 2 minutes and 0.6% recovery after 180 minutes. For midazolam, however, it must be taken into account that the concentrations measured in the present study were far above the ULOQ. The authors believe that this may at least partly be the reason that the MEC was lower than the measured concentrations. This introduced an unknown amount of bias, but the authors do not expect that this measurement bias would explain the high recovery rates. An error in the addition of medication to the system or a laboratory error also were not expected because the experiments were performed on different days for the different systems and all the percentage recovery versus time curves show a similar pattern.

Methylprednisolone concentrations in the first hour were much lower than would be anticipated from the MEC. This most likely was caused by a problem with the mixing of methylprednisolone added to the system with the priming fluid. Another explanation would be very fast binding of methylprednisolone to components of the CPB system and release of drug from those binding sites after 60 minutes. However, it is unknown whether binding of drug to components of a CPB system is a reversible process.

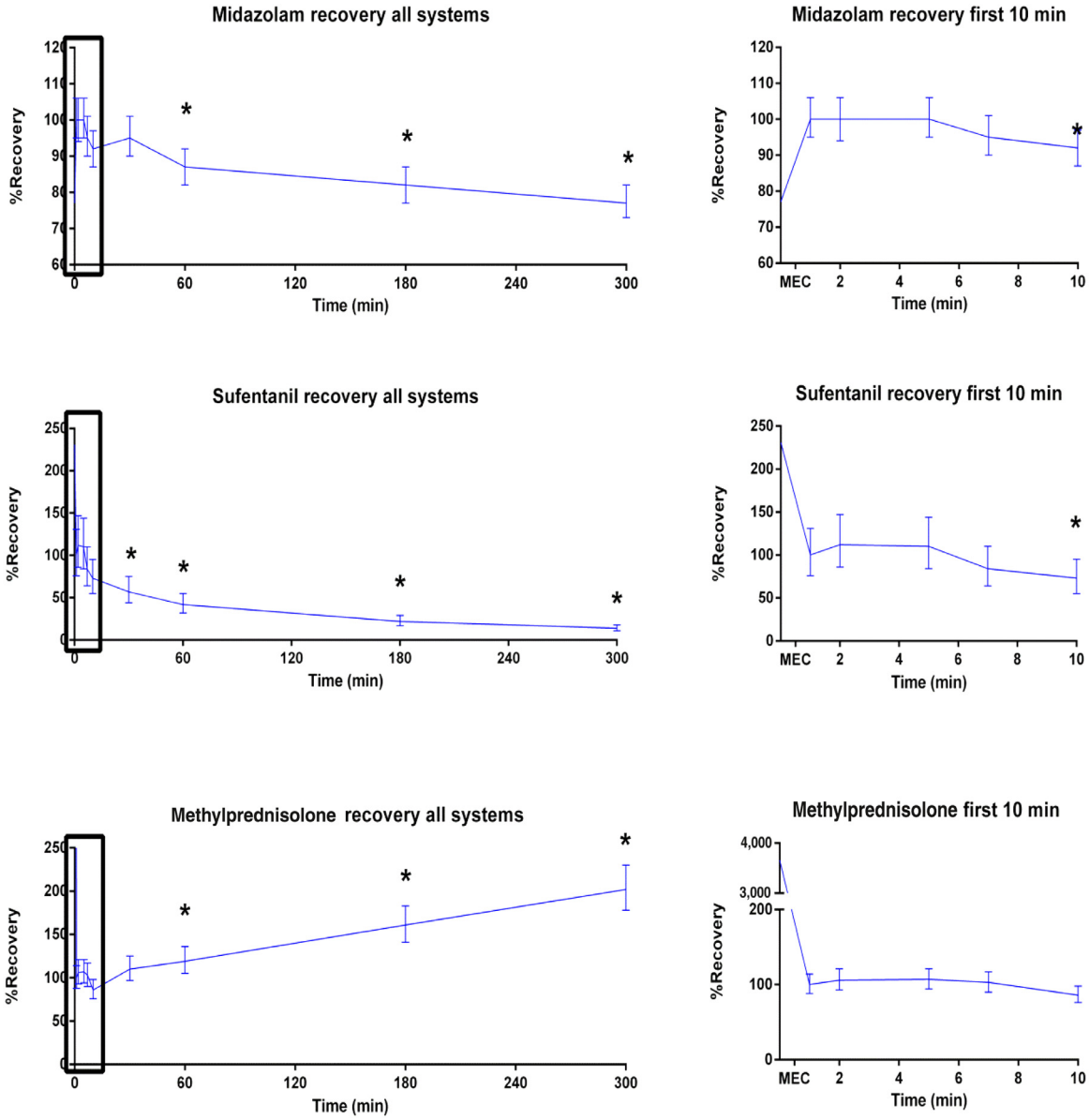

Fig 2. Drug recovery versus time based on estimated marginal means for midazolam, sufentanil, and methylprednisolone, expressed as means and 95% confidence interval based on the linear mixed models.

MEC, maximum expected concentration in case of perfect mixture of drug.

*$p < 0.05$

The substantial differences in recovery found between drugs suggest that drug characteristics influence the interaction with components of the CPB system (Table 3). From previous studies in ECMO systems, recovery of drugs seems to be highly dependent on lipophilicity.[8,9,14,15] In the present study, no correlation between log P and recovery percentage was found. This likely was caused by the recovery profile of midazolam. Significantly decreased recovery of highly protein-bound (>80%) drugs also was shown in a previous publication.[15] We found a generally decreased recovery of highly protein-bound drugs, which may be caused by binding of drug to protein adhered to system components.[15] The similar recovery patterns of propofol and sufentanil suggest that there is a common physicochemical property of both drugs causing this effect. The authors, however, cannot explain why the recovery pattern of midazolam is different in our study because the physicochemical properties known to influence recovery are very similar to those of propofol and sufentanil. Another factor correlated to the percentage recovery at 180 minutes in the present study was pKa. To the authors' knowledge no correlation between pKa and recovery has been described previously. The surface-coated CPB systems are negatively charged, making electrostatic attraction of positively charged molecules a possible mechanism for absorption of drugs to CPB system components. Drugs with a high pKa are unlikely to be dissociated at normal pH, however, which was maintained during the study period.

Table 3
Drug Physicochemical Data[19]

	Blood/Plasma Ratio	Log P	Protein Binding (%)	Vd (L/kg)	pKa
Methylprednisolone	Unavailable	2.06	78	Unavailable	12.58
Sufentanil	0.75	3.4	Unavailable	Unavailable	8.86
Propofol	Unavailable	3.81	95-99	Unavailable for children	10.98
Midazolam	0.75	3.89	97	Children 6 mo-16 y 1.24-2.02	10.98

Abbreviations: Vd, volume of distribution.

A significant difference in drug recovery between the different types of CPB system was found only for propofol. In general, one would expect a larger system to have more binding sites for drugs and thus a lower recovery for drugs with similar properties. The surface area of the oxygenator and the PVC tubing in the authors' pediatric systems are much larger than those in their neonatal and infant systems. In the present study, midazolam and methylprednisolone showed lower recovery in larger systems, although not significantly so. For propofol, however, there was greater recovery in larger systems. The differences are smaller than would be expected if system size were the only factor involved.

Different components of the CPB system are capable of absorbing drugs to their plastics. Differences in drug recovery between different types of oxygenator have been described extensively.[5,16] With the new polymethylpentene and PLP membranes the oxygenator does not appear to be a factor of considerable interest in drug recovery anymore. Based on a study by Preston et al., 80% of drug is lost to PVC tubing, with a small additional amount of drug (of just 5%) lost to the oxygenator.[17] Silicone tubing has been shown to decrease the recovery of drugs compared with PVC tubing.[2,8] The effect of different surface coatings on both oxygenator and tubing on drug recovery has been investigated by several authors,[7,12,13] and those studies suggest different effects for different coatings for different types of drugs. The addition of an arterial filter also may lead to decreased drug recovery.[13] Many factors thus are at play and interact with each other.

It is unclear which differences in system composition play a role in the present study. The interplay of differences in surface area, coating, tubing type, and pump type makes it difficult to draw firm conclusions about the influence of individual system components on drug recovery. In an earlier study by Preston et al., a Terumo Baby Rx oxygenator was used[17]. The Terumo Capiox Fx05 was used in the present study's neonatal systems, which is the same oxygenator with the same membrane, the same coating, and the same surface area, but with an integrated arterial filter. The Baby Rx oxygenator absorbed 3% of fentanyl added to the system in the study by Preston et al., which amounts to 0.6 ng/cm^2. Raffaeli et al. showed that sufentanil absorption is similar to fentanyl absorption in their ECMO systems.[9] Assuming the aforementioned holds true, the amount of drug absorbed in the present study's neonatal systems just by the tubing and the arterial filter would be 4.2 μg of sufentanil at 180 minutes (total uptake of sufentanil 72% at 180 min of 10 μg added to the neonatal systems minus the amount absorbed by the oxygenator). Because the neonatal systems in the present study had silicone tubing, this relatively low amount of absorption seems unlikely.

A similar calculation for the pediatric systems used in the present study is possible. The Capiox Fx15 oxygenator would absorb 9 μg in total because of its larger surface area. After 180 minutes, 76% of the 60 μg of sufentanil added to the system would be absorbed. Further calculation shows that this would mean that the Sorin tubing would absorb 25.1 ng/cm^2 of sufentanil. This, however, would mean that all the sufentanil in the neonatal system in the present study would have to have been absorbed, which is not the case. For a more extensive calculation, the reader is referred to the supplemental materials.

Hynynen et al.[18] described propofol recovery of 25% after 120 minutes of circulation in an adult system. Hammaren et al.[12] and Myers et al.[13] described recovery of 37% and 43%, respectively, after 60 minutes. These values are remarkably similar to those of the present study, even though completely different CPB systems were used. Based on the calculations previously described and the results by Hynynen, Hammaren, and Meyers, it appears that it is very difficult to translate research performed in individual centers to one's own clinical practice because of the amount of factors and interactions at play. It is clear that not all factors influencing absorption to different components of CPB systems are known. A possible lack of generalizability thus may be seen as a limitation to the present study and other studies already performed in this field.

Several authors have found no influence of temperature management on the recovery of drugs in their systems.[16,19,20] Therefore the authors of the present study did not attempt to simulate a cooling protocol.

Despite the significant decrease in recovery of drug from the priming fluid of the CPB system found in the authors' in vitro studies, clinically patients do not wake up on initiation or during CPB. A clinical study of propofol infusions in cardiac surgery in adult patients showed no change or even a decrease in bispectral index values during CPB.[21] This most likely was caused by an increase in unbound drug concentration as a result of decreased protein concentration on initiation of CPB,[21-23] causing a greater amount of drug available for end-organ effect.

The present study has several other limitations. Only complete systems were tested, thus there is no information in the present study as to which amount of drug was absorbed by which system component. Unfortunately this type of research is expensive because of the costs of systems, blood products for

priming, and drug concentration measurements. Also, for purposes of the present study, namely the integration of these in vitro results with the results of the in vivo part of the CPB PHARM study, the authors sought to mimic everyday practice in their hospital as closely as possible, making testing of individual components less useful. For the same reasons, the authors have not performed isolated drug studies. It is not known whether there is competition for binding sites to components of the CPB system for different individual drugs. Although theoretically this is an interesting topic, in clinical practice, patients receive multiple medications at the same time.

Just 3 full systems in each category were used, and although this may seem like a small number, the sample size is comparable with that of other publications performed with both CPB and ECMO systems and which are cited in this article.

Spontaneous degradation may have produced bias in the present study because it causes a decrease in drug concentrations not caused by adherence of drug to components of the CPB system. Previous studies in the authors' hospital have shown that spontaneous degradation over 24 hours for midazolam is 11.4% and for sufentanil is 0%.[9] For propofol, spontaneous degradation in glass bottles with daylight and room temperature is around 5% after 6 hours.[24] For methylprednisolone, no references were found for spontaneous degradation. Because degradation usually is calculated over 24 hours, the effect on the present study's results would be constant over time.

For the calculation of MEC, the authors did not correct for blood-plasma ratio. In previous studies in the authors' hospital, midazolam was shown to have a blood-plasma ratio of 75%.[8] Because the present study's MEC value was calculated in blood, but drug concentrations were measured in plasma, MEC would be underestimated for midazolam. Because propofol is highly bound to red blood cells in vivo, a high blood-plasma ratio would be expected and the present study's MEC would be overestimated.

The authors did not aim for a similar drug concentration in the different types of CPB systems. Instead, the dose of drug that would be administered to a typical patient connected to a neonatal, infant, or pediatric CPB system in the authors' daily clinical setting was added because the goal of the present study was to mimic the authors' everyday practice as closely as possible so that in the future the authors will be able to incorporate CPB system recoveries in a larger population pharmacokinetic model of the influence of CPB on drug concentrations in children.

Because of the high doses of drug added to the authors' systems, drug concentrations were more than the ULOQ for some drug assays, necessitating additional dilution before quantification. The high doses also might have resulted in potential differences in absorption rates, as has been shown for propofol.[12]

In the present study, indications that mixing of drug with the priming fluid is not always complete were observed. This may have clinical consequences if drugs are added to the CPB system during CPB, rather than given directly to the patient.

Despite these limitations, to the authors' knowledge, the present study is the first comprehensive in vitro testing of CPB systems used in pediatric congenital cardiac surgery.

In conclusion, the present study's in vitro experiment with neonatal, infant, and pediatric CPB systems shows a variable recovery of routinely used drugs with significant differences among drugs but not among system categories, except for propofol. The study also demonstrates that the generalizability of this type of research may be limited. The clinical consequences of the present study's research must be investigated further. The decreased recovery of sufentanil and propofol could lead to suboptimal dosing of patients during cardiac surgery with the use of CPB, even though clinically this doesn't show; thus it is important that these findings are integrated with the results of in vivo studies into population pharmacokinetic models to further investigate the clinical relevance of the present study's findings and the implications for perioperative patient care.

Conflict of Interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.jvca.2019.08.029.

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