Effects of an *Ex Vivo* Pediatric Extracorporeal Membrane Oxygenation Circuit on the Sequestration of Mycophenolate Mofetil, Tacrolimus, Hydromorphone, and Fentanyl

Catherine S. Heith, MD; Lizbeth A. Hansen, PharmD; Rhonda M. Bakken, RRT; Sharon L. Ritter, MS, RRT; Breeanna R. Long, RRT; Janet R. Hume, MD, PhD; Lei Zhang, ScM; Danielle B. Amundsen; Marie E. Steiner, MD, MS; and Gwenyth A. Fischer, MD

OBJECTIVES With the expanding use of extracorporeal membrane oxygenation (ECMO), understanding drug pharmacokinetics has become increasingly important, particularly in pediatric patients. This *ex vivo* study examines the effect of a pediatric Quadrox-iD ECMO circuit on the sequestration and binding of mycophenolate mofetil (MMF), tacrolimus, and hydromorphone hydrochloride, which have not been extensively studied to date in pediatric ECMO circuits. Fentanyl, which has been well studied, was used as a comparator.

METHODS ECMO circuits were set up using Quadrox-iD pediatric oxygenators and centrifugal pumps. The circuit was primed with whole blood and a reservoir was attached to represent a 5-kg patient. Fourteen French venous and 12 French arterial ECMO cannulas were inserted into the sealed reservoir. Temperature, pH, PO_2 , and PCO_2 were monitored and corrected. MMF, tacrolimus, hydromorphone, and fentanyl were injected into the ECMO circuit. Serial blood samples were taken from a postoxygenerator site at intervals over 12 hours, and levels were measured.

RESULTS Hydromorphone hydrochloride was not as significantly sequestered by the *ex vivo* pediatric ECMO circuit when compared with fentanyl. Both mycophenolic acid and tacrolimus serum concentrations were stable in the circuit over 12 hours.

CONCLUSIONS Hydromorphone may represent a useful medication for pain control for pediatric patients on ECMO due to its minimal sequestration. Mycophenolic acid and tacrolimus also did not show significant sequestration in the circuit, which was unexpected given their lipophilicity and protein-binding characteristics, but may provide insight into unexplored pharmacokinetics of particular medications in ECMO circuits.

ABBREVIATIONS ECMO, extracorporeal membrane oxygenation; MMF, mycophenolate mofetil; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide

KEYWORDS extracorporeal membrane oxygenation (ECMO); fentanyl citrate; hydromorphone hydrochloride; mycophenolate mofetil; pharmacokinetics; tacrolimus; pharmacokinetics

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Introduction

The use of extracorporeal membrane oxygenation (ECMO), a temporary therapy for cardiac and respiratory failure, is expanding. Previously used when other therapeutic options had failed, ECMO is increasingly being used earlier to allow organs to recover from a variety of diseases and events including transplantation.^{1,2} Patients on ECMO receive both supportive medications like analgesics and sedation to help with ventilation, oxygenation, and patient comfort as well as medications needed to treat the underlying condition such as an organ transplant rejection.³ Opioids, including fentanyl

and hydromorphone, and immunosuppressants such as mycophenolate mofetil (MMF) and tacrolimus are commonly used to achieve these goals. Many analgesics, sedatives, and antimicrobials have been investigated in the context of use with ECMO.⁴ However, no studies have been conducted on immunomodulating medications or hydromorphone, which is a pain medication frequently used in ECMO patients.⁴

There are three main effects of ECMO on drug distribution and clearance. The first effect is a significant increase in circulating blood volume associated with the priming volume of the ECMO circuit. The increased circulating blood volume increases the volume of dis-

tribution 5% to 100% and necessitates more drug to achieve the same serum concentration.⁵ The second effect is drug absorption and sequestration in the circuit tubing and oxygenator. Absorption and sequestration are thought to occur more with lipophilic and highly protein bound drugs.^{6,7} Sequestration effects are also more pronounced at the initiation of ECMO and with each circuit change.⁸ Drug loss in the circuit can decrease serum concentrations of some drugs and lead to treatment failure.⁹ The third effect is altered renal and hepatic blood flow. This effect is thought to be due to the non-pulsatile blood flow associated with ECMO as well as any injury to the organs prior to initiation of ECMO.⁵ These effects place the patient at increased risk for concentration-dependent adverse drug effects.

This study specifically investigates the sequestration effect of the pediatric Quadrox-iD circuit on the clearance of MMF, tacrolimus, hydromorphone, and fentanyl using a pediatric Quadrox-iD circuit and an *ex vivo* circuit model.

Materials and Methods

Exemption. Institutional review board exemption was obtained from the University of Minnesota Institutional Review Board.

ECMO Circuit. Circuits were constructed using the standard University of Minnesota Masonic Children's Hospital protocol with a Quadrox-iD Pediatric oxygenator (Maquet Cardiopulmonary AG, Rastatt, Germany) and Affinity A40 pump (Medtronic, Minneapolis, MN) with Cortiva Bioactive surface 91 inches of ¼ inch heparin-impregnated tubing (Medtronic) and a Micro-temp LT heater (Cincinnati Sub Zero, Cincinnati, OH).

The circuits were primed with 300 mL of Plasmalyte (Baxter Healthcare Corporation, Deerfield, IL) to de-air the circuit. Three hundred milliliters of human whole blood (American Red Cross, Minneapolis, MN) was added to the circuit to exchange the Plasmalyte prime out of the circuit. Albumin prime was not used. With a goal of simulating a 5-kg infant, an assumed infant blood volume of 100 mL/kg was used to fill a Pyrex glass reservoir (Fisher Scientific, Pittsburgh, PA) with 500 mL of whole blood. The total volume of the circuit was 800 mL (500 mL for infant and 300 mL circuit prime washout) prior to addition of MMF (Genentech, San Francisco, CA), tacrolimus (Astellas, Killorglin, Ireland), hydromorphone hydrochloride (Hospira, Lake Forest, IL), and fentanyl citrate (West-Ward Pharmaceuticals, Eatontown, NJ). One hundred units per kilogram of unfractionated heparin (Pfizer, New York, NY) was added to the circuit for anticoagulation. Fourteen French venous and 12 French arterial ECMO cannulas (Medtronic) were inserted into the sealed reservoir.

The circuit flow rate was maintained at 100 to 120 mL/kg/min (500–600 mL/min). Circuit temperature was maintained between 36°C and 37°C. Postoxygenator blood samples measuring pH, PCO₂, PO₂, and

bicarbonate were taken from the circuit and tested on a portable i-STAT (Abbott, Princeton, NJ). The pH was corrected to a goal of 7.2 to 7.5 using 100% carbon dioxide gas, 8.4% sodium bicarbonate, and the ECMO sweep. The PCO₂ was corrected to 25 to 65 mmHg using carbon dioxide gas, and PO₂ was corrected to 100 to 200 mmHg using oxygen gas. Hemoglobin was monitored and remained between 6.5 and 11.6 g/dL. Once parameters were stable and within physiologic range, single doses of each medication were added to the circuit at the preoxygenator site and allowed to circulate for 15 minutes before blood samples were collected.

Drug Administration. All study drugs were obtained, tracked, stored, and distributed by the Investigational Drug Service of the University of Minnesota. Fentanyl, hydromorphone, and tacrolimus were prepared using manufacturer solutions mixed with normal saline. Mycophenolate mofetil was prepared from the manufactured powder for solution that required reconstitution with non-Di(2-ethylhexyl)phthalate dextrose. Doses were calculated based on the standard dose for a 5-kg patient with a dose of 0.033 mg/kg of tacrolimus, 600 mg/m² of MMF (body surface area calculated estimating height for a child in the 50th percentile for weight on the CDC growth chart), 0.01 mg/kg of hydromorphone, and 1 mcg/kg of fentanyl. Drugs were administered in alphabetical order (fentanyl, hydromorphone, MMF, tacrolimus) as a single bolus dose at the time point at which the circuit parameters noted above had been normalized.

Sample Collection and Processing. Postoxygenator blood samples were taken from the ECMO circuit at 7 time points after administration of the medications or the drugs: 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, and 12 hours. Samples for mycophenolic acid (MPA), hydromorphone, and fentanyl were collected into non-heparinized, red-top tubes (Fisher Scientific) and immediately transferred to the lab to be centrifuged at 3500 RPM on a Hettich Universal 320 centrifuge (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) for 10 minutes for the separation of serum and then refrigerated at 4°C. One milliliter of blood was collected into lavender-top K2 EDTA tubes (Fisher Scientific) for tacrolimus measurement from whole blood samples. ARUP Laboratories (Salt Lake City, UT) and Fairview Health Systems (Minneapolis, MN) conducted sample analysis. Medication levels were determined by quantitative liquid chromatography tandem mass spectrometry (Waters 2795 Liquid Chromogram, Milford, MA) in Clinical Laboratory Improvement Amendments–certified laboratories.

Data Analysis. Five ECMO circuits were tested. To assess the variation from the 5 repeated experiments, test-retest reliability was calculated using intraclass correlation. For each drug, its concentration was plotted against time, and a quadratic model was fitted. The

Figure 1. MPA concentration in ECMO circuits over 12 hr: 600 mg/m² of MMF was injected into the ECMO circuit at time 0 and allowed to circulate for 15 min. Concentration was measured in samples from the circuit 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, and 12 hr after drug injection. Each trial is indicated by a different symbol as shown. The graph represents data from 5 independent experiments plotted over 12 hr. Reliability of the MPA concentration was 75%.

○ Circuit 1; △ Circuit 2; + Circuit 3; × Circuit 4; ◇ Circuit 5
ECMO, extracorporeal membrane oxygenation; MMF, mycophenolate mofetil; MPA, mycophenolic acid.

Figure 2. Tacrolimus concentration in ECMO circuits over 12 hr: 0.033 mg/kg (0.165 mg) of tacrolimus was injected into the ECMO circuit at time 0 and allowed to circulate for 15 min. Concentration was measured in samples from the circuit 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, and 12 hr after drug injection. Each trial is indicated by a different symbol as shown. The graph represents data from 5 independent experiments plotted over 12 hr. Reliability of the tacrolimus concentration was 73%.

○ Circuit 1; △ Circuit 2; + Circuit 3; × Circuit 4; ◇ Circuit 5
ECMO, extracorporeal membrane oxygenation.

concentrations at 15 minutes and 12 hours were estimated from the quadratic model. Percent change from 15 minutes was calculated. All analyses were performed using SAS (v9.4; SAS Institute, Cary, NC). Graphs were plotted in R (<http://www.r-project.org>).

Results

The results are shown in Figures 1 to 4. Results for hydromorphone during the first run were removed due to a drug preparation error resulting in the preparation and administration of a 1/10th dose of dilaudid. One result for fentanyl (ECMO circuit 2, 12-hour level) could not be detected due to a lab processing error. There were no additional complications such as circuit instability. MPA concentration in the circuit increased by 15.00% (Figure 1), and the serum concentration of the inactive metabolite mycophenolic acid glucuronide (MPAG) was <5 mcg/mL. Tacrolimus concentration in the circuit decreased by 16.14% (Figure 2), hydromorphone concentration in the circuit decreased by 23.52% (Figure 3), and fentanyl concentration in the circuit decreased by 54.44% over 12 hours (Figure 4). The test-retest reliability of MPA, tacrolimus, and hydromorphone was >70% (75%, 73%, and 71%, respectively), and the reliability of fentanyl was 99%.

Discussion

See Table for a summary of the following discussion.

Fentanyl and Hydromorphone. This is the first pilot study investigating the drug sequestration effect of ECMO on MMF, tacrolimus, and hydromorphone using fentanyl pharmacokinetics as a comparator. Fentanyl has been extensively studied and demonstrates clinically significant sequestration in ECMO circuits.^{10,11} Fentanyl has a high level of lipophilicity (octanol-water partition coefficient of 717:1), which correlates with significant binding to organic materials such as ECMO circuits.^{10,12,13} When fentanyl was administered to extracorporeal circuits, it was demonstrated that 70% to 80% of the drug was sequestered in the circuit.^{10,11} This study produced similar results to previous experiments with a 55.44% decrease in fentanyl over 12 hours and therefore served as a reasonable comparator for the study.

This is the first study investigating the sequestration effect of ECMO on hydromorphone. Based on its low lipophilicity (octanol-water partition coefficient of 1.28:1) and low protein binding (9.5%–14.1%), hydromorphone should not be significantly removed by the ECMO circuit.^{12,13} As expected, hydromorphone was not sequestered as extensively as the fentanyl in the ECMO circuit (23.52% decrease over 12 hours).

Figure 3. Hydromorphone hydrochloride concentration in ECMO circuits over 12 hr: 0.01 mg/kg (0.05 mg) of hydromorphone was injected into the ECMO circuit at time 0 and allowed to circulate for 15 min. Concentration was measured in samples from the circuit 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, and 12 hr after drug injection. Each trial is indicated by a different symbol as shown. The graph represents data from 5 independent experiments plotted over 12 hr. Reliability of the hydromorphone concentration was 71%.

○ Circuit 1; △ Circuit 2; + Circuit 3; × Circuit 4; ◇ Circuit 5
ECMO, extracorporeal membrane oxygenation.

MMF. The results for MMF were unexpected given its high lipophilicity (octanol-water partition coefficient 238:1) and significant protein binding (97%).¹³ To understand the results, it is necessary to further review the clinical pharmacokinetics. When MMF is administered IV, the conversion to MPA, the active metabolite, occurs via very slow esterification both in the serum and, more extensively and rapidly, in the tissues.¹⁴ Tsina et al¹⁵ found that approximately 10% of the MMF is converted to MPA in the blood when the tissue component is not present.

In the body, MPA is then glucuronidated in the liver to the inactive metabolite MPAG and excreted through the biliary system.¹⁴ The supratherapeutic concentrations of MPA throughout the study period are likely secondary to the lack of glucuronidation and elimination through the biliary system in the *ex vivo* setting of this study. This was confirmed by undetectable levels of MPAG throughout the study period and the increase of MPA levels 2 to 3 times in the setting of renal and liver dysfunction (or simulated complete failure in the setting of our *ex vivo* study).^{16,17} Furthermore, the lack of sequestration of MPA may be secondary to the simulated renal dysfunction and hypoalbuminemia. De Winter et al^{18,19} demonstrated decreased albumin binding of MPA in the setting of hypoalbuminemia and

Figure 4. Fentanyl citrate concentration in ECMO circuits over 12 hr: 1 mcg/kg (5 mcg) of fentanyl was injected into the ECMO circuit at time 0 and allowed to circulate for 15 min. Concentration was measured in samples from the circuit 15 min, 30 min, 1 hr, 2 hr, 4 hr, 6 hr, and 12 hr after drug injection. Each trial is indicated by a different symbol as shown. The graph represents data from 5 independent experiments plotted over 12 hr. Reliability of the fentanyl concentration was 99%.

○ Circuit 1; △ Circuit 2; + Circuit 3; × Circuit 4; ◇ Circuit 5
ECMO, extracorporeal membrane oxygenation.

renal dysfunction, which could be reasonably assumed in the *ex vivo* setting of this study.

Though albumin was not measured and represents a limitation of this study, the *ex vivo* nature of this study creates a closed system that limits albumin available for binding, as well as synthesis of additional protein. While protein binding is known to be a factor in sequestration in ECMO circuits, it is unknown whether competition for protein in an *ex vivo* setting, where there is a set restrictive amount of protein available for binding, may alter this effect in the circuit.⁶ Additionally, competition for protein binding is one possible explanation for the effect we saw in this study and is known to occur between drugs in the blood.²⁰ Whether this played a role in our results is unknown. Additionally, the literature available on protein binding with MMF and its metabolites focuses on the protein-binding characteristics at clinically realizable concentrations; the supratherapeutic levels in our *ex vivo* model may alter the protein binding and expected sequestration in the ECMO circuit.¹⁴ Therefore, the unexpectedly low level of sequestration of MPA by the ECMO circuit demonstrated in this study may be secondary to decreased protein binding in the setting of simulated renal and liver failure as well as absolute or relative hypoalbuminemia.

Tacrolimus. The use of tacrolimus is often complicated by notable inpatient and interpatient variability

Table. Medication, Protein Binding, Lipophilicity Expressed as Octanol-Water Coefficient and Percentage (%) Change of Medication After 12 Hr

Drug	Protein Binding, %	Octanol-Water Coefficient (Lipophilicity)	% Change in 12 hr
Fentanyl	80–85	717:1	–55.44
Hydromorphone	9.5–14.3	1.28:1	–23.52
Mycophenolate mofetil	97	238:1	+15.00
Tacrolimus	99	>1000:1	–16.14

ity and narrow therapeutic ranges requiring frequent monitoring to avoid subtherapeutic or toxic levels.^{21,22} Like MMF, tacrolimus is highly lipophilic (octanol-water partition coefficient >1000:1), highly protein bound in plasma (99% over a concentration range of 5–50 ng/mL), has significant presystemic metabolism and elimination through the hepatic and gastrointestinal system, and has been reported to be affected by genetic polymorphisms such as ABCB1.^{13,23}

Tacrolimus, however, is extensively distributed into erythrocytes with only 5% found in plasma.¹³ Hinderling et al²⁴ summarizes the mechanisms and role of erythrocyte binding in pharmacokinetics, and Mateus et al²⁵ describes the role of intracellular binding of medications and bioavailability. The intraerythrocyte binding of tacrolimus is secondary to its lipophilicity, which allows it to penetrate the red blood cell lipid bilayer membrane.^{26–28} This lipophilic-related erythrocyte binding could isolate the lipophilic effect from the organic components of the ECMO circuit that is likely the mechanism of increased sequestration of lipophilic medications. In review of the literature, it does not appear that other intracellular-bound medications have been investigated and further studies are needed to explore the role of intracellular compartments in the pharmacokinetics in ECMO circuits. Additionally, tacrolimus is in the macrolide family and studies investigating azithromycin, a macrolide antibiotic that is also lipophilic, have also unexpectedly found it minimally affected in ECMO.^{3,24,29} Interestingly, the minimal sequestration observed in our study is consistent with the findings reported by Breslin et al³⁰ who found that heart transplant patients on ECMO required a 33% reduced tacrolimus dose to reach therapeutic concentrations.

Limitations. There were multiple limitations to this study largely due to the *ex vivo* nature of the investigation. The administration of multiple highly lipophilic and protein-bound medications into one circuit may have limited our findings. This design was intentional in order to reflect real life practices where multiple drugs are delivered to a patient at one time, and to reflect other ECMO drug pharmacokinetic studies conducted, which also administered multiple medications within a single circuit.^{10,16,17} However, in the case of MMF and tacrolimus, concomitant introduction into the single *ex vivo* circuit with simulated renal and hepatic dysfunction, may have limited the clarity of this study. Further

studies examining these drugs independently and in *in vivo* studies, such as in animal models or in serial measurements of serum concentrations in patients on ECMO, are required to clarify the pharmacokinetics of these medications in the setting of metabolism. Additionally, quantification of albumin and measurement of free versus bound drug may further elucidate the sequestration of MMF and tacrolimus.

Conclusions

In this study, it was demonstrated that fentanyl is still significantly sequestered by newer generation ECMO circuits and requires ongoing dose adjustment in the setting of ECMO. In contrast, hydromorphone is less sequestered by the ECMO circuit than fentanyl and may represent a useful medication for pain control in ECMO patients. MMF and tacrolimus do not appear to be significantly sequestered by the ECMO circuit in this pilot study; however, this effect needs further study in models where renal and liver dysfunction as well as hypoalbuminemia are absent or corrected. Further *in vivo* studies are needed to gain insight on the effects of ECMO on hydromorphone, MMF, and tacrolimus.

ARTICLE INFORMATION

Affiliations Department of Pediatrics, Division of Pediatric Critical Care (CSH, JRH, MES, GAF), University of Minnesota, MN, University of Minnesota Health and Fairview Health Systems (LAH, RMB, RRT, SLR, BRL), Minneapolis, MN, Clinical and Translational Science Institute (LZ), University of Minnesota, Minneapolis, MN, University of Minnesota (DBA), Minneapolis, MN

Correspondence Catherine S. Heith, MD; heith009@umn.edu

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