

Antifungal Extraction by the Extracorporeal Membrane Oxygenation Circuit

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Abstract: Invasive candidiasis is common and often fatal in patients supported with extracorporeal membrane oxygenation (ECMO), and treatment relies on optimal antifungal dosing. The ECMO circuit can extract drug and decrease drug exposure, placing the patient at risk of therapeutic failure. This ex vivo study determined the extraction of antifungal drugs by the ECMO circuit. Fluconazole and micafungin were studied separately in three closed-loop circuit configurations to isolate the impact of the oxygenator, hemofilter, and tubing on circuit extraction. Each circuit was primed with human blood, and flow was set to 1 L/min. Drug was dosed to achieve therapeutic concentrations. Each antifungal was added to a separate tube of blood to serve as a control. Serial blood samples were collected over 24 hours and concentrations were quantified with a validated assay. Drug recovery was calculated at each time point: $(C_t/C_0) \times 100$, with C_t and

C_0 the concentrations at time = t and 1 minute, respectively. After 24 hours of recirculation, mean recovery of fluconazole in the ECMO circuit (95–98%) and controls (101%) was high. In contrast, mean recovery of micafungin was dependent on the time and circuit configuration. Recovery at 4 hours was only 46% when a hemofilter was in-line but was much higher when the hemofilter was removed (91%). By 24 hours, however, micafungin recovery was low in all circuit configurations (26–43%), regardless of the presence of a hemofilter, as well as in the controls (57%). In conclusion, these results suggest that micafungin is extracted by the ECMO circuit, which may result in decreased drug exposure in vivo. **Keywords:** antifungal extraction, extracorporeal membrane oxygenation, fluconazole, micafungin. *J Extra Corpor Technol. 2017;49:150–159*

Extracorporeal membrane oxygenation (ECMO) is a cardiopulmonary bypass device used to support patients with refractory respiratory and/or cardiac failure. Patients supported with ECMO are critically ill and, thus, exposed to multiple drugs. Optimal dosing of drugs in this setting is unknown because ECMO can alter drug pharmacokinetics (PK). Studies of antimicrobials with ECMO (e.g., vancomycin [1–4], gentamicin [5–8], and fluconazole [9]) and sedatives (e.g., opiates [10–13], benzodiazepines [14,15]) generally show an increased volume of distribution (V) and decreased clearance (CL). These alterations in PK are caused by multiorgan dysfunction, the large volume of exogenous

blood required to prime the ECMO circuit, and drug extraction by the circuit.

Drug extraction by the ECMO circuit is thought to be due to nonspecific binding by the components of the circuit (16–20). During ECMO support, nearly all of a patient's blood is drained from the venous system via a large bore cannula and pumped through tubing to an artificial lung (oxygenator) before being returned to the patient. In some cases, the ECMO circuit also contains a hemofilter that can provide hemofiltration or dialysis for patients with renal injury. As drugs transit the ECMO circuit they come in contact with the various components of the circuit (e.g., tubing, pump, oxygenator, and hemofilter) and are vulnerable to extraction. The extent of drug extraction by the ECMO circuit depends on the materials in the circuit and the physicochemical properties of the drug. Nonspecific binding of drugs occurs via two primary mechanisms: 1) hydrophobic interactions and 2) ionic interactions (21). Hydrophobic binding can occur between hydrophobic drugs and a hydrophobic surface,

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while ionic binding occurs when the drug and surface are oppositely charged. Nonspecific binding of drugs can significantly decrease exposure and lead to therapeutic failure.

Circuit-drug interactions have been investigated using *ex vivo* ECMO experiments in which drug is administered to an isolated ECMO circuit (14,16,22–30). Because there is no corporeal metabolism or elimination in this system, decreases in drug concentration are due to either extraction by the circuit or drug degradation. In general, highly lipophilic (e.g., fentanyl) and highly protein bound (e.g., caspofungin) drugs are extensively extracted by the circuit (24,31). However, this relationship is not always predictable. Ciprofloxacin is lipophilic with low protein binding but is not extracted, while meropenem is hydrophilic with low protein binding and is extensively extracted (23,24). This suggests that other factors, such as ionic binding, may play a role. Additionally, the site(s) of extraction within the circuit is not known.

In the present study, the extent of extraction by the ECMO circuit was determined for selected antifungal drugs in an *ex vivo* ECMO system. Antifungal drugs were chosen because fungal infections are common in patients on ECMO, and treatment depends on optimal dosing (32). The contribution of each component of the ECMO circuit to overall extraction was determined by comparing extraction in three circuit configurations with different component combinations.

MATERIALS AND METHODS

Drug selection

Two antifungal drugs were selected for evaluation: fluconazole and micafungin. These drugs were selected based on their frequency of use in clinical practice and differing physicochemical properties (Table 1) (33–35). Drugs used in these experiments were purchased from the Duke Medical Center pharmacy.

Circuit configuration

To determine the impact of each component on drug extraction, the experiment was designed with three circuit

configurations (Figure 1). The Complete Circuit contained tubing, a pump, an oxygenator, and a hemofilter (Table 2). The Oxygenator Circuit was identical to the Complete Circuit except the hemofilter was removed. The Pump Circuit was identical to the Oxygenator Circuit except the oxygenator was removed. Any difference in extraction between the Complete and Oxygenator Circuits was due to the hemofilter. Similarly, any difference in extraction between the Oxygenator and Pump Circuits was due to the oxygenator (Figure 1).

Circuit setup

Circuits were assembled according to the standard practice for Duke Children's Hospital. Circuits were primed with a solution of 1 unit of human packed red blood cells (~350 mL), 0.5 unit of human fresh frozen plasma (~175 mL), and Plasma-Lyte A (Baxter Healthcare, Deerfield, IL) crystalloid (500 mL). Recently expired (<7 days) blood products were used when possible to limit the impact on hospital supply of banked blood. In addition, the following were added to each circuit to complete the prime solution: heparin sulfate (100 units), sodium bicarbonate (30 mEq), and calcium gluconate (6.5 mg). Additional sodium bicarbonate and/or carbon dioxide via the sweep gas were added to the system to maintain physiologic pH (7.2–7.5). To be consistent with standard practice at Duke, albumin was not routinely added to the circuit, and albumin concentrations were low (≤ 1.0 mg/dL). However, if a drug showed extraction in any of the circuit configurations, subsequent experiments were performed, and human serum albumin was added to achieve two additional albumin concentration ranges: low-physiologic (2.2–2.8 mg/dL; typical for a child on ECMO) and physiologic (3.5–3.8 mg/dL). To examine the impact of albumin on extraction, extraction was compared between circuits with low, low-physiologic, and physiologic albumin concentrations.

Because there was no human connected to the circuit, the circuit was completed via a reservoir (double-spiked IV bag with operating volume maintained to prevent air entrainment into circuit). The Quadrox iD integrated heat exchanger was used to maintain a constant temperature of 36°C throughout the study. ECMO flow was set to 1 L/min and was measured post-oxygenator (and posthemofilter shunt for Complete Circuits) using a transonic flow meter. The reservoir return was directed via a 10 fr Biomedicus arterial cannula to simulate the clinical scenario for a 10 kg child.

Control

In parallel with the ECMO circuits, at least three control samples per drug were analyzed to determine the amount of drug degradation over time. Each control sample consisted of a clear, polyvinylchloride plastic tube that was

Table 1. Antifungal drug physicochemical properties and clearance pathways.

Antifungal	Charge	LogP	Plasma Protein Binding (%)*	Molecular Weight (g/mol)	Primary Elimination Pathway
Fluconazole	Neutral	.4	11%	306	Renal
Micafungin	Negative	−.4	99%	1,270	Hepatic

*Fluconazole binds primarily to alpha-1-acid-glycoprotein (33,34). Micafungin binds primarily to albumin (35).

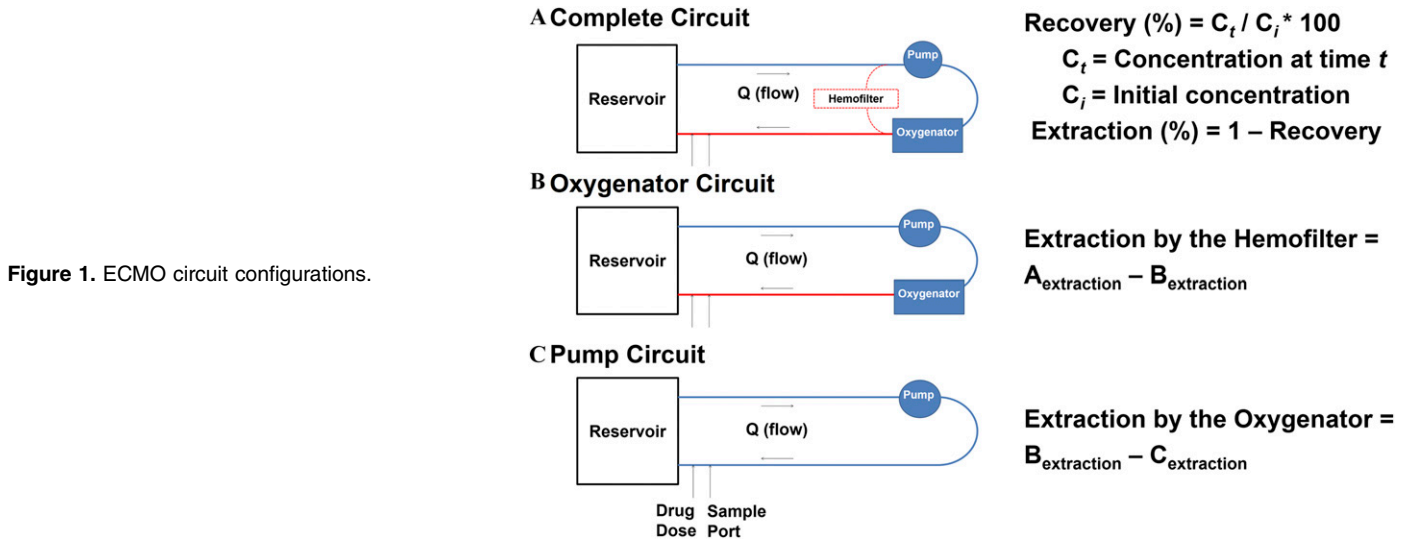


Figure 1. ECMO circuit configurations.

filled with 30 mL of the ECMO prime solution. The prime solution was collected from the ECMO circuit after ≥ 5 minutes of circulation to ensure proper mixing but before drug administration. In this way, the control medium was identical to the medium in the ECMO circuits. Thus when drug was administered, the only difference between ECMO circuits and controls was the ECMO environment (e.g., components, flows). The control tubes were tightly capped in a 36°C static, water bath.

Because micafungin concentrations were noted to decrease in the control samples over time, additional post hoc control experiments were performed to assess the source of loss. Four experimental conditions were studied: 1) Standard Control described above; 2) Silanized Glass Control to determine if micafungin was adsorbed by the polyvinylchloride plastic tubes; 3) Standard Control protected from light to determine impact of light degradation; and 4) Standard Control with crystalloid prime solution to determine the impact of blood metabolism. All tubes except the Standard Control with crystalloid prime were filled with 30 mL of the blood prime solution with albumin added to achieve low physiologic conditions. The crystalloid prime

consisted of Plasma-Lyte A crystalloid (500 mL), heparin sulfate (100 units), sodium bicarbonate (30 mEq), calcium gluconate (6.5 mg), and human serum albumin (25 g). Each of the four experimental conditions was replicated in triplicate.

Drug administration and sample collection

Drug was introduced into the circuit via a port in the arterial cannula just before the reservoir (Figure 1) and downstream of the sampling port at time = 0 and dosed to achieve a therapeutic concentration of 20 mg/mL for both fluconazole and micafungin. The dose was determined based on the drug concentration and volume of the ECMO circuit. Drug was dosed to achieve a comparable concentration in the control samples at time = -5 minutes, and then the tubes were sealed with a tight cap and gently mixed for 5 minutes until time = 0. At time = 0, the controls were returned to the water bath where they remained for the duration of the experiment, except at times of sample collection. Immediately before control sample collection, control tubes were removed from the water bath and gently inverted five times to ensure adequate mixing. Samples

Table 2. ECMO circuit components.

Component	Manufacturer	Model	Material
Oxygenator	Maquet	Adult/Pediatric Quadrox iD*	Polymethylpentane hollow fibers with Bioline† coating
Hemofilter	Sorin	DHF0.2	Polyethersulfone
Pump	Sorin	Revolution Centrifugal	Polycarbonate
Tubing	Sorin	Smart Tubing	Phosphorylcholine coated polyvinylchloride
Cannula	Medtronic	10 ft Biomedicus Arterial Cannula	Carmeda‡ coated polyurethane

*Adult and pediatric oxygenators only differed in surface area (1.8 m² for adult and .8 m² for pediatric). Because the oxygenator was not responsible for clinically significant extraction, and no difference was observed between adult and pediatric oxygenator circuits, adult and pediatric oxygenators were assumed to be equivalent for the purpose of these experiments.

†Bioline coating: heparin + recombinant human albumin.

‡Non-leaching, endpoint attached heparin biosurface.

from the ECMO circuits were collected from a port just upstream of the arterial cannula (Figure 1). In the initial set of experiments, samples were collected from the circuits and controls for 4 hours at the following time points: 1, 5, 15, and 30 minutes, 1, 2, 3, and 4 hours. To better understand drug disposition over an entire dosing interval, concentrations up to 24 hours were measured in subsequent experiments by collecting additional samples at 8, 12, and 24 hours. After collection, samples were immediately placed on dry ice for up to 4 hours before transfer to a -80°F freezer.

Because micafungin concentrations were noted to decrease in the ECMO circuits with in-line hemofilters, post hoc hemofiltrate samples were collected from a subsequent experiment to determine if micafungin was crossing the filter membrane into the hemofiltrate.

Analysis

Drug concentrations were measured using assays that were developed and validated according to FDA guidance (36). Fluconazole concentrations were measured at OpAns Laboratory (Durham, NC) using HPLC/MS-MS. Plasma samples were acidified with formic acid and precipitated with acetonitrile. Samples were centrifuged and diluted with water containing .2% (v/v) acetic acid prior to injection into the HPLC system. Separation was achieved with a Poroshell 120 EC-C18 Column. Fluconazole was quantified using an electrospray ionization source in the positive mode and under the following conditions: gas temperature 300°C , gas flow 10 L/min, nebulizer pressure 50 psi, sheath gas temperature 345°C , sheath gas flow 11 L/min, capillary voltage 3500 V, nozzle voltage 500 V, Dynamic MRM scan type. The lower limit of quantification (LLOQ) was .01 mg/L with a calibration curve range of .01–10 mg/L. Intraday and interday precision (% CV) ranged from 1.4% to 8.5% and 2.8% to 5.8%, respectively. Micafungin concentrations were measured at the University of Texas Health System Fungal Testing Laboratory using HPLC with fluorescence detection. Plasma samples were acidified with phosphoric acid and precipitated with acetonitrile. Samples were centrifuged and diluted with 10 mM ammonium acetate before injection into the HPLC system. Injection into the HPLC occurred under the following conditions: mobile phase with 10 mM ammonium acetate/acetonitrile 60:40 v/v at a flow rate of 1.0 mL/min. Separation was achieved with a Phenomenex-Luna 5 μ C18 Column. Micafungin was quantified by fluorescence. The fluorescence detector excitation and emission wavelengths were 273 nm and 464 nm, respectively, and the LLOQ was .05 mg/L. The calibration curve ranged from .05–25 mg/L. Intraday precision ranged from 1.19% to 7.35% except at the LLOQ where the range was 0% to 12.0%. Interday precision ranged from 4.88% to 10.3%.

Drug recovery in circuits and controls was calculated at each sample time using the following equation:

$$\text{Recovery}(\%) = \frac{C_t}{C_i} * 100$$

$$\text{Extraction}(\%) = 100 - \text{Recovery}$$

where C_t is the concentration at time t and C_i is the initial concentration measured at time = 1 minute. Data are reported as the mean and 95% confidence interval.

Ethics

The Duke University Medical Center Institutional Review Board provided a waiver of review because the protocol met the definition of research not involving human subjects.

RESULTS

The total number of circuits studied, by configuration, are summarized for each drug in Table 3.

Fluconazole

Fluconazole was not extracted by the ECMO circuit (Figure 2A). In three Complete Circuits, the mean (95% confidence interval) recovery of fluconazole was 97.8% (96.3, 99.3) at 4 hours. In the two Complete Circuits that were run for 24 hours, 95.2% (89.6, 100.9) of the initial concentration was recovered. When the hemofilter was removed to create the Oxygenator Circuit, 92.3% (83.1, 101.5) of the initial fluconazole concentration was recovered at 4 hours ($n = 4$). One Oxygenator Circuit was run for 24 hours, and 98.4% of the initial concentration was recovered at 24 hours. When fluconazole was administered to the Pump Circuit ($n = 1$), 105.8% was recovered after 4 hours. Because no extraction was observed in the Complete or

Table 3. Number of circuits by configuration and drug.

Configuration	Fluconazole	Micafungin
A. Complete Circuit (hemofilter, oxygenator, pump, tubing)	3*	10†
B. Oxygenator Circuit (oxygenator, pump, tubing)	4‡	4§
C. Pump Circuit (pump, tubing)	1	1
D. Control	4¶	4

*1 circuit run for 4 hours; 2 circuits run for 24 hours.

†10 circuits stratified by albumin level (g/dL): low (≤ 1 ; $n = 4$), low physiologic (2.2–2.8; $n = 3$); physiologic (3.5–3.8; $n = 3$). 1 circuit run for 4 hours (low albumin); 9 circuits run for 24 hours.

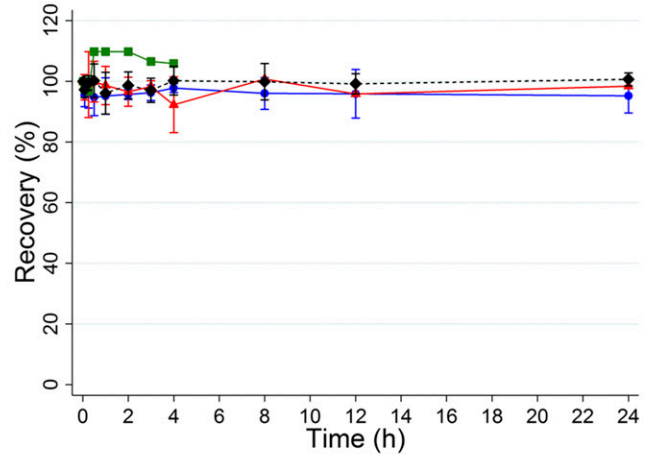
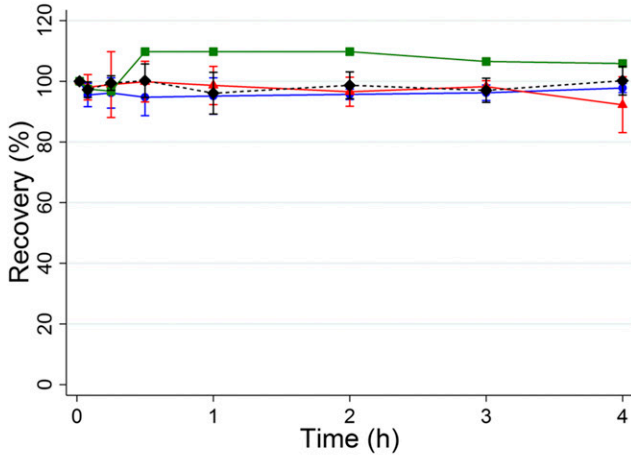
‡3 circuits run for 4 hours; 1 circuit run for 24 hours.

§2 circuits run for 4 hours; 2 circuits run for 24 hours.

¶1 control sampled for 4 hours; 3 controls sampled for 24 hours.

||1 control sampled for 4 hours; 3 controls sampled for 24 hours.

A Fluconazole



B Micafungin

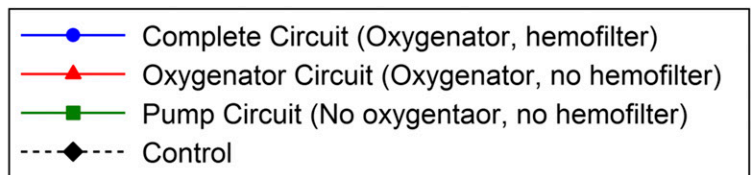
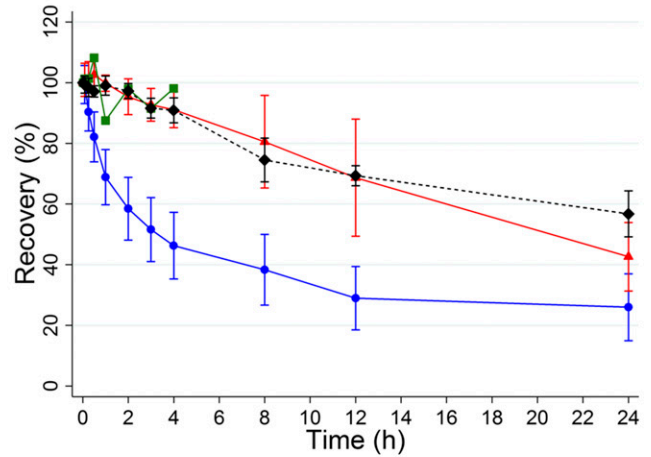
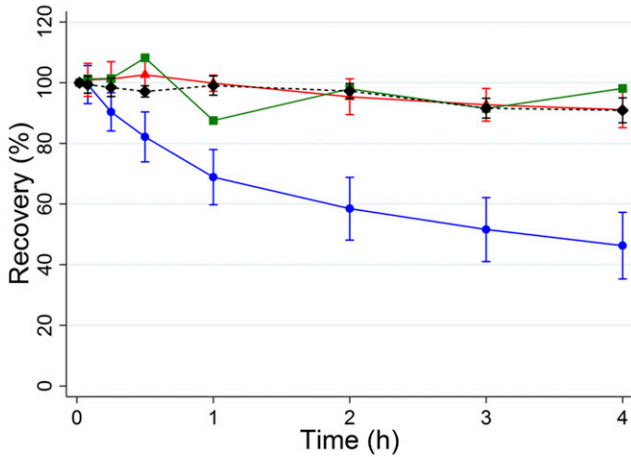


Figure 2. Recovery by circuit configuration for each drug. Panel on the left shows recovery over the first 4 hours after dosing, and panel on the right shows recovery for 24 hours. Values are mean; error bars indicate 95% confidence interval.

Oxygenator Circuits, additional Pump Circuits were not run. In the control samples, 100.2% (95.5, 104.9) of the initial fluconazole concentration was recovered at 4 hours ($n = 4$), and 100.6% (98.5, 102.8) at 24 hours ($n = 3$).

Micafungin

In the Complete Circuit ($n = 10$), micafungin recovery was low at 4 hours (46.3% [35.3, 57.3]). However, when the hemofilter was removed (Oxygenator Circuit, $n = 4$), micafungin recovery at 4 hours was 91.1% (85.2, 97.0) (Figure 2B). Similarly, in the Pump Circuit ($n = 1$) and

Control ($n = 4$) 98.1% and 90.9% (86.8, 95.0), respectively, were recovered at 4 hours. By 24 hours; however, recovery of micafungin was low in the Complete (26.0% [15.0, 37.0]; $n = 9$) and Oxygenator Circuits (42.6% [31.3, 53.9]; $n = 3$) as well as in the Control samples (56.8% [49.2, 64.4]; $n = 3$).

To determine the source of loss in the Control samples, the Control experiments were repeated under four conditions. All four experimental conditions showed substantial loss over 24 hours. The results from the Standard Control were comparable to the results from the Controls above with 54.7% (49.0, 69.2; $n = 3$) recovered at 24 hours

(Figure 3). Results were comparable for both the Silanized Glass Control and the Standard Control protected from light with 56.4% (50.9, 60.1) and 51.6% (49.7, 54.9) recovered at 24 hours, respectively. In the Standard Control primed with crystalloid, recovery was only 11.6% (11.0, 12.3) at 24 hours. Of note, albumin concentration in the crystalloid control samples was low (1.2 g/dL) compared to the other three blood-primed controls where albumin concentration was higher (2.1 g/dL).

Micafungin extraction during the first 4 hours was dependent on albumin concentration (Figure 4). At low albumin concentrations (≤ 1.0 g/dL; $n = 4$) in a Complete Circuit, 29.0% (19.7, 38.4) and 16.1% (1.7, 30.5) of the initial micafungin concentrations were recovered at 4 and 24 hours, respectively. At low physiologic albumin concentrations (2.2–2.8 g/dL; $n = 3$) recovery in a Complete Circuit was 58.4% (53.5, 63.3) and 35.7% (24.7, 46.7) at 4 and 24 hours, respectively. Similarly, when albumin concentrations were in the normal range (3.5–3.8 g/dL; $n = 3$), micafungin recovery was 57.2% (54.8, 59.5) and 26.1% (20.4, 31.9) at 4 and 24 hours, respectively.

Because the hemofilter was responsible for micafungin extraction in the first 4 hours, micafungin concentrations were measured in the hemofiltrate for one circuit to determine if micafungin passively crossed the hemofilter membrane (hemofilter was only in-line and not actively filtering). Concentrations in the hemofiltrate at 1 minute, 1 hour, 2 hours, and 24 hours were only 0.07, 0.06, 0.21 and 0.16 mg/L,

respectively. Concentrations in the plasma samples from the ECMO circuit at the same times were 15.5, 13.1, 11.6, and 8.1 mg/L, respectively. Concentrations in the hemofiltrate were $<2\%$ of concentrations in the circuit suggesting that micafungin does not cross the hemofilter membrane.

DISCUSSION

ECMO can alter drug PK directly and indirectly through a variety of mechanisms. Direct effects include: 1) increased volume of distribution due to the addition of the large volume of exogenous blood required to prime the ECMO circuit, 2) hemofiltration, which is common in patients on ECMO, and 3) extraction of drug by components of the circuit (37). Ex vivo ECMO experiments such as those performed in this study provide insight into extraction via circuit-drug interactions. The degree of interaction with the ECMO circuit is drug-dependent and likely influenced by the physicochemical properties of the drug and circuit components (e.g., oxygenator, hemofilter) (24,31). Results of the present study demonstrate important differences in antifungal drug extraction by the ECMO circuit that can affect dosing recommendations in clinical practice.

Micafungin was highly extracted in the first 4 hours in a Complete Circuit but not in the other circuit configurations. This suggests that extraction was due to the hemofilter. Three possible mechanisms may explain extraction by the

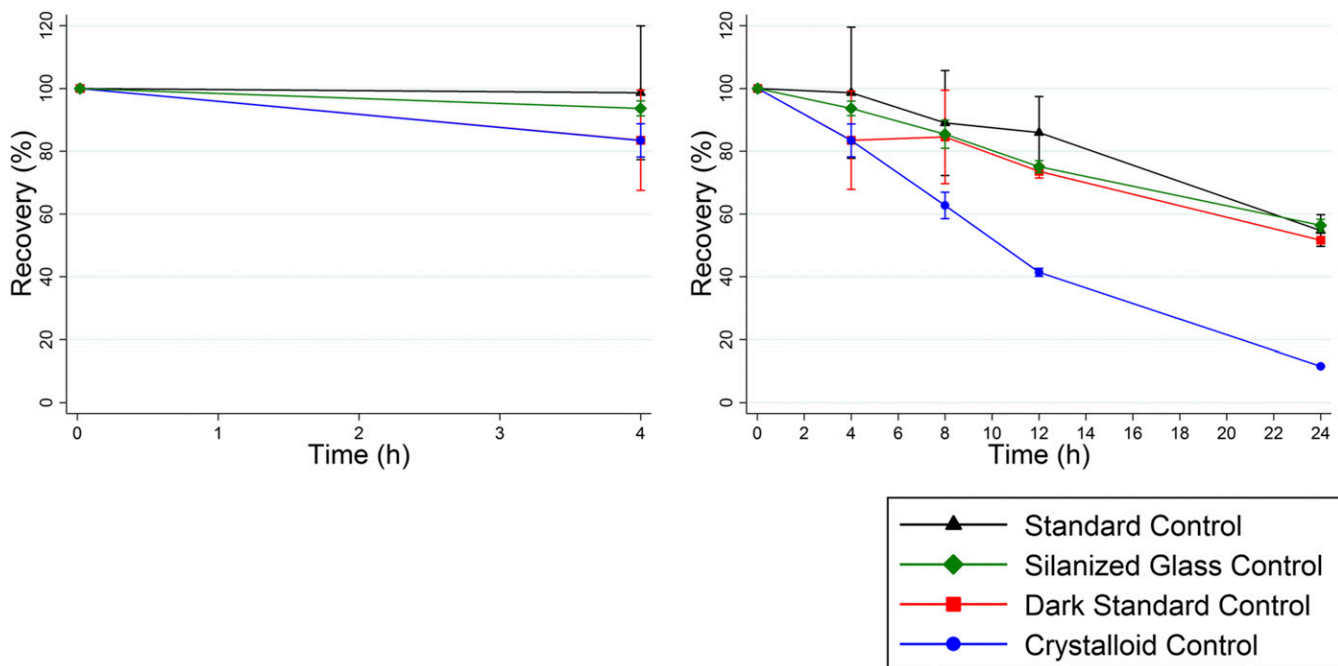


Figure 3. Micafungin recovery in Controls under 4 experimental conditions: 1) Standard Controls, 2) Silanized Glass Controls, 3) Dark Standard Controls (i.e., protected from light), and 4) Crystalloid Controls (i.e., Standard Controls primed with crystalloid). Panel on the left shows recovery over the first 4 hours after dosing, and panel on the right shows recovery for 24 hours. Values are mean; error bars indicate 95% confidence interval.

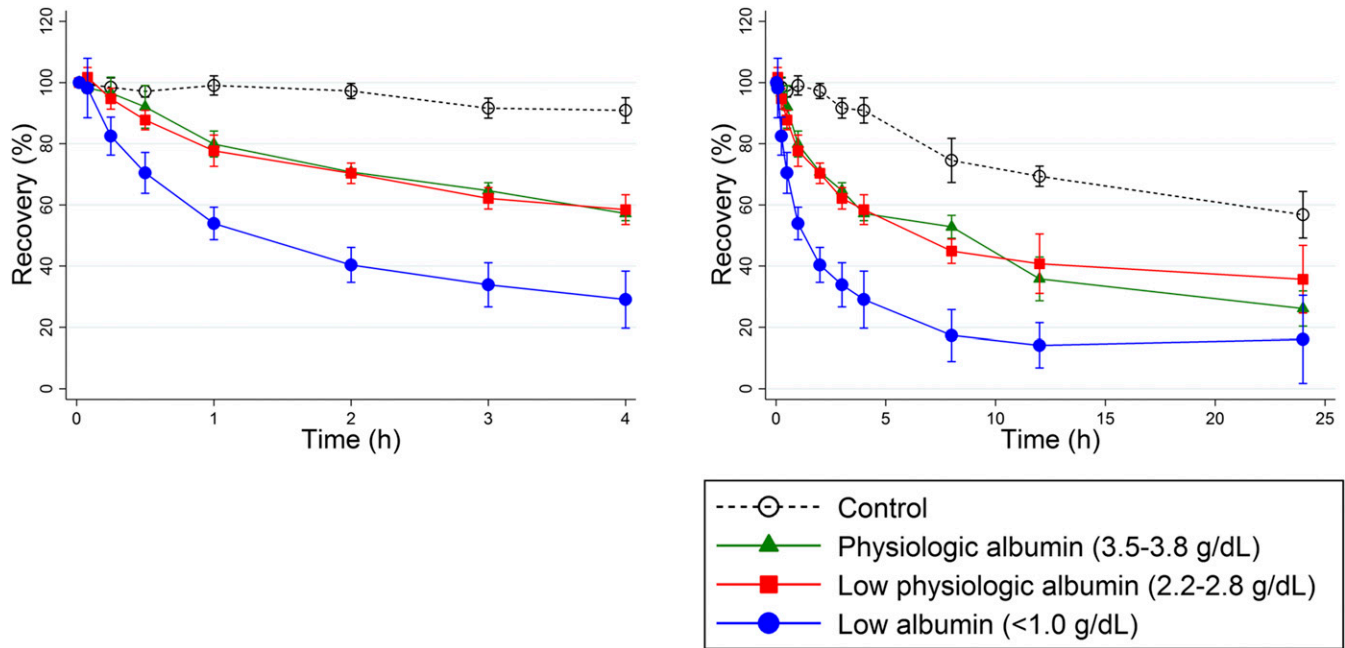


Figure 4. Micafungin recovery in Complete Circuits and Controls stratified by albumin concentration. Panel on the left shows recovery over the first 4 hours after dosing, and panel on the right shows recovery for 24 hours. Values are mean; error bars indicate 95% confidence interval.

hemofilter: 1) diffusion across the hemofilter membrane; 2) areas of stasis in the hemofilter that “trapped” the drug; and/or 3) direct adsorption by the hemofilter. Micafungin would not be expected to diffuse across the hemofilter membrane because it is highly protein bound (>99%) and the hemofilter was not actively filtering during the experiments. This was confirmed by collecting samples of hemofiltrate, which contained virtually undetectable concentrations of micafungin. Although areas of low flow can occur around the hemofilter, inconsistency in the degree of “trapping” and more variability in recovery would be expected if this were the mechanism. Adsorption by the hemofilter appears to be the most likely explanation. This is supported by greater extraction at low albumin concentrations suggesting that the unbound fraction is adsorbed by the hemofilter membrane. However, the polyethersulfone membrane used in these experiments was hydrophobic and had no net charge, making it less likely to interact with a hydrophilic anion such as micafungin. Further studies to understand the mechanism of micafungin loss to the hemofilter are needed.

These results are in contrast to studies of micafungin in continuous venovenous hemofiltration (CVVH) using similar hemofilters that showed no loss when micafungin concentrations were measured pre- and posthemofilter (38,39). However, several important differences were noted between our ECMO ex vivo system and the CVVH studies that could explain this apparent discrepancy: 1) the CVVH studies were pharmacokinetic trials in critically ill adults, so other unmeasured factors inherent to clinical trials may have influenced drug concentrations; 2) flow rates were higher in

the ECMO experiments compared to CVVH and for drugs where extraction is perfusion limited, increased flow results in higher extraction; and 3) the CVVH studies were actively filtering and by removing plasma water but not drug, micafungin was functionally concentrating in the plasma. Differences in protein binding between the in vivo and ex vivo studies could also explain the apparent discrepancy. Also, albumin concentrations were higher in the in vivo CVVH studies. Because micafungin is highly protein bound, small changes in binding can substantially alter the amount of unbound drug available to be adsorbed by the hemofilter.

More important than the hemofilter-related decrease in concentration over 4 hours was the observation that micafungin concentrations decreased over a 24-hour dosing interval in all circuit configurations and the controls. Loss of drug in the controls suggests one of three possible mechanisms: 1) adsorption by the polyvinylchloride of the control tubes; 2) plasma metabolism of micafungin, or 3) drug degradation. The additional post hoc control experiments suggest that adsorption is less likely as concentrations decreased at the same rate in the polyvinylchloride and silanized glass control tubes. Furthermore, nonspecific binding to circuit or control materials should occur quickly (i.e., <4 hours) (17,19–21). Plasma metabolism is not described for micafungin, but peptidases are a major elimination pathway for both caspofungin and anidulafungin, echinocandins with similar structure. However, concentrations were lower in the Standard Control tubes primed with crystalloid compared with those primed with blood, likely due to the lower albumin concentration observed in

the crystalloid primed tubes. Measurement of metabolites could aid in assessing metabolic conversion in plasma but was not possible with our assay. Drug degradation is the most likely mechanism of loss in the controls. Micafungin is known to degrade in light. However, the additional control experiments showed no difference in extraction between the Standard Control and the Standard Control protected from light, suggesting another degradation mechanism. Regardless of the mechanism, mean micafungin recovery ranged from 26% to 57% at 24 hours and suggests that micafungin dosing may need to be increased in patients on ECMO.

In the present study, fluconazole was not extracted by the ECMO circuit. The fluconazole results are consistent with a recent ECMO *ex vivo* study where fluconazole recovery at 24 hours in a system similar to the Oxygenator Circuit and Control used in this study were 96% and 102%, respectively (24). Based on physicochemical properties, fluconazole would not be expected to interact with the ECMO circuit. It is only slightly lipophilic (LogP of 0.4) and should not undergo extensive hydrophobic binding to polymers. The present findings also are supported by an ECMO *ex vivo* study that demonstrated minimal extraction of linezolid, a drug with similar physicochemical properties (LogP of .9, protein binding of 30%) (24). Additionally, fluconazole has a neutral charge at physiologic pH so it would not be expected to undergo extensive ionic binding.

The study has some limitations. We were unable to evaluate drug loss in all three circuit configurations in triplicate for 24 hours. Due to resource constraints we had to prioritize the highest impact experiments. Configurations were designed to run sequentially so that if no drug loss was seen in a configuration (e.g., Oxygenator Circuit), it was reasonable to assume that no loss would be seen in the subsequent configuration (e.g., Pump Circuit) that differed only in the removal of a circuit component (e.g., oxygenator). Because no circuit-related drug loss was observed in the Oxygenator Circuits, we made the assumption that no loss would occur in the Pump Circuits that were identical except for removal of the oxygenator. We only performed a single Pump Circuit experiment for each drug to confirm this assumption. By prioritizing resources we were able to better understand the impact of albumin on micafungin loss in the Complete Circuit and determine why micafungin was lost in the control samples. Another limitation relates to the impact of the hemofilter shunt flow on overall flow in Complete Circuits with the shunt vs. Oxygenator and Pump circuits without a shunt. The shunt results in an ~10% recirculation rate, thereby exposing components (e.g., oxygenator, tubing) to ~10% more flow in a Complete Circuit. We believe that the impact of this on the current experiments was minimal as neither drug appears to be adsorbed by the oxygenator or tubing. However, future experiments with other drugs should consider including the shunt in all circuit configurations.

Treatment of invasive fungal infection in patients on ECMO is challenging because of the tendency of these pathogens to form biofilms on indwelling catheters (40). These biofilms are difficult to treat, and current guidelines recommend both an antifungal agent and removal of indwelling catheters (41,42). Catheter removal for patients on ECMO is nearly impossible because these catheters connect the patient to the ECMO circuit. Although micafungin interacts with the ECMO circuit and may affect *in vivo* exposure, the ability of micafungin to penetrate biofilms makes it an attractive agent in patients on ECMO (43). We recently published an *in vivo* PK study of micafungin in a small cohort of infants on ECMO and showed that appropriate exposures could be achieved with higher doses (44). The differences in extraction between fluconazole and micafungin should not favor the use of one agent over the other. Instead that decision should be based on the clinical scenario, pathogen, and local resistance patterns.

In summary, it is clear that a single physicochemical property (e.g., lipophilicity) is unable to predict which drugs are extracted by the ECMO circuit, and to what extent. Even if older circuits with uncoated, hydrophobic polymers were used, differences in the materials and residuals in the matrix, such as plasticizers, would likely require an empiric correction factor to accurately describe the extent of adsorption (21). The circuit-drug interactions are even more complicated when surface coatings are applied. Although surface coatings are added primarily to increase biocompatibility, the impact on adsorption can be substantial. Studies comparing the adsorption of drugs between tubing with different surface coatings showed significant differences in drug recovery both between coated and uncoated materials, and also between the different types of surface coatings (17,18). Many knowledge gaps remain. It is unknown whether surface coatings on ECMO circuits change over time or if surfaces are saturable after repeated dosing. Furthermore, it is unknown whether endogenous materials (e.g., plasma proteins, platelets) compete for binding sites on circuit components. Future work should explore high-throughput systems to evaluate the interaction between ECMO circuit components and different drugs. Until that time, *ex vivo* experiments remain the best way to define specific circuit-drug interactions. The results from the present *ex vivo* experiments will be used to inform an ECMO compartment in physiologically based pharmacokinetic models of fluconazole and micafungin, and translate the circuit-drug interactions into dosing recommendations in children on ECMO.

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