Argatroban as an alternative to heparin in extracorporeal membrane oxygenation circuits

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We investigated the anticoagulant effects of argatroban, a direct thrombin inhibitor, versus heparin in extracorporeal membrane oxygenation (ECMO) circuits. Three sham circuits were prepared according to our hospital's standard practice and run for six hours simultaneously. Two circuits were anticoagulated with argatroban (one with heparin in the wet prime and one without). One circuit had heparin in the initial prime and was then anticoagulated with heparin. We measured thrombin generation (prothrombin fragment 1+2, D-dimer and thrombin–antithrombin complexes), activated clotting times (ACTs) and partial thromboplastin times (aPTTs), and monitored thrombus formation using thromboelastography. ACTs were >1000 s in each circuit throughout assessment. No clot initiation was detected by thromboelastography. Thrombin generation was decreased in circuits anticoagulated with argatroban versus heparin, despite aPTTs being less prolonged. These results suggest that argatroban may be more efficacious than heparin for anticoagulation in ECMO. Additional studies are warranted to further evaluate argatroban in this setting. *Perfusion* (2004) **19,** 283–288.

Introduction

Extracorporeal membrane oxygenation (ECMO) is a life-saving procedure utilized in patients with severe, but potentially reversible, pulmonary and circulatory disorders.[1,2] It is most often used in neonates and infants for conditions such as primary pulmonary hypertension of the newborn, diaphragmatic hernia, sepsis and congenital heart disease. The procedure involves extending the circulation of blood through an *ex vivo* circuit where blood is oxygenated. As blood must circulate through a nonendothelialized circuit, anticoagulation is necessary to prevent thrombosis of the circuit.[3–6] When thrombotic occlusion develops, it may become necessary to change the circuit which then exposes the patient to increased risk of complications such as cardiopulmonary arrest and infection.

The only anticoagulant in current use for such circuits is heparin. There are, however, significant limitations with heparin, especially in neonates.[7–9] First and foremost is the unpredictable pharmacokinetics of heparin which leads to patients often being over- or underanticoagulated, raising the risk of bleeding and thrombotic complications.[7] Secondly, as neonates are physiologically deficient in antithrombin (AT), the protein through which heparin exerts its effect, therapeutic anticoagulation may be difficult to achieve and maintain.[8] Heparin can lead to heparin-induced thrombocytopenia (HIT), which is very difficult to diagnose in this population of patients as they are often thrombocytopenic to begin with for various reasons. Finally, heparin may cause more bleeding for the same anticoagulant effect as more recently developed anticoagulants, such as low molecular weight heparins (LMWH)[10] and pentasaccharide[11] as measured by anti-factor Xa levels and direct thrombin inhibitors (DTI)[12] as measured by the activated clotting time (ACT).

There are several new anticoagulants that have recently been approved for use in the USA or Europe. Among these is the new class known as DTI. These compounds share the unique property of binding to thrombin directly (that is without AT) and inhibiting both free and clot-bound thrombin.[12] Some are analogs of the leech protein hirudin and are currently approved by the Food and Drug Administration (FDA) for the treatment of HIT [(lepirudin), Refludan, Berlex Laboratories, Montville, NJ, USA][13] or for use in patients with unstable angina undergoing percutaneous coronary intervention [(bivalirudin), Angiomax, The

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Medicines Company, Parsippany, NJ, USA].[14] Another is a synthetic molecule that binds to the active site of thrombin [(argatroban), Argatroban, GlaxoSmithKline, Philadelphia, PA, USA]. Argatroban is currently FDA approved as an anticoagulant for the prophylaxis or treatment of thrombosis in HIT[15] or for use in patients at risk for HIT undergoing percutaneous coronary interventions.[16]

These agents have several advantages over heparin. First, they do not rely on the presence of AT, and, thus, are useful in patients who are deficient, such as neonates and patients on ECMO. Secondly, they have much more predictable pharmacokinetics, leading to a more stable systemic level with less risk of bleeding from supratherapeutic levels and less risk of thrombosis from subtherapeutic levels. In addition, for a given level of anticoagulation as measured by the ACT, they cause less bleeding.[12] This has been documented especially well for bivalirudin, but seems to be a property of all DTI.

For patients on ECMO, a safer alternative to heparin will likely result in improved survival by reducing bleeding and thrombotic complications, and reducing the costs and risks associated with repeatedly changing the circuit. We, therefore, elected to study the effects of argatroban on an ECMO circuit in terms of its ability to prevent thrombin generation as compared to heparin. There has been prior experience with argatroban in extracorporeal circuits in Japan,[17] and a case report utilizing it in ECMO in a patient with HIT.[18] There are additional reports on the use of argatroban in patients undergoing cardiopulmonary bypass in whom heparin could not be used due to the risk for HIT.[19,20] To date, there are no studies comparing the efficacy of argatroban versus heparin in preventing thrombin generation in this setting.

Methods

Three ECMO circuits (Gish Biomedical, Rancho Santa Margarita, CA, USA) were prepared as if they were to be connected to a patient per protocol at our institution. Specifically, two circuits were initially primed with 500 cc Plasma-Lyte A (Baxter Healthcare, Deerfield, IL, USA) and heparin 150 units, which was then flushed through (wet prime). This was followed by the blood prime which consists of 500 cc of washed, irradiated packed red blood cells, 100 cc of platelets, 120 cc of fresh frozen plasma, 50 cc of 25% albumin, calcium gluconate 600 mg, sodium bicarbonate 25 mEq, and 50 cc of THAM solution (Abbott Laboratories, North Chicago, IL, USA). No heparin was used in the third circuit which was otherwise prepared in an identical fashion. The first circuit served as the control and received a bolus dose of heparin 500 units with additional heparin to be infused as a continuous infusion when the ACT fell below 180 s to maintain an ACT of 180–220 s. The second and third circuits received a bolus dose of argatroban 1 mg with a continuous infusion to be given for the same parameters as for the first circuit. The circuits were each managed by an ECMO trained nurse with supervision by one of the investigators (KEY) and run in one day for a period of six hours. Blood samples were drawn at hour 0 (immediately after the blood prime) and at hourly intervals until hour 6. In addition, the nurses and investigator inspected the circuit each hour looking for clot formation in any of the circuitry.

Blood samples were drawn and labeled with the time and circuit number. The following investigations were performed: complete blood count (CBC), ACT, prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen activity (fib), and D-dimers every hour, and prothrombin fragment 1.2 (F1.2), thrombin–antithrombin complexes (TAT), and thromboelastography (TEG) every two hours.

The ACT was performed within 1 min of removing the sample, utilizing the Hemochron Jr. (ITC, Edison, NJ, USA) with results reported in seconds up to 1500 s. The PT, aPTT and TT were performed using an automated coagulation device in the clinical laboratory with standard reagents. Fibrinogen levels were done by the Clauss method.[21] D-dimers, F1.2 and TAT complexes were performed using an enzyme-linked immunosorbent assay (Dade Behring, Deerfield, IL, USA) as per the manufacturer's instructions. TEG (Haemoscope Corp., Niles, IL, USA) was performed within 4 min of withdrawing the blood sample on native whole blood with kaolin activation as per the manufacturer's instructions.

Statistical analysis was performed using a two-way analysis of variance with pairwise comparisons of the three circuits and thrombin generation markers.

Results

We ran three ECMO circuits in parallel with the only difference between circuit 1 and 2 being the use of heparin versus argatroban and the only difference between circuits 2 and 3 being the use of heparin in the wet prime. Thus, circuit 1 serves as the control for circuit 2 and circuit 2 as control for circuit 3. All three circuits functioned properly and continued to

have expected rates of blood flow for the entire six hours. There was no visible evidence of thrombus formation in any of the circuits. Results of the laboratory assays are presented in Table 1. The circuits all maintained supratherapeutic ACTs, aPTTs, PTs and TTs (data not shown) throughout the six hours (probably due to the lack of metabolism of the anticoagulants in the sham circuits), and, thus, no further infusions of heparin or argatroban were needed. There was no clot formation as measured by TEG for any circuit (data not shown). These results likely reflect the pharmacokinetics of heparin and argatroban in a closed circuit. Platelet counts were equal in circuits 1 and 2 and slightly lower in circuit 3 at onset and there was a modest decrease in the platelet count after the first hour, especially in circuits 1 and 2. Fibrinogen levels remained stable in all three circuits throughout the six-hour time period although the levels were lower in the heparin-only circuit, possibly due either to increased consumption of fibrinogen or the variability in the amount of fibrinogen in different units of FFP. D-dimers were not detected in any of the circuits at any time-point, indicating no fibrinolysis (the level of 0.22 is the lower limit of detection in our laboratory).

In the heparin circuit, there was a 5-fold increase in TAT complex (Figure 1 and Table 1) from hour 0 to hour 6, but no increase noted in the argatroban circuits ($p < 0.005$). There was a 2-fold increase in F1.2 (Figure 2 and Table 1) in the heparin circuit, but no increase in the argatroban circuit with the heparin prime ($p < 0.005$) although there was an increase in F1.2 in the argatroban circuit without heparin prime. There was no difference noted between the argatroban circuit with heparin prime and the argatroban circuit without heparin prime with respect to the TAT complex; however, the level of F1.2 was lower in the argatroban circuit primed with heparin versus the argatroban circuit without heparin.

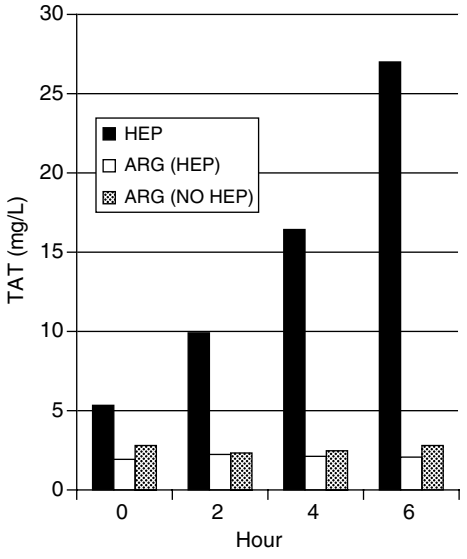

Figure 1 Changes in the TAT complex during the six-hour run of each circuit. Heparin only (HEP), Heparin in wet prime followed by argatroban [ARG (HEP)], and argatroban only (ARG).

Discussion

ECMO is a potentially life-saving procedure used in patients with severe cardiorespiratory disorders. The procedure necessitates the use of anticoagula-

Table 1 Laboratory results from the three ECMO circuits

Anticoagulant	*Hour*	*ACT (s)*	*PTT (s)*	*Fibgn (g/L)*	*Plts (10^9/L)*	*D-dimer (mg/L)*	*TAT (mcg/L)*	*F1.2 (nmol/L)*
Heparin	0	>1000	235	61	137	0.22	5.34	0.84
	2	>1000	235	54	84	0.22	9.91	1.37
	4	>1000	235	52	78	0.22	16.43	1.43
	6	>1000	235	57	82	0.22	27	1.7
Argatroban (heparin prime)	0	>1000	191	137	135	0.22	1.93	0.2
	2	>1000	198	130	95	0.22	2.24	0.2
	4	>1000	235	137	90	0.22	2.12	0.19
	6	>1000	235	157	89	0.22	2.07	0.2
Argatroban (no heparin prime)	0	>1000	144	149	96	0.22	2.8	0.6
	2	>1000	158	140	79	0.22	2.33	0.64
	4	>1000	150	129	83	0.22	2.47	0.57
	6	>1000	151	192	91	0.22	2.8	1.0

Fibgn = fibrinogen, Plts = platelets, TAT = thrombin antithrombin.
$p < 0.005$ for TAT and F1.2 between the heparin and argatroban (heparin prime) circuits.
$p < 0.005$ for TAT and F1.2 between the heparin and argatroban circuits.
No significant difference between argatroban (heparin prime) and argatroban circuits.

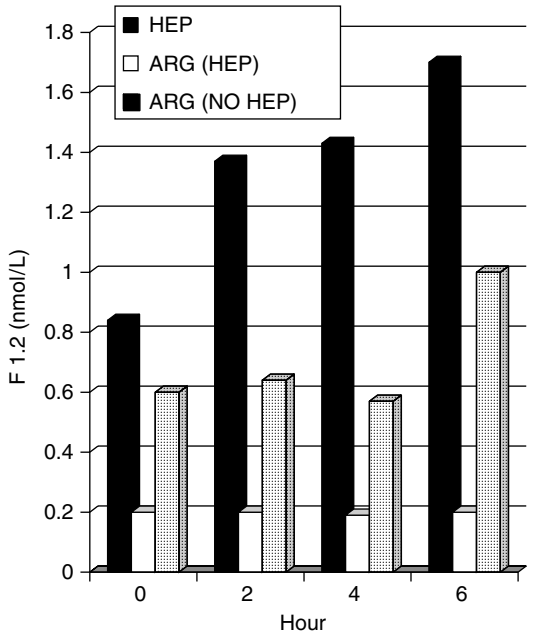

Figure 2 Changes in prothrombin fragment 1+2 during the six-hour run of each circuit. Heparin only (HEP), heparin in wet prime followed by argatroban [ARG (HEP)], and argatroban only (ARG).

tion as blood flows through an artificial extracorporeal circuit. Although the number of patients requiring ECMO has been decreasing in recent years, the length of time on ECMO per patient has increased, particularly in neonates.[22] This added time on ECMO increases the importance of adequate anticoagulation in order to prevent circuit failure and obviate the need for circuit replacement. While ECMO itself leads to consumption of platelets and coagulation factors, additional complications, such as sepsis and organ failure, can exacerbate these problems and further increase the risk for bleeding, thrombosis or both.[3–6] It is, therefore, critical that the level of anticoagulation be therapeutic.

Unfortunately, heparin, the only anticoagulant currently used in ECMO, has significant pharmacologic disadvantages in this setting. First, it binds to numerous plasma proteins (whose levels can greatly fluctuate in sick patients) and to intravenous tubing and circuitry.[23] In addition, AT levels are physiologically low in neonates, resulting in heparin resistance.[7] Perhaps of more importance is the variability in levels in neonates as well as the consumption of AT in circuit thrombi, further complicating the difficulties in achieving and maintaining therapeutic levels of heparin. If levels are too high, there is a serious risk of hemorrhage, including intracranial hemorrhage while subtherapeutic levels can lead to thrombosis, not only in the circuit, but in the patient as well. Thus, a safer alternative to heparin is warranted for patients on ECMO.

We chose to study the effects of argatroban, a novel anticoagulant, in the prevention of thrombus formation in a closed ECMO circuit. Argatroban is currently approved for use in the management of HIT in adults, has been used in Japan in heart bypass circuits, and has been used in selected patients with HIT undergoing cardiopulmonary bypass with success.[17–19] Argatroban has some theoretical advantages over heparin for ECMO patients. In adult studies, it has more stable pharmacokinetics, does not bind to plasma proteins nor rely on AT for its effect, thereby improving its pharmacologic profile and making it more likely to achieve and maintain a therapeutic level. It does not lead to HIT, and inhibits both free and clot-bound thrombin, which improves its ability to prevent clots.[15]

The most important findings of this study are the results of the tests assessing thrombin generation. When prothrombin, the zymogen for thrombin, is cleaved by the prothrombinase complex, a molecular fragment is released (F1.2) and active thrombin is generated. Once thrombin is generated, it is bound in a one-to-one complex with its natural inhibitor AT to form a TAT complex. Thus F1.2 and TAT complex serve as extremely sensitive markers of thrombin generation.[24] Interestingly, when comparing the two argatroban circuits (one with and one without a heparin prime), there was a difference between F1.2 and TAT complex. While the reduction in the TAT complex was equivalent between the two circuits, the F1.2 was lower in the circuit primed with heparin. This is likely due to the presence of small amounts of heparin in the circuit that had the heparin prime. As heparin is a potent inhibitor of the prothrombinase complex (factors V, IX and X), less prothrombin is converted to thrombin, resulting in less F1.2 generated.

In our experiments, despite the equivalent ACTs, PTs, aPTTs and TTs between circuits 1 (heparin with heparin prime) and 2 (argatroban with heparin prime), there was a significant difference in the value of these markers as time progressed. We do not believe the lower levels of fibrinogen in the heparin circuit impacted our results as the formation of thrombin generation markers occurs prior to and without the contribution of fibrinogen. These results indicate that argatroban is more effective at equivalent anticoagulant doses (as measured by clotting assays such as the ACT) in preventing thrombin generation in ECMO circuits. Decreased thrombin generation in the circuit may lead to fewer circuit changes for a given patient, reducing the risks

associated with changing the circuit as well as the cost.

Our study has several limitations. First, only one circuit was run for each arm, and though carefully controlled, we could not account for potential variability in the results had additional circuits been run. Second, these were sham circuits and, thus, the lack of a patient (or animal) attached to the circuit could affect the results by the lack of an ongoing supply of platelets and coagulation factors and the absence of metabolism of the drugs and the by-products of coagulation reactions (i.e., no ability to remove thrombin generation markers from the circulation). It is possible that the behavior of heparin and argatroban could be sufficiently different *in vivo* so as to render our results invalid. Nevertheless, we believe the results of this pilot data provide proof of the concept that argatroban is more effective than heparin at minimizing thrombin generation. However, additional research, especially in an animal model, is needed to confirm our findings before this agent can be recommended for use in patients undergoing ECMO.

In conclusion, argatroban as compared to heparin reduced thrombin generation in sham ECMO circuits suggesting that this agent may be superior to heparin as an anticoagulant in ECMO. Furthermore, it is likely that the more favorable pharmacokinetics will lead to fewer supra- or subtherapeutic ACTs and, thus, reduce the risk for bleeding and thrombotic events in the patient. In addition, there is evidence that DTIs cause less bleeding than heparin for a given ACT, which could further reduce the risk for bleeding. We believe that these results should serve as the basis for additional studies of argatroban with the goal of a clinical trial of this agent for neonates and infants requiring ECMO.

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