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Using Activated Clotting Time to Estimate Intraoperative Aprotinin Concentration

Yusuke Iwata, MD, Toru Okamura, MD, David Zurakowski, PhD, and Richard A. Jonas, MD
Children's National Heart Institute, Children's National Medical Center, Washington, DC

Abstract

Background—Use of aprotinin during cardiopulmonary bypass may be associated with renal dysfunction due to renal excretion of excess drug. We hypothesized that the difference between standard celite activated clotting time (ACT), which is prolonged by aprotinin and kaolin ACT, could provide an estimate of aprotinin blood level.

Methods—Fresh porcine blood was collected from six donor pigs and heparinized. Blood was stored at 4°C, rewarmed and aprotinin was added: 0, 100, 200, and 400 kallikrein inhibitor units/ml. Specimens were incubated at 37°C. Two pairs of ACT tubes (one celite and one kaolin) were measured at 37°C and 20°C using two HEMOCRON 401 machines. A generalized estimating equation (GEE) statistical approach was used to estimate actual aprotinin from differences in celite and kaolin ACT.

Result—There was a significant relationship of the form $y = \exp^{(a+bx)}$ between aprotinin concentration and difference between celite and kaolin ACT at both 37°C ($R^2 = 0.858$) and 20°C ($R^2 = 0.743$).

Conclusion—The time difference between celite and kaolin ACT may be a simple and inexpensive method for measuring the blood level of aprotinin during cardiopulmonary bypass. This technique may improve patient-specific dosing of aprotinin and reduce the risk of postoperative renal complications.

Keywords

Renal dysfunction; complications; CPB; Blood conservation

Introduction

Use of aprotinin remains controversial. In 2006 Mangano et al. reported that aprotinin increases the risk of renal dysfunction after coronary artery surgery [1]. Although some investigators have suggested that using aprotinin in cardiac surgery is safe and that it may even improve kidney function and decrease tubular cell apoptosis after renal ischemia [2,3], many believe that aprotinin can induce renal dysfunction in a dose dependent manner by overloading the tubular reabsorption mechanisms [4]. This situation might arise from administration of aprotinin according to the standard “full-dose Hammersmith” regimen in adults, which is not adjusted according to body surface area or pre-existing renal impairment [5]. Although investigators have tried to find the optimal dose of aprotinin for preventing overdosing, they have been hampered by lack of a simple method for measuring aprotinin concentration in the

Address for correspondence: Richard A. Jonas, MD, Chief, Cardiac Surgery, Co-Director, Children's National Heart Institute, Cohen Fungler Professor of Surgery Children's National Medical Center, 111 Michigan Avenue NW, Washington, DC 20010, rjonas@cnmc.org.

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blood intraoperatively [6]. We hypothesized that intraoperative aprotinin concentration could be estimated by the difference between standard celite activated clotting time (ACT), which is prolonged in the presence of aprotinin, and kaolin ACT which is not affected by aprotinin. The purpose of the current study was to investigate whether the difference between celite ACT and kaolin ACT would allow accurate estimation of aprotinin concentration in blood.

Methods

Fresh porcine blood was collected into four 50 ml tubes from six donor pigs and heparinized (3 IU/ml, porcine Heparin; Baxter, Deerfield, IL). Blood was stored at 4°C until measurement, rewarmed to 37°C and aprotinin was added: 0, 100, 200, and 400 KIU/ml. Specimens were incubated for 30 minutes. Two pairs of ACT tubes (one celite, one kaolin) were measured at 37°C using two alternating Hemochron 401 machines (ACT ranges 0–1500 sec.). The Hemochron 401 has a heater to maintain temperature at 37°C. Usually celite ACT is longer than kaolin ACT. This difference results mostly from the presence of aprotinin. Specimens were then cooled to 20°C for 15 minutes and ACTs were measured in same fashion. Generalized estimating equations (GEE) were used to estimate aprotinin concentration based on the difference between celite and kaolin ACTs taking multiple measurements into account and the Wald test to assess significance [7]. We compared linear and nonlinear models using R-squared and lowest value of the Schwarz Bayesian information criterion to indicate the preferred model. SPSS version 15.0 was used for statistical analysis (SPSS Inc., Chicago, IL).

Results

Kaolin ACT was not significantly affected by aprotinin concentration while celite ACT was prolonged in a dose dependent manner (Figure 1a, b). Aprotinin concentration at 37°C was estimated by a nonlinear model containing kaolin ACT ($P = .008$) and the difference between celite and kaolin ACT ($P < .001$), where $y = \exp^{(4.364 - 0.004 \text{ kaolin} + 0.013 (\text{celite-kaolin}))}$. The kaolin ACT is the standard for the level of heparinization while the difference between celite ACT and kaolin ACT results from the aprotinin effect. Similarly, aprotinin at 20°C was estimated by kaolin ACT ($P = .002$) and the difference between celite and kaolin ACT ($P < .001$), where $y = \exp^{(4.918 - 0.003 \text{ kaolin} + 0.007 (\text{celite-kaolin}))}$. These equations had good fit to the data (37°C, $R^2 = 0.858$ and 20°C, $R^2 = 0.743$) and were used to estimate intraoperative aprotinin concentration at each temperature (Table 1). Comparisons between actual aprotinin concentrations versus the estimated values based on kaolin ACT and celite – kaolin ACTs for each donor pig at both 37°C and 20°C are shown in Figure 2.

Discussion

It has been documented that plasmin is inhibited at a plasma aprotinin concentration of 125 KIU/ml and kallikrein inhibition at a higher level (200–250 KIU/ml) [8]. Thus a concentration of 200–250 KIU/ml was targeted in developing the full-dose Hammersmith regimen [5]. Thus we did not plan to study blood levels greater than 250 KIU/ml. However, Nuttall and colleagues report that the full dose regimen often results in a level of 300 KIU/ml before cardiopulmonary bypass (CPB) and over 200 KIU/ml at the end of aprotinin infusion [6]. Aprotinin has a high affinity for renal tissue and is rapidly eliminated from the blood by glomerular filtration. It is stored in the proximal tubular cells and metabolized by renal lysosomes before excretion as active protein. Therefore aprotinin should be administered as an initial bolus followed by continuous infusion to maintain a targeted blood level. The breakdown of aprotinin can result in kidney dysfunction with albuminuria secondary to overload of the tubular reabsorption mechanisms [8]. We speculate that overdosing with aprotinin results in an excessive blood level of aprotinin, although it is not proven that excessive blood levels or excessive total amount of drug induces renal impairment. Accordingly avoiding excessive blood levels may be

important to avoid renal injury, analogous to the situation with aminoglycoside antibiotics [4].

Traditionally aprotinin concentration has been measured by enzyme-linked immunosorbent assay (ELISA) [9]. O'Connor and colleagues reported a reduction of aprotinin clearance and prolongation of aprotinin half-life in patients with renal insufficiency using the commercially available chromogenic assay kit "Uni-test" (Unicorn diagnostics Ltd., London UK) [10]. But both ELISA and chromogenic assay methods require 2 or 3 hours to determine aprotinin concentration.

It is well-known that aprotinin prolongs celite ACT but not kaolin ACT. Both ACTs are affected by temperature. Dietrich demonstrated that kaolin ACT was not influenced by aprotinin since kaolin bonded nearly completely to aprotinin, however recommended using both ACTs for monitoring to ensure safety [11]. We hypothesized that the difference between celite and kaolin ACT could provide an estimate of the aprotinin concentration in blood. This monitoring method estimates aprotinin concentration in blood as demonstrated. It is suggested that this monitoring method will allow estimation of aprotinin concentration during CPB even at low temperatures and in patients with low body weight or renal impairment. This method has advantages over other assay techniques with respect to measurement time and cost. Presently, this method does not account for all the effects of CPB, and thus it is more accurate to estimate aprotinin concentration before or just after starting CPB to avoid overdosing. We also recommend measuring two pairs of ACTs for each patient and averaging to ensure a more reliable estimation. Nevertheless there is still a possibility of underestimation. We do not recommend increased administration rates of aprotinin when a low level is found regardless of the dosing schedule since this technique is designed only to avoid excessive dosing of aprotinin.

While further investigations are needed, this method may prove useful in avoiding excessive administration of aprotinin that may lead to renal injury.

Conclusion

The difference between celite and kaolin ACT may provide a simple and inexpensive method for measuring the blood level of aprotinin during CPB. This technique may improve patient-specific aprotinin dosing and therefore reduce the risk of postoperative renal complications.

Acknowledgments

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Celite ACT at 37 degrees Celsius

Celite ACT at 20 degrees Celsius

Figure 1.
Celite ACTs at both temperatures were prolonged by aprotinin dose dependently.

Figure 2a

Figure 2b

Figure 2.

Dot plot showing the relationship between actual and estimated intraoperative aprotinin concentration (KIU/ml) for each donor pig at both 37°C and 20°C. Horizontal lines represent the mean of all six animals.

Table 1

Estimated Aprotinin (KIU/ml) based on Kaolin ACT and Difference between Celite and Kaolin ACT*

Difference in ACT Times (Celite-Kaolin) at 37°C							
Kaolin ACT (sec)	0	50	100	150	200	250	300
100	51	100	195	381	744	1454	2841
200	33	65	127	248	484	945	1847
300	22	42	82	161	315	615	1201
400	14	27	54	105	204	400	781
500	9	18	35	68	133	260	507
600	6	12	23	44	86	169	330
700	4	8	15	29	56	110	214
800	3	5	10	19	37	71	139

Difference in ACT Times (Celite-Kaolin) at 20°C							
Kaolin ACT (sec)	0	50	100	150	200	250	300
100	100	139	195	272	381	533	745
200	72	101	142	198	277	388	542
300	53	74	103	144	202	282	395
400	38	54	75	105	147	205	287
500	28	39	55	76	107	150	209
600	20	28	40	56	78	109	152
700	15	21	29	40	57	79	111
800	11	15	21	29	41	58	81

* Estimated aprotinin concentration (KIU/ml) =
exp (4.364-0.004 kaolin ACT + 0.013 (celite-kaolin ACT)) at 37°C
exp (4.918-0.003 kaolin ACT + 0.007 (celite-kaolin ACT)) at 20°C