福大医紀（*Med. Bull. Fukuoka Univ.*）：30(2), 67-72, 2003

Anticoagulative Effects of a Heparin-coated Oxygenator Analyzed Based on the Prothrombin Levels Using the Carinactivase-1 Method

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Abstract: Object: Heparin-coated oxygenators and cardiopulmonary bypass (CPB) circuits are used for the prevention of thrombus formation in cardiac surgery. We review our experience in order to determine whether heparin-coated oxygenators and CPB circuits are effective.

Methods: From November 1999 to May 2001, this blood coagulation study was performed in 25 patients who underwent coronary artery bypass grafting (CABG) using CPB. The patients were randomly divided into two groups. The heparin-coated group (HC group, n=14) used Capiox SX (HP) SX-18 (Terumo, Tokyo, Japan) as the heparin-coated oxygenator. The non-heparin-coated group (NHC group, n=11) used the Affinity NT CVR (Medtronic Inc, Minnesota, USA) as the non-heparin-coated oxygenator. A centrifugal pump (BioMedicusBP-80, Baxter, International Inc., Illinois, USA) was used for all operations. We measured the prothrombin levels by using a Ca^{2+}-dependent prothrombin activator, carinactivase-1 (CA-1).

Results: The results, at the end of CPB, revealed less of a reduction in the prothrombin levels and blood platelet counts for the HC group in comparison to the NHC group. A larger reduction in these levels, correlated with a decrease in the thrombin formation.

Conclusion: The anticoagulation activity was studied in both the HC and NHC groups. Both the % blood platelet counts and the % prothrombin in the HC group were higher than those in the NHC group at the end of CPB. These results suggested that the coagulation activity was more strongly suppressed in the HC group than in the NHC group. Therefore, the heparin-coated oxygenator seemed to be the best device for CPB.

Key words: cardiopulmonary bypass, coronary artery bypass graft, heparin-coated oxygenator, prothrombin, carinactivase-1 method

Introduction

One of the side effects of CPB is thrombus formation in the oxygenator and the circuits. Heparin-coated oxygenators and CPB circuits are considered to be ideal for the prevention of thrombus formation. There are various

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evaluations concerning heparin-coated oxygenators and CPB circuits. Gu YJ et al.[1] reported that the thrombin-antithrombin Ⅲ (TAT) complex remained significantly lower in the heparin-coated CPB circuits than in the non-heparin-coated CPB circuits. Yamanaka et al.[2] reported a reduction in fibrinogen consumption that remained significantly lower in the heparin-coated CPB circuits than in the non-heparin-coated CPB circuits. However, Horikoshi et al.,[3] Masuda et al.[4] and Takahashi et al.[5] reported no statistical differences in the TAT, Fibrinopeptide A (FPA) or fibrinogen between heparin-coated CPB circuits and non-heparin-coated CPB circuits. In order to evaluate the efficiency of using heparin-coated oxygenators during CPB, we measured the prothrombin levels by using Ca^{2+}-dependent prothrombin activator, carinactivase-1 (CA-1). CA-1 was first reported by Yamada et al.[6] in 1996. CA-1 was isolated from the venom of Echis carinatus leucogaster and both recognizes the Ca^{2+}-bound conformation of the Gla-domain in prothrombin as well as activates prothrombin. As a result, this measurement is referred to as the CA-1 method.[7] Furthermore, Yamada et al.[8] reported that the measurement of the prothrombin levels using the CA-1 method is not influenced by heparin. Our paper is the first report of measuring prothrombin levels by the CA-1 method in CPB with heparin-coated oxygenators and circuits.

Subjects and methods

Patients. From November 1999 to May 2001, the 25 CABG patients were selected for this study at the Fukuoka University Hospital. Patients with the following criteria were excluded from this study: emergent and/or repeat CABG, combination procedures, anemia, chronic renal failure, hemodialysis, required intra-aortic balloon pump, acute myocardial infarction, or blood transfusions during CPB. They were randomly divided into two groups consisting those who used a heparin-coated oxygenator and those who did not. In the HC group, a Capiox SX (HP) SX-18 was used as the heparin-coated oxygenator (N=14). In the NHC group, an Affinity NT CVR was used as the non-heparin-coated oxygenator (N=11). The heparin-coated CPB circuits were used for all operations. There were no statistical differences in the preoperative patients' characteristics (age, gender, body weight, body surface area) between the two groups. We obtained informed consent from all patients.

CPB management. The CPB circuit was primed with Ringer's solution, 20% mannitol (5 ml/kg), sodium bicarbonate, low molecular Dextran (5 ml/kg), and heparin (48 mg). The total priming volume was 1,600 ml. The CPB was instituted with a nonpulsatile flow of 2.5 l/min × m^2. A centrifugal pump (BioMedicusBP-80) with tubings, connectors (Baxter International Inc., Illinois, USA) and an arterial line filter (AF-2040 GOLD, Baxter International Inc., Illinois, USA) was used for all operations. Two mg/kg of heparin were given intravenously before the initiation of CPB in both groups. Additional heparin was given during the bypass if the activated clotting time (ACT) decreased to less than 400 seconds. The body temperature was kept at 34℃ throughout the surgery.

Blood sampling. Blood samples were taken at the beginning of anesthesia, at 5 and 60 minutes after the initiation of CPB and at the end of CPB. They were analyzed immediately and/or stored after being centrifuged. The stored plasma was kept at −80℃ until it was assayed.

Laboratory tests. Blood cell counts: The blood cell counts were measured using the K-4500 device (Sysmex, Kobe, Japan). TAT: The measurement of TAT was done by SRL, Inc. (Tokyo, Japan). Fibrinogen: Fibrinogen was measured using the Coagrex-800 (International Reagents Corporation, Kobe, Japan). The fibrinogen reagent used was "thrombo check Fib" (Daiichi Pure Chemicals, Tokyo, Japan). ACT: The Hemocrone 801 (International Technidyne Corporation, New Jersey, USA) and hemocrone test tubes (FTCA510 International Technidyne Corporation, New Jersey, USA) were used. Prothrombin levels: The prothrombin levels were measured by the CA-1 method.[6][8]

Statistical analysis. The results were

expressed as the mean±standard deviation. A statistical analysis was performed using the Student's t-test and a p value of less than 0.05 was regarded as significant. The Mann-Whitney U test was performed when the F test identified a significant difference.

Results

Operative data. The operative data were as follows: The rate of hemodilution was 25.2±3.5% in the HC group and 23.8±2.6% in the NHC group. The CPB time was 99.2±44.8 min in the HC group and 102.6±22.3 min in the NHC group. The aortic clamping time was 65.9±19.6 min in the HC group and 60.8±31.5 min in the NHC group. The number of grafts was 3.1±0.9 in the HC group and 3.1±1.1 in the NHC group. There were no significant differences in the patients' operative data between the two groups.

Red blood cells. The RBC counts were 383.5±54.4×$10^4/\mu l$ in the HC group and 411.5±43.3×$10^4/\mu l$ in the NHC group at the beginning of anesthesia (NS). At the end of CPB, the values were 259.5±36.9×$10^4/\mu l$ in the HC group and 252.5±37.8×$10^4/\mu l$ in the NHC group (NS).

Hemoglobin. It was 12.1±1.8 g/dl in the HC group and 12.9±0.9 g/dl in the NHC group at the beginning of anesthesia (NS).

At the end of CPB, it was 8.1±1.1 g/dl in the HC group and 7.9±1.0 g/dl in the NHC group (NS).

Hematocrit. The hematocrit values were 35.3±4.9% in the HC group and 38.2±2.9% in the NHC group at the beginning of anesthesia (NS). At the end of CPB, it was 23.9±3.0% in the HC group and 23.3±2.8% in the NHC group (NS).

The hemoglobin, hematocrit and red blood cell counts decreased similarly in both groups from the point of initiation to the end of CPB (Table Ⅰ).

The % RBC, % Hb and % Hct in the HC group were higher than that in the NHC group at the end of CPB ($p<0.05$, Table Ⅱ).

Platelet counts. The platelet counts were 18.6±4.2×$10^4/\mu l$ in the HC group and 21.9±3.8×$10^4/\mu l$ in the NHC group at the beginning of anesthesia ($p<0.05$). At the end of CPB, it was 14.0±3.1×$10^4/\mu l$ in the HC group and 14.5±2.7×$10^4/\mu l$ in the NHC group (NS). The blood platelet counts de-

Table Ⅰ. Results of laboratory tests

	Group	Beginning of anesthesia	CPB 5 min	CPB 60 min	End of CPB
RBC ($10^4/\mu l$)	HC Group	383.5±54.4	250.9±41.7	229.7±27.4	259.5±36.9
	NHC Group	411.5±43.3	269.2±40.0	247.2±38.7	252.5±37.8
Hb (g/dl)	HC Group	12.1±1.8	7.9±1.5	7.3±0.9	8.1±1.1
	NHC Group	12.9±0.9	8.3±0.9	7.7±1.0	7.9±1.0
Hct (%)	HC Group	35.3±4.9	23.2±3.7	21.4±2.5	23.9±3.0
	NHC Group	38.2±2.9	24.9±3.0	22.9±2.9	23.3±2.8
Plt ($10^4/\mu l$)	HC Group	18.6±4.2 *1	12.3±2.9	12.3±3.1	14.0±3.1
	NHC Group	21.9±3.8 *1	14.3±2.6	13.8±2.7	14.5±2.7
TAT (ng/ml)	HC Group	4.7±6.6	88.9±62.9	111.6±84.7	224.4±133.7
	NHC Group	2.8±1.9	77.9±53.4	83.3±47.2	257.2±98.8
Fibrinogen (mg/dl)	HC Group	308.5±93.5	190.4±67.0	202.1±66.4	198.0±70.5
	NHC Group	295.4±77.9	183.7±50.5	187.2±64.5	172.7±46.0
Prothrombin (μg/ml)	HC Group	120.6±14.2	86.3±16.0	83.3±20.0	85.2±14.8
	NHC Group	128.7±6.7	85.5±7.9	78.4±16.4	76.4±13.5

*1 $p<0.05$

The data are shown as the mean standard deviation. The p value was determined by the t test. *$p<0.05$. HC group, the heparin coated oxygenator group; NHC group, the non heparin coated oxygenator group; RBC, red blood cell counts; Hb, hemoglobin; Hct, hematocrit; Plt, platelet counts; TAT, thrombin-antithrombin Ⅲ complex.

creased similarly in both groups after the initiation of CPB. The % blood platelet counts in the HC group were higher than those in the NHC group at the end of CPB ($p<0.05$, Table Ⅱ).

ACT. Changes in ACT are shown in Table Ⅲ. In both groups, the ACT values were kept exceeded longer than 400 seconds during CPB. No significant difference was observed in the ACT between the two groups.

Fibrinogen. It was 308.5±93.5 mg/dl in the HC group and 295.4±77.9 mg/dl in the NHC group at the beginning of anesthesia (NS). At the end of CPB, it was 198.0±70.5 mg/dl in the HC group and 172.7±46.0 mg/dl in the NHC group (NS). The fibrinogen and % fibrinogen decreased rapidly in both groups after the initiation of CPB. No significant difference was observed between the two groups (Table Ⅰ, Table Ⅱ).

TAT. Values were 4.7±6.6 ng/ml in the HC group and 2.8±1.9 ng/ml in the NHC group at the beginning of anesthesia (NS). At the end of CPB, it increased to 224.4±133.7 ng/ml in the HC group and to 257.2±98.8 ng/ml in the NHC group (NS).

TAT and % TAT increased similarly in both groups after the initiation of CPB. There was no significant difference between the two groups. (Table Ⅰ, Table Ⅱ).

Prothrombin. It was 120.6±14.2 μg/ml in the HC group and 128.7±6.7 μg/ml in the NHC group at the beginning of anesthesia (NS). At the end of CPB, it was 85.2±14.8 μg/ml in the HC group and 76.4±13.5 μg/ml in the NHC group (NS).

The prothrombin levels and % prothrombin decreased similarly in both groups after the initiation of CPB. The % prothrombin in the HC group was significantly higher than that in the NHC group at the end of CPB ($p<0.05$) (Table Ⅱ). Postoperative blood

Table Ⅱ. Percentages of original values at the beginning of anesthesia

	Group	CPB 5 min	CPB 60 min	End of CPB	
% RBC	HC Group	65.4±5.2	57.1±16.9	67.9±5.7	*
	NHC Group	65.3±4.9	59.9±4.0	61.2±4.5	
% Hb	HC Group	65.4±5.4	57.2±17.0	67.7±5.6	*
	NHC Group	64.7±4.4	59.8±4.4	60.9±4.4	
% Hct	HC Group	65.6±5.2	57.4±17.0	75.9±8.6	*
	NHC Group	65.0±4.9	59.8±3.9	66.2±7.7	
% Plt	HC Group	66.3±6.8	63.1±19.2	75.9±8.6	*
	NHC Group	65.6±7.5	63.0±9.0	66.2±7.7	
% TAT	HC Group	3,410.9±2,775.5	3,683.8±3,434.3	13,169.9±17,906.8	
	NHC Group	3,959.6±4,134.3	4,341.6±4,035.5	13,581.6±10,688.2	
% Fibrinogen	HC Group	61.2±6.9	59.5±22.7	64.0±9.2	
	NHC Group	61.7±9.6	64.1±18.3	59.7±12.7	
% Prothrombin	HC Group	66.4±9.9	57.4±22.5	67.7±10.5	*
	NHC roup	65.0±7.6	59.8±13.8	60.8±11.6	

The data are shown as the mean±standard deviation. The p value was determined by the t test. *$p<0.05$.

Table Ⅲ. Changes in the ACT

		induction of anesthesia	CPB 5 min	CPB 60 min	End of CPB
ACT (sec)	HC group	131.5±10.1	518.4±79.1	416.6±77.0	431.9±78.9
	NHC group	119.7±17.4	493.2±93.1	427.7±76.5	417.1±53.6

The data are shown as the mean±standard deviation. The p value was determined by the t test. No significant difference was found between the two groups. N.S., No significant differences among the groups. ACT, activated clotting time.

loss. The blood loss after 24 hours post-operatively were 483.1±136.0 ml in the HC group, and 575.4±176.3 ml in the NHC group (NS). However, the blood loss in the NHC group tended to increase in comparison to the HC group.

Discussion

Blood was placed in various nonphysiological environments such as foreign surfaces during CPB. Blood damage, hemodilution, the activation of coagulation and the activation of complements have all been reported during CPB.[9] In order to reduce such disadvantages, several investigations regarding CPB equipment have been performed until now.[10] In recent years, heparin and/or silicone coated oxygenators have been used. Technically, there are two kinds of heparin coating methods : one is the ionic-bond method and the other is the covalent-bond method. In this study, we studied the Capiox SX (HP) SX18, which utilizes the covalent bond method. We did not investigate the efficiency of the ionic-bond method oxygenator.

We selected CABG cases that fulfilled the required conditions of minimal cardiotomy suction for bleeding during the operation. In addition, no blood transfusions were performed in any of the patients.

TAT and FPA were increased after the initiation of CPB. This means that thrombin was produced during CPB under heparinization.[1] When thrombin is produced during CPB, prothrombin might be consumed. In previous reports,[1)2)11)-15)] in order to prove the formation of thrombin during CPB, both TAT and FPA were measured. However, the FPA reagent has not been available since July 1998, therefore it could not be measured in this study. The measurement of the prothrombin levels by the CA-1 method is a new examination that is not still commercially available. This is the first study to clearly and directly demonstrate the differences in the prothrombin levels of perfusate using the CA-1 method. Iwahashi et al.[7] reported the normal prothrombin levels using the CA-1 method to be 112.0±20.0 μg/ml in ischemic and valvular patients. In our experience, the prothrombin levels were measured just after sampling and 18 months later on the same samples in 50 cases. We found the results to demonstrate almost the same levels in both groups. The CA-1 method therefore considered to be a reliable examination. In evaluating the antithrombogenic mechanism of the heparin-coated oxygenator, we could not find any previous reports concerning prothrombin levels during CPB. In this study, the prothrombin levels were measured using the CA-1 method to evaluate the heparin-coated oxygenator and circuits for the first time. In this study, TAT showed no significant difference between the two groups.

The prothrombin levels at the end of CPB (HC group 85.2±14.8 μg/ml vs. NHC group 76.4±13.5 μg/ml) were lower than the levels at the beginning of anesthesia (HC group 120.6±14.2 μg/ml vs. NHC group 128.7±6.7 μg/ml). The prothrombin levels showed no significant difference between the two groups.

However, the % prothrombin in the HC group was significantly higher than that in the NHC group at the end of CPB. The heparin-coated oxygenator is considered to suppress the decreasing rate of prothrombin to a greater extent in the HC group than the non-heparin coated oxygenator in the NHC group. As a result, the formation of thrombin was less in the HC group than in the NHC group.

The % blood platelet counts was higher in the HC group at the end of CPB than that in the NHC group. Similar findings have also been reported in previous studies.[4)16)] Using a heparin-coated oxygenator is thus considered to help prevent a decrease in the platelet counts.

Both the short CPB time and open circuits were thus considered to be the main reasons why significant changes occurred in the TAT and fibrinogen levels in both groups. If cardiotomy suction was used, then various coagulation factors could have been activated during this process.

Conclusion

The anticoagulation activity was studied in

both the HC and NHC groups. Both the % blood platelet counts and the % prothrombin in the HC group were higher than that in the NHC group at the end of CPB. These results suggest that the heparin-coated oxygenator suppressed the coagulation activity normally which cannot be suppressed by heparin during CPB. The above-described HC group demonstrated an excellent anticoagulation activity and it is therefore considered to be more useful than the non heparin-coated oxygenator.

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(Received on January 15, 2003,
Accepted on March, 5, 2003)