
A New Method for the Conservation of Platelet Concentration During Extracorporeal Circulation

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Abstract

Pluronic F-68^a, a non-ionic polyol, was investigated for its effect on platelet conservation, platelet function, release reaction, and red blood cell hemolysis during cardiopulmonary bypass (CPB). Three groups of ten dogs each underwent CPB utilizing both normothermia and hypothermia. The control group (bubble oxygenator) did not receive any Pluronic F-68. Experimental group I (bubble oxygenator) and group II (membrane oxygenator) received 2 mg of Pluronic F-68 per milliliter of priming fluid.

After 10 minutes of normothermic CPB, the platelet concentration decreased significantly by 48%. The platelet concentration in group I significantly decreased by 38%, and in group II the decrease was a non-significant 8%. There was also an associated increase in the plasma concentration thromboxane B₂ and platelet factor 4 in both the control group and group I. A significant increase in free plasma hemoglobin was observed in the control group. The increase in group I and II was not significant.

During hypothermia (20°C), a significant change in platelets, thromboxane B₂, and platelet factor 4 was observed in all groups. After re-establishing normothermia, platelet concentration remained

significantly low at 48% in the control group, whereas the platelet concentration in group I increased to 80%, a decrease of 20%, and in group II increased to 92%, a decrease of 8%. Free plasma hemoglobin, platelet factor 4 and thromboxane B₂ remained significantly elevated in the control and group I. There was no significant elevation in platelet factor 4, free plasma hemoglobin, and thromboxane B₂ in group III.

Pluronic F-68, an emulsifying agent, has therefore been shown to be effective in platelet preservation during CPB.

Introduction

Pluronic F-68, a non-ionic polyol, has been used experimentally for the prevention of fat embolism^{1,2} and for the reduction of hemolysis^{4,5,6} during CPB. The beneficial effects of Pluronic F-68 have been attributed to its remarkable surface-active properties, which are thought to stabilize the lipoproteins in circulating plasma and red cell membranes. This study was conducted to demonstrate the experimental benefits of Pluronic F-68 on preserving platelet concentration, function, and release reaction in a series of experimental dogs during cardiopulmonary bypass (CPB).

Materials and Methods

Anesthesia was induced with nitrous oxide and methoxyflurane, or with fentanyl. The dogs were intubated and ventilation was carried out using a Harvard respirator. The arterial and venous pres-

^a Pluronic F-68, Wyandott Chemicals, Wyandott, MI 48192

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tures were recorded using conventional strain gauge transducers and a multi-channel strip chart recorder. The esophageal and rectal temperatures, as well as electrocardiogram were also monitored.

The heart was approached through a median sternotomy. Following systemic heparinization of 3 mg/kg^b, cannulae were placed in the superior and inferior vena cavae through the right atrium, for venous return to the extracorporeal circuit (ECC). A cannula for arterial infusion from the ECC was placed in the aortic root.

The venous return to the ECC was achieved by gravity syphon to a conventional bubble oxygenator^c, (control group and group I) or to a membrane oxygenator^d (group II). The priming fluid of the ECC was composed of one liter of Normosal[®] and 500 cc of dog blood. Groups I and II received 2 mg Pluronic F-68 per cc of prime, while the control group received no Pluronic F-68. Perfusion was carried out using a normothermic high flow technique, a constant 2.4 cardiac index in liters per minute per meter square.

Platelet concentration was obtained with a Coulter ZF Cell Counter.^e The platelet specific protein, low affinity platelet factor 4 (LA-PF₄), was measured in plasma by a radial immunodiffusion assay with a mono-specific antibody against purified LA-PF₄, as described by Niewiarowski⁶. Samples for thromboxane level were drawn into ethylenediaminetetraacetic acid (EDTA) 5 mM and immediately processed. Prior to the preparation of platelet poor plasma (PPP) from platelet rich plasma (PRP), indomethacin (10 µM) was added to prevent artificial generation of thromboxane. Thromboxane B₂ was measured in plasma by radioimmunoassay specific for thromboxane B₂, as described by Lewy⁷. Free plasma hemoglobin was measured by standard colorimetric method.

Platelet concentration and platelet release reaction, an indication of platelet function, and plasma free hemoglobin were examined in experimental dogs during partial bypass (one hour), total bypass (one hour), hypothermia (one hour) and the re-warming phase (30 minutes) during three and one-half hour CPB. (Partial bypass involved ECC

without an oxygenator.) One mg of Pluronic F-68/cc of prime was administered at the beginning of CPB and an additional mg of Pluronic F-68/cc of prime added with the introduction of the oxygenator to the ECC.

Results

Platelet Concentration Two minutes after the initiation of total CPB, the platelet concentration in the control group decreased by 57% ± 6 (Table 1). A further 12% decrease was observed when hypothermia was introduced. At the end of re-warming, the platelet concentration increased by 17% from the depressed values observed during hypothermia. The total decrease in the control group after 3.5 hrs of CPB was 52% ± 7. The Pluronic F-68 with a bubble oxygenator (group I) (Table 2), at 2 minutes after bypass showed only a 27% ± 1 to 30% ± 2 decrease in platelet concentration. In group II (Table 3) with Pluronic F-68 and a membrane oxygenator, the platelet concentration decreased by an average of only 10% ± 6. During hypothermia, there was a significant decrease in platelet concentration in all groups. Group I showed an average decrease of 55% at the end of the hypothermic phase as compared to group II which showed an average drop of 20%. There was a marked trend toward the recovery of both platelet concentration and function in both groups I and II, with a net total loss of 20% and 8%, respectively. These data are summarized in Figure 1.

Platelet Release, LA-PF₄ and Thromboxane B₂ Figures 2 and 3 summarize the events of LA-PF₄ and thromboxane levels in CPB. A significant in-

TABLE 1

	BASELINE	P			
		100%	T	H	R
Minutes	2	80%	43%	30% 35°C	35% 25°C
	4	82%	45%	31% 32°C	41% 28°C
	8	81%	42%	30% 28°C	44% 32°C
	16	83%	43%	33% 25°C	43% 35°C
	32	81%	41%	32% 23°C	48% 37°C
	60	79%	42%	31% 20°C	
	Mean	81%	42%	31%	42%
	S.D.	1.4	1.36	1.1	4.5
	S.E.	0.5	0.5	0.43	2.0

Control platelet count calculated as percent of the dogs platelet pre-bypass: P = partial bypass, T = total bypass, H = hypothermia, R = re-warming.

^b The Upjohn Company, Kalamazoo, MI 49001

^c BOS-5, Bentley Laboratories, Inc., Irvine, CA 92714

^d TMO Pediatrics, Travenol Laboratories, Deerfield, IL 60015

^e Coulter Electronics, Hialeah, FL 33010

TABLE 2

	BASELINE	P		T	H	R
		100%				
Minutes	2	95%	73%	68%	35°C	43% 25°C
	4	92%	75%	63%	32°C	51% 28°C
	8	95%	72%	65%	28°C	52% 32°C
	16	93%	71%	58%	25°C	68% 35°C
	32	91%	73%	51%	23°C	80% 37°C
	60	91%	70%	45%	20°C	
	Mean	91.8%	72.3%	58%		58%
	S.D.	1.7	1.6	8		13.34
	S.E.	0.6	0.65	3		6

Group I with 2 mg Pluronic F-68/cc of prime, platelet count calculated as percent of the dog's platelet pre-bypass in bubble oxygenator. P = partial bypass, T = total bypass, H = hypothermia, R = rewarming.

crease was observed in the control group and group I. A further increase was observed in all groups during hypothermia, with the least increase in group II. During the rewarming phase there was a decrease in all groups, but platelets LA-PF₄ and thromboxane B₂ levels remained significantly elevated in the control and group I. However, in group II which utilized a membrane oxygenator and Pluronic F-68, the plasma levels of LA-PF₄ and thromboxane returned to very near baseline.

Plasma Free Hemoglobin A significant increase in plasma hemoglobin level was first observed (Fig. 4) after 16 minutes on total bypass in the control group. The hemoglobin levels climbed from 20 mg/100 cc of blood to 80 mg/100 cc of blood during CPB initiation and continued to increase throughout the bypass, finally reaching a level of 180 mg/100 cc of blood. The plasma hemoglobin levels in group I were significantly elevated during

TABLE 3

	BASELINE	P		T	H	R
		100%				
Minutes	2	98%	92%	89%	35°C	85% 35°C
	4	97%	91%	86%	32°C	86% 28°C
	8	93%	93%	88%	28°C	87% 32°C
	16	94%	90%	86%	25°C	93% 35°C
	32	92%	92%	79%	23°C	92% 37°C
	60	91%	90%	80%	20°C	
	Mean	94.1%	91%	84.6%		88.6%
	S.D.	2.5	1.1	3.8		3.3
	S.E.	1.0	0.45	1.6		1.5

Group II with 2 mg Pluronic F-68/cc of prime platelet count calculated as a percent of the dog's platelet pre-bypass in a membrane oxygenator. P = partial bypass, T = total bypass, H = hypothermia, R = rewarming.

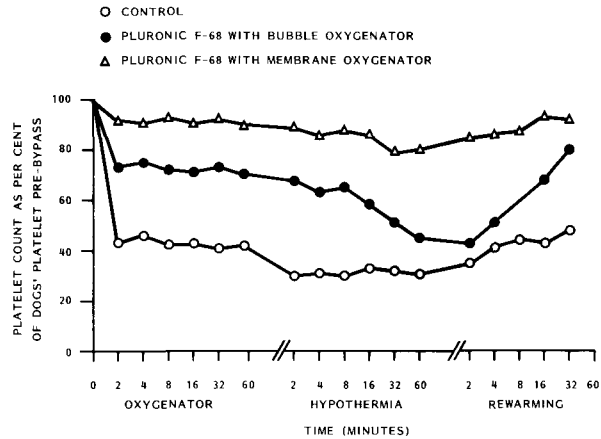


FIGURE 1. The ordinate is platelet count as a percent of dogs platelet pre-bypass. The abscissa is Time in minutes that describes three events: Total bypass, Hypothermia, and Rewarming utilizing membrane or bubble oxygenators. Mean values and S.D. of 10 experiments in each group. ($p = 0.03$).

hypothermia to 65 mg/100 cc of blood and remained at that level until the termination of CPB. There was no significant elevation of free plasma hemoglobin during the entire CPB in group II.

Discussion

Multiple complex changes occur in the hemostatic mechanism during CPB. The literature is re-

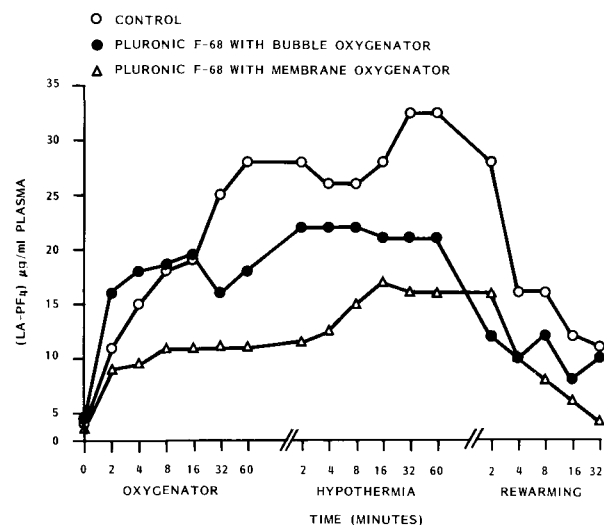


FIGURE 2. The ordinate is low affinity platelet factor 4, (LA-PF₄) in g/ml plasma. The abscissa is Time in minutes that describes three events: Total bypass, Hypothermia, and re-warming utilizing membrane or bubble oxygenators. Mean value and S.D. of 10 experiments in each group. ($p = 0.05$ or less).

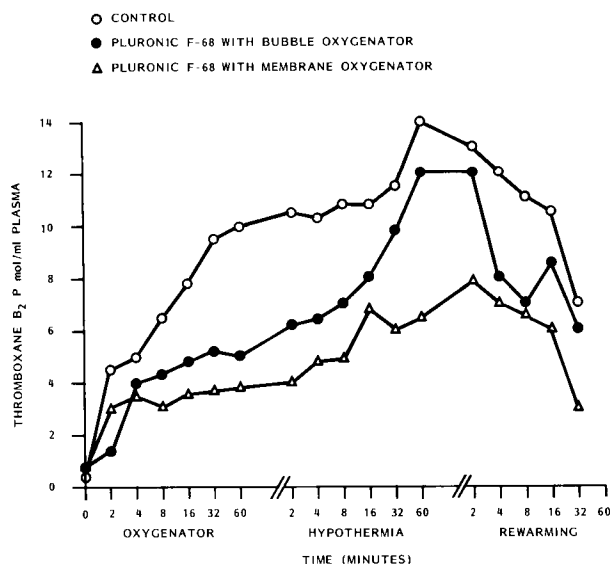


FIGURE 3. The ordinate is Thromboxane B₂ Pmol/ml of plasma. The abscissa is Time in minutes that describes three events: Total bypass, Hypothermia, and Rewarming, utilizing membrane and bubble oxygenators. Mean values and S.D. of 10 experiments in each group. (p = 0.03 or less).

platelet with the pathogenesis of abnormal bleeding. Abnormal bleeding was due, in part, to defective platelet plug formation after CPB⁹. The degree of impairment in platelet function is directly proportional to the duration of bubble oxygenator by-

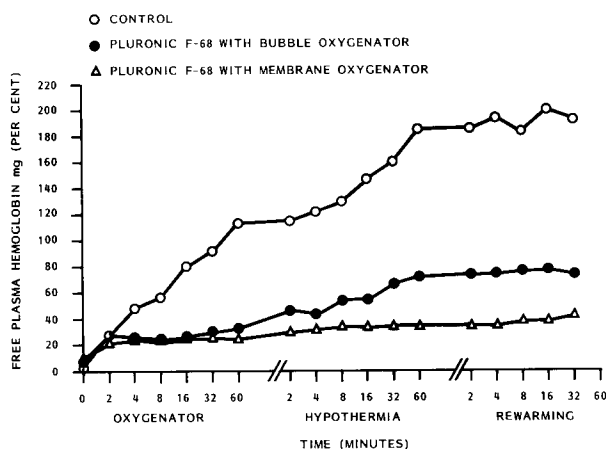


FIGURE 4. The ordinate is free plasma hemoglobin as an index of hemolysis in mg/100 ml of blood or mg%. The abscissa is Time in minutes that describes three events: Total bypass, Hypothermia, and Rewarming utilizing membrane and bubble oxygenators. Mean values and S.D. of 10 experiments in each group (p = 0.05 or less).

pass and probably related to the level of hypothermia. This study does not support the common supposition that abnormal bleeding following CPB generally is due to: (1) reduction in the levels and function of coagulation factors; (2) heparinization with the potential for subsequent inadequate neutralization or protamine excess; (3) qualitative defects in the polymerization of fibrin, and (4) increased fibrinolytic activity and the secondary effects of fibrin-fibrinogen degradation products on coagulation. It has been well established that extensive contact between blood and synthetic surfaces results in pronounced quantitative and qualitative alterations in platelet function. The detection of thromboxane B₂ formation in the CPB indicates prior release⁵ of thromboxane A₂ which is a potent vasoconstrictor and platelet activator. Release of thromboxane A₂ may also contribute to platelet aggregate formation during CPB.

In our study we have demonstrated a method to preserve platelets and inhibit their release reaction by utilizing a detergent, Pluronic F-68, and thereby prevent abnormal hemostasis following CPB. Evidence from our experiments indeed shows a marked preservation of platelet concentration and a significant reduction in the elevation of platelet factor 4 and thromboxane plasma levels. The preservation effect of Pluronic F-68 was even more pronounced when a membrane oxygenator was utilized rather than a bubbler.

Therefore, we feel that the clinical use of Pluronic F-68 will provide a method for preventing the abnormal hemostasis observed following CPB.

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