Ancrod vs. Heparin: The Resulting Effect on Oxygenator Performance During Routine Cardiopulmonary Bypass

AM Benak, V Zulys, B Calvieri, TL Zombolas, MJ Henderson, M Hurdle (by invitation), MFX Glynn

Department of Perfusion and Department of Anaesthesiology
The Toronto Hospital Corporation\Toronto General Hospital Division
Toronto, Ontario, Canada

Key words: Ancrod, anticoagulation, cardiotomy reservoir

ABSTRACT

Ancrod, a thrombin-like enzyme with anticoagulant properties, is extracted from the venom of the Malayan Pit Viper (Agkistron rhodostoma). Recently, Ancrod has been proposed as an alternate anticoagulant for cardiopulmonary bypass (CPB). In order to investigate the effect of Ancrod on the performance of the semi-porous hollow fiber membrane oxygenator now in use during CPB, in-line pressure measurements (pre-and post-oxygenator) and blood gas analysts were carried out in a clinical setting. A control group of 20 patients scheduled for coronary artery bypass grafts were coagulated with heparin and a study group of 20 patients underwent controlled defibrinogenation with Ancrod. There were no significant differences between groups with respect to blood gas values (pO^2, PCO_2) or pressure gradients across the membrane oxygenator and in all cases the intra-operative course was uneventful. Comparison of electron micrographs from various surfaces of the CPB circuit demonstrated less cellular and proteinaceous material were deposited on the study group's circuits than those of the patients anticoagulated with heparin.

This study confirms the efficacy of Ancrod as an alternate anticoagulant for CPB.

INTRODUCTION

Since the advent of cardiopulmonary bypass (CPB), heparin has been used to prevent blood from clotting in the extra-corporeal circuit. In the CPB circuit the potential sites of blood trauma and clot formation are the non-endothelial surfaces of the circuit, the air/blood interfaces and the turbulent flow areas. At the termination of bypass, protamine sulphate is used to neutralize the anticoagulant effect of heparin.

Ancrod (Arvin), an enzyme derived from the venom of the Malayan Pit Viper (Agkistron rhodostoma), prevents blood coagulation by selectively depleting the plasma of fibrinogen for baseline hemostasis and yet too little for thrombosis.

In 1787, Fontana noted that blood stayed fluid in animals killed by vipers. Reid reported[1] how Mellanby, in 1909, showed that viper venom defibrination resulted from in-vivo precipitation of fibrinogen followed by removal of this pseudo-fibrin from the circulation. In 1963, Reid, et.al.[2] made the important observation of the incoagulable blood in humans bitten by the Malayan Pit Viper. Laboratory studies of the blood of these individuals revealed marked hypofibrinogenemia. Despite near absence of fibrinogen, these individuals did not bleed spontaneously or hemorrhage after trauma.

Ancrod has undergone extensive clinical testing in the last decade. Controlled defibrinogenation is used successfully in the treatment of peripheral arterial disease,[3,4,5] in the management of deep vein thrombosis[6] and central retinal vein thrombosis.[7] Ancrod has also been used to prevent thrombosis during hemodialysis. Lowe et.al. studied the use of Ancrod for prevention of deep vein thrombosis in patients who had required internal fixation of femur fractures.[9]

Ancrod has been used as an anticoagulant for long term perfusion with a membrane oxygenator (ECMO) in laboratory settings.[10,11] A study comparing Ancrod to heparin anticoagulation for partial perfusion was conducted in 1977 by Fleming.[12] In a laboratory setting, Fleming had four separate study groups established combining a bubble or membrane oxygenator with either heparin or Ancrod. He had concluded that his study was insufficiently successful to consider the use of Ancrod clinically without a much more intensive study in the handling of this substance for perfusion.

Recently, Ancrod has been used in isolated cases of patients requiring cardiac surgery but having a contra-indication to the use of heparin or protamine. No deleterious effects were noted. All of these patients survived and their recovery was indistinguishable from patients treated with heparin. It is not known which type of oxygenator was used in these cases and the effect of Ancrod had on the performance of the oxygenator.

Address correspondence to: AM Benak, Department of Perfusion, Toronto General Hospital Corp., Toronto General Hospital Division, 200 Elizabeth Street, Toronto, Ontario, Canada

MATERIALS AND METHODS

As part of a larger study documenting the feasibility and efficacy of Ancrod anticoagulation for CPB, 20 patients scheduled for elective aorto-coronary bypass (ACB) surgery, under the age of seventy (70) years, NYHA classification I-III were asked to participate in the study. The protocol was approved by the hospital Research and Ethics Committee. The patients were compared to 20 matched heparin anticoagulated control patients. Patients with primary disorders of hemostasis, as revealed by history or hemostatic screen, were excluded, as were patients on heparin or coumadin. The hemostatic screen included a prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), bleeding time (BT), platelet count and fibrinogen level. Patients for repeat cardiac surgery were also excluded. Fibrinogen levels were determined using a Sherwood[a] fibrinogen analyzer, which measures thrombin-clottable proteins.

Ancrod[b] was infused intravenously prior to the scheduled surgery. The rate of infusion was titrated according to the fibrinogen concentration. The established protocol aimed for a concentration of 0.2-0.6 grams per liter at the time of surgery.

The CPB circuit consisted of polyvinyl chloride (PVC) tubing, a collapsible venous reservoir, a positive displacement roller pump, and a hollow fiber membrane oxygenator with integral heat exchanger.[c] The occlusion of the roller pump was calibrated prior to every case. The standard prime was 2000 milliliters (ml) Ringer's Lactate solution, 100 ml 25% albumin, 100 ml 25 gm mannitol, 100 ml 7.5% sodium bicarbonate solution, 3000 units heparin and 50 mg persantine. The cardiotomy suction return systern consisted of a filtered polycarbonate hard- shell reservoir.[d] No arterial line filters were used in the bypass circuit. Interoperatively the pressure drop across the membrane oxygenator was measured continuously by means of a Cobe-Stockert Pressure Controller[e] and a Uniflow (TM) Disposable Pressure Transducer.[f] Patients were cooled to 25 or 28°C. Control patients received 300 units of heparin per kilogram body weight and additional heparin as necessary to maintain an activated clotting (ACT) time of greater than four hundred (400) seconds.

Post-operatively, the oxygenator and cardiotomy reservoir were sectioned and examined visually both grossly and by means of scanning electron microscopy. Cross-sectional samples were first fixed in universal fixative (pH 7.2 at 20°C) and were rinsed in 0.1M phosphate buffer, followed by post-fixation with 1% osmium tetroxide at 20°C and pH 7.2 buffered with 0.1M phosphate. Samples were then rinsed again with the phosphate buffer and then taken through a gradient alcohol series followed by critical point drying. The samples were mounted, gold coated and examined with a JEOL 35 CF scanning electron microscope of 10 to 15 KV.

a. Sherwood, Model # 0893, St. Louis, MO
b. Arvin, Knoll
c. Capiox II 5.4, Terumo Corp., Tokyo, Japan
d. Model CARDF 3L, Shiley Laboratories, Irvine, CA
e. Cobe Laboratories Inc., Lakewood, CO
f. American Hospital Supply Corp., Irvine, CA

RESULTS

The fibrinogen concentrations dropped to their lowest levels, in both the ancrod and control groups, during hypothermia on CPB but had returned close to their starting levels once the patients had been weaned from bypass.

There were no differences in perfusion techniques as indicated in **Table 1**. The resulting arterial blood gas data indicates no alteration in the efficiency of the microporous hollow-fiber oxygenator (**Table 2**). Pressure drop across the oxygenator stayed consistent in both groups during the initial stages of bypass, that is, the time CPB was initiated until a state of moderate hypothermia was achieved. An elevated pressure drop across the membrane oxygenator in the control group was noted during the rewarming stage of CPB (**Figure 1**).

On gross inspection there was less material accumulation on the various surfaces of the study group's circuitry. On higher magnification, it was again evident that there was much less proteinaceous and cellular material accumulated on the different surfaces examined. The composition of the deposits was not determined.

The cardiotomy reservoir used in all these cases contained four layers of filtering and defoaming screens, most made of different materials (**Figure 2**). The innermost layer is a twenty (20) pores per inch (PPI) polyurethane defoamer. The heparin exposed surface is denoted by " H " in the upper left hand corner. The Ancrod-exposed surface is denoted by " A " in the upper left hand corner. Note the buildup of cellular matter along the grove of the polyurethane sponge in "H" and the cleaner surface in "A" (**Figure 3**). The next surface that blood comes in contact with is the polypropylene depth media. Electron microscopy again reveals what appears to be solid buildup of proteinaceous matter in panel "H", with only finer cellular matter and strands of fibrin formed in panel " A "(**Figure 4**). The third layer the blood comes in contact with is another polyurethane defoamer similar to the innermost layer except that this layer is ten (10) PPI. Once again the electron micrographs were similar to the ones in **Figure 3**. Perhaps one of the most revealing micrographs showed the forty three (43) micron polyester screen outer bag. The surface is shown in panel "A" has less protein and cellular buildup than the surface shown in panel "H " (**Figure 5**).

The first component the blood comes in contact with in the oxygenator is the integral heat exchanger. At first glance, the micrograph "H" has an appearance of a dried up lake or river. The surface is covered with fine cracks, while micrograph "A" appears relatively smooth in comparison (**Figure 6**). Closer magnification does indeed reveal thick cracks in the protein layers with cellular matter settled in some particulate matter. The hollow fiber itself is made of polypropylyne, with a pore diameter of 700 angstroms and the porosity accounted for fifty (50) percent. Internal diameter is 200 microns and wall thickness is 25 microns. Electron micrographs show a smooth but flaking surface in panel "H" and clean hollow fiber of the oxygenator except for strands of fibrin (**Figure 8**). Once again magnification shows that the surface in panel "H " had layers of protein and particulate matter built up over the duration of CPB, whereas the hollow fiber shown in "A" is free of such buildup with only

TABLE I PERFUSION DATA

			Control	Study
BYPASS TIME	range		42 - 168	69 - 133
(minutes)	mean		98.6	88.3
BLOOD FLOWRATE	range	(w)	3.6 - 5.2	4.84 - 5.1
(LPM)		(c)	4.8 - 5.1	4.5 - 4.9
	mean	(w)	5.03	4.92
		(c)	4.71	4.77
GAS FLOWRATE	range	(w)	4.0 - 5.0	4.5 - 6.0
(lpm)		(c)	0.2 - 0.8	0.15 - 0.3
	mean	(w)	4.67	4.8
		(c)	0.31	0.23
GAS-TO-BLOOD	range	(w)	0.96 - 1.38 : 1	0.84 - 1.1 : 1
FLOW RATIO		(c)	0.04 - 0.15 : 1	0.03 - 0.06 : 1
	mean	(w)	0.93 : 1	0.98 : 1
		(c)	0.065 : 1	0.047 : 1
ACTIVATED CLOTTING	range		445 - 831	> 1000
TIME	mean		563	> 1000
(seconds)				

(w) - normothermia (c) - moderate hypothermia

Table II Arterial Blood Gas Results

		Control	Study
ARTERIAL pH	range	7.29 - 7.56	7.31 - 7.46
	mean	7.39	7.39
ARTERIAL pCO2	range	23 - 52	25 - 51
(mmHg)	mean	37.9	36.4
ARTERIAL pO2	range	74 - 304	145 - 359
	mean	196.6	255.5
HEMATOCRIT	range	18 - 28	19 - 27
	mean	23.2	23.8

FIGURE 1 - TRENDS IN PRESSURE DROP ACROSS THE OXYGENATOR DURING THE COOLING AND WARMING STAGES OF CARDIOPULMONARY BYPASS

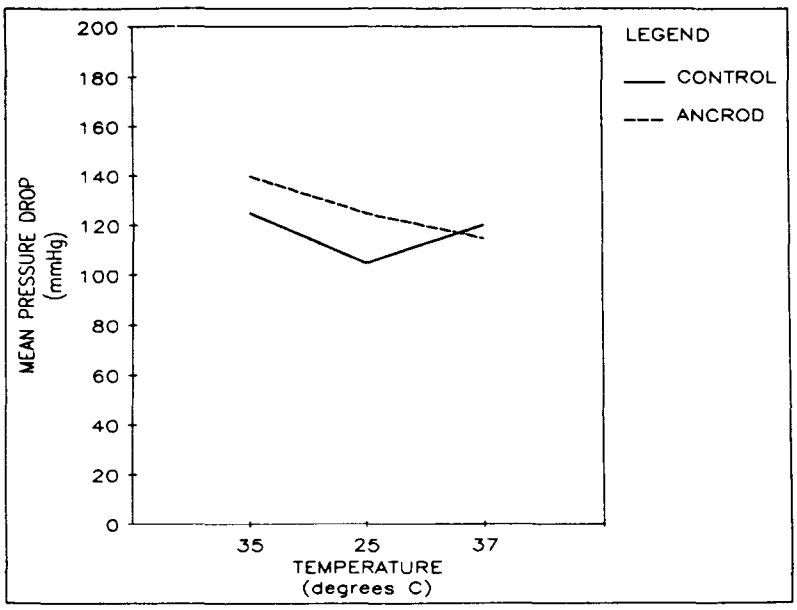


FIGURE 2 - CROSS SECTION OF CARDIOTOMY RESERVOIR

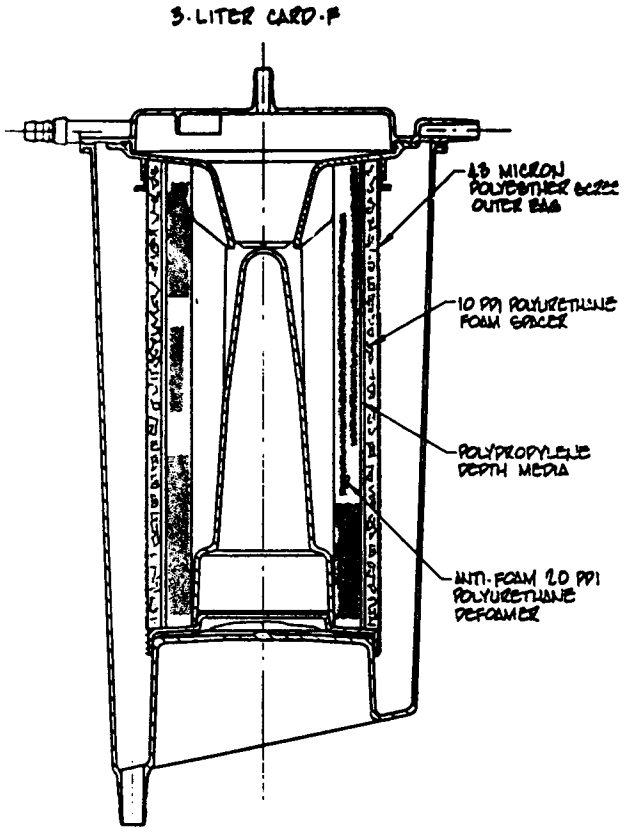
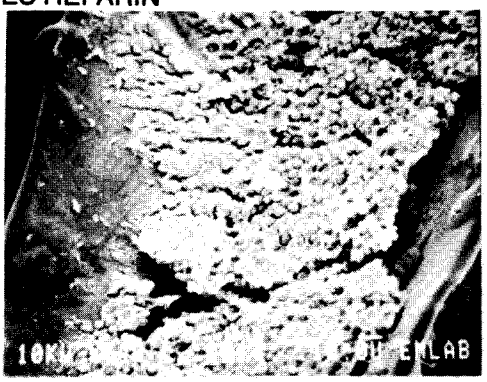


FIGURE 3 - POLYURETHANE DEFOAMER (20 PORES PER INCH) MAGNIFIED 540X. "A" DENOTES ANCROD AND "H" DENOTES HEPARIN



A

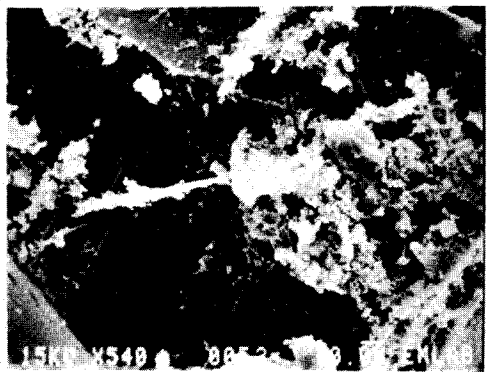


FIGURE 4 - POLYPROPYLENE DEPTH MEDIA MAGNIFIED 1200X. "A" DENOTES ANCROD, "H" DENOTES HEPARIN

H

A

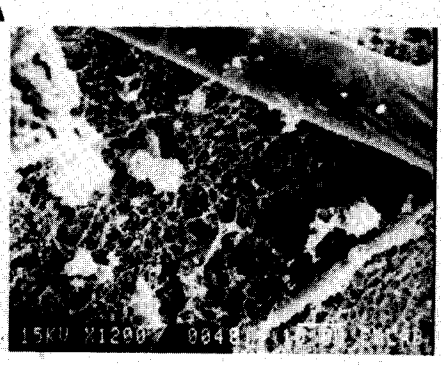
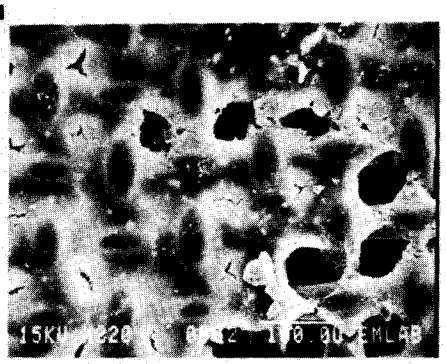


FIGURE 5 - 43 MICRON POLYESTER SCREEN OUTER SAC MAGNIFIED 200X. "A" DENOTES ANCROD, "H" DENOTES HEPARIN

H



A

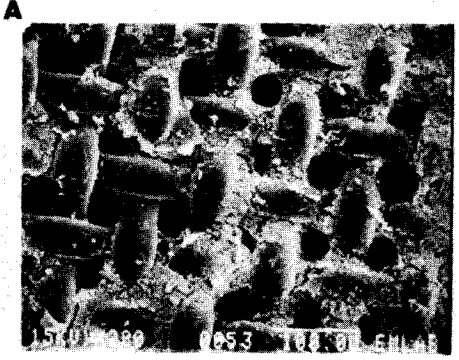


FIGURE 6 - HEAT EXCHANGER TUBE (INNER SURFACE) MAGNIFIED 44X. "A" DENOTES ANCROD, "H" DENOTES HEPARIN

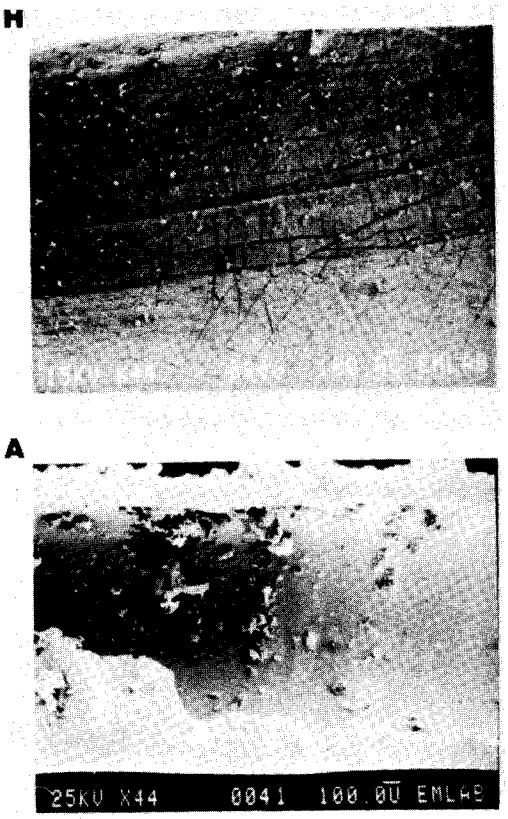


FIGURE 7 - HEAT EXCHANGER TUBE (INNER SURFACE) MAGNIFIED 1500X. "A" DENOTES ANCROD, "H" DENOTES HEPARIN.

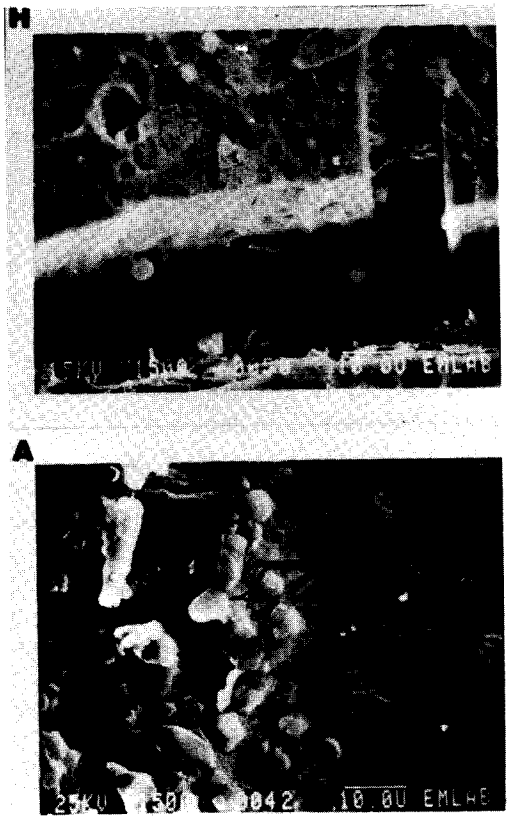


FIGURE 8 - A SINGLE POLYPROPYLENE HOLLOW FILTER (INNER SURFACE) MAGNIFIED 540X. "A" DENOTES ANCROD, "H" DENOTES HEPARIN.

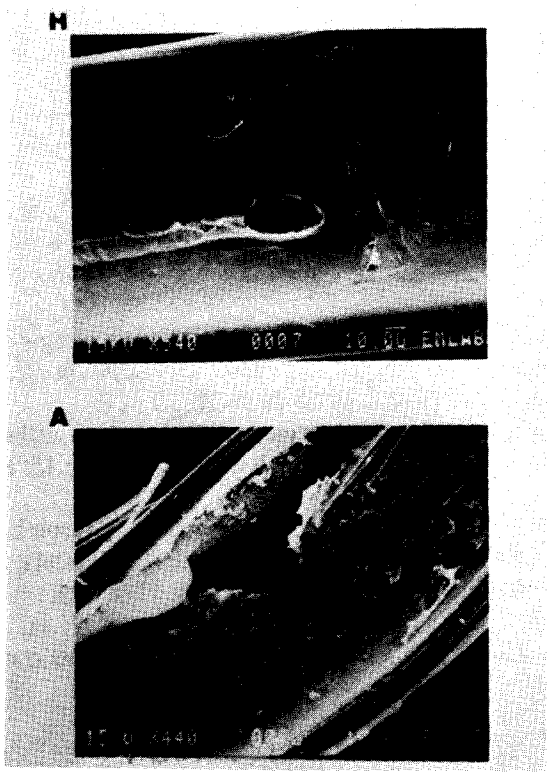
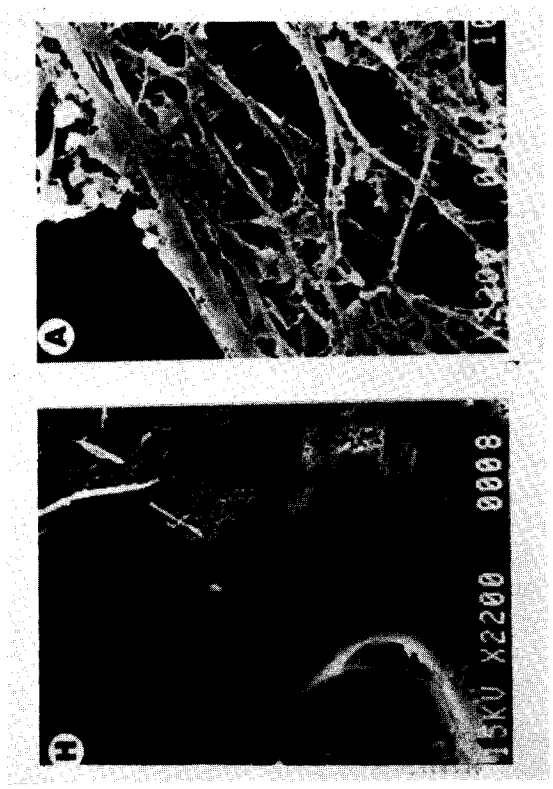


FIGURE 9 - A SINGLE POLYPROPYLENE HOLLOW FILTER (INNER SURFACE) MAGNIFIED 2200X. "A" DENOTES ANCROD, "H" DENOTES HEPARIN.



fibrin strands adhering to the surface material (**Figure 9**).

DISCUSSION

Selective enzyme-substrate specificity is a desirable property for a drug as a theraputic agent. Several specific enzymes have been isolated from a variety of snake venoms.[13]

Ancrod is a thrombin-like enzyme, which separates fibrinopeptide A but not fibrinopeptide B from Fibrinogen (factor I). Its action is not influenced by plasma antithrombin III or heparin. Since Ancrod does not activate factor XIII, the fibrin stabilizing factor which is responsible for the cross-link strands, the development of insoluble clots and thrombins, inhibited by selectively depleting fibrinogen from the plasma.[3] This selective depletion results in end-to-end strand links which are readily dissolved and harvested in the liver.

Heparin, on the other hand, needs the heparin cofactor Antithrombin III for effective anticoagulation. Antithrombin III is a naturally occurring inhibitor of thrombin.

Ancrod causes a progressive reduction in blood vicosity parallel to the fall in fibrinogen concentration. At low fibrinogen levels blood flow becomes Newtonian, that is, the shear force at the vessel wall is not proportional to the velocity gradient. Ordinary blood flow in small vessels is non-Newtonian, that is, much greater shear forces are created as the velocity gradient increases. Intuitively this may help offset the increase in viscosity induced by varying degrees of hypothermia.

Platelet function and deposition are of vital interest during CPB. Intact platelet function is necessary to avoid capillary bleeding, while an increased tendency to aggregation and/or adhesiveness may cause platelet accumulation in the lung and the many surfaces of the CPB circuit. This tendency is more pronounced in heparinized than in defibrinogenated dogs, while both platelet function and levels were better preserved in Ancrod- treated dogs.[14]

Not only did Berglin, et.al., show that platelets were better preserved in Ancrod-treated dogs, he also did scanning electron microscopy of the membrane oxygenators used in his studies. He found that the oxygenator surfaces from heparinized dogs were covered with masses of platelets, leukocytes, erythrocytes and varying amounts of filamentous and amorphous materials. He noted also that the site of thickest and most prominent deposits occurred at the junction where the fibers from the nylon mesh supporting the silicone membrane penetrated the covering surface layer. Conversly, Berglin found the deposits on the membranes used in the defibrinogented group to be smaller and less numerous. Large areas of these surfaces lacked platelets. Although we did not determine the nature and composition of deposits observed in our electron micrographs, our finding more proteinaceous and cellular material deposited on the surface of the heparinized circuit is consistant with Berglin's.

Contra-indications in the use of Ancrod are those that apply to the use of conventional anticoagulants, namely active hemorrage or recent intracerebral hemorrage or infarction. It is not recommended for use during pregnancy since it does cross the placenta and has been associated with an increase in spontaneous abortion, placental separation and teratogenic effects in laboratory animals.[15]

There is increasing interest in the use of ventricular assist devices (LVAD, RVAD, BiVAD) in patients with poor ventricular function post CPB. These patients need to have their anticoagulation state reversed in order to reduce the blood loss, however, some state of anticoagulation must be obtained so that clot does not form on the circuitry. Controlled defibrillogenation with Ancrod may offer a significant advantage to this particular application.

CONCLUSIONS

In summary, this study has shown that controlled defibrinogenation is a viable method of anticoagulation for CPB which does not adversely effect oxygenator performance. Perfusion technique regarding blood gas management need not change from routine methods. As new materials are incorporated into the CPB circuit, such as heparin-bonded surfaces, the use of Ancrod requires special attention and understanding

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