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Heparin-coated devices and high-dose aprotinin optimally inhibit contact system activation in an in vitro cardiopulmonary bypass model

Hans Peter Wendel *, Wolfgang Heller, Michael J. Gallimore

Department of Thoracic and Cardiovascular Surgery, Clinical Research Laboratory University of Tuebingen Calwerstr. 7, 72076 Tübingen, Germany

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1. Introduction

Over the last three decades cardiopulmonary bypass (CPB) surgery has become highly perfected and bypass operations are now considered to be routine operations with a low incidence of mortality. Because the surfaces of the oxygenators used for CPB are not completely haemocompatible however, complications often follow as a result of CPB. With the recent introduction of heparin-coated surfaces for oxygenators, reduced activation of the contact phase of the complement and coagulation system have been demonstrated (Gravlee, 1994; Wendel et al., 1994).

Today postoperative bleeding is still a major complication in heart surgery (Woodman and Harker, 1991). The cause for this is multifactoral: contact of the blood with the artificial surfaces of the CPB machine causes a drop in the platelet count as well as a reduction in platelet functional activity. In addition, there is an activation of F XII and the kallikrein-kinin system resulting in a reinforced activation of the fibrinolytic and complement systems. The high intraoperative and postoperative blood loss requires an average transfusion of two or more stored blood units per patient. Therefore it is particularly important to establish suitable methods for reducing the need for transfusion. Various mechanical methods and therapeutic interventions have been utilised in the last five years the serine proteinase inhibitor aprotinin has been increasingly used (Bidstrup et al., 1993). Many clinical studies have demonstrated that high dose aprotinin therapy in heart surgery significantly reduces postoperative blood loss through inhibition of fibrinolysis along with preventing the adhesive glycoproteins (GP Ib) of the platelet membrane from coming into contact with the different artificial devices (Dietrich et al., 1992; van Oeveren et al., 1990; Fuhrer et al., 1992).

In order to see whether further improvements in minimizing blood activation can be made, we have studied effects on components of the contact system, coagulation markers and cellular elements of blood with their release factors by comparing heparin-coated oxygenators and traditional non-coated oxygenators of the same construction, both with and without aprotinin, in an in vitro CPB model.

* Corresponding author. Tel.: +49-7071-296605; fax: +49-7071-296605.

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2. Materials and methods

In a simulated cardiopulmonary bypass model, we compared 32 Medtronic paediatric membrane oxygenators of the same series (Minimax, Medtronic, Anaheim, CA, USA). The conditions compared were: with or without heparin-coated surfaces (Carmeda® BioActive Surface = CBAS); and with and without aprotinin. In each of the four combinations, 8 oxygenators were tested.

Experimental conditions. 500 ml volumes of fully recalcified fresh ACD whole blood were recirculated through an oxygenator with a roller pump for periods up to 90 min at 28°C. Aprotinin (Trasylol®, Bayer AG, Leverkusen, Germany) was used in a concentration of 250 KIU/ml. Haemodilution, temperature and gas flow were regulated in a similar manner to those used in clinical CPB. The machine was primed with 50 ml 5% glucose solution, 116 ml ringer lactate and 24 ml calcium chloride (10%). Before priming, the oxygenators and tubing were rinsed with 700 ml Ringer lactate for 30 minutes. Eight 10 ml blood samples were taken after 0, 1, 5, 10, 20, 30, 60, and 90 min circulation.

Chromogenic peptide substrate assays for factor XIIa- and kallikrein-like activities (KK) were performed with kits supplied by Unicorn Diagnostics (London, UK). PMN-elastase-α_1-proteinase inhibitor complexes (PMNE-α_1-PI), and platelet factor 4 (PF4) levels were determined using ELISA kits supplied by Merck (Darmstadt, Germany), and Behring Werke AG (Marburg, Germany), respectively. Statistical analysis was performed using the statistics software package SPSS (SPSS Software Inc., Chicago, ILL, USA) using the method of univariate analysis of variance, and p-values ≤ 0.05 were considered significant.

3. Results

Significant increases in kallikrein-like and FXIIa-like activities were seen in all groups after one minute of simulated CPB and continued to increase throughout the period of the study. The highest values were seen in the non-coated oxygenator groups with and without aprotinin. With aprotinin in the non coated oxygenators the values for kallikrein-like activity were lower and for FXIIa-like activity, significantly lower. With the coated oxygenators significantly lower kallikrein-like and FXIIa-like activities were observed both with and without aprotinin. The fall in platelet numbers was significantly less with

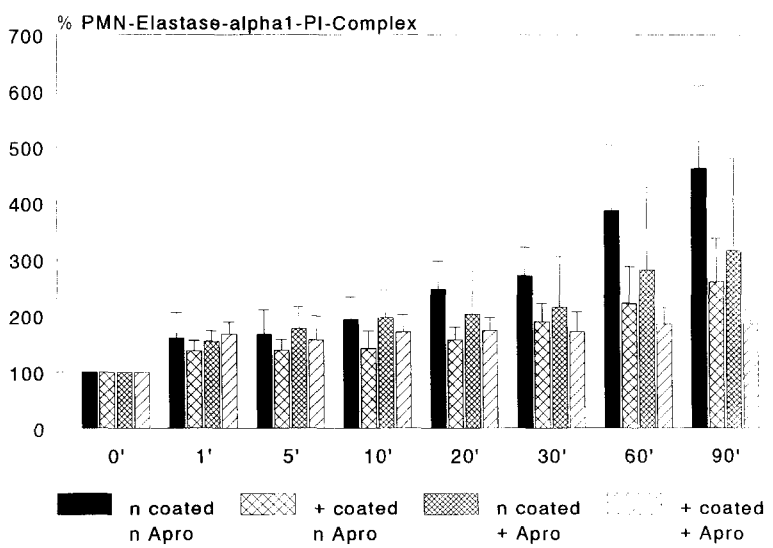

Fig. 1. Mean values ± SD of PMN-elastase-α1-proteinase inhibitor levels in blood samples in the CPB model using heparin coated oxygenators with and without aprotinin and non-coated oxygenators with and without aprotinin. n coated, non-coated; n apro, without aprotinin.

the coated oxygenators and platelet factor 4 levels were markedly elevated with the non-coated oxygenators and unchanged with the coated ones. These results indicate that some platelet adhesion occurred with the coated oxygenators but that the platelets remained intact. Platelet function during CPB was markedly improved when coated oxygenators were used. In the group with coated oxygenators and added aprotinin, the least changes could be detected. PMNE-α_1-PI complex levels rose significantly in all groups during simulated CPB (Fig. 1). The highest values were obtained in the non-coated oxygenator group without aprotinin. In the non-coated oxygenator group with aprotinin significantly lower values were obtained. In the coated oxygenator groups significantly lower values were found and the lowest values were seen in the coated oxygenator group in the presence of aprotinin.

4. Discussion

Leucocyte numbers fall in CPB but the reduction in leucocytes was found to be significantly less in coated compared to uncoated oxygenators. In the present study, the PMNE-α_1-PI complex levels at the end of CPB were markedly lower using the coated oxygenators and significantly lower with aprotinin. We and others have suggested that one of the reasons for the release of PMN elastase from neutrophils is the effect of contact system activation priming the neutrophils for enzyme release (Zimmerli et al., 1989). Our results clearly show that heparinized devices together with aprotinin produce the least changes in blood cells and contact system activation in simulated CPB.

It can safely be postulated that similar beneficial effects will occur in CPB in patients. The simultaneous reduction in contact system activation and effects on white blood cells and platelets suggest that the use of heparin-coated oxygenators together with high dose aprotinin therapy in CPB could have clinical benefits in CPB-related postperfusion syndromes including hyperfibrinolysis and blood loss.

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