PART IX

POSTERS ON DIALYSIS AND RELATED TOPICS
HEPARIN IS UNABLE TO PREVENT CONTACT ACTIVATION BY THREE DIFFERENT MEMBRANES

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Summary

In spite of the anticoagulant activity of heparin platelet deposition and contact activation of coagulation occurs during dialysis. We have studied platelet counts, fibrinogen, platelet factor 4, β-thromboglobulin, thromboxane B₂, FRA, C3d and kallikrein values, whole blood and euglobulin lysis times and membrane areas in haemodialysis using cuprophan and cellulose acetate and in haemofiltration with polyacrylonitrile. Deposits on all the three membranes included leucocytes, platelets and fibrin. The coagulation and fibrinolytic systems are activated more intensively with cellulose acetate and more prolongedly with polyacrylonitrile. Platelet factor 4 and β-thromboglobulin increases suggest platelet activation, only partially dependent on arachidonic acid-mediated pathway as thromboxane B₂ is not increased. The complement system is activated whereas serum kallikrein does not alter, suggesting that platelets rather than factor XII are crucial in contact activation.

Introduction

The activation of the coagulation pathway by any surface other than the endothelium requires the use of anticoagulants during extracorporeal circulation. In dialysis, the choice of the optimal anticoagulant is difficult and for many years there have been no alternatives to heparin. The knowledge that only the inhibition of the late steps in foreign surface-induced activation of the coagulation process may prevent clotting has led to interest in alternative anticoagulants. The fears of adverse effects of full and prolonged heparinisation are probably unfounded, since none of the alleged complications, such as the osteoporotic effect, has ever been proven [1]. Moreover, haemofiltration with membranes largely permeable to free heparin further stimulates the search for alternatives in anticoagulation.

Two main trends have been recently under intensive investigation: the manufacturing of less thrombogenic, heparin-fixing membranes (cellulose and polypeptide with heparin immobilised by covalent bonds [1]), and the study of new
drugs particularly in respect to their inhibitory effect on platelet aggregation [2–5].

The aim of this study was: a) to evaluate the type of blood constituents deposited on dialyser membranes at the end of dialysis, b) to find evidence of platelet activation as inferred from the release of granular constituents, c) to evaluate whether the foreign surface may activate other biological systems and to compare the results obtained during haemodialysis with dialysers of different membranes and geometry and during haemofiltration with flat plate dialysers.

Patients and methods

We studied 71 patients (43 males and 28 females), age 18 to 71, on thrice weekly dialysis, for four months to ten years. Cuprophan flat plate dialysers were used in 54 (group I), cellulose acetate hollow fibre in eight (group II). Nine patients were on haemofiltration with polyacrylonitrile flat plate dialysers (group III). Heparinisation was as follows: group I, 2500U in the last isotonic saline in dialyser washing and continuous infusion of 10 to 15U/min (about 5000U/dialysis); group II, 2500U in the last isotonic saline, 2500U at the start and continuous infusion of 20U/min (about 7000U/dialysis); group III, 7500U in the last isotonic saline and continuous infusion of 20U/min (about 10,000U/dialysis). Heparinisation was monitored by the clotting time (Lee-White), maintained around 10 minutes for group I and between 15 to 20 minutes for group II and III. All patients were evaluated by: light microscopy of membranes and bubble traps, platelet counts and fibrinogen determinations at 15, 30, 120 minutes, platelet factor 4 (PF4) and β-thromboglobulin (BTG), both by RIA pre- and post-dialysis, fibrinogen related antigens (FRA, Thrombowellcotest), lysis time on whole blood with phosphate (Fearnley) and acetate (Gallimore) buffers, lysis area on fibrin plates (Astrup) pre- and post-dialysis, kallikrein (cromogenic substrates) and C3d fragment (radial immunodiffusion).

Results

In keeping with the existing literature, we have found massive deposits of leucocytes, red blood cells and fibrin and, to a less extent, platelets on membranes and in the bubble traps. During dialysis, platelet counts fall maximally at 15 minutes then gradually increase to values higher than basal (Figure 1). A more marked decrease (15 minutes) is seen with cellulose acetate dialysers, although this difference is not statistically significant (27 per cent versus 24 per cent and 15 per cent with polyacrylonitrile and cuprophan, respectively). The granular platelet constituents (PF4 and BTG) increase markedly after dialysis whatever the membrane used, (Figure 1). In contrast, TXB2 is constantly undetected (by RIA) at the end of dialysis. Fibrinogen shows slight variations during dialysis then increases to values higher than basal at the end of dialysis (cellulose acetate 10 per cent, Cuprophan 20 per cent, and polyacrylonitrile 11 per cent) with no significant difference among the three groups (Figure 2). All tests on fibrinolytic activity show a post-dialysis amelioration of basal pre-dialysis hypofibrinolysis and this is more marked with
cellulose acetate (Figure 2). FRA are increased at the end of dialysis whatever membrane is used (Figure 2). C3d is markedly increased at the end of dialysis similarly in all three groups (cellulose acetate from 6.6 ± 3.5 to 15.1 ± 5 U%, cuprophan from 5.05 ± 1.1 to 9.6 ± 2.0 U%, polyacrylonitrile from 4.0 ± 1.3 to 6.8 ± 2.4 U%). It is noted that patients, who regularly use acetate, have basal
Figure 2. Intradialysis fibrinogen and FRA concentrations and euglobulin lysis areas and times with three different membranes.

Statistical significance was found only for euglobulin lysis areas between cellulose acetate and the other two membranes. No significance was observed for the other parameters studied in respect to the three different membranes.

values of C3d higher than those of other groups. Kallikrein slightly decreases with cellulose acetate (from 2.4 ± 1.02 to 1.7 ± 0.7 U/ml) and polyacrylonitrile (from 1.31 ± 0.5 to 0.8 ± 0.1 U/ml) and moderately increase with cuprophan (from 1 ± 0.6 to 1.39 ± 0.9 U/ml). However, the difference between pre- and post-dialysis values is not statistically significant.
Conclusions

In spite of the considerable prolongation of the clotting times, heparin does not confer an absolute thromboresistance in the dialysis circuit as demonstrated by the occurrence of remarkable deposits of blood constituents on membranes [6], easily visible by light microscopy. Activation of platelets occurs as confirmed by the increase in granular constituent release in all three groups studied. The inhibitory effect on platelet activation by drugs, which do not solely block the arachidonic acid-endoperoxides-thromboxane A₂-mediated pathway (such as the pyrimido-pyrimidine compounds [2], prostaglandin E₁ [3] and prostacyclin [4] supports the concept that platelet activation is not only dependent from the arachidonic acid-mediated pathway but is related to changes in intracellular adenylcyclase-cyclic AMP-phosphodiesterase. It is therefore not unexpected that the production of thromboxane A₂ (and the concentrations of its stable catabolite thromboxane B₂) is low, below the threshold sensitivity of the presently available techniques (RIA).

By comparing the three groups of patients, we may conclude that platelet counts fall probably due to microvascular sequestration rather than from adhesion to membranes [5]. This is more marked with hollow fibre dialysers, although these data do not reach statistical significance, and this is probably due to the geometry of the circuit rather than to the type of membrane [6], as similar results were obtained with cuprophan. Platelet counts with polyacrilonitrile show a minor increase at the end of dialysis, in keeping with the hypothesis that the loss of heparin across the membrane may prolong the contact stimulus.

Plasma fibrinogen is not affected by fibrin deposition on membranes (5 to 15mg [5]) or by intravascular consumption. Probably the stimulus induced by the foreign surface contact on this typical 'acute phase' protein accounts for the increment [5], which is even more marked in patients on anti-aggregant drugs, in whom consumption is reduced.

The activation by a foreign surface contact is not only important for its practical implications during dialysis, such as the worsening of the anaemia due to red blood cell entrapment in membrane deposits, reduced filtering capacity and micro-embolisation of platelet-fibrin aggregates, but also accounts for two more general problems.

It represents a continuous and repeated stimulus to the coagulation system which may contribute to the accelerated atherosclerosis already so progressive in uraemic patients [5]. In addition it allows the simultaneous triggering of other plasmatic and cellular enzymatic systems such as fibrinolysis, complement, kinins and lysosomal enzymes and cationic proteins released from neutrophils [7]. Therefore, it is on the multiple stimuli to the coagulation and other biological systems that one may base the criteria of haemocompatibility, a concept that practically sums up the biocompatibility of an artificial organ replacement technique such as dialysis, where the contact of blood to foreign surfaces is extensive and prolonged.

The fibrinolytic system is activated. A more marked acceleration of lysis times is observed with hollow fibre dialysers which is likely to be due to the geometry rather than to the membrane as the same effect is demonstrable with cuprophan
hollow fibre dialysers.

The complement system is also activated in a superimposable fashion in the three groups. A fact that must be kept in mind in view of the possible consequences on the 'leucocyte stress'.

No increase in kallikrein production has been found, at least immediately after dialysis. Patients, who regularly use hollow fibre dialysers, have twice the basal values of the other two groups. As kallikrein production depends directly on the action of activated factor XII on its plasmatic precursor, one may infer that the direct activation of factor XII, although inevitable, is not the main event in the triggering of the coagulation cascade and the other biological systems. It is likely that the statement of Saltzman [8] "It is now customary to view surface-induced thrombosis as chiefly, if not exclusively, a platelet problem" may still be valid, as he recognised in the adhesion of platelets and their attendant activation, the crucial event responsible for both creating the local conditions resulting in heparin lack and for triggering the formation of fibrin, the activation of the fibrinolytic system and the complement cascade.

References

1  Klein E. Clin Nephr 1978; 9: 131
2  Lindsay RM, et al. Lancet 1972; ii: 1287
4  Longmore DB, et al. Lancet 1979; i: 1002