HAEMODIALYSIS WITHOUT HEPARIN

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Summary

Despite the use of heparin, activation of platelets on the artificial surface of dialyser membranes results in thrombus formation, microembolisation and thrombocytopenia. To assess the effects on these events of prostacyclin, three groups of healthy greyhounds were dialysed with heparin, heparin plus prostacyclin or prostacyclin alone. The use of prostacyclin, either alone or with heparin, abolished microembolisation from the dialyser and prevented thrombocytopenia. With prostacyclin dialysis could be carried out without heparin and there was no clotting of blood within the extracorporeal circuit nor any change in tests of haemostasis.

Introduction

Although the use of heparin during haemodialysis prevents gross clotting of blood, interaction between coagulation factors, blood cells and the artificial surfaces of the dialyser membranes and lines results in deposition of fibrin [1]. Such thrombus deposition contributes to residual blood loss within the dialyser due to trapping of red blood cells on the membrane [2] which, in turn, may aggravate the anaemia of dialysis patients [3]. Platelet adhesion and activation occur upon contact between blood and a foreign surface and result in the formation of platelet and white cell aggregates [4] which may be detected in blood returning from the dialyser [5]. In addition, release of antiheparin activity from platelets [6] may encourage fibrin deposition despite the presence of heparin.

Minimising these events by inhibition of platelet function might reduce intradialyser blood loss, prolong dialyser efficiency and prevent systemic circulatory disturbance. Prostacyclin (PGX:PGI₂), which is generated by the vessel wall [7,8], is the most potent antiplatelet agent yet discovered. We describe here the use of synthetic PGI₂ during haemodialysis of healthy greyhounds.
Materials and Methods

Dialysis Techniques

Greyhounds (20–33 kg) were dialysed for 90 to 150 minutes using standard inlet and outlet lines (Travenol), A Cuprophane 1 m² coil dialyser (Travenol, Ultra-Flo II) and Travenol RSP machine after Scribner shunts had been inserted between the carotid artery and jugular vein under thiopentone anaesthesia (25 mg/kg). The femoral artery was cannulated for continuous monitoring of blood pressure. Blood flow to the dialyser was maintained at 200 ml/min and dialysate flow at 400 ml/min. During dialysis, normal saline was infused intravenously at a rate of 15 to 20 ml/min to minimise any rise in haematocrit due to ultrafiltration. Anaesthesia was maintained throughout dialysis by additional doses (2–4 mg/kg) of thiopentone as required.

Anticoagulation and Prostacyclin Administration

Five minutes before dialysis ten animals received an intravenous injection of 10,000 IU heparin (Pularin, Evans) and during dialysis 1,000 IU of heparin/hour was infused into the inlet line of the dialyser. Five of these animals received prostacyclin (1 μg/min equivalent to 30–50 ng/kg/min) by intravenous infusion for five minutes before dialysis in addition to heparin. At the start of dialysis the prostacyclin infusion was resited into the dialyser inlet line and continued at the same rate throughout dialysis. In another five dogs dialysis was carried out with prostacyclin infusion and without heparin. Prostacyclin, 2 μg/min, was infused throughout, having been commenced 30 minutes before dialysis. In four of these animals dialysis and blood sampling were continued for a further 60 minutes after the prostacyclin infusion was stopped at 90 minutes.

Blood Sampling

A blood sample was taken from the arterial limb of the arteriovenous shunt prior to the administration of heparin or prostacyclin, and then, at 5, 10, 20, 30, 60 and 90 minutes during dialysis, samples were withdrawn from the inlet and outlet lines for estimation of haematocrit (micropipette technique) and platelet counts (phase contrast microscopy [9]). Screen filtration pressure (SFP), measured by the method of Swank [10] was recorded in blood samples from inlet and outlet lines at 15, 40, 70 and 90 minutes. Blood (5 ml) was pumped at 12.5 ml/min through a metal screen of 20 μ square pores and the pressure generated in the syringe recorded on a pen recorder via a pressure transducer. Readings obtained after 15 seconds in this test system were used for comparison. In four of the five animals infused with prostacyclin but no heparin in which dialysis was continued after 90 minutes further blood samples were taken at 15 minute intervals.

Rises in haematocrit, platelet counts, percentage extraction of platelets by the dialyser and screen filtration pressure measurements were compared using Student's 't' test.
Results

Dialysis with Heparin or Heparin Plus Prostacyclin

All ten animals completed 90 minutes of dialysis. The initial platelet counts of those receiving heparin only (180 ± 47.8 x 10^9/litre, mean ± SEM) were similar to those of animals receiving heparin plus prostacyclin (158 ± 18 x 10^9/ litre). In dogs which received heparin only the platelet count of blood entering the dialyser fell during dialysis reaching a nadir of approximately 70% of the initial count at 60 minutes. Prostacyclin prevented this thrombocytopenia, and at all stages of dialysis the platelet counts were significantly higher than in dogs treated with heparin alone (Figure 1, upper panel). The platelet counts of blood leaving the dialyser reflected a similar protecting effect of prostacyclin (Figure 1, lower panel).

![Platelet Counts Graph](image)

Figure 1. Mean ± SEM of platelet counts (% of initial) of blood entering (upper panel) and leaving (lower panel) the dialyser in dogs given heparin alone (○-○) or heparin plus prostacyclin (■-■).

Although platelet extraction by the dialyser, expressed as the percentage difference between the platelet counts in the inlet and outlet lines, was similar during the early stages of dialysis for both groups of animals, after 20 minutes prostacyclin reversed such losses while extraction continued and increased up to the end of dialysis in animals which received heparin alone (Figure 2, upper panel).

Prostacyclin-treated dogs showed no rise in screen filtration pressure during dialysis but in heparin-only treated animals the screen filtration pressure of blood leaving the dialyser rose progressively reaching significantly higher levels at 70 and 90 minutes (P < 0.05 and P < 0.02 respectively) (Figure 2, lower panel).

Infusion of prostacyclin was associated with a fall in mean blood pressure of 15 mmHg within two minutes but the blood pressure returned to preinfusion
Figure 2. Mean ± SEM of percentage extraction of platelets by the dialyser (upper panel) and of screen filtration pressure (lower panel) of blood entering (I) and leaving (O) the dialyser. Shaded columns are heparin-only treated dogs and open columns represent dogs treated with heparin plus prostacyclin.

Figure 3. Mean ± SEM of blood pressure in dogs treated with heparin only (○—○) and heparin plus prostacyclin (●—●). The effect on BP of the predialysis infusion of prostacyclin is shown.

values before the start of dialysis. The predialysis blood pressure was similar in both groups of animals, but during dialysis the blood pressure was lower and less stable in those animals which received heparin only (Figure 3).

Dialysis without Anticoagulation
Five dogs were dialysed for ninety minutes with prostacyclin alone and in four of these dialysis was continued for a further 60 minutes after stopping the
prostacyclin infusion. No gross clotting occurred within the dialyser lines or coil during or after stopping the prostacyclin infusion. Thrombocytopenia did not occur during dialysis while prostacyclin was being infused and platelet counts were significantly higher throughout than in animals receiving heparin alone. The platelet count fell rapidly after stopping the infusion (Figure 4, lower panel). With prostacyclin alone dialysis was not associated with a rise in screen filtration pressure but after 60 minutes of dialysis when the prostacyclin infusion had been discontinued the screen filtration pressure of blood leaving the dialyser was significantly elevated (P < 0.01) (Figure 4, upper panel).

During the course of one haemodialysis with prostacyclin alone, tests of whole blood clotting, kaolin-cephalin clotting time, prothrombin time and thrombin clotting time did not differ from pre-prostacyclin infusion values.

**Discussion**

The study demonstrates clearly that, during haemodialysis, prostacyclin prevents thrombocytopenia, reduces platelet losses within the dialyser and abolishes microembolisation as detected by rises in screen filtration pressure. Moreover, using prostacyclin, it was possible to carry out dialysis without anticoagulation with heparin and there was no blood clotting within the dialyser.

In all three groups of animals the extraction of platelets by the dialyser was similar during the early stages of dialysis. Some anti-platelet agents inhibit
the release reaction and irreversible aggregation at lower doses than those needed to affect adhesion and primary aggregation [11]. It is possible that, in our experiments, the dose of prostacyclin was too low to inhibit initial platelet adherence to the membrane but was capable of preventing subsequent aggregation as evidenced by the absence of any rise in screen filtration pressure. In vivo sequestration of platelets and platelet aggregates is probably the major cause of the thrombocytopenia that develops during extracorporeal circulation [12]. Prostacyclin reduces platelet sensitivity to aggregating stimuli, preventing aggregate formation both on the membrane and in the circulating blood with the result that platelets are less likely to be trapped within the microvasculature. This effect is manifest as a sparing of dialysis-induced thrombocytopenia.

During cardiopulmonary bypass rises in screen filtration pressure of the blood returning from the oxygenator have been reported [13], and Dutton and Edmunds [14] have confirmed the presence of large numbers of platelet and white cell aggregates by electron microscopical examination of filters placed temporarily in the cardiopulmonary bypass circuit. Such microembolisation may be responsible, in part, for the pulmonary, cerebral and renal disorders sometimes seen after bypass [15]. In charcoal haemoperfusion of patients with hepatic failure Weston et al [16] have demonstrated elevation of the screen filtration pressure in blood leaving the charcoal column and related this rise to hypotensive crises in some of their patients. Rise in screen filtration pressure during haemodialysis was first documented by Bischof et al [5], and these workers attributed the pulmonary syndrome of hypoxia, hypocapnia and altered ventilation-perfusion to pulmonary vascular sequestration of platelet aggregates returned to the patient from the dialyser [5,17]. The rises in screen filtration pressure and the changes in pulmonary function were reduced by placing a micropore filter in the line returning blood to the patient. In our experiments, the abolition of any rise in screen filtration pressure using an antiplatelet agent is further evidence for the role of platelets in producing this phenomenon, and scanning electron microscopical examination of the screen used in the test has confirmed the presence of platelet aggregates, and reduction in their density with prostacyclin.

The successful completion of haemodialysis without anticoagulation when prostacyclin is used is evidence in support of a paramount role for platelets in foreign-surface-induced activation of coagulation and thrombus formation [11]. The maintenance of a clot-free dialysis circuit after stopping prostacyclin cannot be explained by a persistent effect on platelets as this is short-lived [7,8], and the fall in platelet count and rise in screen filtration pressure attest to the fact that inhibition of platelet function does not persist for long. This lack of blood clotting within the dialyser, despite return of platelet function, may be linked to the finding that prior exposure to cell free plasma results in reduced interaction of some artificial surfaces with whole blood [18].

These experiments demonstrate that the use of an effective antiplatelet agent during haemodialysis, and possibly during other forms of extracorporeal circulation, might permit reduction of the use of heparin during such procedures. A heparin sparing effect would be desirable during haemodialysis of
patients at risk of haemorrhage, and since heparin administered over long periods has been shown to cause osteoporosis [19], reduction in heparin use during dialysis would remove a possible contributing factor in the development of renal osteodystrophy.

The preliminary evidence afforded here, that prostacyclin reduces blood-artificial surface interaction and allows dialysis to be carried out without thrombocytopenia or microembolisation, while not prolonging blood clotting, warrants further study of its effect during extracorporeal haemoperfusion in man.

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Open Discussion

KOPP (Munich) You did not mention the dose of heparin that you administered. Heparin has a coagulation factor releasing effect on platelets which is dose dependent. Heparin in minimal amounts may not release as much platelet factor as heparin in larger amounts.

Secondly, whenever you transfuse a patient with bank blood you use
pre-activated blood in which the coagulation cascade is already stimulated, and where coagulation is not dependent only on the platelets.

WOODS We gave the animal a bolus of 10,000 units. We are sure that this dosage of heparin is a platelet activator. The screen filtration pressure of blood immediately after administering that dose of heparin is high, suggesting that there are platelet aggregates. Heparin increases platelet sensitivity to other stimuli. This is one of the reasons why we think that heparin is not a very good anticoagulant during haemodialysis.

To your second point, blood transfusion is a more important factor during extracorporeal cardiopulmonary bypass for cardiac surgery, where large amounts of blood are transfused. This blood contains a lot of aggregates and the platelets are well and truly activated. This could be another use for prostacyclin. If the blood could be treated with a stable form of prostacyclin prior to transfusion or during the storage procedure, we feel this blood would be more beneficial to the patient and less likely to be injurious.

REMUZZI (Bergamo) Could you give us any idea about the level of prostacyclin in the blood of these animals? Considering the half-life of prostacyclin this could be haemodialysis without active prostacyclin present in blood, but only in the extracorporeal circulation.

WOODS We infuse the prostacyclin cold and at a buffered pH that will leave it stable until it meets physiological conditions. At 1 µg/min its initial level should be 5 µg/ml but we do not know what happens after that. We do know that prostacyclin is very unstable in its present form. At physiological pH it has a half-life of about 2 minutes. Its effect upon platelets lasts for at least another 15 to 20 minutes because the effect on cyclic AMP lasts for about that long, and then platelet function returns. In one of the slides we showed that when we allowed the dialysis to continue in four animals which had only received prostacyclin and then stopped the prostacyclin, platelet function returned. The platelet count fell as the screen filtration pressure rose but the interesting thing was that no clotting occurred. We cannot say what the actual blood levels of prostacyclin were in those animals.