The Membrane Support System and Thrombus Formation on Dialysis Membranes

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Thrombus formation may take place on dialysis membranes, in spite of efficient heparinisation, and cause the regular dialysis patient to have undesirably high blood losses (Lindsay & Kennedy, 1972; Lindsay et al, 1973a). The use of dialysers, which are complicated by in vivo thrombus formation, is associated with a fall in the platelet count of patients undergoing dialysis due to the retention of platelets within the dialysis membranes (Lindsay & Kennedy, 1972; Lindsay et al, 1972a), suggesting that the platelet retention on the dialysis membranes is an important step in the reaction leading to thrombus formation. This hypothesis is supported by the fact that the administration of antiplatelet agents will reduce not only the retention of platelets on the dialysis membranes but also the degree of thrombus formation and patient blood loss (Lindsay et al, 1972b). Because of the effects of platelet retention by dialysis membranes it is important to study factors which may influence it. The nature of the dialysis membrane per se will influence the number of platelets retained by it; for example, with Cuprophan membranes of identical chemical composition, there is a direct relationship between the membrane thickness and the in vitro platelet retention (Lindsay et al, 1973b). Furthermore the thicker Cuprophan membranes appear to be the more thrombogenic in vivo (Lindsay et al, 1973b). The surface geometry of the dialysis membrane will be influenced by the construction of the membrane support system and this study was undertaken to examine the influence of this upon platelet retention.

METHODS

Five types of disposable dialyser were studied. They were the Gambro-Alwall, its successor the Gambro-Lundia, the Rhône-Poulenc parallel flow dialysers, and the EX-03 (Extracorporeal) and UF 100 (Travenol) coils. Three dialysers of each type were examined after use, the respective
dialyses being performed under standard conditions and terminated by standard 'wash-back' procedures. To fix any cellular or proteinaceous deposit a 2% buffered glutaraldehyde solution was pumped slowly through each dialysers' blood manifolds immediately after the 'wash-back' procedure had been completed. Care was taken not to allow air to enter the dialyser between the saline 'wash-back' and the filling with glutaraldehyde. The blood ports were sealed and each dialyser was then left for a minimum of twenty-four hours to allow fixing to take place. The dialyser was then dismantled and membrane samples, 0.6 cm x 0.6 cm, were taken from the arterial and venous ends of the blood compartments and also at a point approximately mid-way along the compartment. With the parallel flow dialysers samples were also taken from the top, middle and bottom layers. Each sample was coated, in vacuo, with approximately 35 nm of gold-palladium and then examined by a scanning electron microscope (Cambridge Stereoscan Mark 2A).

RESULTS

Under low power magnification (x 20) cellular deposits were seen arranged in regular patterns which were obviously related to the geometry of the underlying dialysis membrane supporting system. This feature was constant for each dialyser and is demonstrated in Figures 1-5. The deposits were oval in shape with the coil and Gambro (Alwall and Lundia) dialysers and the spacing between them indicated that they related to the intersections of the coil meshes (Figures 1 and 2) or to the 'high spots' of the Gambro moulded polystyrene support systems (Figures 3 and 4). The deposits were linear with the Rhone-Poulenc dialyser and corresponded to the parallel ridges of its supporting system (Figure 5). With higher power magnification (x 1000 —> x 5000) the deposits were seen to consist of red and white blood cells and platelets (Figure 6) with variable amounts of amorphous proteinaceous material and fibrin-like strands. The regular arrangement of these deposits was fairly uniform along the entire length of the coil dialysis membranes, fibrin formation, ensnaring red cells, only occurring at the junction of the membrane and the blood tubing at the venous or outlet end. Towards the venous end of the Gambro Alwall dialyser the regular pattern of deposit was lost and widespread thrombus formation was seen with fibrin strands trapping large numbers of red blood cells (Figure 7). Very little fibrin formation was seen on the membranes of the Rhône-Poulenc dialyser. With the parallel flow dialysers there was no difference in the pattern of deposits or fibrin formation on the membranes taken from various layers of the dialyser.

DISCUSSION

We have already shown that platelets will adhere to dialysis membranes both in vitro (Lindsay et al, 1973c) and in vivo (Lindsay et al, 1972a). The
Figure 1. Composition photograph (magnification x 20) showing the cellular deposits on the Extracorporeal EX-03 Cuprophan membrane and also the mesh supporting this membrane.

Figure 2. Composition photograph (magnification x 20) showing the cellular deposits on the Travenol Ultra Flo 100 Cuprophan membrane, and also the mesh supporting this membrane.
Figure 3. Composition photograph (magnification x 20) showing the cellular deposits on the Gambro-Alwall Cuprophan membrane and also the pattern of the moulded polystyrene membrane support system.

Figure 4. Composition photograph (magnification x 20) showing the cellular deposits on the Gambro-Lundia Cuprophan membrane and also the pattern of the moulded polystyrene membrane support system.
deposition of platelets on dialysis membranes during haemodialysis may be associated with fibrin formation in spite of heparin anticoagulation (Lindsay et al, 1972b) and thus studies of the interaction between platelets and dialysis membranes are important as membranes with low platelet retention may be less thrombogenic than membranes with a high retention (Lindsay et al, 1973a, 1973b). Other workers have studied thrombus formation on materials used in cardiovascular surgery (eg Teflon, Silastic) and also suggest that platelet retention by these materials is an important step in thrombus formation (Lyman et al, 1968, 1969; Rodman & Mason, 1970a, 1970b). Salzman (1971) has agreed that it is customary to view surface induced thrombosis as chiefly, if not exclusively, a platelet problem.

The nature of the platelet dialysis membrane interaction has been partly studied. As far as the platelet is concerned we have demonstrated that it has an 'intrinsic' ability to adhere to cellulose based dialysis membranes but this reaction is dependent upon the presence of adenosine diphosphate, possibly released from the platelets themselves and from red blood cells, and upon divalent cations (Lindsay et al, 1973c). Furthermore the use of anti-platelet agents such as salicylate and dipyridamole compounds will reduce platelet retention by these membranes (Lindsay et al, 1972b, 1973c). The nature of the dialysis membrane itself will influence the interaction.
Figure 6. Scanning electronmicrograph (magnification x 2000) of the cellular deposit on the Cuprophan membranes of the Rhône-Poulenc dialyser. An aggregation of platelets is seen with some red blood cells. (Reproduced two-thirds original size)

Figure 7. Scanning electronmicrograph (magnification x 1000) of the membrane surface from the venous end of the Gambro-Alwall dialyser. Red blood cells are seen trapped by fibrin strands. (Reproduced two-thirds original size)
The chemical composition of the membrane may be important for fewer platelets to adhere to 'series 10' (copolymer of N-butyl methacrylate and acrylic acid) membranes (Bio-Engineering Unit, University of Strathclyde) than to cellulose-based membranes (unpublished observations). With cellulose-based membranes of identical chemical composition (Cuprophan, J.B. Bemberge, West Germany) there is a direct relationship between platelet retention and the membrane thickness (Lindsay et al, 1973b). It is also likely that other membrane factors such as surface charge (Sawyer & Pate, 1953, Sprinivasan & Sawyer, 1970) or the surface free energy (Lyman et al, 1968) may also influence platelet retention. In this study we have demonstrated that the membrane supporting system influences the retention of platelets, together with red and white cells, almost certainly by influencing the geometric and hydrodynamic characteristics of the membrane surface. Cellular deposits on membranes are seen in regular patterns which correspond to the coil mesh intersection pattern or to the 'high spots' or ridges of the support system in parallel flow dialysers (Figures 1-5). With regard to the supporting meshes of the coils it must be appreciated that while there is no elevation at the points of intersection of the mech strands (Figures 1 and 2) a coil configuration will cause opposing membrane surfaces to be supported by meshes with strands taking different directions. The spaces between the membrane faces directly overlying the mesh strands are presumably narrower than the spaces between areas of unsupported membrane, due to membrane distortion by positive pressure in the blood compartment. It is likely that alterations in blood flow characteristics at these points cause cellular deposition.

The importance of this cellular deposition is twofold. Firstly it is likely that the platelet adhesion — aggregation reaction is followed by the liberation of platelet factors which may lead to thrombus formation downstream should the local conditions encourage this. This is apparent in the case of the Gambro-Alwall dialyser (Figure 7). Thrombus formation, in turn, may lead to excessive blood losses (Lindsay et al, 1973a). Secondly, these cellular aggregates may come off the membrane and form a source of microemboli to the patient. Bischel (1973) has demonstrated that microemboli > 20µ diameter are present in the blood leaving the dialyser and suggested that they may cause pulmonary microembolisation with demonstrable lung function changes. She also demonstrated that filtration of the blood leaving the dialyser by dacron wool or polyester urethane foam filters reduced these pulmonary effects. Examination of these filters after use by scanning electron microscopy showed that these microemboli consisted of platelet-leucocyte aggregates. A source of these cellular aggregates has now been clearly demonstrated.
We submit, therefore, that in the design of membrane supporting systems for dialysers consideration should be given to potential biocompatibility as well as to the efficiency of solute and water transport.

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OPEN DISCUSSION

CAMBI, V (Parma): After how many hours of dialysis treatment did thrombus formation commence?

LINDSAY: In all the examples I have stated, patients have been on routine dialysis, ie from six hours or more. We have done some work on the time factor and have found that thrombus formation can take place 2 hours after dialysis. We have no evidence on any time shorter than this.
A COLOMBI (Lucerne): We know that about half an hour after commencing dialysis, platelets fall to a very low level. What is the reason for the eventual disappearance of thrombocytopenia? Is it because the thrombocytes return from the dialyser, or is it because of some improved regeneration?

LINDSAY: We have noted very significant drops in the platelet count with various types of dialyser. The overall drop in the count is related to the degree of thrombus formation. For example, if you have a dialyser that has a high blood loss because of a lot of thrombus formation, you can then get platelet counts at the end of dialysis about 50 per cent of your normal count. I find the point you make of the platelet count falling and rising later very interesting, because we have found this with another system when passing blood through certain polyurethane filters. In an in vivo situation – haemodialysis – it is quite likely that two things are occurring:

1. The patient's own platelet pool is liberating more platelets into the circulation
2. I also believe that the platelets do disaggregate and come off the membrane.

We have done some in vitro work with 1 litre of blood per hour over a system, and the same thing has happened: the platelet count has fallen off and eventually come back, and obviously they can only come off the surface that they initially adhered to. It is interesting to note that in this situation the platelets still have some function: they will still react to ADP or collagen.

F M PARSONS (Leeds): What marvellous photographs Dr Lindsay! What confuses me is that these aggregations as I understood it occur at the cross-over point of the support membrane. Could you comment on why this happens at that particular point? The membrane is the same there as the gap between the support.

LINDSAY: What concerned me about this point was that if you take a coil you would find that there is no high spot at the point of intersection and one would expect to find a linear deposit of cells as we saw in the Rhône Poulenc Dialyser. One has to remember that with a coil configuration, the membrane tube is going to have opposing surfaces supported by mesh strands each taking a different direction. With the positive hydrostatic pressure you will get membrane distortion. The local geometry changes here are causing the spacing. It is not really the high spot at any mesh intersection.

W R CATTELL (London): Given that you are using dialysers at this time that do not have the best supporting structures that you would like, and given that your problem is platelet aggregation, are you suggesting now that you
should modify your anticoagulation therapy during dialysis, to add what is often referred to as 'antiplatelet agents' such as Aspirin?

LINDSAY: No. We have in fact carried out a trial giving antiplatelet agents to our patients and while no patient has experienced any haemorrhagic or any other side effect, there is the potential danger in have platelet function impaired in a situation where one is giving heparin. I do not think that one should do this, but I do feel that one should be aware of what causes thrombus to form on artificial surfaces, and if it is a platelet induced process, then in the design of new dialysers, one must think about the type of membrane used, the support system, the flow system etc, to make this as weakly thrombogenic as possible.