Effect of Blood Factor Deposits in Reused Dialysers on the Dialysance of Middle Weight Molecules

P KRAMER, D MATTHAEI, J G GO, K WINCKLER, F SCHELER
University Hospital, Göttingen, German Federal Republic

The reuse of dialysers includes the risk of infection transfer, blood compartment bursts and progressive loss of efficiency in eliminating uraemic toxins due to changes of membrane dimension, structure and surface. Fall in dialyser performance may occur even on first use with elapsed dialysis time (Markley et al, 1969). The relative incompatibility of membrane material with blood results in deposition of blood factors on the membrane surface so increasing the resistance to molecular transport. It is conceivable that these deposits might have a predominant effect upon the transport of larger molecules through the membrane. This would be a powerful argument against the reuse of dialysers, since important potential uraemic toxins are thought to range in their molecular size between 200 and 2000 (Babb et al, 1972). Therefore in the present investigation we have studied the elimination of middle weight molecules in reused dialysers.

MATERIALS AND METHODS

Two model middle molecular weight compounds $^{51}$Cr-ethylenediamine tetra-acetic acid (mol. wt. 288) and $^3$H-ouabain (mol. wt. 728.8), which are degraded very little by the human organism, were selected for 'in vivo' dialysance studies with reused flatbed dialysers — Gambro-Alwall, Kiil (Meltec Ltd) and Rhône-Poulenc-B. During haemodialysis the dialysers were held in a vertical position. Blood was propelled into the top by a constant speed roller pump. The pressure gradient from blood to rinsing fluid was maintained at 100 mmHg. A single-pass system with rinsing fluid degassing was used and the dialysate flow was always countercurrent to the blood flow. Immediately after the outset of haemodialysis the patients received an intravenous dose of 12$\mu$Ci (4.1mg) $^{51}$Cr-EDTA and 62.5$\mu$Ci (0.031mg) $^3$H-ouabain. Depending on the individual requirements of the patients 25,000 to 35,000 IU of heparin were infused into the blood inlet of the dialyser at a constant flow rate during dialysis. For determination of the dialysance blood samples were taken after four hours of dialysis time had elapsed and after the blood flow rate had been
adjusted to 150 ml/min as determined by the bubble flow method (Kramer et al., 1972). At the end of the dialysis as much blood as possible was returned from the dialyser to the patient using a combined wash-out and blow-out technique for the AB-Gambro and the Rhône-Poulenc-B dialysers. This technique needs only 150 ml of extra physiological saline and reduces the blood loss in the Rhône-Poulenc-B to 3.65 ± 1.21 ml and in the AB-Gambro-Alwall to 6.86 ± 1.94 ml. The Kill was washed out with 700 ml of physiological saline. Blood loss by this technique was 3.48 ± 1.21 ml in the dialyser and 3.55 ± 0.93 ml in the venous blood lines.

Immediately after the wash-back procedures the blood compartments and rinsing fluid compartments were rinsed with tap water (inflow pressure: 70 mm Hg). The cleaning with tap water was intensified by intermittent clamping of the outflow lines, thereby causing rapid changes of pressure and flow in the dialyser. Rinsing of the blood compartments was continued until the water turned clear. For the Kill and the Rhône-Poulenc-B ten minutes of tap water rinsing was sufficient, whereas for the AB-Gambro up to 40 minutes of tap water rinsing was sometimes necessary. After tap water rinsing the dialysers were filled with 4% formalin for resterilisation and storage. Formalin solution was introduced into the blood compartment from the arterial end of the dialyser with a hydrostatic pressure of 100 mm Hg. Air was removed by tilting the arterial end lower than the venous end. The interval from one use to the next was a mean of 2.4 days. Prior to reuse the blood compartments were rinsed with 500 ml of physiological saline. (For the AB-Gambro rinsing with 1 to 2 litres of saline was necessary.) The blood compartments then were dialysed against tap water at a minimum flow rate of 1000 ml/min for 30 minutes in order to remove the formalin. Before connecting the dialyser to the rinsing fluid line, saline flushed through the blood compartment was tested for the presence of formaldehyde using Clinitest® tablets (Pollard et al., 1967).

RESULTS
With the procedures described above for resterilisation and for pre-use rinsing not a single untoward patient reaction occurred during reuse of the three flatbed dialysers. Figures 1 and 2 show deposits of blood factor on the membrane surface of dialysers used three successive times with tap water rinsing after each use. Figure 1a is a representative cross section of a membrane area which macroscopically appeared free of deposits. Single cells and cellular debris attached to the membrane surface alternate with uncovered areas. Figures 1b and 1c demonstrate the spongy character of deposits which can be seen on the membrane surface on visual inspection. In Figure 1b there is an open space between the deposit and the membrane surface. The deposit is attached to the membrane surface by only a few
Figure 1 a-c. Light micrographs of blood factor deposition on membranes of dialysers used three successive times with tap water rinsing after each use. Magnification x 800
Figure 2 a-b. Electron micrographs of blood factor deposition as shown in Figure 1c. Magnification of Figure 2a x 4,227. Magnification of Figure 2b x 13,334.
cells. In Figure 1c a network of fibrin encloses cells and cellular debris. Several open spaces indicate the former location of red cells, which have been destroyed by tap water rinsing. As shown in Figure 2a even the nuclei and other organelles are disrupted. Figure 2b is a highly magnified electron micrograph which reveals a thin layer (0.1 - 0.3 μm) of dense material on the membrane surface.

![Dialysance of Ouabain, EDTA, Creatinine, and Urea in Re-used AB-Gambro Dialysers](image)

Figure 3. Dialysance of different solutes in reused AB-Gambro Alwall dialysers (mean values ± SD, n = 15)

Although all reused dialysers contained these deposits to varying degrees no decrease of ouabain dialysance was observed even at the third use. The reuse, however, caused a significant decrease of the dialysance of urea (p < 0.01), creatinine (p < 0.01 and EDTA (p < 0.01) in the AB-Gambro dialyser as shown in Figure 3. This dialyser showed an additional decrease of urea (p < 0.05) and creatinine (p < 0.01) dialysance at the third use. These results correspond to the observation that blood loss was highest in the AB-Gambro. The results obtained with the Kili dialyser are demonstrated in Figure 4. There was no change in dialysance of urea, EDTA and ouabain at the second or third use as compared to the first use. A significant decrease of the creatinine dialysance was observed from the first to the second use (p < 0.01) but not from the first to the third use (p = 0.07). Figure 5 demonstrates the results obtained with the Rhône-Poulenc-B dialyser. The dialysance of the four test substances remained unchanged throughout the three uses of this flatbed.
DIALYSANCE OF OUABAIN ■ EDTA □□□ CREATININE □□□□ AND UREA □□
IN REUSED KII DIALYZERS (blood flow 150 ml/min)

Figure 4. Dialysance of different solutes in reused KII dialysers
(mean values ± SD, n = 15)

DIALYSANCE OF OUABAIN ■ EDTA □□□ CREATININE □□□□ AND UREA □□
IN REUSED RHÔNE-POULENC DIALYZERS (blood flow 150 ml/min)

Figure 5. Dialysance of different solutes in reused Rhône-Poulenc-B dialysers
(mean values ± SD, n = 15)

DISCUSSION

These studies demonstrate that the disposable dialysers Rhône-Poulenc-B and AB-Gambro may be reused. In this respect the Rhône-Poulenc-B is superior to the AB-Gambro since in this dialysre reuse caused no reduction of urea, creatinine and EDTA dialysance. These findings contrast with the results of von Hartitzsch et al (1971) who reported a 22% fall in urea and a
16% fall in creatinine dialysance at reuse in four out of six cases. This discrepancy of results may well be explained by different wash-back and rinsing techniques or by different heparin dosage. The reduction of urea, creatinine and EDTA dialysance in the reused AB-Gambro dialyser most probably is due to blood factor deposition as shown in Figure 1 a-c. It is the most interesting finding of this study, that these deposits increase the resistance mainly to the transport of small molecules through the membrane. So far we have two explanations for this phenomenon:

1 The deposits on the membrane surface cause a separation of molecules according to size similar to the gel filtration column, in which the passage of the small molecules is delayed, because in contrast to the larger molecules, they enter the micropores of the dextran gels and take a longer way through it.

2 The deposits reduce the flow velocity of the membrane-close plasma, and thereby the transfer of the small molecules through the membrane decreases because of a reduced trans-membrane concentration gradient.

A powerful argument against the first explanation is that the deposits will become saturated with small molecules and then their transfer should no longer be delayed. The second explanation is more likely. Deposits shown in Figure 1b are found where the two membranes touch each other. From there they protrude into the blood stream and thus decrease the flow velocity on the adjacent membrane surface. In a similar way spotty deposits as shown in Figure 1a are likely to decrease the flow velocity across the membrane surface. Furthermore, a slow flow of plasma must be assumed to occur through the large pores of the deposits as shown in Figure 1c. A reduced flow rate of membrane-close plasma or blood would be the best explanation for the paradoxical findings of this investigation, since it corresponds with the well-known fact that the dialysance of small molecules is much more blood flow dependent than the dialysance of large molecules. Although the reduction of small molecule dialysance was seen only in the AB-Gambro dialyser, it may occur in practice also in the Rhône-Poulenc-B and the Kiil dialyser in the case of low flow dialysis, inappropriate heparin dosage or poor rinsing technique. Especially in home dialysis with dialyser reuse one has to be aware of this complication.

In respect to the 'middle molecular weight hypothesis' (Babb et al., 1972), however, this complication might be desirable. If dialysis is prolonged at reuse the amount of middle weight molecules removed is increased, whereas the amount of small molecules removed is the same as at first use. This might be a desirable effect. We have 14 home dialysis patients dialysing four times per week for ten hours with a Kiil (Meltec Ltd) used four times. All patients are fully rehabilitated with a serum creatinine of $11.8 \pm 3.0$ mg/
100 ml and a serum urea nitrogen of 75 ± 30 mg/100 ml before dialysis. Although the good general condition of these patients is in favour of the 'middle molecular weight hypothesis', there is one disturbing experience, which might be against it. Two of these 14 patients developed haemorrhagic pericarditis. It may well be, that in order to prevent haemorrhagic pericarditis a sufficient amount of small molecules has to be removed together with the middle weight molecules.

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OPEN DISCUSSION

M FERNANDEZ (Ghent): Have you done nerve conduction studies, and have you noticed any improvement or decrease?

KRAMER: No. This was not done for this specific study. I just mentioned that we have to be careful about the middle molecular weight hypothesis, that's all.

FARRELL (Seattle): Just a couple of points on the measurement of the clearances of large molecules. Obviously, you are aware that you do get losses in the statistics as you go up in molecular weight, and I was wondering, did you take mass balance on the ouabain and the other smaller molecules?

KRAMER: Do you think that should be necessary?

FARRELL: Yes. We have had a fair amount of experience of re-use with Kiils, but more so with the Hollow Fiber Artificial Kidney, and we have observed that you do get a drop with say 15 re-uses of the dialyser. You get a drop in urea clearance, but because of the flow dependency it is not proportional to the loss of surface area. However, for middle molecules, for example, inulin, you do get a proportionate decrease as the area is lost, maybe because of the non-flow dependence. A further point is that in the blood channel, flow is laminar and in terms of the overall mass transfer coefficient you are not going to see any effect with variations in blood flow from (say) 150 to 350 ml/min. This would mean that the deposits which you are suggesting may cause a reduction in flow may in fact benefit you, by creating turbulence at the surface of the membrane! So that I think it would be worth checking that it is in fact $^{51}$Cr EDTA and $^3$H labelled ouabain by using a UV spectrophotometer or something like this. Secondly, if you do a dialysis clearance as well as an AV clearance you stand a much better chance from a statistical point of view. Alternatively you can put the dialysate in a closed circuit and thereby watch the build-up of the $^3$H ouabain or Vitamin B12 or whatever compound you choose in the actual dialysate loop: over a short period your blood concentration will remain constant and you can determine your clearance by doing that. Maybe you did take a mass balance, I don't know, but I think it probably is important from the point of view of deposition on the membrane, although this certainly was not obvious from your first two slides on re-use.

KRAMER: Well, concerning your first question, I should think whenever the effective membrane surface was decreased in this dialyser, it should affect
the dialysance of large, middle and small molecules so I do not think this explains our findings. I did not get all your questions, but the second one I think was about the possible degradation of the EDTA and whether I measured ouabain and EDTA. Well, we know that EDTA and ouabain are two substances which are very little degraded by the human organism and we took the samples four hours after the ouabain and the EDTA had been injected. At this time nobody has ever seen any metabolites. This may depend on the difficulty of showing them – we could do it, but it is very difficult because the concentration in the dialysate and in the blood is very low.

B VON HARTITZSCH (Newcastle): I think that this is very interesting but I think that we must put things into perspective. These dialysers are not the most efficient dialysers on the market, having been superseded by the Rhône-Poulenc ID and by the Gambro Lundia, and there are dialysers available which will clear much greater amounts of middle-sized molecules. We found that the Rhône-Poulenc and the Gambro Alwall were not efficient enough for us in terms of urea and creatinine after re-use, and in terms of middle-sized molecules we can use dialysers which will clear twice as much. Dialysers such as the multi-point Kill type dialysers.

KRAMER: I think I agree with you there is not much point in this investigation.

R LINDSAY (Glasgow): I would like to congratulate Dr Kramer on his paper. His work in fact parallels studies of our own on thrombus formation taking place on dialysis membranes. In particular, we have looked a lot at fibrin formation taking place upon the membranes of the Gambro Alwall dialyser. We think we have some ideas into the mechanism of this though I won’t go into that at the present time. One point I would like to take issue with you, however, is that in our experience this thrombous formation traps a lot of red blood cells and the blood loss that we have found with this dialyser using a very accurate $^{51}$Cr red cell label technique is in the order of 36 ml, as opposed to the 6 ml that you have recorded. There seems to me, with the nature of your experiments, that your blood loss figures must have been carried out by haemoglobinometry on a saline wash-through. If there is thrombus taking place on the membrane, you can’t possibly have got the red cells into solution. Furthermore, using haemoglobinometry on a saline wash-through is in itself a fairly inaccurate technique.

KRAMER: We did not use a saline wash-through. We took the whole dialyser apart and washed the membrane in distilled water and then all the haemoglobin or the fibrin thrombi washed off and then these fibrin thrombi
were destroyed. We make sure that all the haemoglobin enters the distilled water and then we measure the haemoglobin in this water. This method has proved to be very accurate — much better than $^{51}$Cr.

LINDSAY: All I would suggest is that we have also looked at the Rhône-Poulenc and the standard Kill, and our figures agree with yours but we find a very high blood loss for the Gambro Alwall. However, it is perhaps fair to say to complete what Dr Von Hartitzsch said, that the new Gambro Lundia dialyser does not seem to have this characteristic problem.

KRAMER: I think I agree with you. We used a different technique — the wash-out and blow-out technique. This reduces the blood loss from 20 ml to 6.9 ml but it is a different technique; otherwise I agree with you.

S SHALDON (London): I am afraid that I think we are lost here. As I understand it, the major importance of this paper is dependent upon one observation on one group of dialysers that there was a maintained clearance of an $^3$H-labelled molecule at the time when urea and creatinine clearances were significantly reduced. I think this is assumption, isn't it? You have shown an AV difference across the dialyser for a $^3$H-labelled marker. You haven't actually proven that the membrane removed that marker. Is it possible that within the interstices of your cellular deposits (which are obviously much greater in the Gambro Alwall) that you have lost the marker? The rest of your story hangs really on that crux, doesn't it? I mean you have not proven to my satisfaction that you have preserved large molecule clearance. You have merely demonstrated that less is coming out in the venous blood and I am not convinced on theoretical grounds that your explanation is justified. I think this may be artifact.

KRAMER: I think for your explanation it would be necessary for EDTA and ouabain to be absorbed. Otherwise, any deposition on the membrane surface would have the same concentration of ouabain and EDTA and I don't think this would affect our dialysance studies. Unless you assume that ouabain and EDTA are absorbed by the deposits, so that you have a very high concentration of ouabain and EDTA on the membrane surface, I don't think it will affect the dialysance.

SHALDON: How many times did you take blood samples at that particular moment in time when you were estimating this clearance data?

KRAMER: We took three samples 15 minutes apart at exactly four hours after the onset of dialysis.
SHALDON: Well, I would have thought that just on the basis of a single transit of your marker, it is conceivable that you were getting progressive loss into your area of dead space. I don’t see how you have to have very high concentration there. I would have thought that at any moment in time you are losing marker, and it is being trapped there, but the quantity in terms of the clearance levels, weren’t very high, were they? The differences in terms of clearances were significant only for ouabain, not for EDTA. I think you said on your slide that EDTA hardly moved. I cannot remember what the ouabain figures went down by. There was quite a large scatter, there were large standard errors, but I can’t accept that this rather unusual physical concept which is against all theoretical basis of membrane diffusion can be accepted in the absence of demonstration of the marker having actually come through the membrane.

KRAMER: I am sorry, but I think that is a misunderstanding. The ouabain dialysance did not go down.

SHALDON: I am sorry I got it the wrong way round – I mean your EDTA Data.

KRAMER: They didn’t go down either. It was just in the Gambro dialyser that there was a difference between first and second using EDTA dialysance but later there is no difference between second and third use.

SHALDON: There was a drop in the EDTA clearance between first and second use?

KRAMER: Just in the Gambro dialyser, but ouabain dialysance didn’t show any change.