PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

The Effect of Dialysis Membranes on Lipid Peroxidation in the Erythrocytes of Patients with Terminal Renal Insufficiency

A. A. Kubatiev, I. A. Rud'ko, T. S. Balashova, and V. M. Ermolenko

UDC 616.61-008.64-036.882-07:616.155.1-008.939.15-39

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 11, pp. 460-462, November, 1994
Original article submitted May 20, 1994

The content of malonic dialdehyde is shown to be significantly increased in the erythrocytes of patients as compared to the control. During hemodialysis using a regenerated cellulose membrane, the level of malonic dialdehyde reliably increased after 30 min of treatment ($p<0.05$) and dropped to the initial level following 180 min of hemodialysis. The level of membrane malonic dialdehyde on the erythrocytes remained at the pre-dialysis level at the 30th and 180th min of hemodialysis when a polysulfone membrane was used. It may be assumed that the activation of lipid peroxidation is due to the interaction of cells with the dialysis membrane and can be considered as one of the markers of biocompatibility.

Key Words: *terminal renal insufficiency; erythrocytes; dialysis membrane; lipid peroxidation*

Anemia is a severe complication of terminal renal insufficiency (TRI). The TRI-associated increase in erythrocyte damage is due to various factors, connected both with the changes of structural-functional properties of the erythrocyte membrane and with the uremia-related disturbance of the general metabolism [3,9]. The data obtained by us and many other researchers present evidence that one of the causes of structural alterations of the erythrocyte membrane and increased hemolysis in TRI patients treated with hemodialysis (HD) is an intensification of lipid peroxidation (LPO) in the erythrocyte membrane [1,7,15]. LPO activation in the erythrocyte membrane in uremic patients treated with HD appears to be related to the effect of oxygen free radicals [8] that can be generated in excess by direct contact of neutrophils with the membrane or via C3a and C5a complement fragments. Practically no increase of oxidative metabolism in the neutrophils was noted when polyacrylonitryl and polysulfone membranes were used during HD [11,13]. These membranes seem to differ from cuprophan membrane in that they absorb the blood proteins in their specific way and possess low complement-activating capacity.

The goal of this work was a comparative study of different dialysis membranes regarding their effect on LPO in the erythrocyte membrane of TRI patients during HD.

MATERIALS AND METHODS

Twelve TRI patients treated with HD aged 31-54 years were examined. In 6 patients TRI developed

Department of General Pathology and Pathophysiology, and Department of Nephrology, Russian Medical Academy of Postgraduate Education, Moscow

0007-4888/94/0011-1156$12.50

as a result of chronic glomerulonephritis, in 5 patients due to chronic pyelonephritis, and in one due to urolithic disease. HD procedures each lasting 4-5 h were performed using capillary C-DAK-4000 dialyzers (Althin Medical Group) with regenerated cellulose membranes, F-40 dialyzers (Fresenius) with polysulfone membranes, and Hemoflow F-5 dialyzers (FREBOR, Belorussia) with polysulfone membranes. Blood of 10 healthy donors served as the control.

Blood was taken from the venous system following transfer through a dialyzer before the addition of heparin, 30 and 180 min from the start of HD, into plastic tubes containing 3.8% sodium citrate. The blood was centrifuged at 640 g for 7 min at 4°C. The platelet-depleted plasma was aspirated and stored at -20°C. The sedimented erythrocytes were washed 3 times with precooled saline (150 mM NaCl, 5 mM Na-phosphate buffer, pH 7.4) and frozen at -20°C prior to the experiment.

The level of malonic dialdehyde (MDA) in the erythrocytes (used as a marker of LPO intensity) was recorded by the fluorimetric method [16], and the protein content was measured after Lowry.

The results were statistically processed using Student's paired test. The differences were considered reliable at $p<0.05$.

RESULTS

The MDA level in the patients' erythrocytes exceeded the control level almost twofold ($p<0.05$). When HD was performed using the regenerated cellulose membrane, the MDA content in erythrocytes insignificantly increased 30 min after the start of the procedure ($p>0.05$) and reverted to the pre-dialysis level 2 hours following the start. In the case of the F-40 and F-5 dialyzers containing the polysulfone membrane, the MDA level remained at the initial level both 30 min and 180 min after the start of HD (Fig. 1).

Our results show the difference in the effect of dialysis membranes (polysulfone and regenerated cellulose) on the process of LPO in the erythrocyte membranes of TRI patients during HD. No activation of LPO was recorded for the use of F-40 and Hemoflow F-5 containing the polysulfone membrane, while moderate LPO activation was observed during HD using C-DAK-400 dialyzers based on the regenerated cellulose membrane. Our results are in agreement with the report [6] in which a decrease was observed in the lipoperoxide substances, attended by a rise of the level of transferrin - the main Fe-binding protein, which, in addition, possesses antioxidant activity - in the

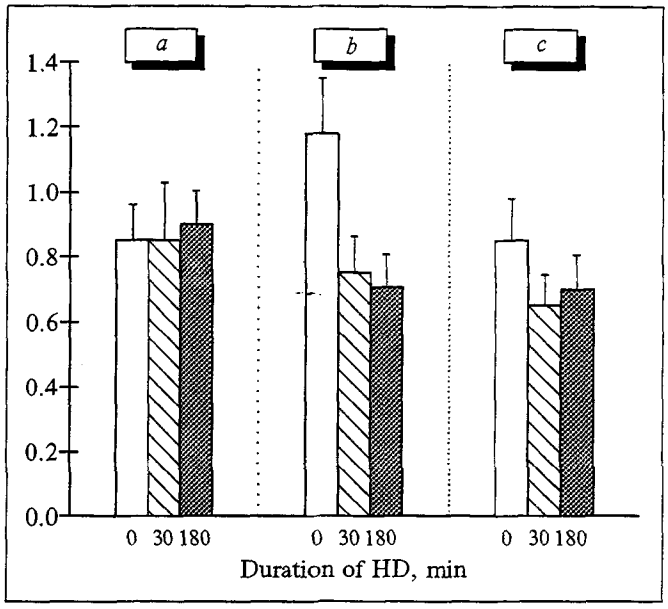

Fig. 1. MDA content (nmol/mg protein) in the erythrocyte membranes of TRI patients treated with HD. *a*) HD was performed using a C-DAK-4000 dialyzer with regenerated cellulose membrane; *b*) an F-40 dialyzer with polysulfone membrane; *c*) an F-5 dialyzer with polysulphone membrane.

patients' plasma. In the mentioned study HD was performed during 6 months using dialyzers with polysulfone membranes (F-60, F-80, F-6, Fresenius). At the same time, the content of lipoperoxide substances remained elevated for the use of a cuprophan membrane [6].

The absence of HD-associated activation of LPO using a polysulfone membrane and the negligible activation in the case of a regenerated cellulose membrane lead us to conclude that these products have a better biocompatibility than the coprophan membrane. In a study of the complement-activating capacity of various dialysis membranes, Bingel *et al.* found that hemophan, a modified dialysis membrane obtained on the basis of regenerated cellulose, induces complement activation to a lesser degree than cuprophan [5]. Hemophan differs from cuprophan in the introduction of quaternary amino groups into the cellulose molecules. In contrast to cuprophan, hemophan, like polysulfone membrane, practically does not induce the expression of Mac-1 adhesion protein on the membrane of monocytes and granulocytes, nor does it induce neutropenia 15 min following the start of HD [14].

The mechanism of LPO activation in the erythrocyte membranes of HD-treated uremic patients remains unclear. Increased production of free radicals in the neutrophils and monocytes of dialysis-treated patients caused by the activation of blood cells by the membrane itself or by mem-

brane-released products, such as endotoxin, may be one of the causes of LPO enhancement in the erythrocyte membrane. As a matter of fact, increased generation of free radicals was observed in the process of HD; however, the content of plasma diene conjugates did not differ from that in healthy persons in the periods between dialysis [12]. A dramatic rise in the plasma MDA and diene conjugates concentration was recorded 20 min following the start of HD performed on the DIP-02-02 dialyzers with cuprophan membranes. However, this effect was absent when using the B2-100 dialyzer (polymethylmethacrylate membrane) or the D-1 dialyzer (cuprophan membrane) [2]. According to these researchers, one of the main causes of LPO activation at the beginning of HD on the DIP-02-02 dialyzer is the activation of monocytes by endotoxin accumulating on the dialysis membrane.

It is worth mentioning here that no difference was revealed in the induction of healthy donor granulocyte aggregation in the presence of autologous donor serum and the serum of dialysis patients whose HD was performed using both cuprophan and non-cuprophan membranes (polyacrylonitryl, polymethylmethacrylate, and cellulose acetate) [4]. According to these scientists, the early (15 min following the start of HD) aggregation of granulocytes on the cuprophan membrane and reduction of the chemiluminescence of the total blood are due to the direct effect of the dialysis membrane on the blood cells rather than to the influence of the serum-derived factors. During the course of HD on a cuprophan membrane, Kolb *et al.* observed a decrease of superoxide anion radical in the granulocytes, while no change was seen when using a polysulfone membrane. It is thought that the inhibition of superoxide anion radical by granulocytes is induced by a preliminary cell activation resulting from interaction with the cuprophan membrane [10].

We are also inclined to think that the LPO activation in the erythrocyte membrane during HD is a consequence of the interaction of the blood cells with the dialysis membrane and may be considered as one of the markers of biocompatibility. Polysulfone membrane proves to be more biocompatible than regenerated cellulose membrane.

REFERENCES

1. T. S. Balashova, I. A. Rud'ko, V. M. Ermolenko, *et al., Ter. Arkh.*, **6**, 66-69 (1992).
2. E. A. Stetsyuk, A. P. Khokhlov, V. N. Sinyukhin, *et al., Urol. Nefrol.*, **4**, 47-50 (1989).
3. L. S. Yudanova, E. V. Yakovleva, N. B. Zakharova, and N. N. Cherneva, *Ter. Arkh.*, **6**, 63-66 (1992).
4. H. Bauer, H. Brunner, H. Franz, and B. Bultmann, *Contrib. Nephrol.*, **36**, 9-14 (1983).
5. M. Bingel, W. Arndt, M. Schulze, *et al., Nephron*, **51**, 320-324 (1989).
6. A. Dasgupta, S. Hussain, and S. Ahmad, *Ibid.*, **60**, № 1, 56-59 (1992).
7. O. Giardini, M. Taccone-Gallucci, R. Lubrano, *et al., Ibid.*, **36**, 235-237 (1984).
8. J. R. Hatherill, G. O. Till, and P. A. Ward, *Agents Actions*, **32**, № 3/4, 351-358 (1991).
9. A. G. Hocken, *Nephron*, **32**, 28 (1982).
10. G. Kolb, C. Nolting, I. Eckle, *et al., Ibid.*, **57**, 64-68 (1991).
11. M. Luciak, and K. Trznadel, *Nephrol. Dial. Transplant.*, **6**, Suppl. 3, 66-70 (1991).
12. E. K. Maher, D. G. Wickens, J. F. A. Griffin, *et al., Ibid.*, **2**, 169-171 (1987).
13. M. Markert, C. Heierli, T. Kuwahara, *et al., Clin. Nephrol.*, **29**, 129-136 (1988).
14. P. Thylen, J. Lundahl, E. Fernik, *et al., Amer. J. Nephrol.*, **12**, 393-400 (1992).
15. K. Trznadel, L. Pawlicki, J. Kedrova, *et al., Free Radic. Biol. Med.*, **6**, № 4, 393-397 (1989).
16. K. Yagi, *Biochem. Med.*, **15**, 212-216 (1976).