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CUPROPHAN INDUCES GREATER CD62 EXPRESSION ON PLATELETS IN HEMODIALYSIS PATIENTS

CUPRAFAN HEMODİYALİZ HASTALARINDA TROMB OS İTLERDE DAHA FAZLA CD62 EKSPRESYONUNA YOL AÇMAKTADIR.

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SUMMARY

During hemodialysis, the contact of blood and membranes result in platelet activation. Previous results on platelets activation during hemodialysis are not uniform. Platelet activation has recently been shown to play a key role in the development of advanced atherosclerotic lesions. Therefore we aimed to assess platelet activation induced by cuprammonium-treated cellulose (cuprophan) and polysulfone dialyzers.in hemodialysis patients.

Twenty-one end stage renal failure patients (9 M and 12 F; aged 43.5 ± 15.8 years, mean ± SD) who were on the chronic hemodialysis for at least three months were included in the study. The patients were sequentially dialyzed by cuprophan and polysulfone membrane, for a month with each membrane. Blood samples were obtained predialysis and postdialysis on the last dialysis session of one-month period with both membranes. As activation marker, CD62 expression of platelets was used. A flow cytometric analysis was performed in whole blood instead of platelet rich plasma used in previous studies to eliminate the activation caused by centrifiigation and washing.

There was no significant change in the percentages of CD62-positive platelets by either membrane. However, postdialysis mean CD62 intensity of a platelet by cuprophan membrane significantly increased in comparison to predialysis value (p=0.01). Polysulfone membrane did not induce any significant change in mean CD62 intensity. Although postdialysis circulating activated platelets (the percentage of CD62-positive platelets x platelet count) by cuprophan were higher compared to predialysis count (p=0.037), there was not any significant difference by polysulfone.

In conclusion: Cuprophan membrane induces platelet activation in chronic hemodialysis patients. There is a need for fitrther studies in terms of clinical implications of this study. However, we suggest that hemodialysis with dialyzers which cause platelet activation, may have an additional effect to morbidity for these patients.

Key words: Hemodialysis, cuprophan, polysulfone, platelet activation, CD62.

ÖZET

Hemodiyaliz (HD) esnasında kanın membranlarla teması trombosit aktivasyonuna neden olur. Hemodiyalizde trombosit aktivasyonu ile ilgili daha önceki bulgular çelişkilidir. Son zamanlarda, trombosit aktivasyonunun ateroskleroz gelişiminde anahtar rol oynadığı gösterilmiştir. Bu nedenle, HD hastalarında kuprofan ve polisülfon diyalizörlerin yol açtığı trombosit aktivasyonunu değerlendirmek istedik.

Enjız 3 aydır kronik HD tedavisi alan 21 son dönem böbrek yetmezliği hastası (9 E, 12 K; yaş 43.5 ± 15.8 yıl, mean ± SD) çalışmaya dahil edildi. Hastalar kuprofan ve polisülfon membranla, her bir membranla bir ay süre ile, ardışık olarak tedavi edildi. Birer aylık tedavilerin son seansında diyaliz, öncesi ve sonrasında kan örnekleri alındı. Aktivasyon belirteci olarak trombositlerdeki CD62 ekspresyonu çalışıldı. Daha önceki çalışmalarda kullanılan, santrifüj ve yıkamanın aktivasyona neden olduğu, trombositten zengin plazma yerine tam kanda akım sitometresi metodu kullanıldı.

Her iki membranla CD62 pozitif trombositlerin oranında anlamlı değişiklik yoktu. Bununla birlikte, kuprofanla diyaliz sonrası her bir trombositteki ortalama CD62 yoğunluğu diyaliz öncesi değere göre anlamlı şekilde arttı (p=0.01). Polisülfon diyalizör CD62 yoğunluğunda anlamlı bir değişikliğe yol açmadı. Kuprofanla diyaliz, sonrası dolaşan aktive trombosit sayısı (CD62 pozitif trombosit oranı x trombosit sayısı) diyaliz, öncesine göre daha yüksek olmasına rağmen (p=0.037), polisülfonla anlamlı değişiklik yoktu.

Sonuç olarak, kuprofan membran kronik HD hastalarında trombosit aktivasyonuna yol açar. Bu bulgunun klinik sonuçları ile ilgili daha ileri çalışmalar yararlı olabilir. Bununla birlikte, tombosit aktivasyonuna neden olan diyalizörlerle HD'in bu hastalarda morbiditeye katkıda bulunabileceğini düşünüyoruz.

Anahtar kelimeler: Hemodiyaliz, kuprofan, polisülfon, trombosit aktivasyonu, CD62.

INTRODUCTION

During hemodialysis, the interaction of blood and artificial membranes result in platelet activation (1). This activation depends on the flow design of the dialysis membrane used (2,3), the composition and the geometry of dialysis membranes (4-6), and on hemorrheological factors derived from blood circulation (7).

Activation of platelets causes surface expression of multiple glycoproteins that are critical for platelet aggregation (8-10) and interaction of platelets with other vascular cells, such as leukocytes and endothelial cells, and artificial surfaces (8,9,11). CD62 (GMP-140, PADGEM, P-selectin) is a 140 kD glycoprotein present in a-granules of platelets which is selectively expressed on the membrane surface of platelets after their activation (12). The detection of certain proteins exposed on the surface of stimulated platelets, is seen as a useful approach to determine early stages of clinical thrombosis (13). Previous results on CD62 expression on platelets during hemodialysis are not uniform. While some authors failed to observe an increase of CD62 expression during hemodialysis on platelets in vivo and in vitro (14-17), others found a significant increase of CD62 expression during dialysis (18-20). Mostly, a platelet rich plasma was used in studies of platelet activation performed in hemodialysis patients. However, centrifugation and washing done during this procedure can also activate platelets (6,21).

Therefore, in this study, the expression of CD62 on platelet membrane surface was measured in whole blood by flow cytometry using a monoclonal antibody against this protein. We aimed to assess and compare, in vivo platelet activation during hemodialysis through cuprophan and polysulfone membranes induced.

Blood sampling

SUBJECTS AND METHODS

Patients

Twenty-one patients on maintenance hemodialysis were selected for the study. Informed consent was obtained from each patient for additional blood sampling. The patients were 9 men and 12 women with a mean age of 43.4 ±15.8 years and a mean time on hemodialysis of 27.2 ± 26.0 months. The primary kidney disease causing chronic renal failure was chronic pyelonephritis in 8 patients, chronic glomerulonephritis in 6, hypertension in 1, autosomal dominant polycystic kidney disease in 1, nephrectomy due to renal cell carcinoma in 1, acute renal failure in 1 and unknown in 3. None of the patients was known to have pre-existing hemostatic disorders unrelated to uremia, infections or had received any medications known to affect platelet function for at least two weeks prior to the study. Fifteen patients were excluded due to unstable clinical conditions, cardiac and vascular instability, positive history for first use syndrome, unstabilized erythropoietin dosage or single needle dialysis.

Study Design

The study design is depicted in **Fig.** 1. It was conducted in a prospective crossover fashion to reduce the individual variability. The patients were sequentially hemodialyzed by cuprophan (Renak-E RE-15H Cuprophan, Kawasumi Laboratories Inc., Tokyo, Japan) and polysulfone hollow-fiber dialyzer (Hemoflow, F7, Fresenius Polysulfone, UF 7.5, Fresenius AG, Bad Homburg, Germany) for a month with each membrane. No hemodialysis prescriptions other than the dialyzer selection were changed during the study period. The blood flow rate was 200 mL/min and the dialysate flow rate was 500 mI7 min in every patient. Anticoagulation was achieved by means of a loading dose and constant infusion of standard heparin. The dose of heparin varied from patient to patient but was held constant for each individual during the study period. A new dialyzer was used for each dialysis session to exclude the effects of reuse. Hemodialysis was performed for 4 hours thrice a week. All patients in this study had arteriovenous fistulas. Arterial and venous fistula set (16G x 1", 35 mm) bloodlines were used (Kawasumi Laboratories Inc, Tokyo, Japan).

Collection of Samples

Blood sampling

In the last hemodialysis during each membrane period, blood samples were taken from arterial lines at the start of hemodialysis, and five minutes after the termination of blood flow through the dialyzer at the end of hemodialysis, without a tourniquet and using a plastic syringe with a 21-gauge needle. The samples for analysis of platelet were carefully transferred into tubes containing 3.8% sodium citrate (Becton Dickinson, Rutherford, N.J., USA).

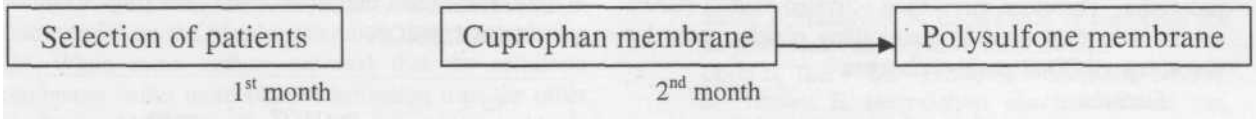

Figure 1. Study design, (n=21).

Platelet Counting

Platelet counting was performed using automated whole blood counter device based on double study principles (Coulter STKS - Coulter Electronics Ltd.,

Northwell, England).

Monoclonal Antibodies

FTTC-conjugated mouse monoclonal antibodies to human platelet glycoprotein Ib (CD42a) [anti-CD42a-FTTC, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA] we used to confirm the particles in the investigation area are platelets and FITC-conjugated mouse monoclonal antibodies to platelet P-selectin (CD62) [anti-CD62-FITC, Immunotech International, Marseille, France] we employed to determine activated platelets.

Flow Cytometric Analysis

For flow cytometric analysis, a whole blood method was used. Within 10 minute of collection, a 5 mL of blood was added to a tube containing 50 mL of Hepes-buffered saline (0.145 mol/L NaCl, 5x10-3 mol/L KC1,1x10-3 mol/L MgSO4, 0.01 mol/L Hepes, pH 7.4) and 5 mL of monoclonal antibody (anti-CD42a-FITC or anti-CD62-FITC or negative isotypic control). The samples were incubated at room temperature for 20 min, and then diluted and fixed by the addition of 0.5 mL of 0.2% formaldehyde in 0.9% NaCl. Within 2 hours of fixation samples were analyzed by flow cytometer (Epics Profile II, Coulter Electronics, Inc., Hialeah, FL, USA).

For one colour analysis, the platelets were distinguished from erythrocytes and leukocytes on the basis of their forward and right angle light scatter characteristics. Debris and electronic noise were excluded by setting the appropriate forward scatter threshold. A gate was set around platelets, so that more than 95% of events were CD42a positive (percentage of CD42-positive particles). 20 000 cells were analyzed for FTTC fluorescence to quantify the percentage of CD62-positive events. Mean channel number is used for FITC intensity of CD62 being an indicator of quantity of CD62 molecules on every platelet (MCN62). We thought that the percentage of CD62-positive platelets might be affected by changes in platelet counts appearing due to hemodialysis procedure. Therefore, circulating activated platelet (CAP) counts were calculated by multiplying platelet count by percentage of CD62-positive platelets.

Statistics

Statistical analysis was performed using the statistical package SPSS. Data are expressed as mean values ± SEM. Platelet activation parameters measured predialysis and postdialysis were compared by paired t-test or Wilcoxon's signed-rank test. A p value below 0.05 was considered significant.

RESULTS

Percentage ofCD42 and CD62 Positive Platelets

The activation of circulating blood platelets was assessed by surface expression of platelet glycoproteins using monoclonal antibodies (see the discussion of materials and methods).

There was no significant change in postdialysis percentages of CD42 positive platelets and CD62 positive platelets compared to predialysis percentages by both membranes.

Intensity ofCD62 on Every Platelet

Postdialysis mean channel number meaning intensity of CD62 on every platelet increased significantly compared to predialysis level by cuprophan membrane (p=0.01). There was no significant change in mean channel number by polisulfone membrane. On the other hand, both predialysis and postdialysis mean channel numbers by polysulfone membrane were higher than those of cuprophan membrane. **(Fig. 2).**

Change in the Count of Circulating Activated Platelets

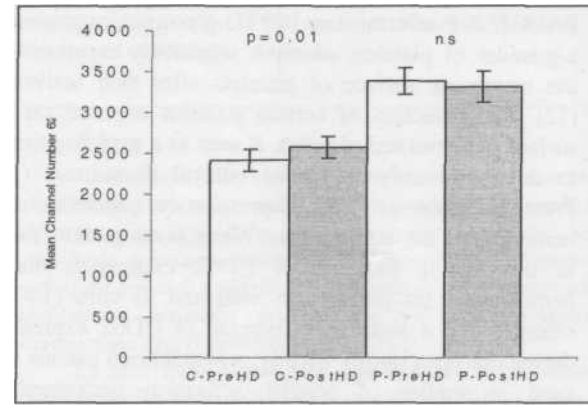

Figure 2. MCN62 values before and after hemodialysis with both membranes (MCN62: Mean channel number 62, C: cuprophan, P: polysulfone, PreHD: predialytic, postHD: post-dialytic, ns: nonsignificant).

Platelets

Postdialysis circulating acticated platelet count, which is calculated by multiplying platelet count and the percentage of CD62 positive platelets, increased significantly in comparison to predialysis level by cuprophan membrane (p=0.037). Despite there was an increase in postdialysis CAPC by polysulfone membrane, it did not reach to a statistical significance **(Fig. 3)**

DISCUSSION

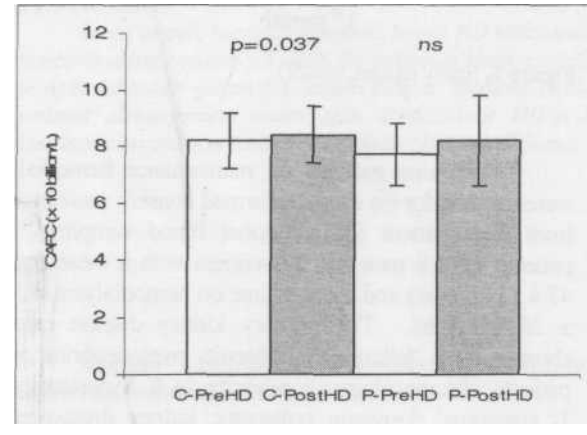

Figure 3. Circulating activated platelet counts (CAPC) before and after hemodialysis with both membranes.

In this study we found that cuprophan membrane induces greater platelet activation compared to polysulfone membrane if the intensity of activation-dependent antibody on platelets (MCN62) is used as an index of platelet activation. Cuprophan membrane also induced a significant increase in postdialysis circulating activated platelet count in comparison to predialysis measurement.

This study also has some limitations. Postdialysis blood sampling time is different from other studies. In the other studies, postdialysis blood sampling time was generally between 5 to 60 minutes during dialysis. So, this study might not show absolute platelet activation during hemodialysis.

In a recent study, Kawabata et al (6) used flow cytometric technique with whole blood. They showed that the percentage of PAC-1 (which recognizes only the conformationally activated GPUb/IIIa) -positive platelets were significantly greater during hemodialysis with regenerated cellulose membrane than with polysulfone. However, changes in the percentage of CD62-positive platelets were not significantly different between hemodialysis with regenerated cellulose and polysulfone.

All other studies using antiCD62 antibodies by flow cytometry about platelet activation in hemodialysis patients until now were usually performed with platelet rich plasma. However, centrifugation and washing itself can cause platelet activation during preparation (6,21). The results of the studies carried out in platelet rich plasma before, are not uniform. While some authors failed to observe an increase of CD62 expression during hemodialysis on platelets in vivo and in vitro (14-17), others found a significant increase of CD62 expression during dialysis (18-20). The contradictory results among these studies could here resulted from the different monoclonal antibodies used and different blood sampling. In addition, using PRP or whole blood can cause different findings. Moreover, the role of the membrane type is controversial for the platelet activation that occurs during HD. While some authors reported that the cellulosic membranes cause more platelet activation than the other membranes (4,22,23), others reported that they could not find any difference (15,24).

In a recent study, Gawaz et al (25) compared cuprophan, hemophan and polysulfone membranes in platelet activation using anti-CD62 and antifibrinogen binding. They showed that platelet activation occurred only with cuprophan. However, they studied in platelet rich plasma.

This study shows for the first time platelet activation according to the increase in intensity of CD62 with whole blood study in dialysis patients. Thus, induction of platelet activation by cuprophan membrane is confirmed even in whole blood study. However, when the patients were dialyzed by polysulfone membrane, they had higher predialysis and postdialysis MCN62 than those of cuprophan membrane. We do not know the explanation of this finding. Perhaps, this might show a chronic platelet activation rather than acute platelet activation during hemodialysis procedure by polysulfone membrane.

Interestingly, platelet activation and platelet derived growth factor release have recently been shown to play a key role in the development of advanced atherosclerotic lesions (26-28). Tveit et al (29) also showed that chronic dialysis patients have high risk for pulmonary embolism, independent of comorbidity. Therefore, the issue of platelet activation by dialysers deserves reconsideration.

While HD with cuprophan causes platelet activation, it does not occur with polysulphon membrane. To estimate the clinical implications of this finding, it needs further studies. However, we suggest that platelet activation by hemodialysis membranes can explain partly the high incidence of atherosclerosis in dialysis patients. Hemodialysis with membranes which cause platelet activation, may have an additional effect to morbidity such as thromboembolic events for the patients undergoing hemodialysis. Using biocompatible membranes as polysulphon can be advantageous at this viewpoint.

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