Effect of Dialyzer Membranes on Beta-2 Microglobulin Production in Thai Hemodialysis Patients

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Abstract

Responses to different types of dialyzer membranes in an Asian population may differ from those of a Caucasian population. Comparative studies on the effects of different dialyzer membranes on beta-2 microglobulin production are also limited. Therefore, we conducted this study to determine the effects of different dialyzer membranes on *in vitro* mononuclear cell production of beta-2 microglobulin in 9 Thai hemodialysis patients. Each patient was dialysed with 4 different types of dialyzer, including cuprophane (CUP), cellulose diacetate (CD), polysulphone (PS), and polyacrylonitrile membrane (PAN), each for a 1-month period in a randomized sequence. Mononuclear cell culture was done by taking an immediate post-dialysis blood sample at the end of the 1-month period. Beta-2 microglobulin production from cell culture was determined 24 hours later. Mononuclear cell culture and determination of beta-2 microglobulin production from the culture were also done in 10 normal controls and 10 predialysis ESRD patients. The beta-2 microglobulin productions (μg/L) were shown as follows;

	Control	CUP	CD	PS	PAN
Normal controls	169±18*	-	-	-	-
Predialysis ESRD	162±29*	-	-	-	-
Hemodialysis pateints	-	268±47	198±4	175±40*	173±20*

(* $p < 0.05$ compared to cuprophane membrane)

Conclusion: polysulphone and polyacrylonitrile membrane induced significantly less beta-2 microglobulin production compared to cuprophane and slightly less compared to cellulose diacetate membrane.

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Biocompatibility in hemodialysis can be defined as the sum of specific interactions between blood and the artificial materials of the hemodialysis circuit. Because the components of the hemodialysis procedure are "foreign" to the body, the interaction of blood with these components is best described as an inflammatory response. When this response is mild and well tolerated, the material can be termed biocompatible. When it is intense, it may adversely affect patient well-being or lead to deleterious outcomes. Importantly, in the chronic hemodialysis patients these interactions are repetitive and occur two to three times a week. Therefore, even mild interactions may, on a chronic basis, lead to adverse clinical sequelae.

Regarding dialyzer membrane, cellulose-based materials were used for the manufacturing of hemodialysis membranes and remain to date the most commonly used membranes. Cuprophane membrane is the prototype of cellulosic membrane. Various modifications to the repeating polysaccharide structure of cellulosic membranes are available. These alterations have resulted in relatively minor changes in the characteristics of the membrane, for example, cellulose acetate membrane, in which the polysaccharide structure is modified by replacement of hydroxyl ions with acetate radicals. In addition, several synthetic materials have been brought to clinical practice and, in general, are distinguished by a decrease in the intensity and specificity of the blood membrane interaction. Hydrophobic synthetic membranes, including polysulfone (PS), polymethylmethacrylate (PMMA), and polyacrylonitrile (PAN), have been recently introduced into the market with the hope that these new membranes will result in better clinical outcomes. However, this remains to be proved, especially in long term outcomes.

During contact of blood with the hemodialysis membrane, several homeostatic reactions are activated. These include the complement cascade[1] and the coagulation cascade[2]. In addition to these protein-mediated pathways, increasing evidence suggests that cellular mechanism can also be activated during hemodialysis. Recent works have documented the activation of neutrophils, leading to the up-regulation of adhesion receptors[3,4], releasing of proteinases and other intracellular enzymes, reactive oxygen species[5], leukotrienes[6], and platelet activating factor, as well as the activation of monocytes leading to the production of monokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF)[7-9]. Monocyte-membrane interaction results in beta-2 microglobulin mRNA transcription and increase beta-2 microglobulin protein production from monocytes[10]. One may take the transcription on the beta-2 microglobulin gene or beta-2 microglobulin production, as well as cytokines production, from monocyte as an index of cell activation or biocompatibility[11].

The clinical sequelae which arise from blood membrane interactions are numerous. Yet, cuprophane membrane, which produce significant complement activation, are still widely used despite the development of more biocompatible membranes such as polyacrylonitrile or polysulphone. A number of adverse events occurring during the hemodialysis treatment have been attributed to the use of bioincompatible membranes. Acutely, hypersensitivity reactions are seen in those individuals who demonstrate higher complement activation following exposure to cuprophane membranes as compared to patients who are asymptomatic[12]. Cytokines, induced during hemodialysis, may be responsible for febrile episodes and catabolic events[13]. More important are the emerging long-term metabolic and clinical consequences of chronic recurrent adverse blood membrane interactions. It is likely that some of the morbidity and mortality which our hemodialysis patients experience is due to the use of bioincompatible membrane[14].

Development of amyloid deposits consisting of beta-2 microglobulin may be another consequence of the use of bioincompatible membranes[15]. Beta-2 microglobulin amyloid arthropathy is a disabling syndrome seen in many chronic hemodialysis patients. Some of its manifestations, such as the development of carpal tunnel syndrome, have become evident in more than 25 per cent of patients after five years of hemodialysis, and increase in incidence even further in those patients maintained on dialysis for a longer duration[16]. Recent studies have indicated that the incidence of this disorder is significantly diminished in patients dialyzed with more biocompatible membranes such as polyacrylonitrile[17]. The possibility that this syndrome may be influenced by the type of membrane is also suggested by the fact that there is increased synthesis of beta-2 microglobulin by peripheral blood mononuclear cells harvested from patients after chronic hemo-

dialysis with cuprophane membranes[18]. In addition to causing disabling arthropathy, high local concentrations of beta-2 microglobulin could depress cytotoxic activity of lymphocytes against virally infected target cells[19]. Beta-2 microglobulin can also activate or suppress T cell line[20]. Therefore, beta-2 microglobulin may play a role in the suppressed immunity seen in hemodialysis patients.

Comparative blood-membrane interactions have been reported in several studies, in which modified cellulosic membranes or synthetic membranes were compared with cuprophane membrane. Few studies have been done to compare between different types of modified cellulosic or synthetic membranes. Moreover, most studies were done in a Caucacian population whose inflamatory response may differ from that of an Asian population. The costs of synthetic membranes are usually greater than those of cellulosic membranes. If the blood-membrane interactions are comparable between modified cellulosic and synthetic membranes, the routine use of synthetic membrane is not justified.

We, therefore, conducted this study to compare blood-membrane interactions induced by cuprophane, cellulose diacetate, polyacrylonitrile, and polysulphone dialyzer by using beta-2 microglobulin production from peripheral blood mononuclear cell culture after dialysis as an index of cell activation.

METHODS

Nine stable chronic hemodialysis patients participated in this study. Each patient was dialysed sequentially with 4 different types of dialyzer, including cuprophane, cellulose diacetate, polysulphone, and polyacrylonitrile dialyzer. The surface area for each type of dialyzer was comparable, 1.5 m^2 for cuprophane and cellulose diacetate, 1.6 m^2 for polysulphone and polyacrylonitrile. Each type of dialyzer had been used for a 1 month period in a randomized sequence before post-dialysis blood samples were taken for mononuclear cell culture. Beta-2 microglobulin production after 24 hours of cell culture was determined. All dialyzers were reused with standard reprocessing technique. For the session that the blood sample was taken for cell cultures, a brand new dialyzer of the same type of membrane was used to avoid effect of dialyzer reprocessing technique on this study. Mononuclear cell culture and determination of beta-2 microglobulin production from the culture was also done in 10 normal controls and 10 predialysis ESRD patients. Except the type of dialyzer, dialysis and water treatment systems were kept in the same condition throughout this study. Peripheral blood mononuclear cell were separated from heparinized blood on Ficoll Hypaque. Peripheral mononuclear cells were suspended at 1 x 10^7 cell/ml in Iscove's modified Dulbeco's media (Sigma, U.S.A.) with 10 per cent fetal calf serum (GIBCO) supplemented with 100 units/ml of penicillin and 100 μg of streptomycin. The peripheral mononuclear cell culture was incubated in 1 ml aliquot at 37°C in 5 per cent CO_2 under a humidified atmosphere for 24 hours[21]. The supernatants were collected for beta-2 microglobulin determination. Beta-2 microglobulin assay was performed by microparticle enzyme immunoassay (MEIA) using IMX set (Abbott Laboratories).

Statistical Analysis

All data were presented as Mean±SD. A comparison of means for multiple measurement was done by using one way Anova and post hoc analysis by using Scheff's method. Student-*t* test was used for a comparison of means from two independent samples. A p value of less than 0.05 was considered statistically significant.

RESULTS

The age was 43±5, 51±7, and 52±6 years in normal controls, predialysis ESRD, and hemodialysis patients, respectively. There were equal numbers of male and female in normal controls and predialysis ESRD groups. There were 6 males and 3 females in the hemodialysis group. During the study, there was no episode of febile reaction or other reaction that could be attributed to the blood-membrane interaction. Four episodes of cramp were reported which were related to over ultrafiltration. Beta-2 microglobulin productions from peripheral blood mononuclear cell culture are shown in the Table. The beta-2 microglobulin productions from normal controls and predialysis ESRD were comparable but less than those of hemodialysis patients, the differences were significant only when compared to the beta-2 microglobulin production after using cuprophane membrane. In hemodialysis patients, the beta-2 microglobulin productions was in the following sequence: cuprophane > cellulose diacetate > polysulphone =

Table 1. Beta-2 microglobulin production (μg/L) from mononuclear cell culture.

Group	Control	Cuprophane	Cellulose Diacetate	Polysulphone	Polyacrylonitril
Normal controls	169±18*				
Predialysis ESRD	162±29*				
Hemodialysis		268±47	198±41	175±40*	173±20*

* $p < 0.05$ compared to cuprophane membrane

polyacrylonitrile. However, the differences were significant only when comparing synthetic membrane, polysulphone and polyacrylonitrile, with cuprophane membrane.

DISCUSSION

Beta-2 microglobulin is a non-glycosylated, low-molecular weight protein (11.8kd) present at the surface of nearly all nucleated cells and is non-covalently associated with the heavy chain of the HLA class I complex(21). In healthy subjects beta-2 microglobulin, resulting from cellular shedding, is found free in the plasma. In its unbound state, more than 95 per cent of the protein circulates in a monomeric form. Beta-2 microglobulin is cleared from blood by glomerular filtration and it is reabsorbed by the proximal tubule where protein break down occurs(22). Plasma beta-2 microglobulin level is closely related to glomerular filtration rate in subjects with normal renal function(23). In patients with renal failure, plasma beta-2 microglobulin is increased and can be elevated up to 50-fold the normal range in those on renal replacement therapy. The rising of serum beta-2 microglobulin level in patients with impaired renal function results mainly from the decrease in renal excretion. In this study, the beta-2 microglobulin production from peripheral blood mononuclear cell culture taken from normal controls and predialysis ESRD patients were almost identical, suggesting against uremic state alone as a strong stimulator for beta-2 microglobulin production. By contrast, in hemodialysis patients, beta-2 microglobulin productions were greater than those produced by both normal controls and predialysis ESRD. The difference became greater and significant when patients were dialysed under cuprophane membrane. As the mononuclear cells for cell cultures were harvested immediately post-dialysis, the increase in beta-2 microglobulin production should result from a stimulation by dialysis membrane. The beta-2 microglobulin production was highest when using cuprophane membrane, followed by cellulose diacetate and lowest when using polysulphone and polyacrylonitrile. Our finding is in concordance with previous studies which demonstrated the strongest inducing effect on beta-2 microglobulin production by cellulosic membranes(10,11,24). This finding indicates a better biocompatibity of synthetic membranes. The differences in beta-2 microglobulin production found in this study are unlikely to have resulted from other effects, for example, dialysis water quality, dialysis technique, or dialysis time, as the conditions were unchanged, and should not be effected by the sequence of dialysis membrane types used in this study as patients were allocated randomly to be dialysed under different membranes. However, we can not demonstrate a significant difference between modified cellulosic membrane, cellulose diacetate, and synthetic membranes, polysulphone and polyacrylonitrile. This may be a result of beta error due to insufficient sample size. Further studies are needed to demonstrate long-term clinical outcomes related to the types or biocompatibility of dialysis membrane. In conclusion: polysulphone and polyacrylonitrile membranes induce significant less beta-2 microglobulin production compared to cuprophane and slighly less compared to cellulose diacetate membrane, suggesting more biocompatibity of polysulphone and polyacrylonitrile membrane.

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ผลของกระบอกฟอกเลือดชนิดต่างๆ ต่อการสร้าง beta-2 microglobulin ในผู้ป่วยฟอกเลือด

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การศึกษาปฏิกิริยาของร่างกายต่อกระบอกฟอกเลือดในผู้ป่วยเอเชียและการศึกษาเปรียบเทียบกระบอกฟอกเลือดชนิดต่างๆ ต่อการสร้าง Beta-2 microglobulin ยังมีอยู่ค่อนข้างจำกัด ด้วยเหตุนี้คณะผู้วิจัยจึงได้ทำการศึกษาโดยใช้ผู้ป่วยไตวายเรื้อรังที่รักษาด้วยการฟอกเลือด จำนวน 9 ราย โดยให้ฟอกเลือดด้วยกระบอกฟอกเลือดชนิดต่างๆ ได้แก่ cuprophane (CUP), cellulose acetate (CD), polysulphone (PS), และ polyacrylonitrile (PAN) แต่ละชนิดเป็นเวลา 1 เดือน ก่อนนำเลือดหลังฟอกเลือดมาทำ peripheral mononuclear cell culture เป็นเวลา 24 ชั่วโมง แล้ววัดระดับ Beta-2 microglobulin ใน supernatant นอกจากนี้ยังได้เปรียบเทียบผลกับคนปกติ 10 รายและผู้ป่วยไตวายระยะสุดท้ายก่อนรักษาด้วยการฟอกเลือดอีก 10 ราย beta-2 microglobulin (μg/L) ที่เกิดขึ้นหลัง 24 ชั่วโมงได้แสดงในตาราง

	Control	CUP	CD	PS	PAN
คนปกติ	169±18*	-	-	-	-
ผู้ป่วยไตวายก่อนการฟอกเลือด	162±29*	-	-	-	-
ผู้ป่วยฟอกเลือด	-	268±47	198±4	175±40*	173±20*

(* $p < 0.05$ เมื่อเทียบกับ cuprophane membrane)

สรุป การฟอกเลือดโดยใช้กระบอกฟอกเลือดที่ทำจาก Polysulphone หรือ Polyacrylonitrile กระตุ้นให้ร่างกายสร้าง beta-2 microglobulin น้อยกว่ากระบอกฟอกเลือดที่ทำจาก cuprophane

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