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THE ROLE OF PAF AND LEUKOTRIENES IN BIOINCOMPATIBILITY OF CUPROPHANE MEMBRANES IN HEMODIALYSIS*

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Inflammatory lipid mediators, PAF and leukotrienes (LTs), are thought to have an important role in biocompatibility in hemodialysis. PAF, LTB4 and LTC4 were studied both in controls (n: 12) and in 11 children on regular hemodialysis (150 minutes) with cuprophane dialyzers. Blood samples were collected initially (0'-precapillary), at first minute (1'-postcapillary) and at one hour after the hemodialysis sessions (210'-venous). Presence of LTs and high levels of PAF in 0' samples compared to levels in controls and significant increases in 1' samples suggested the alterations in PAF and LTs likely originated from the peripheral leukocyte activation. In 210' samples, PAF and LTs levels were decreased but still higher than the levels in 0' samples. This study suggested that PAF and LTB4 may be the control elements in biocompatibility in hemodialysis with cuprophane membranes, and demonstrated that the effects of activation last until the following session. *Key words: hemodialysis, children, biocompatibility, PAF, leukotrienes.*

Clinical hemodialysis (HD) with cellulose-based membranes, especially cuprophane, causes marked alterations in leukocyte and platelet functions and kinetics, and production phenomena that likely contribute to the morbidity of these procedures and to clinical immunosuppression in dialysis patients. A complex array of inflammatory mediators are generated as a consequence of blood contact with HD membranes. Besides complement activation, other mediators involved in cell activation are thought to possibly be responsible for early- and long-term changes in immunity, hypercatabolism, hemostatic mechanisms and susceptibility to infections[1-7].

Recent studies have established that cell membrane-derived new lipid mediators, such as leukotrienes (LTs) and platelet activating factor (PAF), might be generated by complement dependent or independent mechanisms of cell interaction with hemodialysis membranes[8-11]. LTs are biologically active 5-

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lipoxygenase products of arachidonic acid metabolism, and are involved in the mediation of various inflammatory disorders. Of these, LTB4, a potent chemoattractant for leukocytes, and the sulfidopeptide-leukotrienes LTC4, LTD4 and LTE4 which increase vascular permeability and constrict smooth muscle, exert their biological action through specific ligand-receptor interactions[12, 13]. PAF is the common name given to 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl-choline, a biologically active phospholipid. PAF intermits signals between cells and thus functions in a similar fashion to hormones, cytokines, interleukin and other signaling molecules[14-16]. It is now known that LTs and PAF are produced by a wide variety of isolated human cells, upon different immunologic and nonimmunologic stimulation[17, 18].

In this study, we have measured inflammatory lipid mediators just before, at the very beginning of hemodialysis and after the end of the session to clarify the effects of LTs and PAF on bioincompatibility of cuprophane HD membranes.

Material and Methods

Patients: Eleven patients (5 girls and 6 boys) with a mean age of 13.0 ± 3.6 years were included in this study. The patients studied were on regular bicarbonate-buffered HD with cuprophane membranes three times per week, and the sessions were 150 minutes using Gambro AK100 HD machines. The surface area of the dialyzers used (Renak E 08 U) was identical (0.8 m^2). All of the patients included gave informed consent to this study. Twenty healthy children with similar age and gender distribution were studied as the control group.

None of the patients had a history of anaphylactic reaction, autoimmune disease or clinical evidence of acute inflammation, and none had received medical treatment, such as angiotensin converting enzyme (ACE) inhibitors, corticosteroids, benzodiazepines or anti-inflammatory agents, known to have an effect on leukocytes or lipid mediators.

Study Protocol: In patients, blood samples were collected via sampling port proximal to the dialyzer initially (0'), distal to the dialyzer at the end of the first minute (1') of dialysis (to sample blood at first pass before it effects the endothelium and the vascular system), and from a vein an hour after the end of the session (210') to determine LTs, PAF and hemoglobin (Hb), leukocyte count, platelet count, urea, creatinine and C3 levels.

Sample Procedure: Whole blood count was developed by Sysmex K-1000 hemocounter. Plasma creatinine and urea levels were determined by autoanalyzer (Falcon 300), and nephelometry was used to determine serum C3 levels by Backman-Array Protein System analyzer.

PAF Extraction and Purification: PAF was extracted with solid-phase extraction method by XAD-2 Ambetlit columns. PAF was purified with HPLC method by Silasorb Si60, 5μ analytic (250x2.0 mm) columns.

RIA for PAF: The specificity and quantitative measurement of PAF from dried samples were checked using the platelet activating factor (PAF) (^{3}H) scintillation proximity assay system (TRK-990) from Amersham Life Sciences (UK). The dried PAF samples were reconstituted with 100 ml of kit working assay buffer and wirled intermittently in a vortex for about 10 minutes, before measurements. All determinations were done in duplicate.

PAF was measured according to the procedure recommended by the manufacturer. The labeled PAF which remained unbound at the end of the reaction was determined by a β-liquid scintillation counter (TRI-CARB-1600 TR, LSA Packard, Camberra Company) and results expressed as ng PAF/ml plasma. To know the recovery of PAF with the solid-phase extraction method (XAD-2 columns) and HPLC purification, plasma was obtained from five normal subjects and eight patients, mixed with 0.1 ml 0.1 nCi PAF (^{3}H) (Amersham, UK, TRK 743) and processed as described above. The recovery rate was 84.6 ± 2.3 percent (average of 13 experiments).

Extraction and Purification of LTs: LTs were extracted by using Sep-Pak C18 cartridge with solid-phase extraction method. LTs extracted from the plasma samples were separated from the eluent through Ultraphaser ODS C18 (5 micron) analytic column (250x4.6 mm ID) at 280 mm UV in Waters HPLC system.

RIA for LTs: The dried samples were separately solved for LTC4 and LTB4 in a 100 microliter kit buffer. The sensitivity of used kits was 10 pg/ml plasma for LTC4 and 5 pg/ml plasma for LTB4. LTs were measured by RIA kits according to the procedure recommended by the manufacturer. The concentrations of unlabeled LTs in samples were determined by a β-liquid scintillatilon analyzer system (TRI-CARB-1600 TR, LSA Packard, Canberra Company) and results were expressed as ng-ml plasma. The total recovery values of plasma LTs were 78.0 ± 3.6 percent for LTC4 and 84.1 ± 3.2 percent for LTB4. All assaying procedures were performed in duplicate.

Statistical Analysis: Statistical analyses were performed using paired t test to compare the consecutive data of the patients and using independent t test with controls. Data were given as mean ± SD.

Results

The levels of the inflammatory lipid mediators determined in controls and patients at 0', 1' and 210' are given in Table I. PAF levels were found higher in 0' samples compared with controls. PAF levels significantly increased in 1' samples compared with 0' samples, and slightly decreased (210' samples) one hour after the end of the HD sessions when compared with 1' samples. Nevertheless, PAF levels were still higher than controls and 0' samples (Fig. 1).

Table I: Levels of Lipid Mediators in Patients and Controls (ng/ml; mean ± SD)

	PAF	LTB4	LTC4
Control group	0.2 ± 0.1	*	*
0'	1.2 ± 0.7[a]	0.04 ± 0.018[a]	0.038 ± 0.023[a]
1'	4.8 ± 1.4[ab]	0.554 ± 0.296[ab]	0.23 ± 0.139[ab]
210'	3.3 ± 1.9[abc]	0.082 ± 0.068[ac]	0.097 ± 0.058[abc]

a: $p < 0.05$ compared to controls, b: $p < 0.05$ compared to 0', c: $p < 0.05$ compared to 1'.
* less than 0.002 ng/ml.

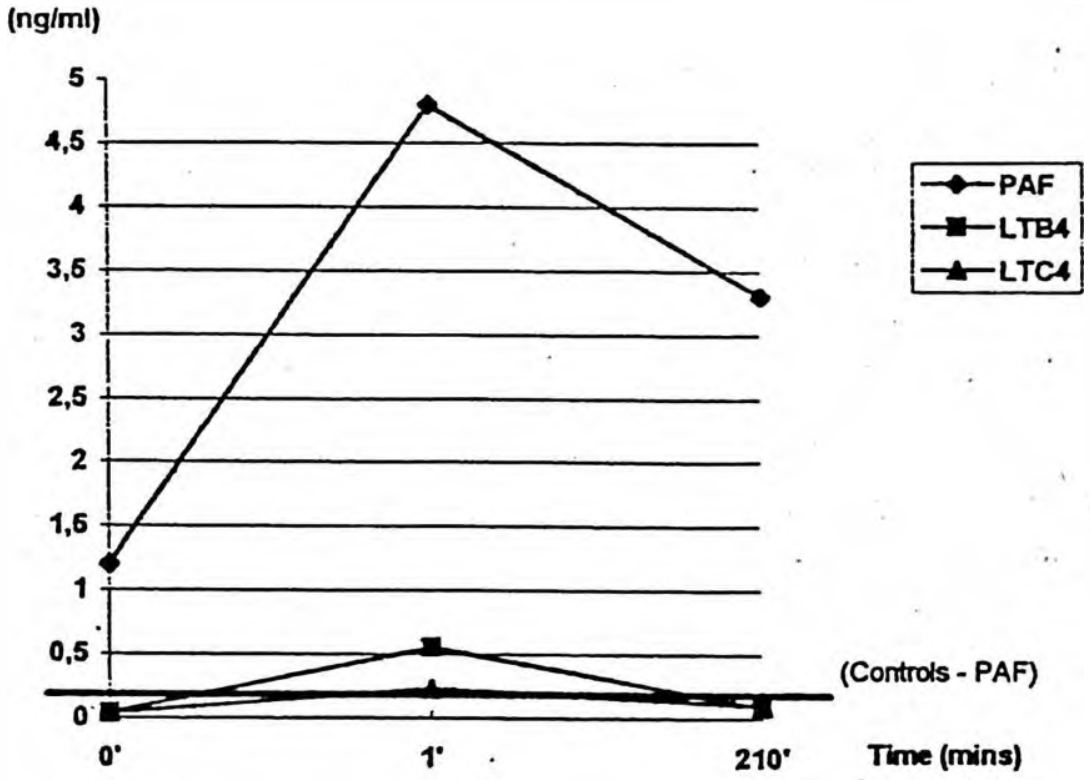

Fig. 1: Graphics of lipid mediators in patients and controls.

LTB4 and LTC4 were measured at detectable levels in 0' samples against the controls. LTs were significantly increased in 1' samples and then decreased to levels higher than the original baselines (Table I) (Fig. 1).

The average C3 level of the control group was significantly higher when compared to the values of patients at all stages. Although a slight decrease was detected in the mean C3 level in 1' samples compared to the initial value, there was no significant difference. C3 levels were found slightly over the initial levels (Table II).

The mean leukocyte count showed a non-significant reduction at 1' compared to 0' samples and controls. However, a rebound leukocytosis was detected in 210' samples, and this increase was statistically significant, (Table II).

Table II: C3 Levels and Leukocyte and Platelet Counts in Controls and Patients Before, During and After the Hemodialysis Session

	C3 (mg/dl)	PNL ($x10^3/mm^3$)	Platelets ($x10^3/mm^3$)
Control group	128.7 ± 29.5	7.7 ± 1.6	275 ± 90
0'	81.9 ± 18.2[a]	6.1 ± 1.7	212 ± 53
1'	77.0 ± 15.0[a]	5.1 ± 0.8[a]	176 ± 51[ab]
210'	84.9 ± 26.5[a]	6.5 ± 2.1[c]	244 ± 54[bc]

a: $p < 0.05$ compared to controls, b: $p < 0.05$ compared to 0', c: $p < 0.05$ compared to 1'.

A significant decrease in platelets in 1' samples occurred when compared to initial count (0') and controls. Similar to leukocytes and C3 levels, the platelet count was increased in 210' samples over the initial levels (0'). The rebound detected in the platelet count was also significant, (Table II).

Discussion

It is known that several reactions are activated during hemodialysis when blood is exposed to artificial surfaces. Cuprophane hemodialysis membranes have been shown to activate several pathways of inflammatory response, as well as cellular elements such as granulocytes, monocytes and platelets. To clarify the bioincompatibility of cuprophane membranes, changes in some biological markers, such as activation of the complement system and degree of leukopenia, are studied as parameters. However, the assessment of bioincompatibility is complex. The role of new lipid inflammatory mediators originated from cell membrane, PAF and LTs, has been studied more recently.

The important role of the complement system in bioincompatibility was noted as the probable cause of granulocytopenia during hemodialysis[4, 19, 20]. The detection of activation of the complement system with C3a and C5a but without C4a, generated from the classical pathway, suggests the activation occurred via an alternate pathway[4, 21]. Maximum complement activation takes place at 15' and lasts till 90' especially with cellulosic membranes[22]. In this study, only C3 levels were determined; the changes we noted during and after the HD sessions were nonsignificant. Since the complement system activation has been well known with cellulosic membranes, the observations in this study may suggest that C3 activation was minimal at the beginning of HD and had finished before the 210' samples were collected. Thus, C3 levels could not be used as an early index of membrane biocompatibility.

Leukopenia, mainly with a reduction in granulocytes and monocytes, also takes place at 15' maximally and may be followed by a rebound during dialysis with incompatible membranes[22, 23]. In this study, a slight decrease in leukocytes was observed at 1' compared to 0' samples, though it was not significant. In 210' samples there was a minimal increase in the leukocyte count. It is likely that

a notable reduction in leukocytes, reported in literature with cellulosic membranes, might have happened after we had bled the patients at 1'. The data we observed showed that neither serum C3 levels nor leukopenia could be an early index to evaluate the membrane biocompatibility.

Although there is some conflicting data in literature, platelet counts have frequently been reported as decreased with cellulosic membranes[24, 25]. We observed significant changes in the mean platelet count at 1' and 210'. Considering the data on cellulose membranes in the literature and the results of this study, the effects of the contact between blood and cuprophane membranes on platelets might begin even in the first minute of contact.

It is known that LTs and especially PAF are generated by complement dependent or independent mechanisms after cell interaction with HD membranes[7]. Furthermore, lipopolysaccharides originated from dialysate solutions may generate PAF via monocyte adhesion to membranes[26, 27]. The data on effects of dialyzers on PAF and LTs is limited to the 1st, 5th and 30th minutes. Alterations in PAF levels were first reported by Ward (1991)[28] within the first five minutes, and at first contact by Tetta et al. (1993)[8]. In this study, the presence of LTs and significantly high levels of PAF in 0' samples compared to controls and the significant increases of LTB4 and PAF in 1' samples compared to 0' samples were noted. An almost ten-fold increase in LTs in the 1' samples, collected subsequent to blood-dialyzer contact and prior to return to the patients' vascular system, suggests cuprophane HD membranes activate the peripheral leukocytes. Since the only contact of 1' samples was with HD membranes and no other contact had taken place with other lipid mediators releasing tissues as endothelium, the detected alterations in PAF and LTs likely originated from the activation of peripheral leukocytes.

Although the levels of the inflammatory lipid mediators were lower in 210' samples compared to the previous samples, they did not return to baseline levels. Therefore it is likely that the lipid mediators activate endothelial cells via three possible pathways: i) by circulating lipid mediators, ii) by lipid mediators in activated leukocytes, iii) by factors, such as PAF and endothelin, released from activated endothelium.

The results we observed in the study may suggest that inflammatory lipid mediators are generated first by peripheral leukocytes after cell interaction with HD membranes and then by endothelium activation. It is likely that endothelium activation continues till the following HD session though it is tapering off. The data in this study suggests that PAF and LTB4 released from stimulated leukocytes may be the control elements in bioincompatibility in HD with cuprophane membranes, and that the effects of activation last at least until the next session.

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