

Determination of serum aluminum, platelet aggregation and lipid peroxidation in hemodialyzed patients

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Abstract

Aluminum (Al³⁺) overload is frequently associated with lipid peroxidation and neurological disorders. Aluminum accumulation is also reported to be related to renal impairment, anemia and other clinical complications in hemodialysis patients. The aim of the present study was to determine the degree of lipid peroxidation, platelet aggregation and serum aluminum in patients receiving regular hemodialytic treatment. The level of plasma lipid peroxidation was evaluated on the basis of thiobarbituric acid reactive substances (TBARS). Mean platelet peroxidation in patients undergoing hemodialysis was significantly higher than in normal controls (2.7 ± 0.03 vs 1.8 ± 0.06 nmol/l, $P < 0.05$). Platelet aggregation and serum aluminum levels were determined by a turbidimetric method and atomic absorption spectrophotometry, respectively. Serum aluminum was significantly higher in patients than in normal controls (44.5 ± 29 vs 10.8 ± 2.5 µg/l, $P < 0.05$). Human blood platelets were stimulated with collagen (2.2 µg/ml), adenosine diphosphate (6 µM) and epinephrine (6 µM) and showed reduced function with the three agonists utilized. No correlation between aluminum levels and platelet aggregation or between aluminum and peroxidation was observed in hemodialyzed patients.

Key words

- Aluminum
- Peroxidation
- Hemodialysis
- Platelets

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Introduction

Chronic renal failure is characterized by a progressive and irreversible loss of renal function. Aluminum intoxication has been a serious problem in some dialysis units (1). Nowadays its pathogenesis is well understood and there is general agreement that aluminum overload occurs due to aluminum from the dialysate and to aluminum used for

phosphate binders (2). This condition generally occurs in long-term dialysis patients and is characterized by speech abnormalities, stuttering, seizures, myoclonus, personality changes, hearing loss, visual hallucinations, and accelerated aging. Aluminum accumulation is also thought to be related to renal impairment, anemia and other clinical complications in hemodialysis patients (1,3). Furthermore, these patients present enhanced

levels of plasma lipid peroxidation compared to healthy individuals (4) and also develop atherosclerosis (5). Some manifestations, such as accelerated aging and atherosclerosis, may be related to the hyperproduction of free radicals (6,7).

Although aluminum has no redox capacity, it can stimulate tissue lipid peroxidation in animal models (8) and the cytotoxic end products of lipid peroxidation may cause platelet dysfunction. Nevertheless, reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide and hydroxyl radicals are generated by platelets and can damage the plasma membranes by triggering polyunsaturated fatty acid peroxidation, membrane receptor and enzyme inactivation, polysaccharide depolymerization and protein cross-linking and fragmentation (9).

Previous studies have reported activation of phospholipase A₂ and phospholipase C by ROS (10). Furthermore, aluminum in combination with fluoride, forming the fluoroaluminate complex (11), activates phospholipase A₂ and β -thromboglobulin release in human blood platelets (12). We have observed that aluminum induces lipid peroxidation and aggregation of human blood platelets (13). All of these events probably constitute the basis for the abnormalities found in chronic renal failure. The aim of the present study was to determine the degree of lipid peroxidation and platelet aggregation and the presence of aluminum in chronic renal failure patients submitted to long-term hemodialysis.

Material and Methods

Reagents

Collagen, adenosine diphosphate and epinephrine were purchased from Chronolog Corporation, Havertown, PA, USA, while malondialdehyde bis(diethyl acetal) was obtained from Aldrich Chemical Company Inc.

(Milwaukee, WI, USA). All other chemicals were of the highest quality available and were obtained from commercial sources.

Patients

The study was conducted at the Dialysis Center of Hospital de Caridade, and included 55 patients (aged 18 to 76 years) on maintenance hemodialysis who gave written informed consent to participate. The study was approved by the Ethics Committee of the University Hospital, UFSC, Florianópolis, SC, Brazil. All patients were submitted to hemodialysis at the same hospital for 4 h, three times a week and the water used was treated by the same dialytic process. A cellulose acetate dialyzing membrane was employed. The etiology of renal failure included chronic glomerulonephritis (15%), hypertensive nephrosclerosis (61%), polycystic renal disease (9%), reflux nephropathy (2%), and indeterminate causes (2%). The water used for the dialysis fluid was treated by deionization. The patients did not ingest acetylsalicylic acid or any other nonsteroidal anti-inflammatory drug for 3 weeks prior to the study and did not receive any aluminum-containing phosphate buffer. None of the patients received blood transfusion during the study or in the preceding week. Heparin was used as the anticoagulant during hemodialysis. Mean patient hematocrit was $27.7 \pm 3.8\%$, and plasma creatinine was 9.6 ± 19.2 mg/dl. The control group consisted of healthy donors (10 males and 10 females) aged 20 to 41 years, all were drug-free with mean hematocrit and creatinine values of $45 \pm 4.4\%$ and 0.8 ± 0.2 mg/dl, respectively.

Platelet isolation

Human blood platelets were collected into plastic tubes containing 3.8% sodium citrate. Platelet-rich plasma was obtained by centrifugation of citrated blood at room temperature for 6 min at 180 g.

Measurement of platelet aggregation

Platelet aggregation was determined by the turbidimetric method of Born and Cross (14) using a Net Lab aggregometer. Aliquots of 450 μ l of a platelet suspension were pipetted into small cuvettes and stirred at a constant speed of 1,000 rpm at 37°C. The aggregation-inducing agents used were 6 μ M adenosine diphosphate, 6 μ M epinephrine, and 2.2 μ g/ml collagen. The extent of aggregation (%) was recorded continuously for 5 min after addition of the agonist.

Measurement of lipid peroxidation (TBARS test)

TBARS tests were carried out on plasma by the method of Buege and Aust (15). Briefly, 200 μ l 12% trichloroacetic acid and 450 μ l 0.3 M HEPES, pH 7.4, were added to the plasma samples. The samples were centrifuged at 900 g for 10 min, and 450 μ l was extracted from the supernatant, to which an equal volume of 0.2 M TBA plus 1% butylated hydroxytoluene was added. This suspension was then incubated at 100°C for 15 min, and cooled to room temperature. Spectrophotometric absorption was then measured at 532 nm and malondialdehyde (MDA) concentration was calculated. Appropriate standard curves were prepared using malondialdehyde bis(diethyl acetal) as the MDA source. TBARS values are reported as nmol/l plasma.

Measurement of aluminum

Serum was removed and stored at -20°C for a period of 60 days. Serum aluminum concentration was determined by atomic absorption spectrophotometry (16).

Statistical analysis

The Mann-Whitney test was employed to estimate differences between groups, and

the Spearman correlation coefficient was determined. Differences were considered to be significant when the probability was $P < 0.05$. The statistical program InStat-2 was used for analysis.

Results and Discussion

The objective of the present study was to determine serum aluminum, peroxidation and platelet aggregation in patients receiving regular hemodialysis treatment. We observed a significant reduction in platelet aggregation in these patients compared with the healthy control group (Table 1). This result is not due to the differences in platelet concentrations of the two groups, since their mean values were not significantly different. On the other hand, the hematocrit value has been shown to interfere with platelet aggregation. It is known that an increase in the value of this parameter can inhibit platelet function *in vitro* since a greater amount of citrate is left in the serum, causing a reduction in calcium levels and consequently leading to platelet inhibition (17). The patients of our study presented hematocrit values of 27.7%, a fact that invalidates this possibility. However, hypofunction of platelet aggregation may have occurred in hemodialyzed patients since a higher tendency towards a reduction in platelet function was observed among them (Table 1). Indeed many chronic renal failure patients present a reduction in platelet function (18-21). However, the

Table 1. Platelet concentration and aggregation.

	Control (N = 20)	Patients (N = 55)
Platelets $\times 10^3/\text{mm}^3$	206.44 \pm 48.2	200.59 \pm 52.4
Aggregation		
Collagen (2.2 μ g/ml)	77.5 \pm 7.2	52.0 \pm 21.0*
Epinephrine (6 μ M)	76.5 \pm 6.3	48.5 \pm 23.0*
ADP (6 μ M)	78.0 \pm 8.4	54.0 \pm 20.5*

Platelets were stimulated with collagen, epinephrine and adenosine diphosphate (ADP). Data are reported as means \pm SD.

* $P < 0.05$ compared to the control group (Mann-Whitney test).

mechanisms involved in this process have yet to be understood. Sreedhara et al. (18) observed a reduction in the availability of GPIIb-IIIa membrane receptors in uremic patients. According to Gralnick et al. (19), the reduced platelet aggregation could be ascribed to a reduction in von Willebrand factor levels. In our study, the primary aggregation response to the three agonists studied displayed normal levels (data not shown), ruling out the possibility of a problem at the receptor level. However, the secondary aggregation response was significantly inhibited, suggesting *in vivo* activation in these patients, with the consequent plasma release of peroxidation products. Indeed, a significant increase in the concentration of plasma MDA was observed in these patients (Table 2).

Among patients presenting chronic renal

Table 2. Serum aluminum and malondialdehyde (MDA) production.

	Control (N = 20)	Patients (N = 55)
Aluminum ($\mu\text{g/l}$)	10.8 \pm 2.5	44.5 \pm 29.0*
MDA (nmol/l)	1.8 \pm 0.06	2.7 \pm 0.03*

TBARS tests were carried out on plasma and aluminum concentration was measured in serum. Data are reported as means \pm SD.

*P<0.05 compared to the control group (Mann-Whitney test).

Table 3. Spearman correlation coefficients between aluminum concentration and malondialdehyde (MDA) production and platelet aggregation.

	Aluminum
MDA	-0.15
Platelet aggregation	
Collagen	0.13
Adenosine diphosphate	0.23
Epinephrine	0.32

No significant correlation was found (P>0.05, N = 55).

failure, whether undergoing hemodialysis or not, several factors such as the lipid composition of platelet membranes (20), lipoprotein abnormalities (6,22), types of dialysis membranes (23) and accumulation of aluminum can trigger platelet activation. It has been reported in the literature that chronic renal failure patients undergoing prolonged hemodialysis show increased levels of aluminum in their sera (24-27). Although this metal does not display redox capacity, it is capable of inducing peroxidation in cerebral tissue (8) and platelet aggregation *in vitro* (13). In addition, a high level of this metal is also associated with pathologies such as Alzheimer's encephalopathy, amyotrophic lateral sclerosis and ageing (28,29). Vecino et al. (29) reported an increase in the cholesterol/phospholipid ratio in the platelets of uremic patients, which was correlated with a hyperreactive state, even though a platelet hypofunction was detected *in vitro*.

In the present study we sought to measure the aluminum levels of hemodialysis patients and the implication such levels could have for hemostasis function. The hemodialysis patients exhibited significantly higher aluminum levels than the controls. However, we found no correlation between high aluminum content and peroxidation or platelet aggregation (Table 3), as reported in other studies (13,30). It is noteworthy that the increased plasma peroxidation events do not reflect the production of MDA exclusively from platelets, but also from other cell elements such as erythrocytes (6) and leukocytes (7). The results reported in the latter study also suggested that patient platelet dysfunction is the result of a multitude of factors rather than the consequence of aluminum accumulation. However, although a clear correlation between aluminum and platelet dysfunction is yet to be demonstrated, it is reasonable to suppose that *in vivo* accumulation of aluminum may increase the effects of ROS occurring in these patients. Although aluminum has no redox capacity it can cause

alterations at the membrane level, facilitating lipid peroxidation. Gutteridge et al. (30) have shown that aluminum salts do not directly stimulate peroxidation, but instead accelerate iron- and hydrogen peroxide-dependent lipid peroxidation in phospholipid liposomes and human erythrocyte membranes. On the other hand, superoxide and hydrogen peroxide are known to induce plate-

let aggregation and these cells are known to produce superoxide and hydrogen peroxide (31).

Our data show that patients undergoing hemodialysis present platelet dysfunction and lipid peroxidation. They also demonstrate that increased levels of serum aluminum do not correlate with lower platelet aggregation.

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