PERIPHERAL BLOOD MONONUCLEAR CELL MEMBRANE LIPID PEROXIDATION IN HAEMODIALYSIS PATIENTS

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Summary

This study shows that a peroxidative damage, similar to that previously detected by the increase of malonyldialdehyde and the decrease of vitamin E in red blood cell membranes of haemodialysis patients, can also be observed in peripheral blood mononuclear cells of uraemic patients undergoing chronic haemodialysis. Such biochemical alterations of immunocompetent cell membranes could play a role in the impairment of immune responses in uraemic patients.

Introduction

We have previously reported in haemodialysis patients a significant increase of red blood cell malonyldialdehyde, a short chain aldehyde which is an intermediate product of the oxidation of polyunsaturated red blood cell membrane fatty acids [1]. Concomitantly a significant decrease in red blood cell vitamin E was observed, attributable to an increased consumption as anti-oxidizing agent. Vitamin E is the most important anti-oxidizing agent protecting red blood cell membrane fatty acids [2]. Parenteral administration of vitamin E in haemodialysis patients induces a significant increase in haematocrit [3]. We also recently reported normal function of the pentose-phosphate shunt in uraemic patients on continuous ambulatory peritoneal dialysis, but significantly reduced red blood cell membrane lipid peroxidation compared to uraemic patients undergoing chronic haemodialysis [4].

In this study the concentrations of malonyldialdehyde and vitamin E in peripheral blood mononuclear cells was measured in a group of haemodialysis patients and in a group of healthy controls.

Materials and methods

Ten uraemic patients undergoing maintenance haemodialysis (5 males and 5 females) were studied. All patients were on a free diet with normal and constant

essential fatty acid intake from the beginning of dialysis treatment and during this study. None of the patients were smokers. During the previous three months none of the patients received transfusions or any other therapy which might interfere with the assays. The mean age was 48 years (range 35–62 years). Primary renal disease included chronic glomerulonephritis (7 cases), interstitial nephritis (1 case), and polycystic kidney disease (2 cases). All patients underwent dialysis three times a week for four hours over an average period of 30 months (range 6–84 months). Dialysate flow rate (Q_D) was 480–500ml/min and blood flow rate (Q_B) was 280–300ml/min. A $1m^2$ parallel plate dialyser and a standard dialysate with an acetate concentration of 38mEq/L were used. Water was purified by passage through deionizing apparatus. The vascular access in all patients was by a Cimino-Brescia arteriovenous fistula. Informed consent to the study was obtained from all patients.

The control group consisted of 10 healthy blood donors (6 males and 4 females) with a mean age of 38 years (range 25–49 years). Three of them had moderate smoking habits (5–6 cigarettes/day), but none had a history of recent acute disease. All controls were on a free diet.

In haemodialysis patients heparinized arterial blood was obtained from the cannula immediately after its insertion. In controls venous blood samples were drawn in heparinized syringes. Blood samples for biochemical tests were stored in sterile plastic tubes and processed within one hour. Isolation and purification of peripheral blood mononuclear cells were performed according to Böyum [5], using a continuous density gradient (d=1077). Cells were collected and washed three times in Hank's salt solution. Isolated peripheral blood mononuclear cells were frozen to disrupt the cells stored at -80°C for two days until analysed. Samples from patients and controls were simultaneously processed in the same experiments. Before the test peripheral blood mononuclear cells were thawed and sonicated in order to ensure complete lysis of cells.

Peripheral blood mononuclear cell malonyldialdehyde was determined according to the technique of Stocks et al [6]. Peripheral blood mononuclear cell tocopherols were measured according to the method of Kajden et al [7]. Peripheral blood mononuclear cell malonyldialdehyde and tocopherols were expressed as μg/mg protein of cell lysate. Cell lysate protein concentration was determined according to Lowry's method [8].

Statistical analysis was carried out using Student's 't' test. Data are reported as mean ± standard deviation and p values less than 0.05 considered significant.

Results

The results are shown in Table I. Compared to controls peripheral blood mononuclear cell malonyldialdehyde was found to be significantly increased in the dialysis patients and peripheral blood mononuclear cell tocopherols significantly decreased.

Discussion

These data suggest an increased peroxidation of polyunsaturated fatty acids in peripheral blood mononuclear cell membranes, as has already been observed

TABLE I. Peripheral blood mononuclear cell malonyldialdehyde and vitamin E levels in haemodialysis patients and in controls

	Patients	Controls
Peripheral blood mononuclear cell malonyldialdehyde (μg/mg of protein cell lysate)	18.68±9.39[a]	11.39±3.28[b]
Peripheral blood mononuclear cell vitamin E (μg/mg of protein cell lysate)	3.00±1.67[c]	4.04±0.39[d]

Student's 't' test: a versus b: $p<0.025$; c versus d: $p<0.05$
Data are expressed as mean ± standard deviation

in red blood cells in haemodialysis patients [1], together with increased consumption of vitamin E as anti-oxidizing agent.

The peroxidation of cell membrane unsaturated fatty acids leads to the formation of fluorescent chromolipids derived primarily from phosphatidyl-ethanolamine and phosphatidyl-serine and high molecular weight protein aggregates derived from spectrine [9]. It has also been shown that addition of malonyldialdehyde to red blood cells in vitro induces the same alterations, at the membrane level, detectable in vivo after proxidative damage [9]. Effects of lipid peroxidation on cell membranes suggest that decreased cell deformability and increased rigidity are the direct consequence of this polymerization leading to altered cell membrane behaviour [9].

It is tempting to speculate that peroxidative damage of peripheral blood mononuclear cell membranes in haemodialysis patients could, by impairing their function, influence immune responses and the expression of functionally relevant membrane determinants.

Vitamin E may thus be a useful supplement in haemodialysis patients in order to reduce the peroxidative damage also seen in peripheral blood mononuclear cell membranes.

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