

POLYMORPHONUCLEAR LEUCOCYTE CHEMILUMINESCENCE AS AN INDEX OF MEMBRANE BIOCOMPATIBILITY

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Summary

A group of patients on chronic haemodialysis was studied during a single treatment with each of the following membranes: cuprophane, cellulose acetate, polyacrylonitrile (PAN). To evaluate their biocompatibility we examined polymorphonuclear leucocyte oxidative metabolism by chemiluminescence. Resting chemiluminescence and zymosan activated chemiluminescence were examined before and after one hour of dialysis. During cuprophane dialysis there is a significant increase in the resting value and a significant decrease in zymosan activated chemiluminescence. With cellulose acetate there is an increase in resting value and no difference in zymosan activated chemiluminescence. With PAN there are no significant variations in either determination. These data suggest the presence of polymorphonuclear leucocyte metabolic alterations during dialysis with cuprophane and cellulose acetate membranes. The lack of alteration with PAN suggests better biocompatibility of this membrane.

Introduction

Polymorphonuclear leucocytes are phagocytic cells and therefore they present a particular reactivity to foreign stimuli. The plasma membrane of the polymorphonuclear leucocytes is the site of stimulus transduction, where most of the surrounding agents act, inducing disturbances in the molecular organization with subsequent activation of various metabolic reactions. These include non-mitochondrial oxidative metabolism which can be excited by both ingested particulate matter (bacteria, zymosan particles etc) and non-phagocytizable stimulants (complement fragments such as C₅a, aggregated IgG, chemicals etc) [1]. This metabolic activation results in the production of large quantities of some highly reactive oxygen species (superoxide anion, hydrogen peroxide, hydroxyl radical, single oxygen, and hypochlorous acid) which on returning to the stable ground state emit energy in the form of photons detected as chemiluminescence [2].

Thus chemiluminescence measurement provides a means of quantifying polymorphonuclear leucocyte oxidative metabolic activity, which can be evaluated in the resting state and during phagocytosis of opsonized zymosan particles.

The present study assesses if polymorphonuclear leucocyte chemiluminescence is affected by the interaction between blood and three different types of dialysis membranes, in order to evaluate differences in biocompatibility.

Patients and methods

Twelve patients (7 males and 5 females) with end-stage renal failure undergoing regular dialysis treatment were studied using three different types of dialysis membranes.

Their mean age was 55 ± 12 years (range 31–70), and duration of dialysis treatment was 53 ± 39 months (range 4–144). Diabetic patients were excluded from the study; all patients had no clinical evidence of infection, and none of them were taking drugs known to affect polymorphonuclear phagocytic activity. All patients were dialysed with an acetate bath, using blood lines of polyvinyl chloride and a Hospal Monitral Monitor. Dialysis membranes successively used for each patient were cuprophan hollow fibre dialyser (GF 80M, Gambro), cellulose acetate hollow fibre dialyser (C-DAK 3500, Cordis Dow), polyacrylonitrile parallel plate dialyser (Biospal 2400S, Hospal).

Blood samples were withdrawn from the arterial line at 0 and 60 minutes from the start of haemodialysis for polymorphonuclear leucocyte isolation. Polymorphonuclear leucocytes were purified as described by Böyum [3]. One part of heparinized blood (10 IU of heparin/ml of blood) was mixed with three parts of Hank's balanced salt solution (HBSS), then layered over Lymphoprep (Pharmacia, Uppsala) to separate and discard mononuclear cells. Erythrocytes were removed using Emagel (Behring) as an aggregating agent. Cells were counted with Bürker haemocytometer, and the final suspension was adjusted to 1×10^6 polymorphonuclear lymphocytes per millilitre HBSS. Cell viability was determined by using trypan blue exclusion and was >95 per cent. Differential cell counting showed polymorphonuclear leucocytes to be >90 per cent.

Chemiluminescence measurement was carried out at 37°C with a Lumino-meter Analyzer (Pico-lite Packard) using $100 \mu\text{l}$ of cell suspension and $50 \mu\text{l}$ of luminol (Sigma) at a final concentration of $1 \pm 10^{-5} \text{M}$, for detection of resting chemiluminescence. Zymosan activated chemiluminescence was assayed by adding to $30 \mu\text{l}$ of Zymosan (Sigma) cell suspension and luminol. Zymosan is a polysaccharide derived from *Saccharomyces* cell walls and was used at a concentration of 6mg/ml , after being opsonized with normal pooled serum. Counts were obtained at three minute intervals up to 30 minutes after Zymosan addition and expressed as count per minute (cpm)/ 10^6 polymorphonuclear leucocytes.

Paired Student's 't' test was used for statistical analysis of data obtained.

Results

Figure 1 shows the time course of polymorphonuclear leucocyte chemiluminescence, both in resting state and during zymosan phagocytosis, for each membrane;

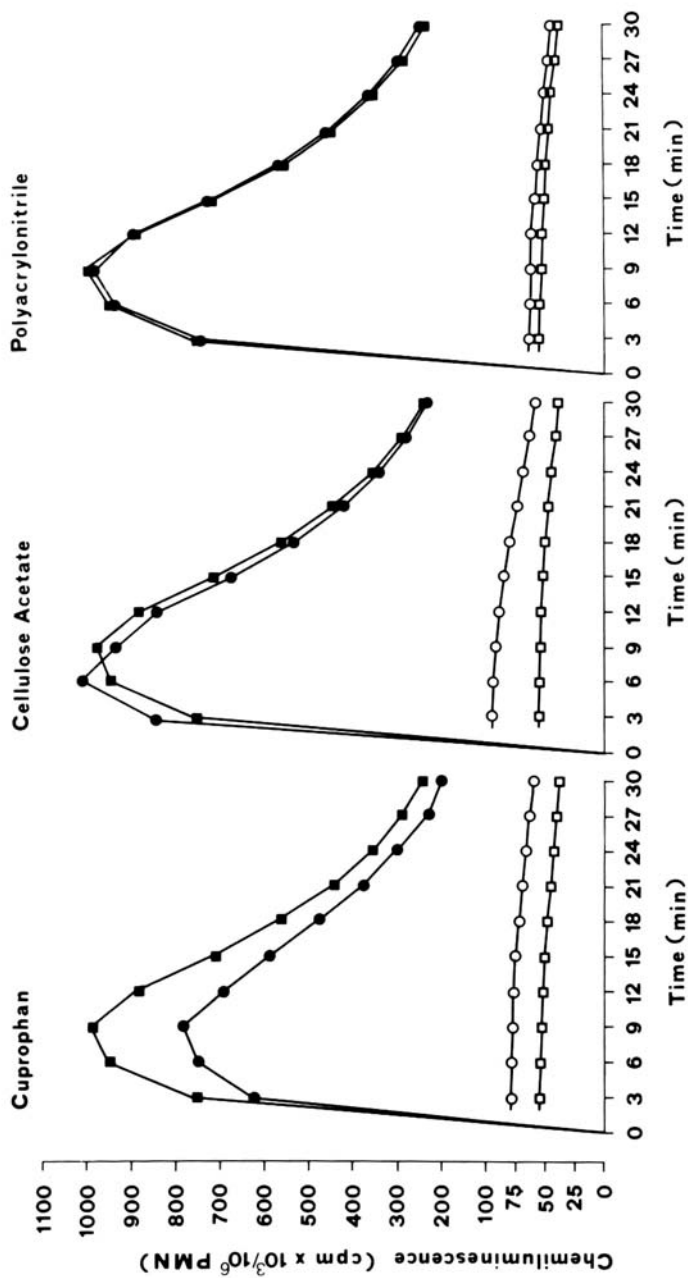


Figure 1. Time course of chemiluminescence, before and after 60 minutes of dialysis, with three different membranes. Open symbols represent data for resting chemiluminescence, while closed symbols refer to zymosan activated chemiluminescence. □ or ■ = before dialysis; ○ or ● = after one hour of dialysis

data obtained prior to start of haemodialysis are compared with those obtained after 60 minutes.

During cuprophane dialysis, resting values of chemiluminescence were higher, at every time interval, than those obtained prior to dialysis (maximum value $78 \pm 15 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes versus $55 \pm 6 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes; $p < 0.01$). Following phagocytosis of zymosan, a marked increase in light emission occurred, with peak values obtained at nine minutes and a gradual decline thereafter; the pattern of response is similar during and prior to cuprophane dialysis but the former curve is lower (peak value $790 \pm 50 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes versus $980 \pm 88 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes; $p < 0.01$).

Cellulose acetate membrane shows an increase in resting state chemiluminescence greater than that obtained with cuprophane (maximum value $95 \pm 17 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes versus $55 \pm 5 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes; $p < 0.01$). After zymosan addition, the peak value obtained during cellulose acetate dialysis is not significantly different from the predialysis value (peak value $985 \pm 90 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes versus $1015 \pm 92 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes), but it appears earlier, at six minutes instead of nine minutes.

During PAN dialysis there are no significant differences either in the resting state (maximum value $63 \pm 24 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes versus $54 \pm 6 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes) or during zymosan phagocytosis (peak value $999 \pm 95 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes versus $987 \pm 92 \text{cpm} \times 10^3/10^6$ polymorphonuclear leucocytes).

Discussion

Several biocompatibility phenomena observed in artificial kidney systems involve polymorphonuclear leucocytes. Previous studies have shown that profound and transient granulocytopenia, resulting from reversible pulmonary vascular leucosequestration, appears during the early phase of haemodialysis with cellulosic membranes, whereas polyacrylonitrile membranes provoke this phenomenon to a much lesser degree [4]. Furthermore, complement activation via the alternate pathway evaluated by C_3a radioimmunoassay techniques has been reported to occur to a lesser extent with PAN than with cellulose acetate and cuprophane [5].

In the present study we found no variations in polymorphonuclear leucocytes chemiluminescence either in the resting state or during phagocytosis, following polyacrylonitrile-blood interaction. These results indicate that this membrane has a lower bio-incompatibility.

Changes in resting chemiluminescence obtained with cuprophane and cellulose acetate indicate the presence of polymorphonuclear leucocyte metabolic alterations due to blood-membrane interaction. This causes an activation of the hexose monophosphate shunt with light emission and could be due to either a direct dialysis membrane polymorphonuclear leucocyte interaction, or a release of both particulate matter and chemicals from dialysers [6] acting on

cellular surface, or finally to C₅a which is a well-known activator of oxidative metabolism and binds avidly to polymorphonuclear leucocyte membrane [7].

Therefore these data with the mechanism involved underline the need to evaluate chemiluminescence on isolated polymorphonuclear leucocytes, since in whole blood, resting chemiluminescence is not detectable owing to interference of platelet or monocytic cell luminescence, or to red blood cell quenching [8].

The decrease in zymosan activated chemiluminescence with cuprophan, but not with cellulose acetate or PAN membrane may be explained by a polymorphonuclear leucocyte degranulation. This has been shown to occur particularly during cuprophan dialysis [9] with loss of various enzymes from the lysosomes; among these enzymes is myeloperoxidase, an intermediate in the chain reactions leading to chemiluminescence.

Our group has previously reported a reduction in granulopoiesis and in neutrophil granulocyte reserve in chronic haemodialysis patients with cuprophan membranes [10]. These observations, together with the deficiency in phagocytic activity observed during cuprophan dialysis, may assist in interpreting the well-known sensitivity to infection on the part of patients on chronic haemodialysis.

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