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Leucocyte Function Tests during Hemodialysis with Different Dialysis Membranes

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Introduction

It has been known for more than 10 years that by using Cuprophan membranes for dialysis a severe granulocytopenia is induced at the beginning of treatment, which reverses after about an hour [9, 11]. The cells are held back in the lung capillaries and partly return to the circulation later on. Dialysis membranes made from non-Cuprophan material like polyacrylonitrile (PAN), polymethylmethacrylate (PMMA) and cellulose acetate (CA) induce a milder granulocytopenia than Cuprophan [14]. It has been assumed that the reason for this phenomenon is an activation of the complement system by the alternate pathway and subsequent aggregation which leads to the removal of leucocytes from the circulation [1]. The sequestration of granulocytes is dependent on a temporarily altered cell function.

The purpose of our study was to examine whether Cuprophan and non-Cuprophan membranes have a different effect on several leucocyte function tests. Aggregation, chemotaxis and chemiluminescence of granulocytes were examined.

Patients and Methods

10 chronic dialysis patients were each dialyzed with hollow fiber dialyzers made from Cuprophan (Asahi AM 10/ AM 20), PAN (Rhone-Poulec AN 69), PMMA (Toray B2 M) and CA (C-DAK 4000). Blood was obtained from the arterial vein before and 15 and 60 min after starting dialysis. For the measurement of whole blood chemiluminescence 5 patients were dialyzed with dialyzers with Cuprophan membranes and PMMA. Blood was taken before and 15, 60, 120 and 240 min after the start of dialysis.

Blood Cell Count. The leucocyte count was performed with the model S Coulter counter.

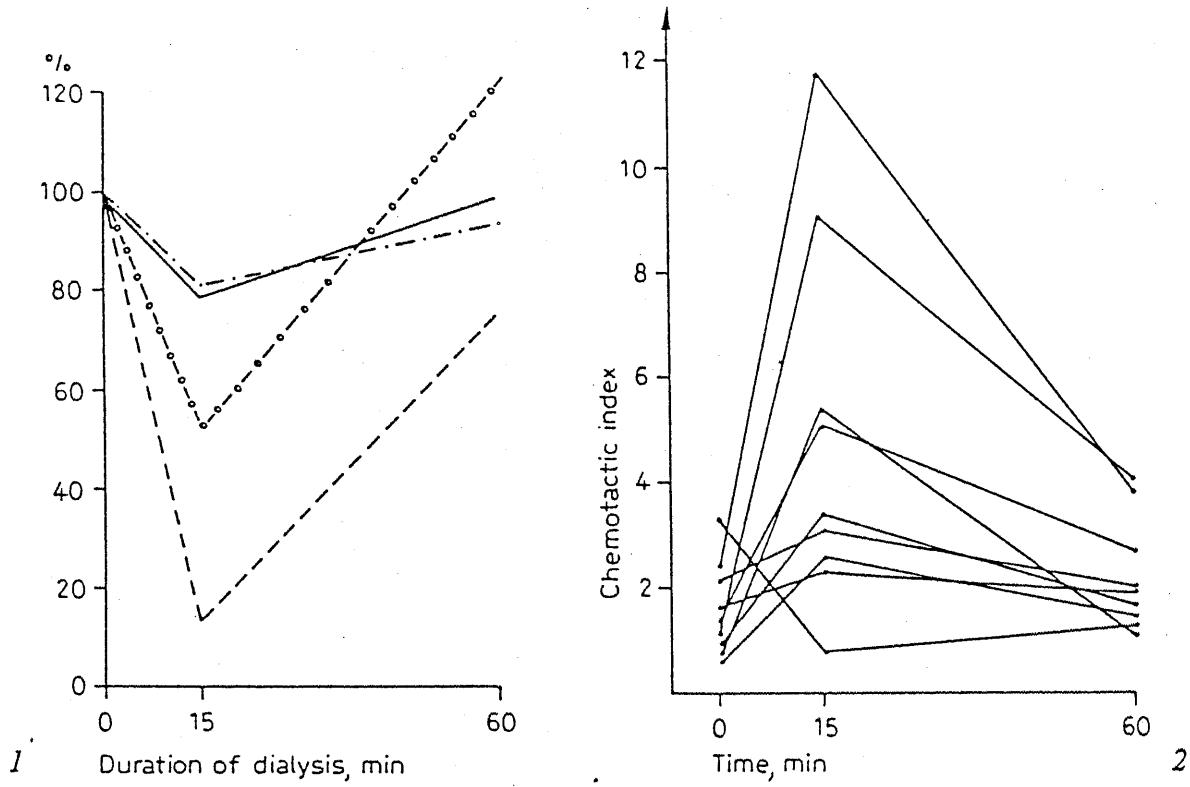

Fig. 1. Blood granulocyte count in percent of predialysis count. – · – · – = Polyacrylonitrile; ——— = polymethylmethacrylate; —— o —— o —— = cellulose acetate; ––– = Cuprophan.

Fig. 2. Chemotactic index during dialysis with the polymethylmethacrylate membrane.

Chemotaxis. For the measurement of their chemotactic ability, granulocytes were isolated by dextran sedimentation. Measurement was done in a serum-free milieu (Hank's solution with human albumin) with N-formyl-methionyl-leucyl-phenylalanine (10^{-8} and 10^{-9} *M*) as a chemotactic agent in a modified Boyden chamber [4].

Leucocyte Aggregation. The aggregation of granulocytes from healthy blood donors by serum from the dialysis patients, before and after zymosan stimulation, was measured. The measurement was done in a commercially available aggregometer. The results were compared with the aggregation of cells by autologous donor sera [5].

Chemiluminescence. Luminol-amplified chemiluminescence in whole blood was measured after the activation of leucocytes by *E. coli* in a Luminometer (Biolumat LB 9500, Berthold, Wildbad, FRG) according to a modification [3] of the method of *Kato* et al. [10]. The specific chemiluminescence, i.e. light emission/leucocyte, was expressed as cpm per leucocyte.

Results

Cuprophan membranes caused granulocytopenia several minutes after the start of dialysis, which returned to normal after about 60 min. CA membranes

Table I. Leucocyte aggregation on percent of predialysis value ($\bar{X} \pm SD$)

Membrane	15 min	60 min
PMMA	79 ± 28.1 n.s.	70.2 ± 36.0
PAN	113 ± 18.0 n.s.	89.2 ± 32.5
CA	140 ± 97.0 n.s.	115.0 ± 56.0
Cuprophan	91 ± 35.0 n.s.	114.0 ± 46.0

PMMA = Polymethylmethacrylate; PAN = polyacrylonitrile; CA = cellulose acetate.
n.s. = Not significant.

caused moderate granulocytopenia. PMMA and PAN membranes had only a slight influence on granulocyte count (fig. 1). The monocyte count was similar to that of the granulocytes. Lymphocyte and platelet counts were not affected.

Chemotaxis. The chemotaxis of granulocytes was not altered significantly by all membranes used. Only PMMA membranes showed a transient but not statistically significant increase of the chemotactic index which disappeared after 60 min of dialysis (fig. 2).

Leucocyte Aggregation. No alteration was seen in the ability of the activated and non-activated sera of dialysis patients, dialyzed with the different membranes, to aggregate the granulocytes of healthy blood donors (table I).

Chemiluminescence. The specific luminol-amplified chemiluminescence in whole blood was reduced after 15 min of dialysis with Cuprophan membranes to 40% of the control value before dialysis ($p < 0.002$) and returned back to control value after 60 min (fig. 3). During dialysis with PMMA membranes the absolute value and the specific value of chemiluminescence was not altered. The difference in specific chemiluminescence between Cuprophan and PMMA membranes at 15 min was also significant ($p < 0.05$).

Discussion

Our results confirm those of others that Cuprophan causes a transient and profound granulocytopenia while non-Cuprophan membranes do not [14].

According to *Craddock* et al. [5] granulocytopenia is caused by an activation of the alternate pathway of the complement system. In animal experiments complement activation leads to an aggregation of leucocytes and loss of the cells into the capillaries of the lungs. Similar reactions are believed to happen at the

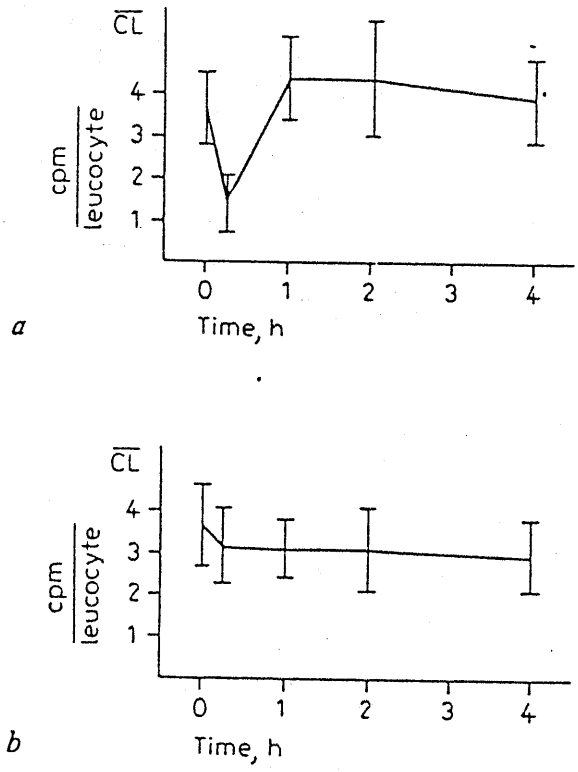

Fig. 3. Specific chemiluminescence during dialysis with the Cuprophan membrane *(a)* and the polymethylmethacrylate membrane *(b)*.

start of dialysis. According to *Craddock* et al. [5] complement activation leads to an increase in adherence and aggregation of neutrophiles. This transient increase in adherence of leucocytes with Cuprophan membranes was reported several times [12, 13].

The hypothesis that leucopenia is caused by complement activation was first questioned by *Aljama* et al. [1] because they found complement activation also during hemodialysis with non-Cuprophan membranes, not followed by profound leucopenia. In our experiments the ability of sera from dialysis patients, dialyzed with Cuprophan and non-Cuprophan membranes, to aggregate leucocytes from healthy donors was not impaired. The aggregation of the leucocytes of patients in early dialysis might therefore be due to a direct influence of the dialysis membrane on the cell, not mediated by serum factors. Besides transient leucopenia, alterations in phagocytosis [8], cell metabolism [15] and chemotaxis of leucocytes [2] have been reported. *Björksten* et al. [2] found diminished chemotaxis of granulocytes in dialysis patients compared to normal persons and not dialyzed uremic patients. In our studies no alteration in chemotaxis could be found. As an additional test of granulocyte function, measurement of chemiluminescence in whole blood was performed. With activation of the oxydative metabolism during phagocytosis, light is emitted by granulocytes which can be amplified with luminol and measured in a scintillation counter. The intensity of

chemiluminescence is strongly correlated with the inital rate of killing of bacteria [7]. For measurement granulocytes do not have to be isolated [6, 10]. The transient decrease, which we encountered in the specific chemiluminescence in the early phase of dialysis with Cuprophan membranes, is in agreement with the results of *Wisson* et al. [16] who found a similar decrease in isolated granulocytes from dialysis patients. We propose two possibilities for the explanation of the decreased chemiluminescence.

(1) the chain of reaction which leads to chemiluminescence in granulocytes is blocked at an yet unknown step either by direct influence on the cell or mediated by a serum factor.

(2) After 15 min of dialysis a population of granulocytes with diminished chemiluminescence dominates the circulation. The observation that in dialysis with PMMA membranes chemiluminescence is not different from control values points to an influence of the membrane material and not dialysis as such.

As leucopenia and diminished chemiluminescence occur and return back to normal at the same time of dialysis, it is likely that they have the same cause.

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