REMOVAL OF URAEMIC TOXINS BY HAEMOFILTRATION WITH DIFFERENT MEMBRANES. THE BENEFIT OF REGENERATING HAEMOFILTRATE USING A NEWLY DEVELOPED SYSTEM


The Department of Nephrology and *Inst. of Biophysics, University of Trondheim, Norway

Summary

Based on earlier results which indicate that certain uraemic toxins (verified by an in vitro assay system) are larger than 10,000 daltons — a new system for treatment of endstage renal failure, SElective DUal Filtration ARtificial Kidney (SEDUFARK) has been developed. This system consists of a multimembrane filter/dialyser unit making removal of small molecules (mol wt < 200) and substances in the mol wt range 10,000 — 40,000 possible without exchange of body fluids. Evaluation of pre and post treatment uraemic plasma with the bio assay showed that SEDUFARK was superior to conventional haemofiltration (CHF) with polyacrylonitrile (PAN) or Gambro Lundia Major High Flux 1.36m² (CPN) membranes as filters.

Introduction

In an earlier paper [1] we have shown that uraemic toxins inhibiting human phagocytes cultured in vitro are of greater molecular weight than 10,000 daltons. These toxins were not removed from uraemic plasma using PAN or cuprophane (CPN) membranes in conventional haemodialysis (HD) [2]. They were, however, transported through PAN membranes used in CHF. Based on these experiments we have constructed a new artificial kidney system which removes both large mol/wt substances (mol wt > 10,000) and small molecules (mol wt < 200) from uraemic plasma while most of the ‘middle mol wt substances’ are returned to the patient. The effect of this system, SEDUFARK, has been compared with that of CHF performed with PAN or CPN membranes.

Materials and Methods

The SEDUFARK system is illustrated in Figure 1. The extracorporeal blood circuit consists of an arterial-venous access site
(A–V), a roller pump (P₁), pressure monitor (Pr₁), a Rhone Poulenc RP6 dialyser serving as filter I (open filter) and a bubble trap (BT₁). The haemofiltrate circuit consists of roller pump P₂ which pumps the primary haemofiltrate (HF₁) through a flowmeter (F₁) into a collecting container (CC). The filtrate in CC was recirculated by roller pump P₃ through an Asahi AHFK K–102 dialyser which served as filter II (a less open filter) where the inlet pressure was monitored by Pr₂. In order to remove small molecules (mol wt < 200) as well as to regulate pH and electrolytes, the filtrate from filter II was pumped through an Asahi AHFK K–101 dialyser, via the bubble trap (BT₂) where venous pressure was monitored, back to BT₁ and to the patient. Before the start of treatment, filter II and the dialyser with their corresponding tubes were primed with physiological saline. During treatment, fluid removal through the dialyser (Figure 1) was avoided by regulating the level of the dialysate tank. The fluid volume in CC during treatment thus gave a continuous measure of the net fluid removal (ultrafiltrate) from the patient. It could be adjusted at any time by changing the relationship between the flow rate of primary (Q_HF₁) and regenerated (Q_HF₂) filtrate. In this comparative study six patients were treated with both SEDUFARK and CHF using PAN and CPN (1.36m²) membranes. CHF was performed in the post dilution mode and lasted for 180–240 min.

Human monocytes differentiated into macrophages in vitro were used for the bio assay and the cells were grown in uraemic plasma or normal serum for 4 days. The capacity of the macrophages to stay attached to glass surfaces (cell survival) and the capacity of the non-detached cells to digest radiolabelled Candida albicans was investigated [3]. Plasma samples obtained pre and post treatment were used as culture media with normal sera as control.
Results

Blood flow rate during SEDUFARK treatment was 200–270ml/min, recirculating flow rate through filter II 400ml/min and dialysate flow 100ml/min. $Q_{HF1}$ (mean ± SEM) was 56.3 ± 2.4ml/min, ultrafiltrate 1733 ± 223ml and treatment time 163 ± 8 min. This means that the average volume of filtrate was 9176ml from which 7443ml were regenerated and returned to the patient. The mean urea clearance was 46.3 ± 2.9ml/min and the mean creatinine clearance 56.6 ± 3.1ml/min. The filtrate volume during CHF (treatment time 180–240 min) was 10733 ± 894ml (PAN) and 10770 ± 785ml (CPN).

The results from the biological tests are given as per cent of the results obtained with sera from healthy donors (Table I).

### TABLE I. Effect of Uraemic Plasma Pre and Post Treatment on Human Phagocytes Cultured in vitro

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell survival</th>
<th>Phagocytosis of $^{125}$I Labelled Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>n</td>
<td>Pre treatment</td>
</tr>
<tr>
<td>CHF with CPN</td>
<td>6</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>CHF with PAN</td>
<td>6</td>
<td>4.0±1.8</td>
</tr>
<tr>
<td>SEDUFARK</td>
<td>6</td>
<td>3.8±0.8</td>
</tr>
</tbody>
</table>

CHF: Conventional haemofiltration. CPN Cuprophan high flux, PAN: Polyacrylonitrile.
Results are given as % of controls (normal serum). Mean ± SEM

Discussion

SEDUFARK is based on selective removal of small molecules (mol wt < 200) and substances with mol wt approximately 10,000–40,000 through a multi-membrane filter/dialyser unit. The limiting factor in this system as used in our trials was mainly filter I. In order to obtain a 'short time treatment' of 3–4 hours per week with adequate urea and creatinine removal, the total filtrate volume should be 18–20L per treatment which means that $Q_{HF1}$ should be kept about 100–120ml/min. The data given in Table I show that SEDUFARK was superior to CHF in improving cell survival as well as in phagocytosis of Candida albicans. CPN were less permeable than PAN membranes for the toxic substance(s) tested in our system. The main difference between CHF using PAN membranes and SEDUFARK is that SEDUFARK returns most of the substances in the mol wt range 200–5,000 to the patient.

Figure 2 gives a theoretical comparison between plasma concentrations of different mol wt solutes after 3 hr treatment with HD using a 1.3m² CPN dialy-
Figure 2. Theoretical comparison between plasma concentrations for different mol wt substances after 3 hr treatment with different artificial kidney systems. \( C_{P_0} \) and \( C_P \) are plasma concentrations pre (\( C_{P_0} \)) and post (\( C_P \)) treatment. All values are based on a 'patient' pool of 20L.

ser, CHF with PAN and SEDUFARK (data for membrane transport are taken from Jørstad et al [4] and Man [5]. The hatched area represents substances discarded using CHF but returned to the patient with SEDUFARK. The toxin tests (Table I) shows the benefit of returning substances in this mol wt range.

The membranes used in these trials were not ideal.

SEDUFARK used with an even more open membrane as filter I (Amicon. XM50) should remove even more high mol wt substances from uraemic plasma (Figures 1 and 2). When the mol wt of the toxin (s) is better known, membranes with a steeper 'cut off' would be best suited for SEDUFARK. A less permeable CPN membrane in the dialyser (Figure 1) resulting in minor alterations in urea and creatinine clearances will reduce to a greater extent the removal of molecules in the 200–5,000 dalton range containing essential substances such as amino acids, vitamins and hormones.

In addition to the removal of haemofiltrate containing essential substances, CHF also has some other drawbacks. The volume of haemofiltrate minus net fluid removal has to be replaced with expensive sterilised solutions of physiological substances demanding precise monitoring of fluid balance. Regeneration of haemofiltrate with cartridge and enzyme systems has been reported both in animal experiments [6,7] and in the treatment of patients [8,9]. However, the use of these systems requires cation replacement, and the regenerated fluid has to be sterilfiltered because of problems with bacterial contamination.

The SEDUFARK system as shown in Figure 1 is too complicated for routine
use, but the different compartments can easily be made into one unit. By optimal combination of membranes with the desired ‘sieve-like’ properties, any given mol wt substances can be removed from uraemic plasma making replacement of body fluid with physiological compounds unnecessary.

Acknowledgment

This work was supported by grants from the Norwegian Research Council for Science and Humanities.

References

6 Shapiro, WB, Fanbert, PF, Fein, PA, Tzeng, T, Salsman, K and Porush, JG (1978) Trans. ASAIO, 24, 185

Open Discussion

FUNCK—BRENTANO (Paris) You told us the rationale for going to this pretty complicated system. Can you comment and give some more details of the procedure you used to get the figures for the toxicity of the uraemic filtrate on cells?

JÖRSTAD The details are illustrated in my extra slide. We are using human monocytes, drawn from healthy donors. The cells were separated by the method of Böyum and dispensed on glass cover slips in normal media. The cells were grown in normal medium from day 1 to day 4. From day 4 to day 8 the cells are cultured in uraemic medium or corresponding normal medium. On day 8 radio-labelled candida albicans are given to the cultures and the cells are given the opportunity to engulf these candida particles for 15 minutes. Then the media are removed and the cells are grown in normal medium from day 8 to day 9, and then harvested. By harvesting, the radioactivity was registered on the cover slips. This radioactivity reflects undigested candida albicans still within the cells. The radioactivity was also registered in the sediment and in the cell-free medium. During digestion the debris of candida particles is transported out of the cells into the medium. The sum of these registrations gives the total radioactivity. By comparing this total radioactivity in the dishes with cells grown in uraemic plasma, with cells grown in normal media, we are getting a relative measure of how many cells detach from day 4 to day 8.

FUNCK—BRENTANO Do you test the influence of different concentrations of plasma?
JÖRSTAD Yes, we have tested different concentrations of uraemic plasma. For instance by using 25% uraemic plasma and 75% normal plasma. The inhibition is still significant.

MATTHAEI (Göttingen) Do you have any correlation between the toxicity of uraemic plasma and clinical signs of uraemia? Did these clinical signs improve after selective haemofiltration treatment?

JÖRSTAD Yes, the macrophages reflect toxicity in parallel with the development of uraemia. The more uraemic the patients are, the more inhibited are the macrophages tested with plasma from these patients. We do not have long term clinical observations with this SEDUFARK system, hence improvement in clinical uraemic symptoms cannot be detected after single treatments.