Investigations on Clinico-Chemical Correlations in Uraemic Polyneuritis

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Introduction

Since Babb and Scribner (1971) first suggested that uraemic neuropathy was due to the retention of middle-molecule toxins (MM) many authors (Christopher et al, 1971; Ginn et al, 1971; Shinaberger et al, 1972; Man et al, 1973a) have supplied clinical evidence in agreement with this hypothesis. Some tentative characterisation of these MM has been made (Dall’aglio et al, 1972; Dzurik et al, 1973; Man et al, 1973a; Bergstrom et al, 1974).

In order to characterise the solutes responsible for uraemic neuropathy, we have chosen as a first goal to correlate the neurologic status of our patients and the results of chemical analysis and biological testing made on the dialysate content.

We assumed that retention of toxic solutes in the MM range causes neuropathy, and that patients with severe neuropathy due to inadequate dialysis retain these solutes in their blood.

During the first polyacrylonitrile (PAN) dialysis, we presumed that the transfer of MM from plasma to dialysate would yield a concentration of neurotoxic substance sufficient for analysis. Subsequent dialysates from the same patient would show a decreasing concentration of significant solutes which might be identified in successive fractions.

Isolated solutes were used for the experimental reproduction of neuropathy in animals.

PATIENTS AND METHODS

Patient Selection

Three patients having severe neuropathy probably due to inadequate dialysis on Cuprophan were selected for the study.
We have chosen, as reference, five patients adequately dialysed on Cuprophan (De Palma et al, 1972) and free of neuropathy.

Urine Collection
The urines of five healthy subjects with a normal protein intake, were collected during three days to make a pool of samples.

The urine was collected in two other healthy subjects, for 12 days. During the first six days, they were on a 0.5 g/kg body wt/day protein diet and on a 2.5 g/kg body wt/day protein diet during the six subsequent days.

Dialysis Techniques
The neuropathic patients were treated with a new artificial kidney equipped with a PAN membrane and having a clearance for MM (Vit B₁₂ = mw 1355) twice that of the same kidney equipped with Cuprophan (Man et al, 1973b). The dialysate delivery system consists of a closed-loop closed-batch of 50 litres, allowing the collection of dialysate for analytical procedures.

The dialysis duration was three hours, three times a week.

Sample Preparation
The dialysates were sterilised by filtration and lyophilised. Dry samples were stored at 4°C under nitrogen. For analysis, dry samples were dissolved in distilled water to a concentration of 10 g per 100 ml, giving the standard solution.

The urines collected from the five healthy subjects were ultrafiltered on PAN membranes. The urines collected from the two healthy subjects on low- and high-protein diets were prepared according to the following procedure.

To exclude high-molecular-weight solutes, urine was ultrafiltered across PAN (cut off: 30,000 mw). In order to increase the concentration ratio between MM and small molecules, this initial standard solution was dialysed for 15 min with a Cuprophan dialyser against the same volume of water. Then two fractions were obtained: the dialysed fraction A (FA) and the dialysate fraction B (FB). With this procedure, the final concentration ratio of FA to FB was one for urea (mw: 60) and three for vitamin B₁₂ (mw: 1355). The two fractions were both lyophilised and prepared as the earlier standard solution of patient dialysate.

WI 38 Cells Test
The test consists of determining the titre of samples at which a standard structural alteration of a sheet of WI 38 cells is observed. The cell response was read after one and two days' incubation in a maintenance medium at 37°C (Figure 1).
Gel Chromatography

Gel chromatography of the samples was performed on Sephadex G 15. The absorption was measured at 254 nm.

Figure 1. Micrograph of WI-38 culture cells: left – normal structure, right – altered structure.

A region for exclusion from approximately 1300 to 1500 mw was selected as being representative of MM. The results are expressed in integrated absorbance units (IA) per kilogramme body weight which represents the peak surface divided by body weight. The production was expressed as IA/kg body wt/week (Figure 2).

Determination of Conjugated $\alpha$-amino Nitrogen

Urine $\alpha$-amino nitrogen was measured by the difference of nitrogen concentration before and after 18-hr hydrolysis at 110° C by 6N HCl. The conjugated $\alpha$-amino fraction is characteristic of polypeptides.

RESULTS

Clinical Results

A rapid neurological improvement was observed in all three patients. At the beginning of treatment two of them had a paraplegia, and the third needed a walking stick. After one month, they were all able to walk with a stick and three months later to walk without any help.

The motor nerve conduction velocity (MNCV) of the peroneal nerve was not measurable at the onset of the treatment and reappeared over a 12-month period in one patient.

In all of them the cubital MNCV increased as the clinical condition improved. The muscular mass of the legs increased significantly as indicated by the increase of the circumference of the triceps and the quadriceps.
Despite the fact that plasma urea and creatinine remained high as shown in Table I, the general condition of the patients after six months of therapy with PAN membrane was satisfactory. The patients felt better and noticed that their weakness had diminished.

TABLE I. Mean Pre-dialysis Concentrations (mg/100 ml) after Six Months Treatment.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Body weight (kg)</th>
<th>Urea</th>
<th>Creatinine</th>
<th>G.S.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP</td>
<td>50</td>
<td>118 ± 2</td>
<td>10.9 ± 0.15</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>ML</td>
<td>53</td>
<td>319 ± 10</td>
<td>15.4 ± 0.35</td>
<td>0.92 ± 0.16</td>
</tr>
<tr>
<td>GA</td>
<td>55</td>
<td>156 ± 8</td>
<td>9.9 ± 0.24</td>
<td>0.39 ± 0.18</td>
</tr>
</tbody>
</table>

Figure 2. Chromatograms (Sephadex G 15, absorbance: 254 nm) of a neuropathic patient dialysate (M.P.) and of a healthy subject urine (M.G.). Shaded area represents the selected MM region.

Such a rapid improvement in such a short time has never been recorded with standard dialysis techniques. This improvement is very similar to what happens after successful renal transplantation (Ibrahim et al, 1974).

Dialysates

**WI 38 Cells Test**

Figure 3 shows the effect of the first PAN dialysate on WI 38 cells test in the three patients with severe neuropathy and in the five reference patients without neuropathy.

The dialysate of patients with polyneuritis shows a high WI 38 cells titre, while the dialysate activity of the reference patients is equal to that of normal urine pool ultrafiltrate.
Gel Chromatography

The first dialysate chromatogram of the patients with severe neuropathy shows a MM content much higher than that of the control dialysate (Figure 4.).

Figure 3. Toxicity of the first PAN dialysate obtained from neuropathic patients and reference patients.

Figure 4. Middle-molecule content, measured as integrated absorbance per kilogramme of body weight, in the first PAN dialysate obtained from neuropathic patient and control patient.

Urine

WI 38 Cells Test

The WI 38 cells titre of the normal urine pool PAN ultrafiltrate was low. In healthy subject MG, on a varied protein diet, most of the urine PAN
ultrafiltrate activity was found in Cuprophan dialysis fraction A, whereas a small activity was found in Cuprophan dialysate fraction B. WI 38 cells responded better to the fraction which was supposed to contain the higher level of MM. (Table II).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Low protein diet</th>
<th>High protein diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial solution</td>
<td>52 Dil</td>
<td>86 Dil</td>
</tr>
<tr>
<td>(Urine Pan Ultrafiltrate)</td>
<td>30 Dil</td>
<td>50 Dil</td>
</tr>
<tr>
<td>Fraction A</td>
<td>19 Dil</td>
<td>25 Dil</td>
</tr>
</tbody>
</table>

**Gel Chromatography**

The urine of healthy subjects showed the same chromatographic patterns as the dialysate of neuropathic patients. The MM peak obtained with normal urine was comparable to that obtained with dialysate from neuropathic patients (Figure 2).

In subject MG there was a significant linear correlation between steady urea excretion and MM production ($\gamma = 0.302x + 0.086, r = 0.738, p < 0.01$). The results obtained with conjugated $\alpha$-amino nitrogen were similar ($\gamma = 5.03x + 0.73, r = 0.830, p < 0.01$). However in the other subject, the correlation was very poor ($\gamma = 2.48 - 0.33x, r = -0.058, NS$).

**DISCUSSION**

Clinical observations as well as in vitro measurements are consistent with the idea that links exist between neuropathy and middle molecule body retention.

It has been already demonstrated that adequate removal of MM can improve extremely neuropathic patients. The delay in this improvement has been shortened in our three patients treated with the RP6 dialyser equipped with PAN membrane and a closed-batch system. This rapid improvement raises the question whether it is due to the high permeability of the membrane alone or associated with a protective effect against "wash-out" due to the closed-batch system.

The effect of the PAN membrane was clearly demonstrated in patient GA. With Cuprophan on the closed-batch system, the dialysate activity was low in the WI 38 cells test (10 Dil), but dialysate obtained with the PAN membrane two days later had a very high activity (160 Dil).

The results are consistent with the hypothesis that MM are retained in excess in neuropathic patients and that their removal is dependent on membrane permeability.
The MM fraction which was present in the first dialysate of neuropathic patients was also present in the urines of healthy subjects.

As has been pointed out by Peters and Gotch (1974), we have found a significant linear correlation between MM production and urinary urea excretion in one subject out of two. Thus individual patterns may exist between urea production and MM production.

Adequate removal of middle molecules of 1,300 – 1,500 mw seems to represent the main condition for prevention of uraemic neuropathy.

Conclusion

The analysis of the first dialysate from extremely neuropathic patients by WI 38 cells test and chromatography indicates that uraemic neuropathy is strongly related to the body retention of MM.

The MM fraction contains 1,300 – 1,500 mw solutes which are present in the urine of healthy subjects. The clinical outcome of studied patients is consistent with the hypothesis that solutes of 1,300 – 1,500 mw range found in dialysate are toxic and responsible for polyneuritis. This does not exclude the role of complementary factors such as plasma-solute depletion or deficient metabolic function of the kidney.

References


Bergstrom, J, Gordon, A, Furst, P and Ryhage, R Seventh Annual Contractors' Conference of the Artificial Kidney Program of the National Institute of Arthritis, Metabolism and Digestive Diseases. (in press)


Open Discussion

A P ROODVOETS (Holland) Do you think it would be possible to treat or prevent polyneuropathy by treating a patient, say, once a month with the Rhone-Poulenc and the rest of the time with conventional dialysers?

DRUEKE Yes, but we have no experience with such a schedule.

Dr COHEN One of your slides showed a rather high level of blood urea and creatinine but a low level of blood guanidinosuccinic acid (GSA) and I wondered how you explain that GSA dialysed very effectively whereas the other two did not on short-term dialysis with PAM.

DRUEKE I don’t think the level of GSA was low but I can’t explain the difference.

COHEN It raises the question whether small molecular weight material is protein bound.

DRUEKE I agree with this suggestion.

Dr. TEITELMAN Have you any idea about amino acid or fatty acid loss with this more permeable membrane?

DRUEKE Unfortunately we have no personal data about that.