Changes in Activity of Selected Lysosomal Enzymes in Peritoneal Macrophages of Renal Failure Patients on Peritoneal Dialysis

Władysław Sulowicz, Tadeusz Cichocki, and Zygmunt Hanicki

Department of Nephrology and Department of Histology, Medical Academy, Krakow, Poland

Activity of acid phosphatase (AP), beta-glucuronidase (GR), N-acetyl-beta-D-glucosaminidase (GZ), and peroxidase (P) was assessed using a semiquantitative cytochemical method in peritoneal macrophages of 30 patients with end-stage renal failure treated by intermittent peritoneal dialysis and of 30 control patients with normal renal function.

The dialysed patients showed a significantly higher activity of GR and P at the beginning of the treatment as compared with the respective activities observed in the control group and a further significant rise of these activities after 4 months of dialysis. Activity of AP at the beginning of the treatment was insignificantly lower than in the control group and the difference became significant at the end of the investigated period. There was no significant difference between the dialysed patients and the control group in the activity of GZ assessed at the beginning of the dialytic treatment and after 4 months of dialysis. A significant decrease in that activity was, however, observed in the course of dialysis.

KEY WORDS: Acid phosphatase; beta-glucuronidase; N-acetyl-beta-D-glucosaminidase; peroxidase; peritoneal macrophages.

Disturbed immune response of both humoral and cellular type has been demonstrated in uraemic patients (1-6). Most information regarding the functional state of cells involved in the immune reactions in patients with end-stage renal failure (ESRF) had been obtained by studying cells from peripheral blood. Cells from outside the vascular bed have not been studied in this respect.

Since peritoneal dialysis is an increasingly used treatment for ESRF, and can often be complicated by peritonitis, there is an increasing interest in the cells participating in the defense mechanisms of the peritoneal cavity (7-10). Macrophages are the predominant cells in the peritoneal cavity in both normal persons and those on peritoneal dialysis (11-13). Together with neutrophils, they constitute the first line of defense against bacterial invasion in dialysed patients.

Correspondence to: Władysław Sulowicz, Dept. of Nephrology, Medical Academy, 31-501 Krakow, Poland.

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Thirty patients (19 male, 11 female) aged 17 to 63 years (mean = 39.7) treated by intermittent peritoneal dialysis were included in the study. In most patients, chronic glomerulonephritis was the cause of the renal failure. The patients were dialysed 3 times a week for 9 h. Twelve fluid exchanges during each dialysis were carried out manually using 2000 mL of dialysate in 0.5 L bottles (manufactured by Polfa Lublin) containing 132 mmol/L of sodium, 1.75 mmol/L of calcium, 0.75 mmol/L of magnesium, 101 mmol/L of chloride, 35 mmol/L of acetate, and 1.5% glucose. The pH of the solutions ranged from 6.0 to 6.3. The fluids were prewarmed to body temperature and 2 mL of 0.1 N NaOH and 10 mg of heparin were added before instillation. The duration of each exchange was 45 min. If necessary higher glucose concentrations and potassium supplementation were used in the following dialysate exchanges. Peritoneal macrophages were collected twice: immediately after starting dialysis (i.e., in the period of severe metabolic disturbances) and after 4 months of treatment (i.e., when the metabolic functions were, in part at least, back to normal). None of the patients developed peritonitis during the period of the study. They did not receive steroids or cytotoxic drugs. Mean serum urea and creatinine levels before dialysis were 39.6 mmol/L and 1150 μmol/L, respectively, at the beginning of the treatment, falling to 31.3 mmol/L and 997.2 μmol/L after 4 months.

From the first dialysate exchange (always 1.5% glucose), 100 mL of dialysate was collected in sterile siliconized containers, centrifuged, and supernatant discarded to obtain a final mL volume of condensed dialysate. The number of cells in 1 μl of condensed
The reaction products formed with all the above substrates were coupled to hexazotized pararosaniline hydrochloride (Sigma).

Peroxidase activity was demonstrated according to Graham and Karnovsky (22) with 3,3-diaminobenzidine (Sigma) as electron donor (Figure 4).

Activity of the studied enzymes was assessed using a semiquantitative method. In 100 macrophages per smear the intensity of the colour reaction was arbitrarily estimated according to a 0 to 4 scale, and the activity index (score) was then calculated. The observations were blind and always performed by the same person. The precision of cytochemical methods used was tested by comparing the results obtained in 10 smears prepared from the same sample of dialysate. The variability coefficients (V) were 4.1% for AP, 3.5% for GR, 4.9% for GZ, and 8.8% for P.

The results were also compared with those obtained in 30 patients constituting the control group (14 males, 16 females), aged 30 to 72 years. Control pa

### Table 1

<table>
<thead>
<tr>
<th>Groups examined</th>
<th>Fluids volume</th>
<th>Numbers of cells in 1 μl</th>
<th>Total numbers of cells in obtained fluids</th>
<th>Macrophages %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with duodenal ulcer or gall stones</td>
<td>50–170 mL*</td>
<td>24.5 ± 16.6</td>
<td>6 × 10⁵–6.4 × 10⁶</td>
<td>64.9 ± 11.9</td>
</tr>
<tr>
<td>(n = 10)</td>
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<td></td>
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<tr>
<td>Patients with ovarian cyst</td>
<td>0.65–10 mL</td>
<td>8370 ± 7216</td>
<td>2.8 × 10⁶–6.3 × 10⁷</td>
<td>65.1 ± 11.9</td>
</tr>
<tr>
<td>(n = 10)</td>
<td></td>
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</tr>
<tr>
<td>Patients with ascites (n = 10)</td>
<td>3000–10 000 mL</td>
<td>181.7 ± 181.6</td>
<td>3 × 10⁷–4.5 × 10⁹</td>
<td>67.5 ± 12.1</td>
</tr>
<tr>
<td>Dialysed patients immediately after the</td>
<td>1500–2000 mL</td>
<td>57.2 ± 49.1</td>
<td>1 × 10⁷–5.7 × 10⁹</td>
<td>63.0 ± 18.1</td>
</tr>
<tr>
<td>beginning of the treatment (n = 10)</td>
<td>1st exchange</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialysed patients after 4 months of</td>
<td>1600–2000 mL</td>
<td>63.4 ± 47.3</td>
<td>1.6 × 10⁷–2.4 × 10⁸</td>
<td>61.2 ± 14.5</td>
</tr>
<tr>
<td>treatment (n = 30)</td>
<td>1st exchange</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 200 mL of 0.9% NaCl was instilled into peritoneal cavity.

dialysate was counted and converted for cell count and total cellularity of noncondensed dialysate (Table 1). After additional centrifugation, smears were made, stained by May-Grünewald Giemsa method and the differential cell count estimated (Figure 1). Additional smears were made for cytochemical examinations.

The activity of lysosomal enzymes in peritoneal macrophages was detected according to the following methods:

1. Acid phosphatase according to Barka and Anderson (19) with Naphthol AS-BI phosphoric acid (Sigma) as substrate (Figure 2).
2. Beta-glucuronidase according to Hayashi et al. (20) with Naphthol AS-BI beta-D-glucuronic acid (Sigma) as substrate (Figure 3, left).
3. N-acetyl-beta-D-glucosaminidase according to Hayashi (21) with Naphthol AS-PC N-acetyl-betaD-glucosamine (Sigma) as substrate (Figure 3, right).

![Figure 1](http://www.pdiconnect.com/)

**Figure 1**—Cells from peritoneal dialysate. Macrophages, lymphocyte and neutrophil visible (May-Grünwald Giemsa).

![Figure 2](http://www.pdiconnect.com/)

**Figure 2**—Peritoneal cells from ascites with strong positive acid phosphatase reaction (19).
patients had a normal renal function, and the peritoneal fluid was collected from 20 patients during an abdominal surgery (6, gall stones; 4, duodenal ulcer; 10, ovarian cyst) and in 10 patients as a result of ascites releasing tap in the course of heart failure. Detailed characteristics of the quantity of collected fluids and of isolated cells were presented in Table 1.

The results were analysed statistically using Student’s t test.

RESULTS

The obtained results are summarized in Table 2.

ACID PHOSPHATASE (AP)

Score value of AP activity in peritoneal macrophages of dialysed patients at the beginning of the treatment was insignificantly lower from that found in the control group. After 4 months of dialytic treatment, the score value decreased to a level significantly lower than that in the control group \( (p < 0.01) \), although as compared with the initial value found in the dialysed patients, the difference was not statistically significant.

BETA-

GLUCURONIDASE (GR)

As compared with the control group, score value of GR activity in peritoneal macrophages of dialysed patients was significantly higher both at the beginning and after 4 months of the treatment \( (p < 0.001) \). The increase in the score value during the treatment was also statistically significant \( (p < 0.01) \).

N-ACETYL-BETA-D-GLUCOSAMINIDASE (GZ)

Score values of GZ activity in peritoneal macrophages of patients with end-stage renal failure calculated at the beginning of dialytic treatment and after 4 months of dialysis did not significantly differ from that observed in the control group. There was, however, a statistically significant decrease in the GZ activity in the course of the treatment \( (p < 0.05) \).

PEROXIDASE (P)

As compared with the control group, score values of P activity in peritoneal macrophages obtained of patients with renal failure at the beginning and after 4 months of the treatment were significantly higher \( (p < 0.001) \). There was also a statistically significant difference between the values found at the beginning of the dialytic treatment and 4 months later, when the activity was higher \( (p < 0.01) \).

DISCUSSION

Assessment of cytochemical properties of macrophages, which reflect their intracellular metabolism, are of a considerable importance for an indirect estimation of their functional state. The value of such studies is emphasized by results of cytophotometric investigations demonstrating that the amount of coloured product formed in the cytochemical reaction reflects the true activity of the enzyme in the cell (23). Previous studies on cells involved in immune reactions in patients with end-stage renal failure have shown that dialytic treatment can exert a favourable effect on the disturbed functions of lymphocytes (24-29), granulocytes (30,31) and monocytes from peripheral blood, as well as of macrophages from skin exudate (32). It is also known that peritoneal dialysis, especially continuous ambulatory peritoneal dialysis, is more effective in that respect than hemodialysis (26, 27).

Results of this study demonstrate differences in the activity of the investigated enzymes in peritoneal macrophages at the beginning and after 4 months of
intermittent peritoneal dialysis. Score values assessed at the end of the tested period were lower in case of AP and GZ (insignificant and significant difference, respectively), and significantly higher in case of GR and P. The differences between the enzymes may result from different mechanisms of their intracellular synthesis, or can be related to their diverse biological roles. A change of enzyme activity in the course of treatment is probably caused by a complex mechanism. Such change may result from an altered function of macrophages, or reflect an influx of younger cells to the peritoneal cavity replacing the previous population washed out in the course of repeated dialysis (18). The latter possibility would imply the presence or domination of another subpopulation of macrophages in the peritoneal cavity of patients after prolonged dialytic treatment. This is not unlikely, especially since subpopulations of macrophages with different activities of such enzymes as AP, GR, cathepsin D, p-nitrophenyl phosphatase, lysozyme, cytochrome c oxidase, and peroxidase were demonstrated in laboratory animals (33).

There are only few data concerned with the activity of lysosomal enzymes in human peritoneal macrophages. They show a higher AP activity than monocytes from peripheral blood (34), and that the activity of AP and neutral protease is increased in patients with endometriosis (35, 36). In patients with endstage renal failure treated by intermittent peritoneal dialysis, an increase in GR and P and a decrease in AP activity was observed, as compared with the control group (17, 18). It was also demonstrated that in the course of bacterial peritonitis the activities of GZ and P were significantly higher and lower, respectively, as compared with the activities observed in the same patients in the period of dialytic treatment without inflammatory complications (17, 18). In our study, a rise in P activity was accompanied by a fall in GZ activity in the course of dialytic treatment-these inverse changes cannot result from subclinical infections; they may, however, be due to a loss of some dialysable substances which influence the activity of cells (28). Moreover, dialysis itself can modify the enzymatic activity of macrophages, leading to differences between the values found at the beginning of the treatment and after 4 months of dialysis.

It seems therefore that peritoneal dialysis can influence the local immune response on macrophage population by a multidirectional effect. This effect can also have a practical significance, since inflammatory processes in the peritoneal cavity are relatively frequent complications during peritoneal dialysis.

### REFERENCES


### Table 2

Activity of AP, GR, GZ and P in Peritoneal Macrophages of Dialysed and Control Patients

<table>
<thead>
<tr>
<th>Groups examined</th>
<th>AP score</th>
<th>GR score</th>
<th>GZ score</th>
<th>P score</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control group (n = 30)</td>
<td>x</td>
<td>288.3</td>
<td>88.1</td>
<td>172.0</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>83.5</td>
<td>41.9</td>
<td>95.9</td>
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<tr>
<td>II. Dialysed patients immediately after the beginning of treatment (n = 30)</td>
<td>x</td>
<td>253.2</td>
<td>128.8</td>
<td>182.7*</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>66.4</td>
<td>39.2</td>
<td>69.9</td>
</tr>
<tr>
<td>III. Dialysed patients after 4 months of treatment (n = 30)</td>
<td>x</td>
<td>219.5</td>
<td>167.4</td>
<td>143.7*</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>75.6</td>
<td>63.2</td>
<td>47.7</td>
</tr>
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<table>
<thead>
<tr>
<th>Statistical significance</th>
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</thead>
<tbody>
<tr>
<td>I:II t</td>
</tr>
<tr>
<td>p ns</td>
</tr>
<tr>
<td>I:III t</td>
</tr>
<tr>
<td>p &lt;0.01</td>
</tr>
<tr>
<td>II:III t</td>
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<tr>
<td>p &lt;0.01</td>
</tr>
</tbody>
</table>

* The examinations were performed in 20 patients.
ns = Not significant.


