Inhibitory effects of plasma dialysate on protein synthesis in vitro: influence of dialysis and transplantation

Christine Delaporte, Françoise Gros, and Takis Anagnostopoulos

ABSTRACT An in vitro technique was designed to measure the protein synthesis rate in the presence of plasma dialysate from severely uremic patients. Some of them were treated with a poor nitrogen diet, hemodialysis, or transplantation. The data observed with plasma of such patients were compared to those obtained with plasma dialysate of control subjects. Each plasma sample was dialyzed for 24 hr to extract the molecules having molecular weights less than 12,000 and the dialysate was lyophilized. Protein synthesis was studied in vitro, using a cell-free system from mouse Krebs II ascites cells. The cells were lysed, and the 30,000 × g supernatant (“S-30 lysate”) was used to test the protein synthesizing activity of the plasma dialysate from control and uremic animals. The rate of protein synthesis was monitored by measuring the incorporation of a radiolabel (3H-leucine) into trichloroacetic acid-precipitable material in the presence of increasing amounts of plasma dialysate. In the presence of dialysate plasma from uremic patients as well as from hemodialyzed (HD) patients sampled before dialysis, there was a decrease in the 3H-leucine incorporation rate when the amount of added dialysate increased in the incubating medium. In severely uremic patients a difference between a high or low nitrogen diet could not be demonstrated. Though the count level of incorporated leucine with plasma from HD patients was always lower than that recorded in the presence of non-HD patients, the difference was not significant. However both these incorporation curves were significantly lower than the control curve (P < 0.01). Plasma specimens from HD patients were obtained at the beginning and end of a dialysis session. The comparison between the pre- and postdialysis incorporation curve showed an obvious improvement in the leucine incorporation rate with postdialysis plasma dialysate. In all transplanted patients but one, the incorporation curve was similar to the control curve. Therefore, it could be concluded 1) that uremic plasma inhibits protein synthesis, 2) predialysis plasma has the same inhibitory effect on in vitro protein synthesis as plasma from non-HD patients, 3) dialysis removes a great part of this inhibitory effect which appears to be related to dialyzable molecules, and 4) successful transplantation restores a normal pattern of in vitro protein synthesis. Am. J. Clin. Nutr. 33: 1407–1410, 1980.

The alterations of protein metabolism in renal failure have been reviewed elsewhere (1, 2). They may be related to the prescription of poor protein diet and/or to the abnormal accumulation of “uremic toxins” in the patient’s blood. In previous studies in chronically uremic children, we have demonstrated the occurrence of changes in muscle cell protein metabolism. These included a decrease in the amount of the noncollagenous proteins (“alkali soluble proteins”) (3) and an increase of the free amino acid pool associated with modification in its qualitative composition (4). These alterations were more accentuated in patients treated with low nitrogen diets and in children undergoing long-term hemodialysis. More recently, we have demonstrated an inhibitory effect of uremic ultrafiltrate on in vitro protein synthesis (C. Delaporte and F. Gros, unpublished observation). The purpose of this study was to investigate the effects of plasma dialysate on the in vitro protein synthesis in the presence of plasma obtained from uremic patients 1) receiving a low or high protein diet, 2) treated with hemodialysis, and 3) after transplantation.

Methods Preparation of the plasma

Twenty-four plasmas were tested: 12 from severely uremic patients (nine children, three adults) with a
plasma creatinine higher than 5.1 mg/dl and six from children treated by long-term hemodialysis. In the dialysis patients, the plasma samples were obtained just before a dialysis procedure and in two subjects, a second sample was taken at the end of the dialysis session. Six plasmas were obtained from transplanted patients. Plasma samples were obtained early in the morning after an overnight fast. The plasma of patients receiving therapy other than 25-OH-cholecalciferol were discarded. The plasma of transplanted patients was sampled 48 hr after ingestion of corticotherapy.

Eleven of the 12 severely uremic children underwent a dietary survey. Calculation of dietary nitrogen and calorie intake were based on an analysis of the actual food ingested during a 3-day period each month over an average period of 6 months (3 months to 1/2 years). Five children consumed a low-protein diet with a mean nitrogen intake equal to 50% of the recommended dietary allowances (RDA) (5) for their stature age (range 24 to 64% of RDA). The six other patients ingested at least 100% of the RDA.

As molecules known to be retained in uremic blood were not of high molecular weight, the study was not made with the total plasma, but with its dialysate. One milliliter of plasma was dialyzed for 24 hr against 25 ml of water. The dialysate with molecules of mw < 12,000 was lyophilized and resuspended in 1 ml of water.

Preparation of the cell-free system

A cell-free system from Krebs II ascites cells was used to study protein synthesis in vitro. Its preparation was achieved by modifying the Mathew's technique (6). Briefly, the ascites cells were lysed in an hypotonic medium and homogenized with a glass Dounce homogenizer. The homogenate was centrifuged at 30,000 × g for 15 min to remove the nuclei and the cell membranes. The supernatant ("S-30 lysate") was preincubated at 37 C for 30 min in the presence of 1) the 20 t-aminos acids naturally occurring into proteins and 2) energetic material: ATP, GTP, creatine phosphate, and creatine phosphokinase. After incubation, the newly formed proteins were discarded by centrifugation at 15,000 × g for 15 min. Then, the S-30-lysate was passed through a G-25 Sephadex column to collect the heavy material which contained the molecules necessary for the protein synthesis. After distribution in aliquots, the S-30 lysate was rapidly frozen and stored at -90 C until time for analysis.

3H-Leucine incorporation assay

The 3H-leucine incorporation rate in the presence of uremic plasma dialysate was tested in an incubating medium of 122 µl per assay made up as follows: 50 mM Tris-HCl-pH 7.5, 60 mM potassium chloride, 3 mM magnesium acetate, and the naturally occurring t-aminos acids in the proportion defined by Borsook et al. (7) excepted t-leucine which was added as radioactive label: 2 µl 3H-leucine (specific activity 100 mCi/mole), 25 mM ATP, 6 mM GTP, 200 mM creatine phosphokinase, 75 mM reduced glutathione, and increasing amounts of plasma dialysate from 0 to 30 µl. The reaction was started when 20 µl of S-30-lysate were brought into the tubes. After an incubation of 90 min at 37 C, the reaction was stopped by placing the tubes in ice and the non-incorporated 3H-leucine was chased by washing with molar unlabeled leucine. The tubes were again incubated at 37 C for 20 min. The proteins were precipitated with cold trichloroacetic acid, the precipitate was filtered through a Millipore filter (for size 0.45 µ) and the β emission was counted by liquid scintillation.

Results

3H-leucine incorporation rates in the presence of plasma from severely uremic patients and from hemodialyzed subjects are shown in Table 1 and schematically represented in Figure 1. They were compared with those noted in the presence of plasma dialysate from seven healthy subjects.

In the presence of increasing amounts of plasma from untreated uremic patients, there was no increase in the rate of 3H-leucine incorporation; actually, a small and linear decrease was observed (Fig. 1). The addition of increasing amounts of predialysis plasma dialysate from patients submitted to a long-term hemodialysis treatment also produced a decrease of the 3H-leucine incorporation rate, if anything more pronounced than in non-hemodialyzed patients (Fig. 1). However, the difference between effects of the plasma from both groups was not statistically significant. The 3H-leucine incorporation rates were significantly lower than observed with plasma from normal subjects (Fig. 1) when 15 µl or more of plasma dialysate (P < 0.01) were used.

Despite a marked decrease of 50% of the incorporated leucine into proteins in the presence of small amounts (5 µl) of added dialysate from two patients on a poor nitrogen diet, the difference between the curves obtained with plasma dialysate from patients on a low- and high-protein diet was not statistically significant.

The effects of plasma taken from uremic subjects before and after a dialysis procedure on 3H-leucine incorporation are represented on Figure 2. From the comparison between the pre- and postdialysis incorporation curves, it was obvious that dialysate from postdialysis plasma enhanced the incorporation of 3H-leucine into trichloroacetic acid-precipitable material, presumably protein, by about the same amount.

Five of the six plasma dialysates from transplanted patients exhibited the same 3H-leucine incorporation as that of control ultrafiltrate. One transplanted patient exhibited
TABLE 1

<table>
<thead>
<tr>
<th>Added dialysate (μl)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control dialysate</td>
<td>7840</td>
<td>8005</td>
<td>8760</td>
<td>9055</td>
<td>10 400</td>
<td>8730</td>
<td>7680</td>
</tr>
<tr>
<td>(cpm ± SD, n = 7)</td>
<td>450</td>
<td>605</td>
<td>500</td>
<td>490</td>
<td>610</td>
<td>640</td>
<td>440</td>
</tr>
<tr>
<td>Severely uremic patients</td>
<td>7840</td>
<td>7830</td>
<td>7620</td>
<td>7440</td>
<td>7300</td>
<td>7060</td>
<td>6950</td>
</tr>
<tr>
<td>(cpm ± SD, n = 12)</td>
<td>450</td>
<td>660</td>
<td>545</td>
<td>460</td>
<td>490</td>
<td>470</td>
<td>425</td>
</tr>
<tr>
<td>Hemodialysed patients</td>
<td>7840</td>
<td>7150</td>
<td>6970</td>
<td>7360</td>
<td>7000</td>
<td>6880</td>
<td>6560</td>
</tr>
<tr>
<td>(cpm ± SD, n = 6)</td>
<td>450</td>
<td>450</td>
<td>280</td>
<td>320</td>
<td>290</td>
<td>230</td>
<td>295</td>
</tr>
</tbody>
</table>

FIG. 1. \(^3\)H-leucine incorporation into trichloroacetic acid-precipitable material as a function of the amount of added plasma dialysate in the incubating medium. Control plasma dialysate: full line, upper curve (●—●), plasma dialysate from severely uremic patients: dotted line, middle curve (○—○); plasma dialysate from hemodialyzed patients: full line, lower curve (●—●). Symbols indicate mean and bars ± 1 SD.

FIG. 2. Effect of a dialysis procedure on the \(^3\)H-leucine incorporation rate into proteins in the presence of plasma dialyzed from two hemodialyzed patients before (■) and after (□) a dialysis procedure. Full line, patient no. 1; dotted line, patient no. 2.

a “uremic” pattern; his renal function was similar to that of the five others, but his general condition was poor with recurrent extrarenal infections (Fig. 3).

Discussion

The observation of a diphasic curve of \(^3\)H-leucine incorporation into trichloroacetic acid-precipitable material in the controls is consistent with the following interpretation. Amounts less or equal to 20 μl of added plasma dialysate from healthy subjects exhibited an increasingly activatory effect on protein synthesis as compared to the incorporation rate in the absence of dialysate (zero abscissa, Fig. 1). By contrast, amounts of plasma dialysate greater than 20 μl depress protein synthesis. That could be ascribed to
Experiments in which pre- and postdialysis plasma was added into the incubating medium strongly suggest that a major portion of the inhibitory molecules were removed from the plasma by dialysis. Although information on the structure of such molecules is lacking, it is reasonable to conclude that they could pass across the commercially available dialysis membranes; i.e., they are of low or middle molecular weight. Preliminary studies in our laboratory suggest that the inhibitory effect of in vitro protein synthesis is mainly carried by a peptide molecule of a MW <700 (C. Delaporte and F. Gros, unpublished observations).

As one might have anticipated, the inhibition of protein synthesis by uremic plasma dialysate was reversible after transplantation. Such an observation was not surprising since a functional transplantated kidney removes the catabolites accumulated in plasma during uremia. This single exception reported in this paper was probably related to extrarenal causes which may hinder the full recovery of metabolic processes regulating protein synthesis.

References