The Permeation of Essential Amino Acids through Different Dialysis Membranes

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Abnormalities in protein metabolism in patients with terminal renal failure are not always fully corrected by adequate maintenance haemodialysis (Aviram et al, 1971). The permeation of amino acids through cellulose membranes and therefore the loss of amino acids into the dialysate fluid are discussed by several authors as a possible contribution to the persisting protein catabolism and the development of hypoproteinaemia and muscle wasting in many patients on regular dialysis treatment (Giordano et al, 1968; Giovannetti & Maggiore, 1964; Young & Parsons, 1966; Rubini & Gordon, 1968; Ginn et al, 1968; Fürst et al, 1970; Norée et al, 1971).

These data, however, show a rather wide range in the calculated or measured loss of amino acids into the dialysis bath. Giordano et al (1968) reported the remarkable loss of up to 20 g but Aviram et al (1971) calculated only a loss of a small percentage of the minimal daily requirement. Apart from enhanced catabolism of protein and changes in plasma levels of amino acids or other abnormalities in protein synthesis in uraemia, the different results could be due to the different dialysis equipment used in these experiments.

The purpose of this study was to provide some data about the possible difference in mass transfer rate of essential amino acids (EAA) through commercially available cellulose membranes that are in routine use for dialysis treatment.

MATERIALS AND METHODS

The mass transfer rates through Nephrophane, Cuprophane PT 150, Visking Cellophane PT 300 and Neflex DVF 30 B have been studied for the 8 essential amino acids using a modified Muir-test cell as described earlier (Muir & Ross, 1967; Klinkmann et al, 1968).

Standard solutions with a concentration of 200 mg/l were prepared for each individual EAA and for creatinine. Distilled water was used as dialysis
bath fluid. The circulation speed was 150 ml/min for the various test solutions. The bath fluid circulated at 500 ml/min through the test cell during the one hour of the experiment. The whole test cell assembly was maintained at a constant temperature of 37°C.

The EAA content in the test solution and in the dialysate was determined by using a Beckman automatic amino acid analyser. Creatinine was determined by using the alkaline picrate method.

The mass transfer rates obtained from the test cells are expressed in μMol/cm²/min and have been extrapolated to mMol/m²/h for better comparison with routinely used dialysers (Table I).

These in vitro results have then been adjusted from the 200 mg l solution to the normal plasma levels for the individual amino acid (Müting, 1958) in order to calculate the possible loss of amino acids per m² membrane and hour of dialysis.

The relation between the mass transfer rates through the 4 different membranes and the molecular weight has been investigated for each EAA using the regression analysis (r > 0.5 for a statistically significant correlation).

Table I. Mass transfer rates (mMol/m²/h) through 4 cellulose membranes for essential amino acids (EAA) and creatinine

<table>
<thead>
<tr>
<th>Amino-acid</th>
<th>Nephrophone</th>
<th>Cupropane</th>
<th>Visking-Cellophane</th>
<th>DVF-30B</th>
<th>Molecular-weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>14.28</td>
<td>13.81</td>
<td>10.85</td>
<td>7.30</td>
<td>117.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>17.32</td>
<td>16.00</td>
<td>11.59</td>
<td>3.70</td>
<td>119.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>13.91</td>
<td>7.85</td>
<td>5.35</td>
<td>2.48</td>
<td>131.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>16.95</td>
<td>12.16</td>
<td>10.57</td>
<td>3.72</td>
<td>131.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>19.08</td>
<td>14.26</td>
<td>10.43</td>
<td>8.75</td>
<td>146.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>10.46</td>
<td>12.45</td>
<td>8.44</td>
<td>6.97</td>
<td>149.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10.08</td>
<td>8.11</td>
<td>4.68</td>
<td>0</td>
<td>165.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>11.04</td>
<td>10.56</td>
<td>5.72</td>
<td>6.31</td>
<td>204.2</td>
</tr>
<tr>
<td>Creatinine</td>
<td>14.20</td>
<td>10.32</td>
<td>8.01</td>
<td>3.46</td>
<td>113.12</td>
</tr>
</tbody>
</table>

RESULTS

Table I shows the transfer rates in mMol/m²/h for the EAA and for creatinine through the different membranes in our dialysis test cell. The 4 membranes show a remarkable difference in the transfer of the EAA. In accordance with the decrease of creatinine transfer, the transfer of the EAA decreases significantly in the sequence Nephropane, Cupropane PT 150, Visking Cellophane PT 300 and Neflex DVF 30 B.

The graphic representation of these results (Figure 1) demonstrates
this significant decrease in the mass transfer rate dependent on the specific membrane. The creatinine transfer rate is taken as the reference line. The actual transfer of each individual EAA is almost identical with that of creatinine in each membrane investigated.

A clear relationship between the molecular weight and the mass transfer rate cannot be seen from this data for the group of the EAA. The regression analysis for this expected correlation is only significant ($r = 0.54$) in the Nephrophane experiment (Figure 2).

Neither for Cuprophane (Figure 3), Visking Cellophane (Figure 4) nor for Neflex DVF 30 B (Figure 5) the regression analysis is significant.

In order to compare these in vitro results with the actual loss of the EAA during dialysis treatment the test cell data have been converted from mMol/m²/h into mg/m²/h after adjusting the concentration of the EAA in
Figure 3. Regression line for the correlation between molecular weight and mass transfer rate through Cuprophan PT 150 (double determination of each essential amino acid)

Visking - Cellophane

Figure 4. Regression line for the correlation between molecular weight and mass transfer rate through Visking Cellophane PT 300 (double determination of each essential amino acid)

DFV 30B

Figure 5. Regression line for the correlation between molecular weight and mass transfer through Neflex DFV 30 B (double determination of each essential amino acid)
Table II. Calculated loss of essential amino acids through cellulose membranes in mg/m²/h, assuming normal plasma levels

<table>
<thead>
<tr>
<th>amino acid</th>
<th>Nephrophane</th>
<th>Cuprophane</th>
<th>Visking-Cellophane</th>
<th>DVF-30 B</th>
<th>molecular-weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>267.5</td>
<td>259.0</td>
<td>203.0</td>
<td>137.0</td>
<td>117.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>114.0</td>
<td>105.0</td>
<td>71.2</td>
<td>24.2</td>
<td>119.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>227.5</td>
<td>88.0</td>
<td>38.6</td>
<td>41.3</td>
<td>131.2</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>77.5</td>
<td>56.0</td>
<td>48.6</td>
<td>17.1</td>
<td>131.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>264.5</td>
<td>197.5</td>
<td>144.8</td>
<td>121.5</td>
<td>146.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>62.3</td>
<td>74.5</td>
<td>50.5</td>
<td>41.4</td>
<td>149.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>125.0</td>
<td>94.3</td>
<td>54.2</td>
<td>0</td>
<td>165.2</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>169.5</td>
<td>161.0</td>
<td>87.8</td>
<td>96.9</td>
<td>204.2</td>
</tr>
<tr>
<td>Σ</td>
<td>1307.8</td>
<td>1035.3</td>
<td>698.7</td>
<td>479.4</td>
<td></td>
</tr>
</tbody>
</table>

the test cell to the normal plasma level (Table II).

These calculated data show that during routine dialysis treatment one can expect the greatest loss for valine and lysine through all 4 membranes. In the case of tryptophane one can certainly not compare the in vitro results with clinical results because of the extensive binding of tryptophan to albumin in uraemic patients. The mass transfer rate for the remaining EAA and therefore their possible loss during dialysis treatment differs remarkably between the 4 membranes (see Table I and Table II).

COMMENTS

Different manufactured cellulose membranes show a remarkable difference in the mass transfer rate for the EAA. We are able to confirm the clinical results of Shinaberger and Ginn (1967) that the actual dialysance of each of the individual EAA is almost identical with that of creatinine in each membrane investigated.

Probably the transfer of EAA through the cellulose membranes depends on the shape and size of the pores of the individual membrane in combination with the specific structure of the amino acid molecules.

A statistically significant correlation as one could expect between the mass transfer rate and the molecular weight of each EAA was only found for Nephrophane — the cellulose membrane with the thinnest wall and the largest pores (Klinkmann et al., 1968).

The calculated loss of EAA through an unsupported dialysis membrane per square meter an hour varies from 1.3 g in Nephrophane, 1.0 g in Cuprophane, 0.7 g in Visking Cellophane to 0.5 g in Neflex DVF 30 B.

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These figures are comparable to the amino acid loss into dialysate determined under clinical conditions by Giordano et al (1968).

In all 4 membranes Valine and Lysine show the greatest transfer rate among the EAA. This confirms the results of Aviram et al (1971) and Norée et al (1971).

In conclusion, these in vitro results should be interpreted carefully for the actual in vivo situation. But despite the specific protein catabolism in uraemia and during dialysis treatment we are sure that the loss of EAA during dialysis treatment depends on a certain amount on the dialysis membrane and possibly on the technical construction of dialysis hardware used for the dialysis treatment. Whether these differences in mass transfer for EAA through different membranes will be of any clinical significance in long term dialysis treatment remains to be investigated.

REFERENCES

Giovannetti, S. and Maggiora, Q. (1964) Lancet, i, 1000
Muir, W. M. and Ross, D. S. (1967) Proceedings of the VIIth International Conference on Medical and Biological Engineering, 499
OPEN DISCUSSION

R DZURIK (Bratislava): Professor Klinkmann, you have got a rather bad correlation, or no correlation at all, between the molecular weight and dialysance. I wonder whether you would not get a better correlation if you had calculated not only the molecular weight but also the hydration of the aminoacids?

KLINKMANN: Well, I think you are right, but so far as in vitro loss is concerned it has also been shown by Gyulassy that there is no direct correlation. Of course, if you take into account the water binding you probably would get a better correlation. But I guess that knowing the ultrastructure of these different membranes would be essential. Even the varying ultrastructure pores and the different steric configurations might be a great influence on the rate of permeation.