Kinetic studies of dipeptide-based and amino acid-based peritoneal dialysis solutions

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Background. Dipeptide-based peritoneal dialysis solutions may have potential advantages compared with the glucose or amino acid-based solutions. Dipeptides may hydrolyze in the peritoneal cavity, generating constituent amino acids and thereby increasing the osmolality of the dialysate. Dipeptides can also be a valuable source of amino acids, which are poorly soluble in water, such as tyrosine.

Methods. Dwell studies in rats were performed during four hours with dipeptide solutions containing five dipeptides (Gly-His, Ala-Tyr, Thr-Leu, Ser-Phe, Val-Lys), 8, or 16 mmol/L of each dipeptide (low or high dipeptide group). Dwell studies were also performed with a 1.1% amino acid solution (Nutrineal®). The model of dipeptide hydrolysis (hydrolysis rate, \( K_H \)), diffusive (rate constant, \( K_{BDD} \)) and convective transport as well as transport of constituent amino acids consisted of mass balance equations, written for each dipeptide and amino acid.

Results. Peritoneal volume with the amino acid solution decreased much faster than that with the high and low dipeptide solutions. \( K_H \) for all dipeptides did not differ between the high and low dipeptide groups. In the low dipeptide group, \( K_H \) was 0.004 ± 0.004 mL/min (mean ± SD) for Gly-His (the lowest) and 0.088 ± 0.048 mL/min for Thr-Leu (the highest). \( K_{BDD} \) was higher than \( K_H \) for all dipeptides, the average being 0.2 ± 0.05 mL/min.

Conclusions. Dipeptides are hydrolyzed in the peritoneal cavity, generating constituent amino acids. However, the hydrolysis rate appears to be several times lower than the dipeptide diffusive transport rate from dialysate to blood. Due to the higher molecular weight and intraperitoneal generation of amino acids, the dipeptide-based solutions provide more sustained ultrafiltration than the amino acid solution. The plasma concentration of amino acids at 60 minutes, in relation to the dose of amino acids delivered between 0 and 60 minutes, is considerably higher during the dwells with amino acid-based solution than during dwells with the dipeptide-based solutions.

Key words: dialysate, osmotic agents, end-stage renal disease, malnutrition and PD, fluid homeostasis.

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In peritoneal dialysis, a sterile electrolyte solution together with an osmotic agent is instilled into the peritoneal cavity with the aim of removing uremic toxins, preserving electrolyte homeostasis and preventing accumulation of fluid. However, the solutions presently in use are far from ideal. Glucose, which is the most commonly used osmotic agent, has several drawbacks. Due to its low molecular weight, it is rapidly taken up by diffusive transport which renders it relatively inefficient, especially in patients who are “high transporters,” that is, who have a peritoneal membrane with a high permeability for small molecules, necessitating the use of frequent exchanges with solutions having high glucose concentrations to prevent fluid accumulation [1]. The glucose taken up from the dialysis solution may increase the total energy intake, lead to obesity and changes in lipoproteins that are potentially atherogenic [2]. Moreover, the high concentration of glucose in peritoneal dialysis solutions could be detrimental because it may adversely affect the peritoneal membrane through glycosylation of proteins or by other mechanisms [3].

A mixture of amino acids has also been successfully introduced as an alternative osmotic agent in peritoneal dialysis solutions [4]. By using an amino acid solution for one or two exchanges a day, the number of exchanges with glucose-containing solutions may be reduced. Amino acids are absorbed from the dialysis fluid [5], and this may counteract dialytic protein and amino acid losses and provide an extra supply of amino acids, which may be advantageous as regards nutrition [6, 7], considering that protein malnutrition is a common problem in patients with end-stage renal disease (ESRD). Amino acids are also reported to be more biocompatible than glucose regarding local effects on the peritoneal membrane [6, 8]. However, amino acids are relatively small molecules (on average about 140 daltons) and therefore have no advantage over glucose as osmotic agents [9]. Moreover, low solubility of some amino acids may be a limiting factor in making an adequate amino acid solution from the
nutritional point of view [10]. One such amino acid is tyrosine, which is considered essential in patients with renal failure due to reduced generation from phenylalanine [11]. However, dipeptides of tyrosine, such as alanly-tyrosine and glycyl-tyrosine have a higher solubility [12]. Glycyl-tyrosine has been added to amino acid solutions for parenteral nutrition of patients with renal failure to increase the supply of tyrosine more than is possible with free tyrosine in the solution [2, 13].

After a protein-rich meal, part of the protein is absorbed from the gut as dipeptides, which are rapidly hydrolyzed into their constituent amino acids [13]. Similarly, after parenteral administration, dipeptides are rapidly metabolized and free amino acids are produced [10, 12].

Dipeptides might be useful as osmotic agents, since they have about twice as high molecular weight as amino acids and should therefore diffuse more slowly out of the peritoneal cavity. They may even be hydrolyzed into the constituent amino acids in the peritoneal cavity, thus generating additional osmotically-active molecules, which might further enhance ultrafiltration. Tyrosine-containing dipeptides may also be of benefit, since they may be present in the dialysis fluid at higher concentrations than are possible with free tyrosine.

In the present study, in a rat model we investigated the diffusive and convective transport properties of the peritoneum, using a dialysis solution containing an equimolar mixture of 5 dipeptides at two concentrations, and compared the results with those obtained from a solution containing free amino acids. We also determined to what extent the dipeptides were hydrolyzed to their constituent free amino acids in the peritoneal cavity, the rate of disappearance of dipeptides and amino acids from the peritoneal cavity, and the rate of appearance of amino acids in plasma.

### METHODS

#### Solutions

The following solutions were used: (1) an amino acid solution (Nutrineal®, AA solution) containing 15 amino acids in varying proportions and with a total amino acid concentration of 86 mmol/L (its composition is given in Table 1); (2) a weak (Dip 8) solution containing the five dipeptides, glycyl-histidine (Gly-His), alanyl-tyrosine (Ala-Tyr), threonyl-leucine (Thr-Leu), seryl-phenylalanine (Ser-Phe) and valyl-lysine (Val-Lys) in equimolar concentrations (8.4 mmol/L), with a total concentration of dipeptides of 42 mmol/L [alanine-tyrosine was a kind gift from Professor Peter Fürst (Hohenheim University, Stuttgart, Germany) and all other dipeptides were purchased from Sigma (St. Louis, MO, USA)]; (3) a strong (Dip 16) solution containing the same five dipeptides in double the amounts (16.8 mmol/L) with a total concentration of 84 mmol/L dipeptides.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Molecular weight</th>
<th>Concentration mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histidine</td>
<td>155.2</td>
<td>4.60</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>131.2</td>
<td>6.48</td>
</tr>
<tr>
<td>Leucine</td>
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<td>7.77</td>
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<tr>
<td>Lysine</td>
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<tr>
<td>Methionine</td>
<td>184.7</td>
<td>4.60</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>165.2</td>
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<tr>
<td>Threonine</td>
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<td>5.42</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>204.2</td>
<td>1.32</td>
</tr>
<tr>
<td>Tyrosine</td>
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<td>1.66</td>
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<tr>
<td>Valine</td>
<td>117.1</td>
<td>11.90</td>
</tr>
<tr>
<td>Alanine</td>
<td>89.1</td>
<td>10.67</td>
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<tr>
<td>Arginine</td>
<td>174.2</td>
<td>6.15</td>
</tr>
<tr>
<td>Glycine</td>
<td>75.1</td>
<td>6.79</td>
</tr>
<tr>
<td>Proline</td>
<td>115.1</td>
<td>5.17</td>
</tr>
<tr>
<td>Serine</td>
<td>105.1</td>
<td>4.85</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>86.03</strong></td>
</tr>
</tbody>
</table>

Amino acids included in dipeptide solution are marked in bold.

The three solutions had the following electrolyte composition: sodium, 132 mmol/L; calcium, 1.25 mmol/L; magnesium, 0.25 mmol/L; lactate, 40 mmol/L; and chloride, 105 mmol/L.

The Dip 8 solution contained half as many moles of dipeptides per liter as the number of moles of free amino acids in the amino acid-based solution (that is, the osmolality of the amino acid-based solution was approximately twice as high). Both the amino acid and the Dip 8 solutions had similar total amino acid contents. The Dip 16 solution had similar total molar concentration as the amino acid-based solution (that is, the osmolality was approximately the same) but the amino acid content was twice as high.

Seven of the amino acids in the dipeptide solutions were essential amino acids, that is, leucine, histidine, lysine, phenylalanine, threonine, valine and tyrosine (tyrosine is an indispensable amino acid in uremia), which may be of potential nutritional benefit in future solutions. Market availability and prices were considered when selecting the dipeptides to be used. By studying two dipeptide solutions, one hypo-osmolar with the same amino acid content as in the amino acid solution and one iso-osmolar with double the amino acid content, we could analyze the properties of dipeptides as osmotic agents and as providers of amino acids.

#### Animals

Twenty-four male Sprague-Dawley rats with an average body weight of 300 g were divided into three groups (with 8 rats in each group). Each rat was anesthetized with a single intraperitoneal injection of 50 mg/kg pure pentobarbital sodium (Pharmacia, Uppsala, Sweden). The fur over the abdominal wall was closely shaved. The animal was laid in a supine position and was kept at

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Table 1. Composition of constituent amino acids in amino acid-based (Nutrineal) solution.
37°C with a heating pad (CMA/Microdialysis, Stockholm, Sweden). Isotonic saline, 1 mL/hour, was injected subcutaneously to prevent hypovolemia. A multiholed silastic catheter (0.8 mm internal diameter; Venoflon, Helsingborg, Sweden) was inserted percutaneously in the left lower quadrant of the abdomen for dialysis fluid infusion and sampling. The experiment was started by giving an intraperitoneal injection of 25 mL of 1.1% amino acid dialysis solution (Nutrineal® AA solution) or the weak (Dip 8) or strong (Dip 16) dipeptide solution, containing 18.5 KBq131 I-human serum albumin (RISA), Isopharma AS, Kjeller, Norway) as a peritoneal volume marker. The solutions were prewarmed to 37°C before injection.

A priming dose of human serum albumin (final concentration 0.2 g/L) was added to the solutions to minimize the adhesion of tagged albumin to the surface of the catheter. After infusion, the dialysis solution remained in the peritoneal cavity for four hours. Dialysate samples and plasma were measured with reverse-phase HPLC, 120 minutes, respectively.

The peritoneal volume was flushed back and forth five times the net volume change in time plus the amino acid dialysis solution (Nutrineal® AA solution) or the weak (Dip 8) or strong (Dip 16) dipeptide solution, containing 18.5 KBq131 I-human serum albumin (RISA), Isopharma AS, Kjeller, Norway) as a peritoneal volume marker. The solutions were prewarmed to 37°C before injection.

Amino acid and peptide concentrations in dialysate and plasma were measured with high-pressure liquid chromatography (HPLC; Waters, Milford, MA, USA).

Amino acid and peptide concentrations in dialysate and plasma were measured with reverse-phase HPLC, essentially as described by Suliman, Anderstam and Bergström for amino acids [14]. Briefly, samples were deproteinized with 5-sulfosalicylic acid, filtered and stored at −70°C. The determinations of amino acids and dipeptides were carried out using fluorometric detection (Ex = 340 nm and 450 nm). The method was based on automated precolumn derivatization with orthophthalaldehyde/hydroxy/3-mercaptopropionic acid. Mobile phases used were solvent A [12.5 mmol/L phosphate buffer, pH 6.93, and tetrahydrofuran 99:1 (vol:vol)] and solvent B [methanol, acetonitrile and deionized water 35:15:50 (vol:vol)]. A chromatographic run lasted for 54 minutes. The HPLC system (Waters Associate) consisted of a Waters system controller 600E and two M-45 pumps, an auto-sampler WISP-712B with a cooling system to optimize the temperature at +4°C and a Waters 470 fluorescence detector. The amino acids and dipeptides were separated on a C18 column, 4.6 × 150 mm (YMC Co. Ltd, Kyoto, Japan). Chromatograms were integrated in a computerized data system (Chromatography Data System AB, Stockholm).

Calculations

Intraperitoneal dialysate volume (Vd) was estimated from the dilution of RISA with corrections made for the elimination of RISA from the peritoneal cavity and the sample volume [15–18]. Note that the intraperitoneal volume at the end of the dwell was directly measured. The RISA elimination coefficient (KF) was calculated using a formula reported previously [15–18]. Since only a very small amount of free iodine was found in the RISA solution [19], we did not take the free iodine into account in the KE calculation. The actual calculations of KE and time course of Vd were performed using the computer program PERTRAN (Baxter Novum, Karolinska Institutet, Stockholm, Sweden), available at internet address: http://www.ibib.waw.pl/peritone. The peritoneal fluid absorption rate (QA, mL/min) was estimated as the coefficient of RISA elimination from the peritoneal cavity, KE. The transcapillary ultrafiltration rate (Qf) was calculated as the net volume change in time plus KE (Appendix II).

The kinetic model developed for dipeptide transport and hydrolysis as well as transport of amino acids is shown in Figure 1 and described in Appendix I (Appendices I, II and III may be viewed in the online version of the journal: http://dx.doi.org/10.1046/j.1523-1755.2001.00499.S).

The method of estimating kinetic parameters is described in Appendix II.

The amount (mass) of a particular amino acid transported from dialysate during the dwells with the amino acid-based solution, between 3 and 120 minutes of the dwell, was calculated as follows:

\[ \Delta M_A = V_B(3)C_{DA}(3) - V_B(120)C_{DA}(120) \]

where V_B(3) and V_B(120) are dialysate volumes at 3 and 120 minutes, respectively. C_{DA}(3) and C_{DA}(120) are amino acid concentrations at 3 and 120 minutes, respectively.

The amount of total amino acids in the dialysate in dwells with dipeptide-based solutions was calculated as the sum of the amount of dipeptide (µmol) and of constituent free amino acids (µmol). This calculation is valid only for amino acids contained in dipeptides in fresh dialysis solution. The amount of a particular amino acid transported from the dialysate in dwells with dipeptide-based solution could therefore be calculated as:

\[ \Delta M_A = V_B(3)[C_{DD}(3) + C_{DA}(3)] - V_B(120)[C_{DD}(120) + C_{DA}(120)] \]

where C_{DD}(3) and C_{DD}(120) are dipeptide concentrations at 3 and 120 minutes, respectively, and C_{DA}(3) and C_{DA}(120) are concentrations of free constituent amino acids at 3 and 120 minutes of the dwell. The reason for choosing measurements at 120 minutes instead of 240 minutes,
Fig. 1. Kinetic model of diffusive and convective transport of dipeptides and amino acids. Nomenclature is: $V_D$, peritoneal volume; $C_{DD}$ and $C_{DA}$, dialysate and blood dipeptide concentrations, respectively; $C_{BA}$ and $C_{BA}$, concentrations of amino acids in dialysate and blood plasma, respectively; $K_H$, the hydrolysis rate of dipeptide; $K_{BDD}$ and $K_{BDA}$, diffusive mass transport rate coefficients of dipeptides and amino acids, respectively; $Q_A$, fluid absorption rate; $Q_V$, ultrafiltration flow rate. This model does not include exchanges between the blood and extravascular compartments.

The relative changes in the concentration of amino acids in plasma were calculated as:

$$\frac{\Delta C_{DA}}{\Delta M_A} = \frac{C_{BA}(60) - C_{BA}(0)}{V_D(3)[C_{DD}(3) + C_{DA}(3)] - V_D(60)[C_{DD}(60) + C_{DA}(60)]}$$

$C_{BA}$ at 60 minutes was chosen, $C_{BA}(60)$, because the maximal concentration of amino acids in plasma occurred at about 60 minutes. The results are expressed as mean ± SD. Comparisons of normalized plasma amino acid concentrations were performed, using ANOVA and Student $t$ test for comparison of kinetic parameters. A $P$ value of less than 0.05 was considered significant.

RESULTS

Peritoneal volumes ($V_D$) over time curves, normalized by $V_D$ at 3 minutes [$V_D(3)$] for the three dialysis solutions, are shown in Figure 2. Dwell with the amino acid-based solution exhibited an earlier maximum $V_D$ than with the Dip 16 solution. The subsequent volume decrease was more rapid than that with the Dip 16 solution and similar to that with the Dip 8 solutions. The average volume decreases between 90 and 240 minutes of the dwells were 3.4 mL and 3.5 mL with Dip 8 and Dip 16 dipptide-based solutions, respectively, and 4.8 mL with the amino acid-based solution.

In Figure 3, the time courses of the concentrations of particular dipeptides and constituent amino acids in the dialysate during dialysis with the dipptide-based strong (Dip 16) solution are shown. The time courses of dipeptides and amino acids obtained with Dip 8 showed similar patterns (data not given). The dipeptides disappeared approximately exponentially from the dialysate, while the concentrations of the constituent amino acids increased, due to their transport between blood and dialysate and the cleavage of dipeptides occurring in the peritoneal cavity.

The kinetic model allowed for a fairly accurate quantitative description of the complex processes of dipptide hydrolysis in the peritoneal cavity, characterized by the hydrolysis rate coefficient ($K_H$), and dipptide diffusion from the peritoneal cavity to plasma, characterized by the diffusive mass transport coefficient ($K_{BDD}$) (Fig. 1 and Appendix I). Since the plasma dipptide concentrations were negligible, the convective dipptide transport by
ultrafiltration was neglected. Convective flow of dipeptides from the dialysate was expressed as a product of the fluid absorption rate ($Q_A$) and the dipeptide dialysate concentration ($C_{DD}$). The generation rate of amino acids from dipeptides in the peritoneal cavity was described as the product of dipeptide hydrolysis rate ($K_D$) and $C_{DD}$. The model also included transport of constituent amino acids between dialysate and plasma by diffusion (mass
transport rate coefficient, \( K_{\text{BDA}} \) and convection from plasma to dialysate, characterized by the sieving coefficients, \( S_A \), as well as convective absorption from the dialysate, in which the sieving coefficient was assumed to be one. The method for estimating a model’s parameters is given in Appendix II.

Table 2 shows the average values of diffusive mass transport coefficients \( (K_{\text{BDA}}) \) for 10 amino acids that were estimated from eight experiments with the amino acid-based dialysis solution. The \( K_{\text{BDA}} \) values for different amino acids did not differ substantially (maximum value of 0.334 ± 0.096 mL/min for glycine and a minimum value of 0.242 ± 0.056 mL/min for phenylalanine). However, increasing molecular weight was accompanied by decreasing values of \( K_{\text{BDA}} \) (Fig. 4).

The diffusive mass transport coefficients for dipeptides \( (K_{\text{BDD}}) \), estimated without any assumption concerning amino acid transport, and \( K_{\text{BDD}} \) estimated with \( K_{\text{BDA}} \) taken from amino acid-based dialysis studies (Table 2), were calculated using data from the dwell studies with weak and strong dipeptide-based dialysis solutions (Table 3). The difference between \( K_{\text{BDD}} \) and \( K_{\text{BDD}} \) and the methods used in estimation of their values are described in Appendix II. As shown in Table 3, the diffusive mass transport coefficients for dipeptides, \( K_{\text{BDD}} \) or \( K_{\text{BDD}} \), tended to be higher with the Dip 16 than with the Dip 8 solution. Another characteristic feature is the dependence of \( K_{\text{BDD}} \) \( (K_{\text{BDD}}) \) on dipeptide molecular weight. In Figure 4, the diffusive mass transport coefficients for amino acids \( (K_{\text{BDA}}) \) and that for dipeptides \( (K_{\text{BDD}}) \) estimated in dwell studies with weak \( (\text{Dip 8}) \) solution are presented in reference to the corresponding molecular weights. \( K_{\text{BDD}} \) estimated in dwell studies with Dip 8 solution is probably better suited than that of Dip 16 for comparison with \( K_{\text{BDA}} \), because the concentrations of amino acids in the amino acid solution and of dipeptides in the Dip 8 solution are more similar.

The hydrolysis rate coefficients for the five dipeptides are presented in Table 4. It should be noted that the lowest \( K_{\text{B}i} \) values were found for Gly-His with both the weak and the strong dipeptide-based solutions.

For each dipeptide, \( K_{\text{BDD}} \) \( (K_{\text{BDD}}) \) was much higher than \( K_{\text{B}i} \) \( (K_{\text{B}i}) \); Tables 3 and 4). This means that transport of dipeptides out of the peritoneal cavity occurs at a much higher rate than the rate of hydrolysis. Statistically, \( K_{\text{B}i} \) and \( K_{\text{B}i} \) as well as \( K_{\text{BDD}} \) and \( K_{\text{BDD}} \) showed no significant differences, and both methods of estimating kinetic parameters for dipeptides seem to be equally accurate.

Since the concentration of dipeptides in blood was negligible, there was no convective transport of dipeptides from blood to dialysate with ultrafiltration flow and the only convective transport was by fluid absorption from the peritoneal cavity. The fluid absorption rate \( (Q_A) \) was estimated by the volume marker elimination rate \( (K_k) \). The average values of \( Q_A \) were estimated as 0.026 ± 0.008 mL/min in dwells with the weak \( (\text{Dip 8}) \) dipeptide-based solution and 0.031 ± 0.005 mL/min in dwells with the strong \( (\text{Dip 16}) \) dipeptide-based solution. The average values of \( Q_A \) did not differ statistically between the groups. These values of \( Q_A \) were much smaller than the values of \( K_{\text{BDD}} \) \( (K_{\text{BDD}}) \), reflecting that the convective transport of dipeptides was very small as compared to the transport by diffusion.

Table 5 shows the amounts (masses), \( \Delta M_A \), and the fractional amounts of amino acids transported from the dialysate, \( \Delta M_A/M_A(3) \), where \( M_A(3) \) is the amount of amino acids at 3 minutes of the dwell. These fractional amounts were quite similar in the dwells with amino acids and in the dwells with strong and weak dipeptide-based solutions.

The time courses of blood concentrations of amino acids exhibited an initial rise with maximum values achieved after 30 or 60 minutes, followed by a gradual fall, as shown in Figure 5. This pattern was observed in all dwell studies. Histidine, lysine, threonine and serine showed very similar plasma amino acids concentration time courses during dwells with the amino acid solution and Dip 8 solution.

We also wished to determine whether the increase in plasma concentrations in relation to the uptake of amino

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Molecular weight</th>
<th>( K_{\text{BDA}} ) mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>75.01</td>
<td>0.334 ± 0.096</td>
</tr>
<tr>
<td>Alanine</td>
<td>89.1</td>
<td>0.282 ± 0.069</td>
</tr>
<tr>
<td>Serine</td>
<td>105.1</td>
<td>0.266 ± 0.066</td>
</tr>
<tr>
<td>Valine</td>
<td>117.1</td>
<td>0.243 ± 0.053</td>
</tr>
<tr>
<td>Threonine</td>
<td>119.1</td>
<td>0.255 ± 0.134</td>
</tr>
<tr>
<td>Leucine</td>
<td>131.2</td>
<td>0.251 ± 0.072</td>
</tr>
<tr>
<td>Lysine</td>
<td>146.2</td>
<td>0.275 ± 0.128</td>
</tr>
<tr>
<td>Histidine</td>
<td>155.2</td>
<td>0.251 ± 0.061</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>165.2</td>
<td>0.242 ± 0.056</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>180.2</td>
<td>0.234 ± 0.061</td>
</tr>
</tbody>
</table>

Fig. 4. Relationship between diffusive mass transport coefficients for amino acids \( (K_{\text{BDA}}) \) as well as dipeptides \( (K_{\text{BDD}}) \) and molecular weight of corresponding amino acids or dipeptides.
acids differs, depending on whether the amino acids are given in the solution in free form or as dipeptides. To study this, the amount (mass) transported from the dialysate of histidine, lysine, threonine and serine, during dwells with the amino acid solution were compared to the $\Delta M_A$ of the same amino acids during dwells with the Dip 8 solution. As shown in Table 5, the $\Delta M_A$ was substantially higher for each of these amino acids with Dip 8 than with the amino acid-based solution (for histidine 2.83 times higher, $P < 0.0001$; for threonine, 3.47, $P = 0.0001$; for serine, 1.84, $P = 0.0005$; and for lysine, 2.45, $P = 0.0019$). However, despite these large differences, the plasma concentrations were very similar during the two dwells.

Another way to show the relationship between the plasma concentration of an amino acid and the dose of that amino acid transported from the peritoneal cavity is shown in Table 6. Here the increases in plasma amino acid concentrations at 60 minutes, $\Delta C_{BA}(60)$, representing values close to the maximum, are divided by the mass of amino acids transported from the peritoneal cavity between 3 minutes and 60 minutes ($\Delta M_A$). For all amino acids except phenylalanine, the mean $\Delta C_{BA}(60)/\Delta M_A$ in the amino acid-based dwell studies was higher than in the dipeptide-based dwell studies. The difference was statistically significant for glycine, histidine, leucine and valine.

**DISCUSSION**

The reduction in dialysate volume between 90 and 240 minutes with the strong (Dip 16) dipeptide-based solutions was less rapid than that with the amino acid-based solution (Fig. 2), in spite of the initial osmolality being very similar. This seems to be caused by the higher molecular weight of the dipeptides and generation of constituent amino acids from hydrolysis of dipeptides in the peritoneal cavity.

The reason why the weak dipeptide-based solution results in a similar pattern of peritoneal volume changes as the amino acid-based solution (Fig. 2), despite having half the molar concentration, may be that: (1) the diffusive mass transport coefficient for dipeptides ($K_{BAO}$) is smaller than the $K_{BAO}$ for amino acids (Fig. 4); and (2) dipeptides are hydrolyzed in the peritoneal cavity (although at lower rate than the rate of their transport from dialysate; compare Tables 3 and 4), which generates free amino acids during the dwell. The driving force for changes in peritoneal volume is the difference in concentration of an osmotic agent between dialysate and plasma. In the case of dipeptide-based dialysis, this driving force can be approximately expressed as the concentrations (molar) of dipeptides plus concentrations of constituent...
Table 5. Average masses ($\Delta M_A$) fractional average masses [$\Delta M_A/M_A(3)$] of amino acids transported from the dialysate between 3 and 120 minutes of the dwell with the amino acid-based solution (AA sol) as well as weak (Dip 8) and strong (Dip 16) dipeptide-based solutions

<table>
<thead>
<tr>
<th>Dip 8</th>
<th>Dip 16</th>
<th>AA sol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta M_A$</td>
<td>$\Delta M_A$</td>
</tr>
<tr>
<td></td>
<td>$\mu$mol</td>
<td>$\mu$mol</td>
</tr>
<tr>
<td>Glycine</td>
<td>118.4 ± 23.3</td>
<td>215.7 ± 25.6</td>
</tr>
<tr>
<td>Histidine</td>
<td>120.7 ± 23.9</td>
<td>219.6 ± 25.9</td>
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<tr>
<td>Alanine</td>
<td>107.9 ± 24.0</td>
<td>229.1 ± 30.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>107.5 ± 23.1</td>
<td>230.0 ± 31.3</td>
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<tr>
<td>Throneine</td>
<td>169.5 ± 58.2</td>
<td>318.3 ± 66.6</td>
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<tr>
<td>Leucine</td>
<td>157.5 ± 21.4</td>
<td>318.6 ± 63.1</td>
</tr>
<tr>
<td>Serine</td>
<td>90.4 ± 23.8</td>
<td>260.8 ± 48.1</td>
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<tr>
<td>Phenylalanine</td>
<td>88.2 ± 24.6</td>
<td>230.2 ± 60.3</td>
</tr>
<tr>
<td>Valine</td>
<td>121.9 ± 42.4</td>
<td>351.7 ± 82.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>126.8 ± 53.2</td>
<td>340.2 ± 62.3</td>
</tr>
</tbody>
</table>

Table 6. Increase in amino acid concentration in plasma between 0 and 60 minutes of the dwell normalized by the mass of amino acids delivered between 3 and 60 minutes of the dwell, $\Delta C_{AA}/\Delta M_A$ (mean ± SD), with the amino acid-based solution (AA sol) as well as weak (Dip 8) and strong (Dip 16) dipeptide-based solutions

<table>
<thead>
<tr>
<th></th>
<th>AA sol</th>
<th>Dip 8</th>
<th>Dip 16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$mol</td>
<td>$\mu$mol</td>
<td>$\mu$mol</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.08 ± 1.80*</td>
<td>1.09 ± 0.77</td>
<td>1.46 ± 0.39</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.84 ± 2.37*</td>
<td>2.14 ± 0.57</td>
<td>1.99 ± 0.47</td>
</tr>
<tr>
<td>Alanine</td>
<td>3.74 ± 2.53</td>
<td>2.69 ± 2.39</td>
<td>0.98 ± 1.02</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.39 ± 2.42</td>
<td>3.05 ± 1.70</td>
<td>2.08 ± 0.53</td>
</tr>
<tr>
<td>Throneine</td>
<td>7.06 ± 5.95</td>
<td>2.28 ± 0.82</td>
<td>2.87 ± 0.96</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.42 ± 2.95*</td>
<td>1.77 ± 0.28</td>
<td>1.83 ± 0.82</td>
</tr>
<tr>
<td>Serine</td>
<td>3.30 ± 1.93</td>
<td>2.82 ± 1.35</td>
<td>2.16 ± 0.66</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.95 ± 1.22</td>
<td>2.48 ± 1.30</td>
<td>1.49 ± 0.53</td>
</tr>
<tr>
<td>Valine</td>
<td>6.55 ± 3.27*</td>
<td>3.69 ± 2.04</td>
<td>3.07 ± 0.83</td>
</tr>
<tr>
<td>Lysine</td>
<td>6.05 ± 2.76</td>
<td>5.67 ± 2.16</td>
<td>4.06 ± 1.56</td>
</tr>
</tbody>
</table>

*Significant differences between AA sol and Dip 8 and Dip 16.

amino acids in the dialysate, minus the concentrations of corresponding amino acids in blood plasma. As the diffusive mass transport parameters of the dipeptides and amino acids depend on their molecular weights (Fig. 4) and the hydrolysis rates of the dipeptides are similar (except that of Gly-His), we chose Thr-Leu, which has a representative molecular weight of dipeptide and constituent amino acids (Tables 2 and 3) as an example to illustrate the changes in the peritoneal volume driving force. The driving force of this single dipeptide in dwells with Dip 8 solution divided by the value of the driving force at 3 minutes was constantly higher after 60 minutes than that generated by threonine or leucine in the amino acid-based solution. At 180 minutes, it was 0.35 ± 0.10 (mean ± SD) mmol/L for threonine-leucine, 0.23 ± 0.12 mmol/L for threonine and 0.20 ± 0.10 mmol/L for leucine.

The average values of $K_H$ and $K_B$ as well as the values of $K_{HD}$ and $K_{BD}$ showed no significant differences. This analysis confirms that the accuracy of estimating kinetic parameters for amino acids has little effect on the estimation of dipeptide kinetic parameters. No significant differences were noted in the hydrolysis rates ($K_H$ or $K_B$) between the weak (Dip 8) and strong (Dip 16) dipeptide-based solutions for the various dipeptides. The hydrolysis rates, ($K_H$ or $K_B$), were of the same order for all dipeptides, except Gly-His, which had much lower values (Table 4).

The decline of dipeptide concentration as shown in Figure 3 is due to dipeptide diffusion from the peritoneal cavity and dipeptide hydrolysis. The contribution of diffusion alone to this decline can be assessed by a ratio of diffusive mass transport coefficient for dipeptide, $K_{HD}$, to the sum of $K_{RBD}$ and the hydrolysis rate, $K_H$. Using this assessment it can be shown that diffusion contributed from 73% (for Ser-Phe) to 98% (for Gly-His) to the dipeptide concentration decline, whereas 27% and 2%, respectively, of the decline was due to hydrolysis.

The higher concentration curve for glycine than for histidine in the dialysate, shown in Figure 3, is difficult to explain, since hydrolysis of the Gly-His dipeptide should result in the generation of equimolar amounts of the two amino acids and diffusive transport of histidine should be lower than that of glycine, due to its more than twice higher molecular weight. One possibility might be that histidine removal from the peritoneal fluid is enhanced by its adsorption to peritoneal membrane structures. A higher concentration of serine than phenylalanine was also observed; in this case the much lower molecular weight of serine might lead to more rapid removal by diffusion than for phenylalanine.

In Figure 4, the pattern of decreasing $K_{RBD}$ and $K_{BD}$ with increasing molecular weight is clearly seen. This observation is in general agreement with thermodynamic theory, that solutes of higher molecular weight diffuse more slowly than solutes of lower molecular weight. It is interesting to note that $K_{RBD}$ and $K_{BD}$ from the dwells with the strong (Dip 16) dipeptide-based solution tended to be higher than $K_{RBD}$ and $K_{BD}$ from the dwells with the weak (Dip 8) dipeptide-based solution (Table 3). The reason for this difference is not clear; however, a similar phenomenon was observed in a study with 1% and 2.7% amino acid-based solutions in uremic patients [5].
An unexpected finding was that, in the case of four amino acids (histidine, lysine, threonine and serine), the absolute amount of amino acid transported from dialysate during the dwells with the weak (Dip 8) dipeptide-based solution was about three times higher than that observed during the dwells with the amino acid-based solution, although the plasma concentration of these amino acids was similar during the dwells with the weak dipeptide-based solution and during those with the amino acid-based solution (Fig. 5). Moreover, for all amino acids except phenylalanine, the increase in plasma concentration at 60 minutes in relation to the dose delivered between 0 and 60 minutes was considerably higher during the dwells with the amino acid-based solution than during those with the two dipeptide-based solutions (Table 6). From these findings it can be inferred that dipeptide-based solutions may provide a method of delivering amino acids during dialysis with a smaller change in plasma amino acid concentrations than during dialysis with an amino acid-based solution.

The reason for this difference between amino acid and dipeptide-based solutions is not clear. A possible explanation may be that amino acids, in the form of dipeptides, delivered to the splanchnic area from dialysis fluid are taken up in the liver more efficiently (with less delivery to the peripheral circulation) than the corresponding free amino acids. Another explanation could be that dipeptides are not immediately hydrolyzed in blood (as can be inferred from undetectable dipeptide blood concentrations), but rather are rapidly stored in the blood vessel walls (or peritoneal tissue) and slowly hydrolyzed [20].

Conceivably, some of the adverse effects of amino acids, such as loss of appetite, nausea and vomiting, are related to the concentrations of amino acids in blood, which may have effects mediated in the splanchnic area or in centers regulating appetite and satiety in the central nervous system [21, 22]. If this is the case, dipeptide-based PD solutions should be advantageous because they permit delivery of larger amounts of amino acids with fewer side effects than do amino acid-based solutions.

In summary (1) intraperitoneal dialysate volume decreases more slowly between 90 and 240 minutes during a dwell with dipeptide-based solutions than with amino acid-based solutions; (2) dipeptides are hydrolyzed in the peritoneal cavity, but at a much lower rate than the rate of diffusive transport from the peritoneal cavity; (3) diffusive mass transport coefficients for amino acids ($K_{BDA}$) and for dipeptides ($K_{BDD}$) show a clear dependence on their molecular weights; (4) hydrolysis rate coefficients for dipeptides in the peritoneal cavity differ between various dipeptides, which may be due to different affinities of particular dipeptides to the hydrolysis enzyme; and (5) dipeptide-based solutions may be a better way, as compared to amino acid-based solutions, to supply amino acids with a moderate increase in plasma amino acid concentration.

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APPENDIX I

Kinetic model for dipeptides and its constituent amino acids

Following installation of the dipeptide-based dialysis solution in the peritoneal cavity in rats, measurements of dipeptide and amino acid concentrations were performed in dialysate and blood. Since dipeptides were not detected in blood it could be assumed that they were rapidly hydrolyzed in blood (or deposited in tissues) and their concentration in blood was therefore assumed to be zero.

A schematic diagram of diffusive transport of dipeptides, generation of amino acids by hydrolysis of dipeptides and diffusive transport of constituent amino acids is shown in the upper left part of Figure 1. On the right side are presented corresponding differential equations of the kinetic model. In the lower section of Figure 1, the diagram and corresponding equations for convective transport of dipeptides and amino acids are shown. The sum of diffusive and convective transport yields the total transport.

The mass balance equation for dipeptides is therefore as follows:

\[
\frac{d(V_s C_{CD})}{dt} = - (K_D + K_{BDA})C_{CD} + Q_s C_{DD} - Q_D C_{PD} \quad (\text{Eq. 1})
\]

where $C_{CD}$ is the dipeptide concentration in dialysate. The fluid absorption rate ($Q_s$) is estimated by the RISA elimination coefficient ($K_D$). $V_s$ and $K_s$ were evaluated as described in the Calculation section. The net coefficient, $K_D + K_{BDA}$, was estimated using equation 1. This equation does not allow an estimation of $K_D$ and $K_{BDA}$ separately, and for this purpose a kinetic model for amino acids is needed.

After hydrolysis, each dipeptide molecule generates two molecules of constituent amino acids. Therefore, each dipeptide produces two equal input flows for constituent amino acids in the peritoneal cavity, each equal to $K_D C_{DD}$.

Amino acids are transported between the dialysate and plasma by diffusion and the diffusive flow for each amino acid can be described as a product of the diffusive mass transport coefficient $K_{BDA}$ and the concentration difference between plasma and dialysate for this amino acid. The convective flow of a particular amino acid from plasma to dialysate can be expressed as a product of the ultrafiltration flow rate, $Q_s$, amino acid sieving coefficient, $S_s$, and the average amino acid concentration, $C_A$, calculated as an arithmetic mean of blood, $C_{BA}$, and dialysate, $C_{DA}$, amino acid concentrations, $C_A = (C_{BA} + C_{DA})/2$. The convective flow of amino acids from the peritoneal cavity can be described as a product of the fluid absorption rate, $Q_s$, and $C_{DA}$; no sieving of amino acid in the convective flow from peritoneal cavity was assumed ($S_s = 1$). The mass balance equation for each amino acid produced in the peritoneal cavity by dipeptide hydrolysis is therefore as follows:

\[
\frac{d(V_s C_{DA})}{dt} = K_D C_{CD} + Q_s S_s C_A - K_{BDA}(C_{DA} - C_{BA}) - Q_D C_{DA} \quad (\text{Eq. 2})
\]

Note that each mass balance equation for a particular dipeptide (Eq. 1) is accompanied by two mass balance equations for constituent amino acids (Eq. 2) with an identical amino acid generation rate, $K_D C_{DD}$.

APPENDIX II

Estimation of kinetic parameters

Integration and rearrangement of equation 1 yields:

\[
Y(t_s) = (K_D + K_{BDA})X(t_s) \quad (\text{Eq. 3})
\]

where
where \( t_i \) and \( t_F \) are the initial and consecutive, \( n \)-th, time points in which measurements have been made, respectively. \( Y_i(t) \) and \( X_i(t) \) were calculated using the available measurements, and then \( K_H \) and \( K_{BDD} \) was estimated using linear regression.

Integration and rearrangement of equation 2 yields

\[
Y_i(t) = K_{BDD} X_i(t) + S_i X_i(t) + K_H X_i(t) \quad \text{(Eq. 4)}
\]

where

\[
Y_i(t) = V_d(t) C_{d0}(t) - V_d(t) C_{i0}(t) + Q_A \int_{t_i}^{t_F} C_{dA} \, dt
\]

\[
X_i(t) = -\int_{t_i}^{t_F} C_{d0} \, dt
\]

\[
S_i X_i(t) = \int (C_{dA} - C_{d0})dt
\]

\[
X_i(t) = \int C_A dt = -X_i(t)
\]

\[
Y(t), X_i(t) \text{ and } X_i(t) \text{ could be calculated using available measurements, } X_i(t) \text{ was calculated with the following estimate of ultrafiltration flow rate } Q_U:
\]

\[
Q_U = \frac{dV_d}{dt} + Q_A \approx \frac{V_d(t) - V_d(t_{n-1})}{t_n - t_{n-1}} + K_H
\]

Using equation 4 and multiparameter linear regression, the parameters, \( K_{BDD}, S_i \) and \( K_H \) were estimated. Having estimated \( K_H \) with equation 4, and \( (K_B + K_{BDD}) \) with equation 3, \( K_{BDD} \) was calculated. The integrals in equations 3 and 4 were calculated, using linear interpolation between data points.

These calculations showed that some kinetic parameters pertaining to amino acid transport in the dipeptide-based dwells had unacceptable values. For some amino acids, \( K_{BDD} \) became negative and \( S_i \) outside of an acceptable range of 0 to 1 (data not shown). Nevertheless, kinetic parameters pertaining to dipeptide hydrolysis, \( H_{BDD} \), and diffusive transport between dialysate and blood, \( K_{BDD} \), showed acceptable values and are reported in this study.

To obtain more reliable estimates of parameters \( K_{BDD} \) and \( S_i \), the data from separate experiments in eight rats with amino acid-based dialysis solution were used. In this estimation, Equation 4, with \( K_B \) equal to zero, was applied using linear regression, and this resulted in a reliable estimate of diffusive mass transport coefficients for a certain amino acid, \( K_{BDD} \). However, this procedure yielded sieving coefficient values for a certain amino acid, \( S_i \), out of the 0 to 1 range, and subsequent numerical simulations showed that convective transport of amino acids had a negligible effect on the overall amino acid transport, in which diffusion plays a dominant role. For this reason, convective transport of amino acids was not taken into account and only \( K_{BDD} \), estimated by using data from the dwell studies with the amino acid-based dialysis solution, was used in equation 4 to estimate \( K_B \). With convective transport of amino acids neglected, \( S_i X_i(t) = 0 \), and \( K_{BDD} \) denoted as \( K_{BDD}^* \), equation 4 can be rearranged as follows:

\[
Y_i(t) = K_{BDD}^* X_i(t) \quad \text{(Eq. 5)}
\]

where

\[
Y_i(t) = V_d(t) C_{d0}(t) - V_d(t) C_{i0}(t) + Q_A \int_{t_i}^{t_F} C_{dA} \, dt
\]

\[
- K_{BDD}^* \int (C_{dA} - C_{d0})dt
\]

Finally, equations 3 and 5 were used in a linear regression parameter estimation procedure that yielded kinetic parameters \( K_B \) and \( K_{BDD}^* \) (Tables 3 and 4). \( K_B \) and \( K_{BDD}^* \) are shown together with \( K_H \) and \( K_{BDD} \) estimated without correction for amino acid transport parameters. The reliability of estimating the dipeptide hydrolysis rate coefficient, \( K_H \), (or \( K_B^* \)) and the diffusive mass transport coefficient for dipeptide, \( K_{BDD}^* \) (or \( K_{BDD} \)), is enhanced by the fact that for every dipeptide \( K_B \) was estimated twice, using equation 5, for two different amino acids generated from the single dipeptide. The reported values for \( K_B \) or \( K_H \) are the arithmetic average of these two estimates.

### APPENDIX III

**Nomenclature**

- \( V_d \): peritoneal volume in mL or L.
- \( C_{d0} \): dipeptide concentration in dialysate in \( \mu \text{mol}/\text{L} \) or \( \text{mmol}/\text{L} \).
- \( C_{dA} \): amino acid concentration in plasma in \( \mu \text{mol}/\text{L} \) or \( \text{mmol}/\text{L} \).
- \( C_A \): arithmetic mean of amino acid concentrations in plasma and dialysate in \( \mu \text{mol}/\text{L} \) or \( \text{mmol}/\text{L} \).
- \( C_{dA} \): fluid absorption rate in mL/min.
- \( K_H \): rate coefficient of dipeptide hydrolysis in mL/min. Estimated without any assumption concerning amino acid transport coefficients.
- \( K_B \): rate coefficient of dipeptide hydrolysis in mL/min. Estimated with assumption that diffusive mass transport coefficient of amino acids, \( K_{dA} \), in dwells with the dipeptide-based solutions is the same as in dwells with the amino acid-based solution.
- \( K_{BDD} \): coefficient of diffusive mass transport between dialysate and plasma of amino acid in mL/min. Estimated using data from dwells with the amino acid-based solution.
- \( K_{BDD}^* \): coefficient of diffusive mass transport between dialysate and plasma of dipeptide in mL/min. Estimated without any assumption concerning amino acid transport coefficients.
- \( M_i \): mass (amount) of amino acid in \( \mu \text{mol} \).
- \( \Delta \): change (difference).
- \( C_A \): sieving coefficient of amino acid (dimensionless).
- \( S_i \): sieving coefficient of dipeptide (dimensionless).

**REFERENCES**


*Werynski et al: Kinetic studies of peritoneal dialysis solutions*