Kidney International, Vol. 52 (1997), pp. 1663–1670

Dialytic nutrition: Provision of amino acids in dialysate during hemodialysis

CHARLES CHAZOT, EHSAN SHAHMIR, BELEN MATIAS, STEWART LAIDLAW, and JOEL D. KOPPLE

Division of Nephrology and Hypertension and Division of Oncology, Harbor-UCLA Medical Center, UCLA Schools of Medicine and Public Health, Los Angeles, California, USA

Dialytic nutrition: Provision of amino acids in dialysate during hemodialysis. Maintenance hemodialysis (MHD) patients are frequently malnourished, a condition associated with high morbidity and mortality. Amino acid (AA) losses in dialysate may enhance protein malnutrition in patients with low food intake. We studied the possibility of providing AA in dialysate during MHD to either prevent AA losses or as a nutritional supplement. Six clinically stable men were studied during three hemodialysis treatments. The first treatment was performed using the usual dialysate (0XAA). The two other treatments were performed using a dialysate containing an amount of AA equal to normal plasma AA concentrations (1XAA) or to three times the normal plasma AA concentrations (3XAA). During the OXAA treatment, the total AA losses were 10.0 ± 0.9 (SEM) grams (g) and the total AA concentrations in plasma decreased by 49 ± 4%. During the 1XAA treatment, the total AA balance was +0.8 ± 1.8 g and there was no significant change in the postdialysis plasma total AA. With the 3XAA treatment, the patients gained 36.9 ± 4.1 g of AA during the hemodialysis treatment and the plasma total AA levels increased by 45 ± 9%. No side effects were observed. These findings indicate that it may be feasible to provide AA supplements to MHD patients by adding AA to hemodialysate.

It is well recognized that there is a high prevalence of protein-calorie malnutrition in maintenance hemodialysis (MHD) patients [1]. Malnutrition, particularly as indicated by low serum albumin levels or reduced dietary protein intake, is of great concern because it is associated with a high incidence of morbidity and mortality [2, 3]. Although several factors contribute to malnutrition in MHD patients, decreased ingestion of food due to anorexia, intercurrent illnesses, and/or socio-economic conditions are major causes of this disorder [1, 2, 4]. Losses of amino acids into hemodialysate may contribute to protein malnutrition, particularly in patients with low nutrient intakes. Attempts to increase the intake of foods by counseling the patient, increasing the dialysis dose or providing food supplements often do not raise the dietary nutrient intake to the desired levels. Studies of intradialytic parenteral nutrition (IDPN) suggest that it may improve nutritional status and clinical outcome [5–7], although large scale, prospective, randomized clinical studies of this treatment have not been carried out. However, IDPN is expensive, and, in the United States, the Health Care Financing Administration has severely restricted the medical conditions for which IDPN is reimbursable [8]. Since the molecular weights of amino acids are sufficiently small to allow substantial clearances by dialyzer membranes, we examined whether adding amino acids to hemodialysate could either eradicate the net losses of amino acids or actually provide a nutritional supplement of these amino acids during hemodialysis. The results of this study indicate that the addition of amino acids to hemodialysate can be used both to prevent losses and to provide amino acids to the patient.

Key words: amino acids in dialysate, hemodialysis, nutrition, morbidity and mortality, dialysate.

Received for publication June 9, 1997
and in revised form July 28, 1997
Accepted for publication July 30, 1997

METHODS

Patient characteristics

Six male MHD patients were studied. Their mean age was 50.9 years (range, 30.6 to 61.6 years), duration of hemodialysis was 50 months (12 to 160 months), height was 174 ± 3.9 (SEM) cm, and body wt was 75.8 ± 5.4 kg. The relative body wt [9] was 92.7 ± 5.2%; three patients had a relative body wt less than 90% [9]. Body mass index was 25.0 ± 1.1 kg/m^2, and serum albumin was 4.1 ± 0.1 g/dl (range, 3.8 to 4.2 g/dl). The patients underwent hemodialysis three times weekly for three hours (in 4 patients) or four hours (in 2 patients) using a single-pass technique; reused polysulfone dialyzers and bicarbonate buffered dialysate were employed. End-stage renal disease was caused by IgA nephropathy in two patients and by malignant hypertension, diabetic nephropathy, polycystic kidney disease and unknown factors in one patient each. Two patients were receiving antihypertensive medicines (nifedipine alone in one patient or combined with minoxidil and clonidine in the other). All received phosphate binders. Five patients were routinely given erythropoietin injections, and all were receiving injections of 1,25-dihydroxycholecalciferol. No patient had received glucocorticoid therapy during the study or for at least three months before the study.

Experimental design

Each patient was studied during three hemodialysis treatments that were separated by at least one week. For a given patient, the three studies were conducted on the same day of the week. The day of the week on which the study was performed was Monday (after a 2–5/6 day interval) in Patient 5, and Wednesday, Thursday or Friday (after a 1–5/6 day interval) in the other five patients. All patients were fasted from 10:00 p.m. the night before the study

until the completion of the blood drawing at the end of hemodialysis the next day. Hemodialyses were prescribed to last for 240 minutes and began between 7:00 and 9:00 a.m. and ended between 11:00 a.m. and 1:00 p.m. Blood was drawn at the exact beginning and end of the hemodialysis treatment for measurement of urea and amino acids. The grand mean of the average urea reduction ratio during the three hemodialyses in each patient was 70.3 ± 1.0%.

In all patients, blood flow was set at 400 ml/min and dialysate flow was maintained at 500 ml/min. The rate of blood flow and dialysate inflow was determined by a pump that was periodically calibrated. The dialysate inflow was measured by a flow meter that was also calibrated periodically. All hemodialyses were performed with cellulose triacetate CT 190 G®, 1.9 m^2 dialyzers (Baxter Healthcare, Deerfield, IL, USA). Dialyzers were not reused in the study. The standard composition of all dialysates used in the study was as follows: D-glucose monohydrate 200 mg/dl, sodium 138 to 139 mEq/liter, potassium 1 to 3 mEq/liter, chloride 104 to 106 mEq/liter, bicarbonate 35 mEq/liter, calcium 2.5 to 3.5 mEq/liter, magnesium 0.7 to 1.5 mEq/liter. The first hemodialysis treatment of the study was performed using the patient's usual dialysate, without any amino acids added (referred to as 0XAA). For each patient, the order of administration of the two hemodialyses with amino acids added was determined randomly. The composition of dialysate used with these latter two hemodialyses was identical to the 0XAA hemodialysate with the following exceptions: during one hemodialysis, the dialysate contained 20 amino acids in concentrations that were similar to that present in plasma from normal postabsorptive adults (that is, the plasma amino acid concentrations after an overnight fast, referred to as 1XAA). The other hemodialysis was performed with a dialysate that contained amino acids in concentrations similar to three times normal postabsorptive plasma values (referred as to 3XAA).

In order to attain these amino acid concentrations in the delivered dialysate, the 20 amino acids were purchased separately as a dry powder (Ajinomoto Corporation, Japan) and mixed together. An amount of 46.4 and 139.2 g of this amino acid mixture was added to 3.7 liters of the acid dialysate concentrate in order to attain the 1XAA and 3XAA concentrations, respectively, in the diluted delivered dialysate. The enriched concentrate was thoroughly stirred for two hours, filtered and stored at 4°C until the hemodialysis was performed the next day. A sample from the enriched acid dialysate concentrate was drawn at the beginning of the dialysis treatment and cultured for bacteria. During the dialysis treatment, one volume of the acid concentrate was added to 34 volumes of water purified by reverse osmosis and to 1.36 volume of bicarbonate solution concentrate by a Baxter 550 single pass delivery system.

All spent dialysate was collected in the following manner: the spent dialysate flowed into small 10 liter holding tank. A proportioning pump (Masterflex®, Cole-Parmer Instrument Co, Niles, IL, USA) continuously transferred dialysate from the holding tank into either a 10 liter jug or a large vat. The ratio of the volume of dialysate transferred to the jug versus the vat was kept constant at 1 to 18. The jug was packed in ice to reduce bacterial growth. At the end of hemodialysis, the total volume of dialysate outflow in the chilled jug and the vat was weighed. The dialysate in the jug was stirred, and three 30 ml aliquots of spent dialysate each were taken and quickly frozen at −20°C until analyzed for amino acids. During dialysis with 1XAA and 3XAA, a 10 ml aliquot of fresh dialysate was collected at the beginning, midpoint (120 min) and end of hemodialysis for amino acid analyses, and was also stored at −20°C until analyzed.

Blood was collected in heparinized tubes at the exact onset and termination of hemodialysis. The blood was centrifuged within five minutes of collection at 1300 × g for 10 minutes. Plasma was quickly separated and deproteinized by adding 100 μl of 4.5% sulfosalicylic acid to 1.0 ml of plasma, mixed with a vortex mixer, and centrifuged at 4°C for 10 minutes. The supernatant was pipetted and recentrifuged for 10 minutes at 4°C if flecks of white precipitate were observed in the solution. The supernatant was then stored at −20°C until analysis. For each hemodialysis, amino acids were measured in three specimens of fresh hemodialysate (with the 1XAA and 3XAA hemodialyses), in triplicate in each collection of spent dialysate, and in the deproteinized plasma obtained at the beginning and end of each hemodialysis. Almost all specimens were analyzed for amino acids within seven days of collection.

Amino acid measurements were performed with a Beckman Model 6300 Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA, USA) using a lithium buffer system. For analyses of the hemodialysate containing the 1XAA and 3XAA amino acid concentrations, cysteine, citrulline and ornithine were not measurable because of interferences from the chromatographic peaks of the large quantities of amino acids added to the dialysate. Urea in plasma and dialysate was measured by the urease method (Abbott Laboratories, South Pasadena, CA, USA). For the 0XAA hemodialysis, the amino acid (AA) balance across the dialyzer was considered to be equal the total amino acids in the spent dialysate and was calculated as follows:

Total AA in spent dialysate

= spent dialysate AA concentration (mg/ml)

× spent dialysate volume (ml)

For the hemodialyses (HD) performed with the 1XAA and 3XAA treatments, the quantity of amino acids delivered to the dialyzer was calculated as follows:

Fresh dialysate AA concentration (mg/ml)

× Fresh dialysate flow (ml/min) × Duration of HD (min)

Fresh dialysate flow was calculated as the volume of spent dialysate (liters) minus total ultrafiltration (liters) divided by the duration of hemodialysis treatment (minutes). Total ultrafiltrate was taken as the patient's weight change during the dialysis. It was assumed that the weight of dialysate or the weight change of the patient was equal to the liters of dialysate or of ultrafiltrate.

The amino acid balance with the 1XAA and 3XAA hemodialyses was calculated as follows:

AA balance across dialyzer =

Total AA delivered to dialyzer

minus total AA in spent dialysate

Statistical analyses

The results are expressed as mean ± SEM (SEM). The individual amino acid levels and the total essential, total semi-essential, total

Table 1. Expected and measured amino acid concentrations in fresh hemodialysate used for the 1XAA and 3XAA treatments

	Expected values[a]	Measured values			
		1XAA[b]		3XAA[b]	
	μmol/liter	*μmol/liter*	*mg/liter*	*μmol/liter*	*mg/liter*
Total essential amino acids					
Histidine	80	91 ± 3[c]	14 ± 0	248 ± 9	38 ± 1
Isoleucine	60	68 ± 2	9 ± 0	183 ± 5	24 ± 1
Leucine	130	139 ± 6	18 ± 1	379 ± 10	50 ± 1
Lysine	180	210 ± 19	43 ± 4	506 ± 37	104 ± 8
Methionine	30	33 ± 2	5 ± 0	91 ± 5	14 ± 1
Phenylalanine	60	62 ± 2	10 ± 0	178 ± 5	29 ± 1
Threonine	150	159 ± 9	19 ± 1	409 ± 18	49 ± 2
Tryptophan	50	—[d]	—[d]	—[d]	—[d]
Valine	270	339 ± 22	40 ± 3	825 ± 47	97 ± 6
Total	960	1102 ± 58	158 ± 9	2819 ± 119	405 ± 18
Semi-essential amino acids					
Tyrosine	28	28 ± 1	5 ± 0	48 ± 4	9 ± 2
Total nonessential amino acids					
Alanine	300	357 ± 39	32 ± 9	877 ± 55	78 ± 5
Arginine	100	114 ± 6	20 ± 2	302 ± 5	53 ± 1
Asparagine	50	18 ± 4	3 ± 1	91 ± 11	9 ± 4
Aspartic acid	15	15 ± 1	2 ± 0	28 ± 4	2 ± 1
Glutamic acid	90	98 ± 5	14 ± 1	241 ± 21	38 ± 3
Glutamine	500	548 ± 20	80 ± 3	1476 ± 20	216 ± 3
Glycine	320	319 ± 10	24 ± 1	929 ± 24	70 ± 2
Proline	200	208 ± 15	23 ± 2	598 ± 33	66 ± 4
Serine	100	110 ± 3	12 ± 1	294 ± 15	31 ± 2
Taurine	55	67 ± 7	8 ± 1	152 ± 12	19 ± 1
Total	1730	1942 ± 124	218 ± 9	4939 ± 99	582 ± 9
Grand Total Amino Acids[e]	2718	3058 ± 162	382 ± 18	7777 ± 213	995 ± 26

[a] Expected values for the 1XAA amino acid concentrations
[b] 1XAA and 3XAA: the fresh dialysate contains one times and three times the normal postabsorptive amino acid concentrations
[c] Mean ± SEM of fresh hemodialysate concentrations from six patients
[d] Tryptophan was not measured
[e] Refers to the sum of total essential, total semi-essential and total nonessential amino acid concentrations in fresh dialysate

nonessential, and the grand total of the amino acid levels in dialysate and plasma were each averaged for the six hemodialysis studies carried out with each of the three treatments. The possibility that the dialysate AA balances or plasma AA concentrations with the three separate treatments (that is, 0XAA, 1XAA, 3XAA) were significantly different from each other was tested by multivariate analysis of variance for repeated measures [10] (SPSS, Chicago, IL, USA). The differences between plasma pre- and postdialysis AA concentrations were analyzed with the paired *t*-test. Linear regression analyses were used to determine the relationships between the AA balances across the dialyzer and the AA concentrations in plasma or fresh dialysate, the molecular weight of the AA, the urea reduction ratio and the quantity of urea cleared into the dialysate. A P value < 0.05 was considered to be statistically significant.

RESULTS

The average duration for the 18 hemodialysis treatments was 237 minutes (range 217 to 241 min). The hemodialyses with the amino acids in dialysate were well tolerated by all patients. The bacterial cultures from 12 acid dialysate concentrates containing the amino acids for the 1XAA and 3XAA treatments demonstrated no bacterial growth.

The mean amino acid concentrations measured in fresh hemodialysate containing 1XAA and 3XAA are shown in Table 1. The grand total amino acid concentrations in the fresh dialysate averaged 3058 ± 162 μmol/liter and 7777 ± 213 μmol/liter with the 1XAA and 3XAA concentrates. In general, the measured amino acid concentrations in fresh dialysate were similar to the expected values with both the 1XAA and 3XAA preparations. The similarity of these amino acid levels to one times or three times the normal plasma concentrations is even more evident if the rather substantial range of the normal values for postabsorptive plasma amino acid concentrations are taken into consideration. Indeed, the mean of the ratios of the measured to the expected concentrations for all of the individual amino acids was 1.06 ± 0.04 and 2.75 ± 0.1 for the 1XAA and 3XAA dialysate preparations, respectively.

There were several exceptions to these close relationships. The tyrosine concentrations with the 3XAA dialysate were lower than expected, probably because tyrosine is rather insoluble in water, and some tyrosine may have precipitated in the acid concentrate before it was diluted. Tryptophan was added to the dialysate, but the tryptophan concentrations in dialysate were not assessed because of technical difficulties associated with its measurement. Citrulline and ornithine were not added to the dialysate because these amino acids are not found in human proteins, are part of the urea cycle, and are readily synthesized from arginine. Cysteine was not included in the amino acid preparation because it is increased in uremic plasma [11] and it is chemically rather unstable; thus, methionine, which is its metabolic precursor, was added.

Table 2. Amino acid balance during hemodialysis treatments with dialysate containing 0XAA, 1XAA and 3XAA

	0XXA[a]	1XAA[a]	3XAA[a]
	mg		
Essential amino acids[b]			
Histidine	−312 ± 25[c,d]	−34 ± 129	1586 ± 103[d,f]
Isoleucine	−192 ± 25[d]	103 ± 69[e]	1023 ± 78[d,f]
Leucine	−330 ± 35[d]	261 ± 130[e]	2153 ± 203[d,f]
Lysine	−1119 ± 181[d]	−411 ± 284	4408 ± 568[d,f]
Methionine	−156 ± 8[d]	68 ± 31[e]	588 ± 53[d,f]
Phenylalanine	−213 ± 31[d]	63 ± 68[e]	1201 ± 114[d,f]
Threonine	−355 ± 42[d]	−105 ± 310	2020 ± 309[d,f]
Valine	−534 ± 34[d]	703 ± 321	3537 ± 582[d,f]
Total	−3211 ± 320[d]	647 ± 86[e]	16516 ± 1856[d,f]
Semi-essential amino acids			
Cysteine[g]	−258 ± 38[d]		
Tyrosine	−124 ± 16[d]	84 ± 37[e]	391 ± 78[d,f]
Total	−390 ± 47[d]		
Nonessential amino acids			
Alanine	−1012 ± 67[d]	91 ± 127[e]	3097 ± 655[d,f]
Arginine	−403 ± 42[d]	255 ± 240[d,e]	2134 ± 180[d,f]
Asparagine	−416 ± 49	−171 ± 121	−6 ± 389
Aspartic acid	−19 ± 2	−63 ± 92	9 ± 74
Citrulline[g]	−625 ± 109[d]		
Glutamic acid	−282 ± 47[d]	−10 ± 155	1367 ± 257[d,f]
Glutamine	−2242 ± 228[d]	41 ± 454[e]	7015 ± 971[d,f]
Glycine	−684 ± 74[d]	23 ± 146[e]	2726 ± 361[d,f]
Ornithine[g]	−324 ± 71[d]		
Proline	−561 ± 87[d]	−259 ± 222	1959 ± 361[d,f]
Serine	−322 ± 31[d]	80 ± 89[e]	1025 ± 154[d,f]
Taurine	−130 ± 10[d]	72 ± 84	647 ± 181[d,f]
Total	−6618 ± 578[d]	59 ± 977[e]	19972 ± 2441[d,f]
Grand Total Amino Acids[h]	−9953 ± 848[d]	790 ± 1798[e]	36879 ± 4137[d,f]

[a] Dialysate containing no amino acids (0XAA) or containing about one time (1XAA) or three times (3XAA) the normal postabsorptive plasma amino acid concentrations

[b] Tryptophan was not measured

[c] Mean ± SEM from six men studied with all three hemodialysis treatment. A minus sign preceeding the mean value indicates that this quantity of amino acids was, on average, lost from the patients; the absence of a minus sign before the mean value indicates that this quantity of amino acids, on average, was gained by the patients

[d] Different from 0 ($P < 0.05$)

[e] $P < 0.005$ for difference in AA balance between 0XAA and 1XAA treatments

[f] $P < 0.02$ for difference in AA balance between 3XAA and the 0XAA and 1XAA data

[g] Cysteine, citrulline and ornithine, that were not added in dialysate (see text) were not measured in 1XAA and 3XAA dialysate because of the interferences of the added amino acid peaks on the chromatograms

[h] Refers to the sum of total essential, total semi-essential and total nonessential amino acid balances

The amino acid balances across the hemodialyzers during treatment with the 0XAA, 1XAA and 3XAA dialysate concentrations are shown in Table 2. When no amino acids were added to hemodialysate, there were statistically significant losses of all individual amino acids as well as total essential, total semi-essential, total nonessential and the grand total of amino acids. The total amino acid losses with the 0XAA dialysis were 9,953 ± 848 mg. As has been reported previously [12], the mean losses into dialysate of individual amino acids were highly correlated with the average predialysis plasma concentrations of the respective amino acids ($r = 0.88$, Fig. 1), but not with the molecular weights of the amino acids. No correlation was observed between the total amino acid losses during the 0XAA hemodialysis and either the urea reduction ratio or the urea content in the spent dialysate.

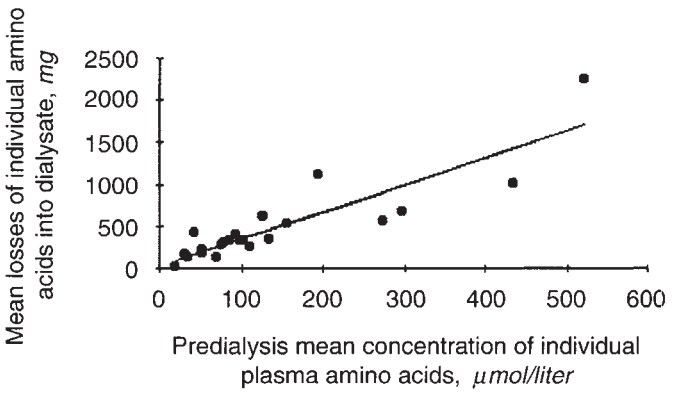

Fig. 1. Relationship between the mean predialysis plasma concentrations of individual amino acids (μmol/liter) and the mean losses of individual amino acids into dialysate (mg/hemodialysis treatment). Each symbol represents the mean predialysis plasma and total dialysate content for an individual amino acid from six maintenance hemodialysis patients. Data were obtained from patients who had been fasted from the previous evening until the end of the hemodialysis treatment. No amino acids were added to the fresh dialysate entering the dialyzer. $y = 3.2x + 30.5$; $r = 0.88$; $P < 0.001$.

During hemodialysis with the 1XAA concentrations, the losses of individual amino acids, except for arginine, as well as the losses of total essential (+ 647 ± 861 mg), total nonessential (+59 ± 977 mg) and the grand total amino acids (+790 ± 1,798 mg) were not significantly different from zero (Table 2). There were substantial individual variations in the grand total amino acid balances among the six patients, from −5.8 to +7.4 g. The balances for several individual amino acids and also for total essential and total nonessential amino acids varied substantially (see variances for the balances with the 1XAA treatment that are listed in Table 2). The grand total amino acid balances in the six patients did not correlate with either their grand total amino acid concentrations in the fresh 1XAA dialysate or their grand total predialysis amino acid concentrations in plasma (data not shown).

With the 3XAA dialysate, there was a statistically significant uptake of all of the individual amino acids that were added to the fresh dialysate, except for asparagine and aspartic acid. There was also a large and highly significant uptake of the total essential (16,516 ± 1,856 mg), total nonessential (19,972 ± 2,441 mg) and total amino acids (36,879 ± 4,137 mg; Table 2). For each individual AA and for total essential AA, total nonessential AA and the grand total of AA, there were significant differences in the amino acid balance among the three hemodialysis treatments, except for the aspartic acid and asparagine balances (Table 2). Balances for the amino acids were significantly more positive with the 3XAA dialysate as compared to the 1XAA and 0XAA dialysates. Also, the amino acid balances with the 1XAA dialysate were often significantly more positive as compared to the 0XAA dialysate (Table 2).

There was a highly significant correlation between the mean uptake of individual amino acids during hemodialysis with the 3XAA hemodialysate and the average concentrations of the individual amino acids in the fresh dialysate (Fig. 2). The mean

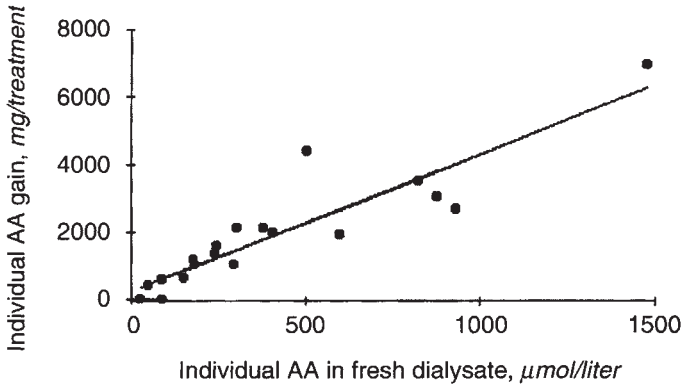

Fig. 2. Relationship between the mean individual amino acid concentrations in fresh dialysate (μmol/liter of dialysate) and the mean balance for individual amino acids (mg/hemodialysis treatment) during the course of a four-hour hemodialysis treatment. Hemodialysis was performed using fresh dialysate that contained amino acids in approximately three times the postabsorptive plasma amino acid concentrations of normal adults (3XAA). Each symbol represents the mean dialysate concentration and balance for an individual amino acid from six maintenance hemodialysis patients. Patients were fasted from 10 p.m. the night before the study until the end of the hemodialysis treatment on the study day. $y = 4.1x + 245$; $r = 0.91$.

uptake of individual amino acids also was correlated with the predialysis individual plasma amino acid concentrations ($r = 0.92$, $P < 0.0001$). This latter relationship may largely reflect the fact that the predialysis plasma concentrations of individual amino acids were highly correlated with the amino acid concentrations in the fresh 3XAA dialysate. Indeed, the amino acid concentrations in the 3XAA hemodialysate were patterned after the postabsorptive plasma amino acid levels of normal adults, which are not markedly different from the postabsorptive plasma amino acid concentrations of MHD patients. No correlation was found between the amino acid balances and the molecular weights of individual amino acids. Also, the correlation coefficients for relationship between the total amino acid balance and the predialysis plasma grand total amino acid concentrations, the urea reduction ratio and the amount of urea nitrogen removal by the hemodialysis (g) were respectively 0.60, 0.57 and −0.69. These correlations were not statistically significant, possibly because only six patients were evaluated.

The plasma amino acid concentrations at the onset and termination of the three hemodialyses and the percent changes in these values are shown in Table 3. The concentrations of essential AA, semi-essential AA, nonessential AA and total AA in plasma at the beginning of the three treatments were not statistically different according to the multivariate analysis of variance. With the 0XAA treatment, all individual amino acid concentrations, as well as total essential, total semi-essential and total nonessential amino acids, decreased significantly, with the exception of aspartic acid. The plasma grand total amino acids also fell significantly, by 1473 ± 148 μmol/liter (−49 ± 4%, $P < 0.03$). With the 1XAA hemodialysis, there were no significant changes in the plasma concentrations of total essential or nonessential amino acids, the grand total of amino acids or the individual amino acids, except for a decrease in isoleucine, methionine, tyrosine, aspartic acid, cysteine and citrulline (these latter two amino acids were not added to the dialysate) and total semi-essential AA (Table 3).

In contrast, during the 3XAA hemodialysis, there were statistically significant increases in the plasma concentrations of total essential and nonessential amino acids, the grand total of amino acids and most individuals amino acids (Table 3). The increase in the plasma grand total amino acid concentrations with the 3XAA dialysate averaged 1121 ± 138 μmol/liter (+45 ± 9%, $P < 0.001$). The plasma amino acid concentrations that did not increase were often the ones that were not added to the hemodialysate (that is, cysteine, citrulline and ornithine) or in which the dialysate concentrations tended to be lower than the targeted dialysate concentration for that amino acid (that is, tyrosine, asparagine and aspartic acid with the 3XAA dialysate (Table 1)). Also, the increase in plasma alanine ($P = 0.069$) did not reach statistical significance.

The magnitude of the increases in the mean concentrations of individual plasma amino acids during hemodialysis with the 3XAA dialysate were correlated with the amino acid concentrations in the fresh dialysate ($r = 0.78$, $P < 0.001$), with the balance of the respective amino acids across the dialyzer ($r = 0.70$, $P < 0.001$) and with the predialysis plasma concentrations of individual amino acids ($r = 0.92$, $P < 0.0001$). The postdialysis plasma levels of individual amino acids with the 3XAA hemodialysis were also correlated with the predialysis plasma amino acid concentrations ($y = 1.4\,x + 5$; $r = 0.95$, $P < 0.0001$). Therefore, one may be able to predict the postdialysis amino acid concentrations precisely from either the quantity of amino acids added to the hemodialysate or from the quantities taken up during the hemodialysis procedure.

Thus, the magnitude of the change in plasma total essential and total nonessential amino acid concentrations, the grand total amino acid levels and many individual amino acid concentrations were significantly different according to the dialysate employed. These changes were frequently significantly more positive with the 3XAA dialysate as compared to the 1XAA and 0XAA dialysate and were often more positive with the 1XAA dialysate as compared to the 0XAA dialysate (Table 3). As expected, the mean postabsorptive plasma amino acid concentrations at the onset of the three different hemodialyses were similar. In contrast, the postdialysis plasma amino acid levels varied greatly according to the amino acids levels in the dialysate. The postdialysis plasma amino acid levels with the 1XAA hemodialysis were essentially unchanged from the postabsorptive predialysis plasma values, whereas the postdialysis plasma amino acid concentrations with the 3XAA treatment were about one and one-half times as great as with the 1XAA hemodialysis ($P < 0.001$) and were almost three times as great as with the 0XAA treatment ($P = 0.001$; Table 3).

DISCUSSION

The results of this study indicate that the addition of amino acids to hemodialysate may prevent amino acid losses during hemodialysis treatment and, if provided in sufficient quantities, may provide nourishment to the patient. Six men who were postabsorptive and who underwent hemodialysis with the high-flux cellulose triacetate CT190® dialyzer without amino acids in the dialysate, sustained losses of total amino acids that averaged 10.0 ± 0.9 g per dialysis. In contrast, when these patients were treated with hemodialysis in which the dialysate contained amino acids at concentrations similar to the normal postabsorptive plasma amino acid levels, there were no significant losses or gains in total essential, total nonessential or the grand total amino acid

Table 3. Pre- and postdialysis plasma amino acid values for the three hemodialysis treatments[a]

	0XAA[b]			1XAA[b]			3XAA[b]		
	Predialysis	Postdialysis	Change	Predialysis	Postdialysis	Change	Predialysis	Postdialysis	Change
	μmol/liter		%	μmol/liter		%	μmol/liter		%
Essential amino acids[c]									
Histidine	79 ± 6[d]	40 ± 3[g]	−49 ± 2	84 ± 6	77 ± 4[j]	−8 ± 4	83 ± 8	123 ± 11[h,m]	52 ± 9
Isoleucine	52 ± 6	32 ± 3[h]	−37 ± 4	62 ± 6	49 ± 5[h]	−21 ± 4	59 ± 6	78 ± 7[g,l]	32 ± 5
Leucine	86 ± 10	56 ± 6[h]	−33 ± 6	105 ± 10	99 ± 12	−6 ± 6	100 ± 12	158 ± 14[g,l]	63 ± 11
Lysine	195 ± 27	115 ± 13[h]	−40 ± 2	192 ± 22	186 ± 19[j]	−1 ± 4	204 ± 26	302 ± 22[g,l]	56 ± 14
Methionine	31 ± 2	15 ± 1[g]	−50 ± 2	29 ± 2	21 ± 2[g]	−27 ± 4	30 ± 3	35 ± 2[i,l]	21 ± 8
Phenylalanine	52 ± 8	29 ± 3[h]	−42 ± 4	56 ± 5	51 ± 6[k]	−10 ± 5	57 ± 7	80 ± 6[h,m]	58 ± 17
Threonine	134 ± 11	59 ± 4[g]	−56 ± 2	132 ± 17	113 ± 11[j]	−13 ± 4	120 ± 7	200 ± 8[f,l]	69 ± 7
Valine	156 ± 16	78 ± 9[g]	−50 ± 2	229 ± 56	246 ± 33[k]	−17 ± 10	210 ± 33	492 ± 39[f,l]	154 ± 29
Total	783 ± 69	423 ± 33[g]	−45 ± 2	887 ± 107	842 ± 82[j]	−4 ± 3	863 ± 91	1468 ± 94[f,l]	76 ± 12
Semi-essential amino acids									
Cysteine	111 ± 19	14 ± 2[h]	−76 ± 2	70 ± 11	28 ± 9[i]	−58 ± 13	115 ± 26	32 ± 9[h]	−72 ± 7
Tyrosine	35 ± 4	26 ± 4[h]	−58 ± 5	30 ± 3	19 ± 4[g]	−39 ± 7	34 ± 4	27 ± 4	−19 ± 10
Total	146 ± 20	40 ± 5[g]	−72 ± 2	76 ± 15	38 ± 8[i,k]	−51 ± 13	129 ± 28	53 ± 9	−60 ± 6
Nonessential AA									
Alanine	389 ± 17	158 ± 9[f]	−42 ± 20	287 ± 54	214 ± 31[j]	−19 ± 9	258 ± 53	345 ± 52[m]	49 ± 18
Arginine	94 ± 11	43 ± 4[g]	−53 ± 3	110 ± 22	73 ± 6	−27 ± 7	102 ± 12	148 ± 10[i,m]	64 ± 35
Asparagine	43 ± 7	13 ± 0[h]	−67 ± 4	44 ± 10	29 ± 3	−14 ± 19	34 ± 6	42 ± 4	42 ± 28
Aspartic acid	20 ± 4	10 ± 4	−41 ± 21	12 ± 0	6 ± 1[i]	−54 ± 6	18 ± 4	9 ± 2	−48 ± 6
Citrulline	126 ± 25	42 ± 8[h]	−64 ± 5	122 ± 23	66 ± 16[i]	−45 ± 8	170 ± 29	87 ± 33[i]	−55 ± 6
Glutamic acid	75 ± 4	60 ± 7	−17 ± 13	78 ± 11	91 ± 10[k]	22 ± 11	68 ± 5	124 ± 8[f,l]	82 ± 8
Glutamine	522 ± 36	305 ± 25[f]	−42 ± 2	513 ± 33	495 ± 33[j]	−2 ± 6	559 ± 40	729 ± 44[f,l]	31 ± 3
Glycine	293 ± 21	160 ± 11[g]	−45 ± 4	262 ± 28	253 ± 23[j]	−2 ± 4	291 ± 25	444 ± 22[g,l]	56 ± 10
Ornithine	99 ± 12	48 ± 5[h]	−51 ± 4	85 ± 13	55 ± 8	−29 ± 6			
Proline	273 ± 41	80 ± 7[h]	−69 ± 3	206 ± 27	176 ± 16[k]	−6 ± 16	239 ± 49	306 ± 35[i,m]	38 ± 11
Serine	104 ± 13	58 ± 6[h]	−43 ± 5	84 ± 11	95 ± 18	19 ± 27	84 ± 8	123 ± 15[h,m]	51 ± 8
Taurine	70 ± 11	34 ± 5[i]	−42 ± 15	73 ± 7	71 ± 6	2 ± 11	86 ± 16	134 ± 5[i,m]	80 ± 26
Total	2076 ± 115	1000 ± 64[f]	−49 ± 5	1786 ± 141	1571 ± 85[j]	−9 ± 5	1842 ± 207	2435 ± 134[g,m]	39 ± 6
Grand total amino acids[e]	3005 ± 179	1463 ± 94[f]	−49 ± 4	2750 ± 262	2451 ± 162[k]	−8 ± 5	2834 ± 291	3956 ± 205[f,l]	45 ± 9

[a] Blood was obtained at the exact onset and termination of the hemodialysis procedure
[b] Dialysate containing no amino acids (0XAA) or one times (1XAA) or three times (3XAA) normal postabsorptive plasma amino acid concentrations
[c] Tryptophan was not measured
[d] Mean ± SEM
[e] Refers to the sum of total essential, total semi-essential and total nonessential amino acids
[f,g,h,i] $P < 0.0001$, < 0.001, < 0.01, < 0.05 differs from the respective predialysis value
[j,k] $P < 0.01$, < 0.05 differs from the respective postdialysis 0XAA value
[l,m] $P < 0.001$, < 0.01 differs from the respective postdialysis 0XAA and 1XAA values

balance (+0.8 ± 1.8 g amino acids per dialysis). When hemodialysis was performed using dialysate that contained three times the normal postabsorptive plasma amino acid concentrations, the patients gained an average of 36.9 ± 4.1 g of amino acids per hemodialysis.

Other researchers have found slightly lower losses of amino acids in fasting patients when they underwent hemodialysis with an amino acid free dialysate. Kopple et al reported 6.3 g (range 4.5 to 7.1 g) of amino acid losses during an 11 hour dialysis with the less efficient Kiil dialyzers using a Cuprophan membrane [13]. Wolfson, Jones and Kopple described amino acid losses of 8.1 ± 1.1 g (SEM) with a five hour dialysis with various low flux dialyzers [12]. Ono, Sasaki and Waki observed amino acid losses of 6.6 ± 0.5 g with a five hour dialysis using a cuprammonium rayon hollow fiber dialyzer [14]. Gutierrez, Bergström and Alvestrand reported an amino acid removal of 7.9 ± 0.4 g with a Baxter CA170 G or Gambro GFS*Plus* 20 dialyzer [15]. Ikizler et al described similar amino acid losses in male patients who ate a small meal and who underwent dialysis using a low flux cuprophan dialyzer (7.2 ± 0.9 (SEM) g) and a low flux polymethyl methacrylate dialyzer (6.1 ± 0.5 g) [16]. When the polysulfone membrane was reused six times, the free amino acids losses rose to 12.2 ± 1.5 (SEM) g.

In patients who are postprandial, Kopple et al reported 8 to 10 g of amino acid losses during an 11 hour hemodialysis with the Kiil dialyzer [13]. The slightly higher amino acid losses with the 0XAA dialysate in the postabsorptive patients in the present study may reflect the fact that the cellulose triacetate dialyzer is a high flux membrane (sieving coefficient: 0.55 ± 0.03 for β2-microglobulin). Indeed, when Ikizler et al [16] used a high flux polysulfone dialyzer with similar parameters of dialysis kinetics (surface area, blood and dialysate flow rates, duration of dialysis treatment), only slightly lesser quantities of amino acids were removed, 8.0 ± 0.9 g. The cellulose triacetate dialyzer used in the present study has a surface area of 1.9 m^2 and a pore size of 100 angstroms. The polysulfone membrane used in the studies of Ikizler et al has a surface area of 1.8 m^2 and a pore size distribution of 60 to 80 angstroms.

The 49 ± 4% decrease in the plasma grand total amino acids levels during the 0XAA hemodialysis in the present study was somewhat greater then that reported previously (32% decrease in

the study of Wolfson et al [12]; 34% by Ono et al [14]; 23% by Guttierez et al [15]; and 31% by Ikizler et al [16]). These rather minor differences in the fall in plasma amino acids levels may reflect the fact that in some of the other studies, patients may not have been fasting [14, 16], postdialysis samples were drawn 15 minutes after the termination of dialysis [16], or less efficient hemodialyses were employed [13–15]. The fact that our patients lost slightly more amino acids during the 0XAA hemodialysis may have contributed to the greater decrease in their plasma amino acid concentrations. Several grams of peptides and protein are also lost during hemodialysis treatment [13, 16]. Thus, the total free and bound amino acid losses during hemodialysis are even greater than are indicated by these studies.

In contrast to these findings, when amino acids were added to hemodialysate in an amount similar to normal postabsorptive values, there was not only no gain or loss of amino acids across the dialyzer, but plasma amino acids levels did not change significantly during the dialysis ($-8 \pm 5\%$). When amino acids were added to the dialysate in about three times the normal postabsorptive plasma concentrations, the amino acid balance during the hemodialysis procedure was not only strongly positive but plasma total amino acid levels rose by $45 \pm 9\%$.

The amount of amino acids that was taken up with the 3XAA hemodialysis was not unexpected because of the known sieving characteristics of the cellulose triacetate membrane and the dialysance predicted from the membrane characteristics, the blood and dialysate flow rates, the molecular weights of the amino acids, and the dialysate and estimated mean plasma concentrations of the amino acids. Creatinine has a molecular weight of 113, which is close to the average molecular weight of the studied amino acids (that is, 139 daltons). The creatinine clearance given by the manufacturer for the CT190 G® is 266 ml/min at a blood flow of 400 ml/min and a dialysate flow of 500 ml/min. Using these values, and according to the mean of the grand total amino acid concentrations in the predialysis and postdialysis plasma and the grand total amino acid concentrations in the fresh dialysate, the calculated total quantity of amino acids transferred from dialysate to plasma should be about 39 g per treatment, a number similar to what we obtained.

In the present study, the gain in individual amino acids with the 3XAA hemodialysis was closely correlated with the concentrations of the respective amino acids in the dialysate (Fig. 2). This finding suggests that it may be possible to accurately predict the amino acid balance and the change in plasma amino acids concentrations during hemodialysis from the quantity of amino acids in the fresh dialysate and the kinetic characteristics of the hemodialysis procedure. Thus, it may be possible to accurately regulate the balance and postdialysis concentrations of specific amino acids by modifying the quantity that is added to the dialysate.

The potential benefits of hemodialytic nutrition are not well defined, and further studies will be necessary to determine whether it may improve the clinical status of patients. Maeda et al added amino acids to hemodialysate and reported an increase in plasma amino acid concentrations obtained postdialysis as compared to the predialysis values [17]. However, the clinical benefits of this therapy were not clearly demonstrated. Feinstein et al gave amino acids and glucose by dialysis to three patients with acute or chronic renal failure [18]. Slow blood and dialysis flow rates were employed. These authors also showed a substantial uptake of glucose and amino acids from dialysate, but the study was not designed to examine the effects of dialytic nutrition on clinical outcome.

The rise of plasma amino acids with the 3XAA hemodialysis is roughly similar to the average increase in plasma amino acids concentrations that occurred after a meal in nondialyzed patients with chronic renal failure that was reported by Garibotto et al [19]. However, in this latter study, the patients were given 4 g/kg of ground beefsteak that provided about 1 g protein/kg body wt. The greater increment in plasma amino acids per g of amino acids absorbed in the present study probably reflects the physiological effects of the route of administration. Thus, the amino acids in the dialysate were transferred directly to a peripheral vein, whereas after a meal the food remains in the stomach for up to several hours, is slowly digested and absorbed from the intestine and most of the organic nutrients are processed by the liver before entering the peripheral circulation. Also, the stimulation of hormonal secretion by these two routes of nutrient administration may be quite different. Although these differences in the route of administration might affect the utilization of the nutrients for anabolic or other biological processes, the finding that parenteral nutrition provided through a systemic vein is nutritionally valuable [20] suggests that dialytic nutrition should be beneficial as well.

In this preliminary study, we administered the amino acids in dialysate without large amounts of carbohydrates in order to examine the amino acid balances during hemodialysis in a more independent fashion. In future studies, it would be of value to examine the nutritional and metabolic effects of administering in dialysate both amino acids and substantial amounts of other energy sources. In particular, it could be valuable to know whether this combined treatment will enhance the utilization of amino acids and improve the nutritional status of malnourished maintenance hemodialysis patients.

In summary, the present study suggests that substantial amounts of amino acids may be administered to patients through hemodialysate. These amino acids do not need to be sterilized or administered with sterile bags, tubing or needles, and there is no need for personnel who are trained in the preparation and the administration of intravenous solutions (such as pharmacists). Therefore, it may be feasible to provide these nutrients more inexpensively in hemodialysate than by intradialytic parenteral nutrition, even though with the 3XAA dialysate only about 26% of the amino acids added to the dialysate were taken up by the patient.

ACKNOWLEDGMENTS

The project was supported in part by Baxter Healthcare, Renal Division. This work was presented in part at the Annual Meeting of the 28th American Society of Nephrology, November, 1995, San Diego, California and published in abstract form (*J Am Soc Nephrol* 6:574, 1995).

Reprint requests to Joel D. Kopple, M.D., Division of Nephrology and Hypertension, Harbor UCLA Medical Center, 1000 West Carson Street, Torrance, California 90509, USA.

REFERENCES

1. KOPPLE JD: Effect of nutrition on morbidity and mortality in maintenance dialysis patients. *Am J Kidney Dis* 24:1002–1009, 1994
2. ACCHIARDO SR, MOORE LW, LATOUR PA: Malnutrition as the main factor of morbidity and mortality of hemodialysis patients. *Kidney Int* 24:199–203, 1983

3. OWEN WF, LEW NL, LIU Y, LOWRIE EG, LAZARUS JM: The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *N Engl J Med* 329: 1001–1006, 1993
4. BERGSTRÖM J: Why are dialysis patients malnourished? *Am J Kidney Dis* 26:229–241, 1995
5. CANO N, LABASTIE-COEYREHOURQ J, LACOMBE P, STROUMZA P, DI COSTANZO-DUFETEL J, DURBEC JP, COUDRAY-LUCAS C, CYNOBER L: Perdialytic parenteral nutrition with lipids and amino acids in malnourished hemodialysis patients. *Am J Clin Nutr* 52:726–730, 1990
6. CAPELLI JP, KUSHNER H, CAMISCIOLI TC, CHEN SM, TORRES MA: Effect of intradialytic parenteral nutrition on mortality rates in end-stage renal disease care. *Am J Kidney Dis* 23:808–816, 1994
7. CHERTOW GM, LING JL, LEW NL, LAZARUS JM, LOWRIE EG: The association of intradialytic parenteral nutrition administration with survival in hemodialysis patients. *Am J Kidney Dis* 24:912–920, 1994
8. KOPPLE JD, FOULKS CJ, PIRAINO B, BETO JA, GOLDSTEIN J: Proposed Health Care Financing Administration guidelines for reimbursement of enteral and parenteral nutrition. *Am J Kidney Dis* 26:995–997, 1995
9. FRISANCHO AR: New standards of weight and body composition by frame size and height for assessment of nutritional status of adults and the elderly. *Am J Clin Nutr* 40:808–819, 1984
10. HAND DJ, TAYLOR CC: *Multivariate Analysis of Variance and Repeated Measures*. London, Chapman & Hall, 1987
11. SMOLIN LA, LAIDLAW SA, KOPPLE JD: Altered plasma free and protein-bound sulfur amino acid levels in patients undergoing maintenance hemodialysis. *Am J Clin Nutr* 45:737–743, 1987
12. WOLFSON M, JONES MR, KOPPLE JD: Amino acid losses during hemodialysis with infusion of amino acids and glucose. *Kidney Int* 21:500–506, 1982
13. KOPPLE JD, SWENDSEID ME, SHINABERGER JH, UMEZAWA CY: The free and bound amino acids removed by hemodialysis. *Trans Am Soc Artif Int Organs* XIX:309–313, 1973
14. ONO K, SASAKI T, WAKI Y: Glucose in the dialyzate does not reduce the free amino acid loss during routine hemodialysis of non-fasting patients. *Clin Nephrol* 21:106–109, 1984
15. GUTIERREZ A, BERGSTRÖM J, ALVESTRAND A: Hemodialysis-associated protein catabolism with and without glucose in dialysis fluid. *Kidney Int* 46:814–822, 1994
16. IKIZLER TA, FLAKOLL PJ, PARKER RA, HAKIM RM: Amino acid and albumin losses during hemodialysis. *Kidney Int* 46:830–837, 1994
17. MAEDA K, KAWAGUCHI S, MANJI T, SAITO A, KOBAYASHI A, ITO K, SASA R, NISHI H, SHINTANI T, YOKOYAMA I, YAMAMOTO Y, OHTA K: Clinical evaluation of amino acid addition into dialysate. *J Med Assoc Thailand* 61(Suppl 1):88–96, 1978
18. FEINSTEIN EI, COLLINS JF, BLUMENKRANTZ MJ, ROBERTS M, KOPPLE JD, MASSRY S: Nutritional hemodialysis. *Progr Artif Organs* 421–426, 1983
19. GARIBOTTO G, DEFERRARI G, ROBAUDO C, SAFFIOTI S, PAOLETTI E, PONTREMOLI R, TIZIANELLO A: Effects of a protein meal on blood amino acid profile in patients with chronic renal failure. *Nephron* 64:216–225, 1993
20. LIPMAN TO: Efficacy and safety of total parenteral nutrition. *Nutrition* 6:319–329, 1990